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SPECIAL BULLETIN

American Institute of BAKING

400 E. ONTARIO ST. CHICAGO 11, ILL

Number 80 July, 1954

VITAMIN D FORTIFICATION OF BREAD

In April, 1953 special bulletin No. 75 from American Institute of Baking carried the following recommendations of its Scientific Advisory committee:

1. That the baking industry refrain from using its prerogative to add vitamin D to bread since bread is not considered the best possible vehicle for this nutrient; or

2. That those bakers who consider it desirable to use vitamin D refrain from using it in quantities that would permit claims greater than 400 U.S.P. units per pound; and

3. That bakers see to it that their product furnishes sufficient calcium to permit simultaneous claims for calcium should they add vitamin D at levels up to 400 U.S.P. units per pound of bread.

These recommendations were challenged in an article published August 15, 1953. As a result of this challenge the Institute requested the opinion of the Food and Nutrition Board of the National Research Council. Recently the Institute has been advised that at the April meeting of the Food and Nutrition Board, an ad hoc committee on Addition of Vitamin D to Bread made its report and the Board adopted the following statement:

> The Board has recognized vitamin D and calcium as optionally added ingredients in enriched flour and bread since the early hearings on enrichment of flour. Sunshine plays an important role in supplying vitamin D, but natural foods contain little of this vitamin. For this reason, its addition to certain staple foods is important in preventing rickets. It is generally recognized that milk is the most logical vehicle for carrying vitamin D. However, it is fortunate that

> food technology has demonstrated that other foods, especially bread, can be used as an effective vehicle and that such foods can be enlisted when and if it becomes necessary to do so. Since milk, including fluid, evaporated, and special milk formulae, as a carrier of vitamin D has been so successful in reducing the incidence of rickets in this country and since there is no indication of an increase in rickets -- in fact, there seems to be a continuing decrease -- the Board believes it is unnecessary at the present time to call upon bread to aid in the correction of deficiencies of vitamin D in the general population or significant segments of the population.

This statement of the Board would seem to confirm the Scientific Advisory committee's view that the use of vitamin D in bread, at the present time, contributes little to the nutritional welfare of the American public.

American Pharmaceutical Association. 1970

National Formulary, Ed. XIII

American Pharmaceutical Association, Washington, D.C.

Anon.

Jama
Vol. 198: 47

Third International Congress of Nephrology

Peritoneal Dialysis Removes Vitamin D

A group of investigators at the National Heart Institute, Bethesda, Md, have demonstrated, the first time, that vitamin D can be removed from the body by peritoneal dialysis.

The technique may thus prove to be effective in treatment of patients who absorb an excess of the vitamin during vitamin D therapy (hypervitaminosis D).

Significant amounts of vitamin D as well as calcium were removed during two peritoneal dialyses in a 54-year-old woman with hypercalcemia secondary to hypervitaminosis D, R. I. Henkin, MD, told the Third International Congress of Nephrology.

The removal of calcium from extracellular fluid by peritoneal dialysis has been previously demonstrated, and treatment of hypercalcemia by this technique has been accepted for several years.

"The removal of vitamin D by peritoneal dialysis has not been previously demonstrated, however," Dr. Henkin said.

The patient, who eventually died from acute renal failure, had received slightly more than 100 million units of vitamin D orally and intramuscularly over a period of 3½ months for vitamin D resistant osteomalacia.

Vitamin therapy was discontinued when hypercalcemia was noted. Three months after therapy was discontinued, she experienced a series of grand mal seizures. Serum calcium concentration was 20.4 mg/100 ml, and serum vitamin D concentration was greater than 3,500 units/100 ml.

Peritoneal dialysis was promptly begun, using a calcium-free, balanced electrolyte solution to which 4% glucose was added.

A total of 27,000 units of vitamin D were removed during the first dialysis. Serum vitamin D concentration was reduced to 1,340 units/100 ml—still abnormal, but considerably below the pre-therapy level.

A total of 59,000 units of vitamin D were removed during the second dialysis. Serum concentration of the vitamin at the conclusion of the second dialysis was not obtained.

The patient showed marked clinical improvement after both the first and second dialysis, although relapse followed both treatments. The patient's BUN gradually rose after the second dialysis to above 150 mg/100 ml, and she died on the fourth day after dialysis.

The mechanism by which vitamin D diffuses across the peritoneal membrane is not clear, Dr. Henkin said, since in man, almost all vitamin D is bound to beta lipoprotein. Beta lipoprotein could not be detected in the dialysate, suggesting that the vitamin D apparently diffused across the peritoneal membrane in a free form.

Dr. Henkin's co-workers are M. Lotz, MD, and F. C. Bartter, MD.

THE LANCET

The Need for Vitamin-D Supplements

Bone is a highly specialised form of connective tissue which has mechanical rigidity. The rigidity is due to orderly deposition of calcium salts on an uncalcified matrix, the osteoid. The bones in adults, and even more so the growing bones in children, undergo constant remodelling, with the simultaneous deposition and resorption of calcium salts. The rate of deposition of calcium salts as bone depends primarily on the activity of osteoblasts in the formation of osteoid. The processes involved in the mineralisation of osteoid are ill-defined, but mineralisation is certainly a function of the osteoblasts. A fundamental requirement is an adequate concentration, or supply, of calcium and phosphate ions in the extracellular-fluid compartment. If there is a reduction in the concentration of calcium and phosphate ions in that compartment, or if there is a defect in the osteoblast mineralisation process, then the newly formed osteoid will not be mineralised. If calcium salts, for some reason, are not deposited on newly formed osteoid tissue, the result is an excess of uncalcified matrix and loss of structural rigidity; these are the characteristic features of the disease due to vitamin-D deficiency—in adults osteomalacia, in children rickets.

Vitamin D is the name given to a group of steroid compounds with antirachitic properties. Cholecalciferol (vitamin D_3), the naturally occurring compound in man, is formed in the skin from 7-dehydrocholesterol by ultraviolet-light irradiation. In the absence of sufficient endogenous synthesis of cholecalciferol—particularly in northern latitudes with limited ultraviolet light in solar and sky radiation—the dietary intake of vitamin D becomes important, and in this situation the consequent rickets or osteomalacia is usually termed nutritional. Since the primary defect is one of endogenous cholecalciferol synthesis, a more appropriate term is ultraviolet-deprivation rickets or osteomalacia.

The problem of ultraviolet-deprivation osteomalacia or rickets in countries such as Britain is not new. The early reports of rickets date from the middle of the seventeenth century.[1,2] The appearance of rickets in England at that time coincided with the growth of cities during the industrial revolution: the pall of coal-smoke that hung over these cities blocked solar ultraviolet-irradiation and endogenous cholecalciferol synthesis. Infantile rickets was common in Britain until 1945, when addition of cholecalciferol to National dried-milk virtually eliminated the disease. In the past decade, however, reports of rickets among indigenous White children and immigrant Asian children have appeared with increasing frequency. The incidence of radiological rickets among urban schoolchildren was lately reported as about 4%.[3] The increasing incidence of infantile rickets has been attributed to mothers' unawareness of the need for vitamin-D supplements once dried-milk feeds cease.[4] In immigrant Asian children, among whom the incidence of rickets is the greatest, there is evidence of cholecalciferol deficiency[4]; additionally, in this population, dietary factors also play a role.[5-8]

Cholecalciferol deficiency is a problem in adults as well as in children—indeed, the cholecalciferol status of the general British population may verge on deficiency.[9,10] In the past decade it has been established that ultraviolet-deprivation osteomalacia is common among elderly women.[11-14] In this population group exposure to sunlight is low, since elderly people, particularly when living alone, tend to be more apathetic and reluctant to go out of doors; physical disabilities may also limit mobility. Ultraviolet-deprivation and inadequate dietary vitamin-D intake in the elderly are also possibly involved in another bone problem—osteoporosis. This is the commonest bone disease in old age, and foremost among its causes are impaired calcium absorption and negative calcium balance.[15] Nordin[16] proposed that cholecalciferol deficiency in the elderly, although it may be severe enough occasionally to cause osteo-

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malacia, "is probably responsible for or at least aggravates the steady deterioration in bone status which we tend to accept as an ageing process". NORDIN's concept would suggest that the effect of cholecalciferol deficiency in the elderly depends on the degree of deficiency; a relatively mild degree of cholecalciferol deficiency manifests itself as osteoporosis while a severe degree of cholecalciferol deficiency presents as osteomalacia.

Ultraviolet-deprivation and consequent endogenous cholecalciferol deficiency is a problem of our time which merits urgent attention. There are three ways in which this problem, and its metabolic bone consequences, can be tackled. Firstly, vitamin-D supplements should be given to the elderly, particularly the house-bound who are at high risk. This proposal is consistent with the recommendation of the Panel on Nutrition of the Elderly,[17] which also suggested that, for this population group, fortification of liquid milk may be necessary. Secondly, the vitamin-D fortification policy for foods available in Britain needs to be radically revised, so that values are comparable to those in other northern countries such as the United States and Canada. (The existing fortification programme in Britain covers essentially infant foods; little other vitamin-D fortification is undertaken, except in margarine.) Thirdly, an educational programme should be instituted to inform mothers that, once dried-milk feeding ceases, children under 5 need vitamin-D supplements, and to make everyone, and particularly the immigrant population, aware of the dietary sources of vitamin D and the importance of an adequate intake.

In at the Deep End

IN 1959, MALLESON described a novel method of treating examination phobias.[18] He suggested that continual avoidance of the feared situation reinforced the phobia, and that, if the patients deliberately thought about the awful consequences of failure whenever examination fears were aroused, the phobias were considerably improved. In learning-theory terms, their fears were said to be "reactively inhibited". This treatment would now be called flooding-in-fantasy, or implosion[19]—as opposed to flooding-in-practice, in which the patient is exposed to the actual phobic stimulus. It is only lately that the potentialities of this method of treatment have been systematically investigated. One reason is that, in the years after MALLESON's paper, more attention was paid to another form of behaviour therapy, desensitisation.[20] This treatment, involving gradual exposure to the phobic stimulus in a setting of mental and muscular relaxation, appeared to contradict MALLESON's approach. When, however, desensitisation was shown to be of limited efficacy in many patients[21] and often too time-consuming to be suitable for widespread use, other forms of treatment were re-examined. Flooding is one of the most effective of these. (The treatment has many other names, but perhaps flooding is the most appropriate for a subject which lends itself so readily to aquatic metaphors.) The important principle of treatment that MALLESON described has remained the same. The patient's chief symptoms are deliberately induced in a setting where avoidance is prevented.

Flooding was soon shown to be useful in volunteers with phobias,[22] and investigations were extended to include psychoneurotic patients in clinical practice. The approach has been used principally in the treatment of phobic and obsessional disorders, and in chronic anxiety. The most impressive results have been with phobic patients. Flooding-in-practice is at least of equivalent efficacy to desensitisation, and improvement is more rapid.[23-25] There is some indication that more complex phobias such as agoraphobia are particularly helped,[23] as are other phobias in which there is much pre-existing anxiety.[24] The length of the treatment session is important; for best results the patient should be calm and relaxed at the end of each session. Preliminary results in the treatment of obsessional compulsive disorders are also encouraging. Patients with these disorders carry out their obsessional rituals in order to relieve persistent anxiety. If their rituals are deliberately prevented their anxieties initially increase, but later they improve, with a reduction in obsessional behaviour.[26,27] The technique has also been used with chronically anxious patients by DONN. He induces even greater anxiety in these patients by using the pharmacological stimulus of intravenous sodium lactate, first described by PITTS and MCCLURE.[28] His preliminary results are encouraging,[29,30] and the method merits further investigation.

There are many questions which need answering before flooding and similar therapies (such as "modelling"[31] and "shaping"[32]) can be used on a widespread scale in clinical practice. The actual mechanism of improvement is still open to question. Psychiatric symptoms cannot be regarded purely as

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Fed. Regist. 38: 20737, 1973

FINAL ORDER

Therefore, on the basis of the foregoing findings of fact, discussion, and conclusions of law drawn therefrom: *It is ordered*, That the stay of effective date of § 80.1, which stay was promulgated December 14, 1966 (31 FR 15730), be ended and that § 80.1 be modified to read as follows:

PART 80—DEFINITIONS AND STANDARDS OF IDENTITY FOR FOOD FOR SPECIAL DIETARY USES

§ 80.1 Dietary supplements of vitamins and minerals; definition, identity, label statements.

(a) The dietary supplements of vitamins and/or minerals for which definitions and standards of identity are prescribed by this section are prepared and offered as tablets, capsules, wafers, or other similar uniform units; in powder, granular, flake, or liquid form; or in the physical form of conventional foods; and purport to be or are represented for special dietary use by man to supplement his diet by increasing the total dietary intake of one or more of the essential vitamins and/or minerals specified in paragraph (f) of this section. The dietary supplements of vitamins and/or minerals are henceforth referred to as "dietary supplements" in this section.

(b) Classifications of dietary supplements.

(1) A dietary supplement shall contain only those vitamins and/or minerals listed in paragraph (f) of this section and shall be offered for its vitamin and/or mineral content only in the following combinations, with the provision that any vitamin or mineral defined as optional in paragraph (f) of this section may be omitted:

(i) All vitamins and minerals.

(ii) All vitamins.

(iii) All minerals.

(iv) All vitamins and the mineral iron.

(v) Inclusion of the optional ingredients vitamin D and/or phosphorus in a multivitamin, multimineral, or multivitamin and multimineral supplement does not require inclusion of any additional optional ingredients. Inclusion of the optional ingredients biotin and pan-

tothenic acid and/or copper and zinc in such products does not require inclusion of vitamin D and/or phosphorus when the latter two nutrients are optional. The inclusion of any of the other optional ingredients (biotin or pantothenic acid or vitamins and copper or zinc for minerals) requires the inclusion of both such optional ingredients if the product is a multivitamin or multimineral supplement, and requires the inclusion of all four such ingredients if the product is a multivitamin and multimineral supplement.

(2) A dietary supplement may also be composed of any single vitamin or mineral listed in paragraph (f) of this section.

(3) Folic acid is optional for liquid dietary supplements because of instability of the vitamin in liquid preparations. Liquid dietary supplements containing multivitamins but not containing folic acid shall bear the following statement on the label, "This product does not contain the essential vitamin folic acid" which shall immediately follow the listing of vitamins, and minerals as prescribed in paragraph (i) of this section.

(4) Addition to or amendment of the list of permissible combinations of vitamins and/or minerals contained in paragraph (b)(1) of this section may be proposed by the Commissioner of Food and Drugs, on his own initiative, or upon petition by an interested person in accordance with the procedures set forth in Part 2 of this chapter. Any such petition shall be submitted in the form set forth in § 2.65 of this chapter, and shall include scientific data of a human nutritional and/or technological nature to support such addition or amendment as being consistent with the definition and purpose of dietary supplements as described by this section. The Commissioner, upon request, may extend the effective date of this section with respect to any particular product or class of products pending consideration and any administrative or court proceedings relating to any such petition, and may set a new effective date upon completion of the matter.

(5) The provisions of this section shall not apply to any food which contains or consists of any vitamin or mineral listed in § 125.1(b) of this chapter, or any combination thereof, provided that all of the following requirements are met: (i) No such nutrient is contained at a level of 50 percent or more of the adult U.S. RDA per serving for that nutrient. (ii) No direct or implied representation is made on the label, in labeling, or in advertising that the product is a dietary supplement or is adequate or appropriate for supplementing the daily diet with essential nutrients, and (iii) The product is labeled pursuant to the provisions of § 1.17 of this chapter.

(c) General definitions.

(1) Subject to good manufacturing practices, dietary supplements described in this section shall contain in the specified daily quantity no less than the lower limit nor more than the upper limit of each nutrient specified in paragraph (f) of this section for the group(s) for which the supplement is offered.

(2) For the purposes of this section, the term "daily quantity" means the quantity of a dietary supplement that shall be specified in the labeling of the dietary supplement for consumption in a period of 1 day, and which shall be an amount or number of units reasonably suitable for and practicable of consumption in 1 day.

(3) In the event a dietary supplement is offered for more than one specific group, the specified daily quantity for each group shall be stated separately on the label.

(d) The different groups described in § 125.1(b) of this chapter shall be designated as follows: For labeling purposes:

(1) Infants.

(2) Children under 4 years of age.

(3) Adults and children 4 or more years of age.

(4) Pregnant or lactating women.

(e) Section 80.1 does not apply to:

(1) Other standardized foods.

(2) Foods the composition of which is defined by other regulations or statutes.

(3) Any food represented for use as the sole item of a meal or of the diet.

(4) Foods represented for use solely under medical supervision to meet nutritional requirements in specific medical conditions.

(5) Raw agricultural commodities (including marine products) and fabricated and other conventional foods to which single or multiple vitamins and/or minerals are added to improve nutritional quality, unless the total level (including any naturally occurring amounts) of any added vitamin or mineral per single serving attains or exceeds 50 percent of the U.S. Recommended Daily Allowance (U.S. RDA) for adults and children 4 years or more of age as specified in § 125.1(b) of this chapter, in which case the provisions of both this section and § 1.17 of this chapter shall apply. If the provisions of both this section and § 1.17 apply to a food, the labeling of such food shall conform to the labeling established in this section except that the labeling established in paragraph (c) of § 1.17 of this chapter, including the order for listing vitamins and minerals established in paragraph (c)(7)(iv) of that section, shall be used in lieu of the labeling established in paragraph (i)(1) of this section.

(6) Raw agricultural commodities (including marine products) which by their nature are sources of a specific vitamin(s) and/or mineral(s) at a level that, if it had been added, would have brought them within the definition of a dietary supplement. Such shall meet all the requirements of this section if they are represented as dietary supplements. Such foods may also be used as a source of specific vitamins and/or minerals in dietary supplements meeting the requirements of this section.

(7) A food with nutrients restored to pre-processing levels or added pursuant to § 1.8(e) of this chapter so that it is not nutritionally inferior to the food for which it substitutes and which it resembles.

(f) Permissible qualitative and quantitative composition of dietary supplements.

(1) The following table sets forth the permissible qualitative and quantitative composition of dietary supplements of vitamins and/or minerals:

U.S. GOVERNMENT RECOMMENDED DAILY ALLOWANCES (U.S. RDA's) AND PERMISSIBLE COMPOSITIONAL RANGES FOR DIETARY SUPPLEMENTS OF VITAMINS AND MINERALS

	Unit of Measurement	Children under 4 years of age [1]—U.S. RDA			Adults and children 4 or more years of age—U.S. RDA			Pregnant or lactating women—U.S. RDA		
		Lower Limit		Upper Limit	Lower Limit		Upper Limit	Lower Limit		Upper Limit
Vitamins										
Mandatory										
Vitamin A	International Units	1,250	2,500	2,500	2,500	5,000	5,000	5,000	8,000	8,000
Vitamin D [1]	do	200	400	400				400	400	400
Vitamin E	do	5	10	15	15	30	45	30	30	60
Vitamin C	Milligrams	20	40	60	30	60	90	60	60	120
Folic acid [2]	do	0.1	0.2	0.3	0.2	0.4	0.4	0.4	0.8	0.8
Thiamine	do	0.35	0.70	1.05	0.75	1.50	2.25	1.50	1.70	3.00
Riboflavin	do	0.4	0.8	1.2	0.8	1.7	2.6	1.7	3.0	3.4
Niacin	do	4.5	9.0	13.5	10.0	20.0	30.0	20.0	20.0	40.0
Vitamin B_6	do	0.35	0.70	1.05	1.00	2.00	3.00	2.00	2.50	4.00
Vitamin B_{12}	Micrograms	1.5	3.0	4.5	3.0	6.0	9.0	6.0	8.0	12.0
Optional										
Vitamin D	International Units				200	400	400			
Biotin [3]	Milligrams	0.075	0.150	0.225	0.150	0.300	0.450	0.300	0.300	0.600
Pantothenic acid.	do	2.5	5.0	7.5	5.0	10.0	15.0	10.0	10.0	20.0
Minerals Mandatory										
Calcium	Grams	0.125	0.500	1.000	0.125	1.000	1.500	0.125	1.300	2.000
Phosphorus [4]	Do	0.125	0.500	1.000	0.125	1.000	1.500			
Iodine	Micrograms	35	70	105	75	150	225	150	150	300
Iron	Milligrams	5	10	15	9	18	27	18	18	60
Magnesium	do	40	200	300	100	400	600	100	450	900
Optional										
Phosphorus [5]	Grams							0.125	1.300	2.000
Copper	Milligrams	0.5	1.0	1.5	1.0	2.0	3.0	1.0	2.0	4.0
Zinc	Do	4.0	8.0	12.0	7.5	15.0	22.5	7.5	15.0	30.0

[1] When labeled for use by infants, a dietary supplement shall contain not less than the lower limit designated for a nutrient in this column nor more (nad 100% of the infant U.S. RDA for a nutrient as prescribed in § 125.1(b) except that the level of biotin, when used, shall be 0.06 mg per daily recommended quantity.

[1] Optional for adults and children 4 or more years of age.

[2] Optional for liquid products.

[3] Lower limit may be 0.06 milligram until December 31, 1976.

[4] Optional for pregnant or lactating women. When present, the quantity of phosphorus may be no greater than the quantity of calcium.

(2) The U.S. Recommended Daily Allowances (U.S. RDA's) have been derived by the Food and Drug Administration from the "Recommended Dietary Allowances," published by the Food and Nutrition Board, National Academy of Sciences—National Research Council, and are subject to amendment from time to time as more information on human nutrition becomes available.

(g) Acceptable ingredient sources for dietary supplements:

(1) A vitamin or mineral used in a dietary supplement may be supplied by any suitable chemically synthesized or naturally produced substance which is not a food additive as defined in section 201(s) of the act; or if it is a food additive as so defined, it shall be used in conformity with regulations established pursuant to section 409 of the act.

(2) Any safe and suitable substance may be used as a preservative, stabilizer, flavor, sweetener, color, seasoning, carrier, base, or vehicle, or to facilitate preparation of the vitamin and mineral substances. A dietary supplement shall be prepared so that any such substance contained therein does not exceed the amount reasonably required to accomplish its intended physical or technical effect, and so that the biological availability of the vitamin(s) or mineral(s) is not impaired by the presence of such substance. Any such substance shall not be a food additive or color additive as defined in section 201 (s) or (t) of the act; or if it is a food additive or color additive as so defined, it shall be used in conformity with regulations established pursuant to section 409 or 706 of the act.

(h) Nomenclature.

(1) The common name of a dietary supplement shall consist of a term descriptive of the vitamin and/or mineral composition of the product, as established in paragraph (h)(2) of this section, together with a phrase or phrases designating the group(s) for which the supplement is represented, as established in paragraph (h)(3) of this section (e.g., "multivitamin and multimineral supplement for children under 4 years of age"). The name of the dietary supplement shall appear prominently and conspicuously on the principal display panel(s) of the label. The letters of the phrase(s) designating the consumer group(s) for which the product is represented shall be no less than one-third the size of those used in the term descriptive of the composition of the product. In addition to the common name prescribed by this paragraph, a dietary supplement may be labeled with a proprietary name: *Provided*, That it is not false or misleading in any particular.

(2) The terms used to describe the vitamin and/or mineral composition of dietary supplements listed in paragraphs (b) (1) and (2) of this section shall be as follows: (i) "Multivitamin and multimineral supplement" for a dietary supplement containing all vitamins and minerals, (ii) "multivitamin supplement" for a dietary supplement containing all vitamins, (iii) "multimineral supplement" for a dietary supplement containing all minerals, (iv) "multivitamin and iron supplement" or "multivitamin supplement with iron" for a dietary supplement containing all vitamins and the mineral iron, (v) "———supplement" for a dietary supplement containing a single vitamin or mineral listed as a mandatory or optional ingredient in the table contained in paragraph (f)(1) of this section (the blank to be filled in with the name of the vitamin or mineral).

(3) The phrases used to designate the group(s) for which a dietary supplement is intended shall be as follows: (i) "For infants"; (ii) "For children under 4 years of age"; (iii) "For adults and children 4 or more years of age"; and (iv) "For pregnant or lactating women".

(i) Format for listing vitamins and minerals, and ingredients: (1) Immediately following the name and group designation on the principal display panel, as required by paragraph (h) of this section, or on the information panel (21 CFR 1.8d) if insufficient space is available on the principal display panel, the label shall bear a listing in tabular form of each of the vitamins and/or minerals supplied by the specified daily quantity of the dietary supplement, such daily quantity being specified at the top of the list. The vitamins and/or minerals shall be described by the names appearing in paragraph (f) of this section, shall appear in the order listed in paragraph (f) of this section, and shall be grouped and identified separately as "vitamins" and/or "minerals" without reference to "mandatory" or "optional." The quantity of each vitamin and/or mineral present in a specified daily quantity of the dietary supplement shall be stated as a part of this list and expressed in percentage of the U.S. RDA for each specific group for which the supplement is offered. The quantity of each vitamin and/or mineral present in the specified daily quantity of the dietary supplement shall also appear in the tabular listing in terms of the units of measure specified in paragraph (f) of this section.

(2) For determining the percentage contents of the U.S. RDA's present in the dietary supplement, the quantitative content of the following vitamins shall be calculated in terms of the following chemically identifiable reference forms:

REFERENCE FORM

Vitamin	Name	Empirical formula	Molecular weight
Vitamin C	*L*-Ascorbic acid	$C_6H_8O_6$	176.12
Folic acid	Pteroyl monoglutamic acid	$C_{19}H_{19}N_7O_6$	441.41
Niacin	Nicotinic acid	$C_6H_5NO_2$	123.11
Riboflavin	Riboflavin	$C_{17}H_{20}N_4O_6$	376.37
Thiamine	Thiamine chloride hydrochloride	$C_{12}H_{17}ClN_4OS.HCl$	337.28
Vitamin B_6	Pyridoxine	$C_8H_{11}NO_3$	169.18
Vitamin B_{12}	Cyanocobalamin	$C_{63}H_{88}CoN_{14}O_{14}P$	1,355.40
Biotin	*D*-Biotin	$C_{10}H_{16}N_2O_3S$	244.31
Pantothenic acid	*D*-Pantothenic acid	$C_9H_{17}NO_5$	219.23

(3) The following synonyms may be added in parentheses immediately following the name of the vitamin in the statement described in paragraph (i)(1) of this section:

Vitamin	*Synonym*
Vitamin C	Ascorbic acid.
Folic acid	Folacin.
Riboflavin	Vitamin B_2.
Thiamine	Vitamin B_1.

(4) A separate list of ingredients in the product shall be included on the label pursuant to the requirements of Part 1 of this chapter. Such list shall include the natural source or chemical form of each individual nutrient present in the dietary supplement.

(j) When the dietary supplement is in liquid form and contains alcohol, the label shall state the percent-by-volume of alcohol present.

(k) A dietary supplement containing one or more nutrients, subject to deterioration below the labeled value before consumption shall bear on its outside wrapper or container, as well as on the label of its immediate container, the statement: "Expiration date ———", the blank to be filled in by the month and year. The expiration date shall be the date selected by the manufacturer, packer, or distributor of the dietary supplement on the basis of tests or other information showing that the dietary supplement, until that date, under the conditions of handling, storage, and use prescribed by directions appearing on its label, or, in the absence of such prescribed directions, under customary or usual conditions of handling, storage, and use will contain not less than the quantity of each vitamin and/or mineral, as set forth on its label, when consumed.

(l) All labeling information required by this section shall comply with the conspicuousness required by section 403(f) of the act and § 1.8d of this chapter. In addition, the following labeling requirements shall be met.

(1) The list of nutrients required by paragraph (i)(1) of this section shall appear in uniform type size.

(2) The synonyms permitted by paragraph (i)(3) of this section, if used, and the list of ingredients required by paragraph (i)(4) of this section shall appear in uniform type size, and in type size no larger than that used for the list of nutrients required by paragraph (i)(1) of this section.

(m) In addition to the requirements of this section, the labels and labeling for a dietary supplement is subject to the prohibitions contained in § 125.2(b) of this chapter.

Effective date. Labeling may be changed to comply with this regulation beginning August 2, 1973. All labeling for dietary supplements that is ordered after

December 31, 1973, and all labeling used for such products shipped in interstate commerce after December 31, 1974, shall comply with the provisions of this section; and if not so labeled, such product shall be deemed to be misbranded under section 403(a), (g) and (j) of the act.

(Secs. 201(n), 401, 403(a) and (j), 701(a) and (e), 52 Stat. 1046, 1048, 1055-1056, as amended by 70 Stat. 919; 21 U.S.C. 321(n), 341, 343(a) and (j), 371(a) and (e))

Dated: July 25, 1973.

A. M. SCHMIDT,
Commissioner of Food and Drugs.

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38: 6951, 1973

PART 1—REGULATIONS FOR THE ENFORCEMENT OF THE FEDERAL FOOD, DRUG, AND COSMETIC ACT AND THE FAIR PACKAGING AND LABELING ACT

Nutrition Labeling

In the FEDERAL REGISTER of January 19, 1973 (38 FR 2125), the Commissioner of Food and Drugs promulgated a new section on nutrition labeling under Title 21, § 1.17 Food; nutrition labeling. A period of 30 days was provided for additional comments suggesting technical changes or corrections. More than 200 comments were received from industry, consumer, and professional groups, trade associations, and individuals. Each of the suggested modifications and request for clarifications has been reviewed, and the Commissioner's conclusions are as follows:

1. Requests to make nutrition labeling mandatory were again received from consumer groups. In the preamble to the regulation as published on January 19, 1973, the Commissioner stated his conclusion that current information is insufficient to adopt a mandatory nutrition labeling regulation at this time. No additional information has been brought to the attention of the agency to alter this decision

2. Consumer comments again requested complete ingredient labeling and labeling of the percentage of ingredients. The Commissioner has issued a separate policy statement on labeling of all ingredients in standardized foods in the FEDERAL REGISTER of January 19, 1973 (38 FR 2137), and is publishing in this issue of the FEDERAL REGISTER a new Part 102 which establishes a procedure for including the percentage of characterizing ingredients as part of the common or usual name of a food.

3. Comments were submitted questioning the legality of § 1.17. This matter was fully discussed in the preamble to the regulation as published on January 19, 1973. See "Federal Power Commission v. Texaco," 377 U.S. 33 (1964); "United States v. Storer Broadcasting Co.," 351 U.S. 192 (1956); "Securities Exchange Commission v. Chenery," 332 U.S. 194 (1946); "Abbott Laboratories v. Gardner," 387 U.S. 136 (1967); "Ciba Geigy v. Richardson," 446 F. 2d 466 (2d Cir. 1971).

4. Comments were received which stated that the Food and Drug Administration does not have authority to regulate advertising of food products. The Commissioner agrees with this statement. Section 1.17 in no way attempts to regulate claims made in advertising. If a nutrition claim is made in advertising, however, § 1.17 provides that the label of the product must comply with requirements of that section. Once nutrition claims are made for a food, the consumer who wants to use the product for its nutrient content is entitled to examine a label which is complete enough to provide information at the time of purchase and use upon which an intelligent determination may be made as to the total nutrient content of the food.

5. A number of comments requested further clarification of the circumstances under which nutrition labeling becomes mandatory, and in particular what constitutes a "nutrition claim."

The Commissioner has adopted no rigid rule in determining exactly what type of claim will require nutrition labeling. In general, any claim designed to state or give the impression that a food is a good source of nutrition generally, or of a particular nutrient, will require compliance with § 1.17. Thus, mention of any nutrient (i.e., vitamins, minerals, protein, calories, carbohydrates, fat, fatty acids, or cholesterol), or any claim that the food is nutritious, will fall within § 1.17. In close cases, the content of the claims and the impression intended or produced in the public will be controlling. Since a manufacturer or distributor can easily avoid nutrition claims if he chooses to do so, close cases will ordinarily be decided in favor of requiring compliance with §1.17.

6. Questions have been raised as to whether a food may be labeled in compliance with § 1.17 rather than existing Part 125, pending the uniform effective date and implementation of the changes in Part 125 and § 80.1 published as tentative orders on January 19, 1973 (38 FR 2143, 2152). In publishing new § 1.17, the Commissioner concluded that it is in the public interest that transition to labeling under § 1.17 begin immediately. Accordingly, any food meeting the requirements of § 1.17 will be exempt from existing Part 125 to the extent that § 1.17 and the tentative order for new Part 125 so provide, effective as of January 19, 1973. Similarly, any food meeting the requirements of the tentative orders for new Part 125 and § 80.1 will be exempt from existing Part 125 to the extent that existing Part 125 is changed, effective as of the same date.

7. One comment requested a clear statement that nutrition labeling does not apply to foods designed for use by animals other than man. Section 1.17 applies only to food for human use, as is clearly shown by its provisions. The Commissioner concludes that it is unnecessary explicitly to state this fact in the regulation itself.

8. Questions have been raised whether an industry-wide trade advertisement for a type of food requires nutrition labeling for all members of the sponsoring group. The Commissioner has concluded that, while nutrition claims in labeling an advertising of individual manufacturers will require nutrition labeling for a product, manufacturers will not at this time be subject to nutrition labeling solely because of advertising programs of trade associations or educational organizations. Thus, a nutrition claim for a class of processed foods, or for a specific commodity, when made by a trade organization or educational group, will not in itself require any manufacturer or distributor of the food mentioned to use nutrition labeling if the manufacturer or distributor or his brand(s) is not identified. However, if such material is used by a manufacturer or distributor to promote a specific brand, the fact that the material was prepared and used by the trade or educational organization for general information programs will not exempt such brand from nutrition labeling.

9. Several comments asked that restoration of nutrient levels not be considered an addition of nutrients that would require full nutrition labeling, when such restoration provides only nutrient levels contained in the product prior to processing. The Commissioner has given this comment very careful consideration, and concludes that it is not feasible for a number of reasons. First, there are extremely few foods for which nutrient restoration occurs only up to levels found in the product prior to processing. Any exemption for these products would therefore be so limited as to be illusory, and would not be of any significant assistance to those companies concerned with the problems of meeting the new nutrition labeling requirements. Second, any product for which restoration of nutrients is made would undoubtedly refer to that fact in labeling or advertising, which would independently require use of nutrition labeling. Again, the result would be that any exemption would, for all practical purposes, be nonexistent. Third, any such exemption, if it did result in a significant number of foods with restored nutrients without nutrition labeling, would deprive consumers of important nutrition information about the foods they consume. In this respect, foods with restored nutrients are no different than fortified or enriched foods. Finally, the comments submitted with respect to this issue fail to substantiate any particular products for which this requested exemption would be appropriate. If in fact restoration is found to present a special problem for any particular food, the Commissioner will entertain a petition directed specifically to that problem at that time.

10. Numerous manufacturers asked that they be allowed to state only the caloric content on products which contain no vitamins or minerals and 50 calories or less. Others asked that the exact caloric content be stated when the total caloric content of a serving is less than 50 calories. These correspondents pointed out that at low calorie levels, e.g., 4 calories per serving, the manufacturer would be required to label the product either as zero or as 10 calories, both values being potentially misleading to the consumer. One of the strong consumer positions received following publication of the proposal on March 30, 1972, asked for a clear statement of the vitamin and mineral content of foods which make claims for low calories, and for those which are frequently consumed between meals and provide calories but offer little in terms of other nutrients. The Commissioner concludes that the provision requiring full nutrition labeling when caloric content was stated is reasonable and should be retained.

11. Requests were received from consumers and consumer groups to require full nutrition labeling when cholesterol content is stated. Their comments suggested that foods represented as useful in modified diets because of their cholesterol content should be identified as to their complete nutrition value, as required when fatty acid information is stated in labeling, because consumers need more complete nutrition information to determine how a food can fit into their diet.

Comments from manufacturers asked that the requirement for complete nutrition labeling whenever fatty acid information is present be deleted. They contended that the fatty acid information alone, or with caloric information, was all that was wanted or needed by consumers.

The Commissioner has reviewed this question, and concludes that the original exemption from full nutrition labeling when cholesterol content is provided should be removed. Many of the foods on which either or both fatty acid and cholesterol information will be stated make important contributions of several nutrients to the diet, and the consumer will be best served if full nutrition labeling is required. The requirement for full nutrition labeling when fatty acid information is given is retained.

12. Some manufacturers have placed statements on the labels of their products offering consumers nutrition information if they write to the company. These manufacturers expressed concern that they would no longer be able to carry out such information programs unless the products bear full nutrition labeling. Trade associations pointed out that, unless this approach were permitted, the consumer would receive less nutrition information.

The purpose of nutrition labeling is to provide consumers with information at the points of purchase and use to compare products, to evaluate nutritional claims which have been made for a product, and to prepare a nutritious diet. Providing information independent of the label would not meet these objectives at the point of purchase. However, some companies may not wish voluntarily to adopt nutrition labels, and others may be concerned about placing nutrition information on their labels where nutrient values may be changing or too uncertain to permit label declaration in advance. Where companies have developed what they feel is adequate current information, but would prefer to carry out additional studies to support a more permanent label statement, it would seem appropriate to permit them to offer such information to consumers by a uniform label statement. This has been provided for in the regulation, by permitting a manufacturer to state "For nutrition information write to _____ _____," without requiring full nutrition labeling. Any information provided in response to the label statement is labeling that is subject to the format and compliance requirements for nutrition labeling. Any product with an added nutrient or for which any other nutrition claim is made on the label or in labeling or advertising may not use this approach to avoid nutrition labeling.

The Commissioner recognizes that this limited exception could be subject to abuse. The agency will maintain surveillance over this matter to determine whether it should be retained. If such label statements become a substitute for full nutrition labeling, and are used by companies which have sufficient label space and nutrition data to utilize full nutritional labeling, the Commissioner will promptly revoke this exception.

To conform with this change, the provision in § 1.17(f) relating to labeling statements offering additional nutrition information upon request has been deleted. This deletion results in no change in substance. A food that is subject to § 1.17, as well as one that is not subject to § 1.17, may include an offer to supply additional nutrition information on request. A food not subject to § 1.17 would be limited to furnishing the nutrition information specified in § 1.17, but a food subject to § 1.17 could furnish any additional truthful nutrition information it might choose.

13. A request was made that food be permitted to contain 50 percent of the U.S. RDA, and that a dietary supplement contain more than 50 percent. The Commissioner has concluded, however, that any product with nutrients added so that it contains half the nutrient level established as the U.S. RDA is properly regarded as a dietary supplement rather than as a food (unless such fortification is specifically provided for in a standard of identity, nutrition guideline, or other applicable regulation). Accordingly, no change has been made in this provision in § 1.17(a).

14. One comment reflected concern that § 1.17 may prohibit the sale of all food which naturally contains 50 percent or more of the U.S. RDA for a given vitamin or mineral unless the food conforms to § 80.1 or another standard of identity. Such an interpretation is incorrect. Those products in which the naturally occurring level of a nutrient equals or exceeds 50 percent of the U.S. RDA, and those foods in which addition of a nutrient(s) is permitted or required by regulation, e.g., a standard of identity or nutritional quality guideline, or is

otherwise exempted by the Commissioner, are not prohibited and need not conform to the requirements of § 80.1. Only those foods in which a nutrient has been added so that it equals or exceeds the 50 percent of the U.S. RDA must conform to the requirements of § 80.1, as well as § 1.17. This matter was discussed fully in the preamble to this regulation as published on January 19, 1973, and in the discussion of the proposed findings for § 80.1 (38 FR 2152, 2157), also published on January 19, 1973.

15. Comments were received from many manufacturers asking that, in addition to the use of a serving as a basis for nutrition labeling and fatty acid and cholesterol labeling, the designation of a "portion" be permitted. The term "portion" was defined in several ways: The amount reasonably consumed in 1 day; a quantity expressed in common household terms but not necessarily related to a serving, e.g., 2 ounces of margarine, or 1 cup of flour; or the complete container when that quantity would usually be used as an ingredient in the preparation of a meal component, e.g., a can of tomato paste used in preparing a meat sauce.

The Commissioner recognizes that there are many foods for which servings cannot easily be stated. However, nutrition labeling statements based on unreasonably large quantities could confuse consumers and make comparisons between products difficult or impossible. In seeking reasonable statements of serving sizes the Commissioner concludes that, for most products, the requirements for serving size as stated in the final regulation published on January 19, 1973, shall be maintained. Manufacturers may express a "portion" for a food which is used only as an ingredient in other foods; and shall do so in an easily identifiable serving quantity in household terms, e.g., cupfuls or teaspoonfuls or ounces, or a complete container where this information is not misleading to consumers.

16. A comment was received stating that the use of the phrase "adult male engaged in light physical activity" indicated that § 1.17(b) was intended to apply only to this segment of the population. This is erroneous. The phrase is used only as a guide in attempting to define a serving.

17. Several comments suggested that the serving or portion size should be standardized so that labels will reflect a uniform approach from which consumers may readily make comparisons. The Commissioner agrees that serving and portion sizes should be uniform. Under the regulation it is incumbent upon industry and consumers to work together to devise uniform serving and portion sizes. If this does not materialize, the Commissioner will establish a procedure for adopting uniform serving and portion sizes that will be applicable to all foods.

At least one comment expressed concern that requiring declaration of nutrient content on the basis of a serving or portion would understate the nutritional value where it may be consumed at each of three meals during the day. The Commissioner recognizes that repeated consumption of a food item with a moderate or low nutritional content may well result in a significant nutrient contribution to the daily diet. Accordingly, § 1.17(b) has been revised to provide that, where a food is commonly consumed more than once a day, the label shall state the nutrient content on a serving or portion basis and may, in addition, also state the nutrient content when consumed on a daily basis. For example, it may be appropriate for bread to be shown per two slices (a serving) and per six slices (per day), and for milk to be shown per glass (a serving) and per quart (per day). In order to justify any such statement, reliable data, such as statistically valid dietary surveys, must be available to show that the food is in fact customarily consumed more than once daily and the total daily amount usually consumed.

18. It has been suggested that nutrition labeling should be provided both with respect to the food in the package and with respect to the food as prepared for consumption after cooking or other home preparation. Section 1.17(b) has been modified to permit this information. Where this optional form of declaration is used, the specific method of preparation or cooking must be specified. Manufacturers are also encouraged to provide consumers with additional information on methods of cooking or preparation which will result in maximum retention of nutrients. The Commissioner has concluded that requiring nutrient declaration on the basis of the product as consumed is not feasible because, for many products, there are numerous variations of cooking or other methods of preparation, and enforcement would not appear to be feasible.

19. Consumer groups and individual consumers expressed great concern that some deviations from the standard format are permitted. They asked that the label have the basic information on the seven nutrients plus protein present every time that nutrition labeling is used. Consumer groups also preferred to have zero listed when less than 2 percent of the U.S. Recommended Dietary Allowance (U.S. RDA) of a nutrient is present.

The comments from manufacturers asked that a more flexible position be taken on the question of listing the percent of the U.S. RDA. It was suggested that if no vitamins or minerals are present in a food, a statement "contains no significant amounts of nutrients" or "contains no nutrients" be permitted. Some manufacturers also asked that, for foods containing only a single component contributing only calories, only the caloric content and the amount of this component be required, e.g., corn starch containing only carbohydrate and no vitamins or minerals.

The Commissioner has concluded that a standard format is desirable whenever reasonable, since this will assist those developing consumer information and educational programs. This matter was fully discussed in the preamble to the January 19, 1973, regulation. The exception permitted in the regulation, when no more than three of the vitamins or minerals are present at levels of 2 percent or greater, was based in part on the results of consumer studies suggesting that a few positive values intermingled with zeros or asterisks could be misunderstood. A statement that the other nutrients are not present is required immediately following the listing of the nutrients by percent U.S. RDA.

The Commissioner concludes that a change in the basic format proposed in the January 19, 1973, regulation is not warranted. Accordingly, the format is retained. However, several technical changes suggested in order to make the basic format more readable, and reduce possible confusion, are reasonable and have been adopted. The optional statement "per serving" has been added to the heading "Nutrition Information" in order to make it clear that the basis of the information is on a serving. The term "Calories" can be used as an alternate term for "Caloric content." Similarly, the term "content" may be eliminated as a required part of the headings for the protein, carbohydrate, and fat statements. The placement of fatty acid information has also been clarified by stating that it must follow the statement on fat, rather than be adjacent to the statement.

Several manufacturers pointed out that the statement "Percentage of the U.S. RDA for fat, protein, vitamins, and minerals" could be shortened by deleting the portion "for protein, vitamins, and minerals," as it was obvious that these were what was stated. The Commissioner agrees that this latter phrase is not needed, and the regulation has been corrected.

In stating the exception to the complete format of the U.S. RDA percentage of seven nutrients, the fact that, with protein, there are eight items to be listed was overlooked. In order to correct this error, the section relating to the exception has been changed to read that if five or more of the eight nutrients required are present at less than 2 percent of the U.S. RDA the alternate labeling approach may be used.

20. The question of stating calories to the nearest calorie, rather than in 10 calorie increments, has been carefully considered, and the Commissioner agrees that at calorie levels less than 50 calories a more correct statement of calories should be permitted. The regulation has been slightly modified in § 1.17(c)(3) to permit the use of 2-calorie increments up to 20 calories and 5-calorie increments above 20 calories up to and including 50 calories. Ten-calorie increments will be used above 50 calories. At the lower caloric levels compliance will be more difficult, but careful monitoring of protein, fat, and carbohydrate content can provide a means of determining if real misrepresentations are being made by the low calorie statements.

Several objections were raised concerning the 2, 5, and 10 percent of the U.S. RDA intervals used to state the protein, vitamin, and mineral levels. Some persons felt that 5 or 10 percent intervals should be used, while a few felt that 1 percent intervals over the entire range should be permitted. The 2 percent intervals at the lower level of nutrients was incorporated on the basis of consumer, industry, and nutrition educator comments on the original proposal, and are reasonable. Using smaller intervals over the entire range will in the opinion of the Commissioner only result in consumer confusion.

21. Several manufacturers objected to the double listing for protein, both as grams of protein and as a percent of the U.S. RDA, under § 1.17(c)(4). They were particularly concerned about a product which contains no protein, and which would have to list zero twice, or zero and an asterisk, on the label.

The Commissioner concludes that there is a need to maintain the basic nutrition labeling format, and there appears to be no special hardship in the situation mentioned. In many of the instances where protein content is zero the manufacturer will be able to use the limited listing plus the statement that specific nutrients are not present, thus eliminating the second zero statement from the listing under the percentage of the U.S. RDA.

22. Several manufacturers and trade associations raised questions about the procedure to be used for establishing a value for the U.S. RDA of protein in a food. Some asked for a third standard of 30 grams of protein to be used with those animal protein foods with the highest biological quality. They pointed out that the use of 45 grams of protein as a standard for all proteins equal to or better than casein put the better quality proteins at a disadvantage.

A review of the proposed standard does not support such a change at this time. As the Commissioner stated in the preamble to the regulation as published on January 19, 1973, further extension of subdivisions of protein types does not appear justified. As experience with nutrition labeling is gained, and additional information is obtained concerning simpler procedures for evaluating protein quality, the Commissioner will evaluate the procedure for establishing the U.S. RDA value for labeling protein and make any appropriate modifications.

Several manufacturers also requested the Commissioner to establish a standard conversion factor for converting nitrogen content to protein as part of the analytical method for protein in food. The factor of 6.25 times the nitrogen content is commonly used to calculate protein, but scientists recognize that this factor gives larger protein values than are determined by more accurate and complex procedures for certain food proteins. The alternate conversion factors for specific proteins are available in the literature, and could be used when use of the common conversion value of 6.25 results in significant overstatement of the protein content.

In reviewing this matter, the Commissioner is of the opinion that for labeling purposes use of the 6.25 conversion factor is satisfactory. However, methods for determining protein content listed in the Official Methods of Analysis of the Association of Official Analytical Chemists, 11th Ed., 1970 (AOAC), give more correct factors for the conversion of nitrogen to protein, and when a conversion factor other than 6.25 is given in the AOAC methods that value shall be used.

23. Many manufacturers stated that they felt that the calculation of caloric content by the Atwater method as required by the regulation is more accurate than required. They suggested that the more commonly used values of 4, 4, and 9 calories per gram of protein, carbohydrate, and fat, respectively, be used as these are actual standard values derived from the Atwater values.

The reference to the Atwater method to determine caloric content was included in the final regulation in order to provide manufacturers with a standard guide for calculating calories. This procedure was developed to account for those factors which led to caloric contents less than expected due to reduced absorption or biological availability. The Commissioner is aware that for most foods, the caloric factors of 4, 4, and 9 calories per gram of protein, carbohydrate, and fat, respectively, will give a satisfactory statement for caloric content. However, there are situations where the calculation of caloric content using the 4, 4, and 9 calories per gram values will misrepresent the caloric content of the product. In such situations, where more accurate values are given in the reference cited in the regulation, USDA Handbook 74 (1955), it would appear reasonable for manufacturers to use the correct values. The agency will follow this procedure outlined above in determining caloric content in order to establish if a product is in compliance. Manufacturers can identify those situations where the use of the 4, 4, and 9 calorie per gram values will result in a label value more than 20 percent greater than the actual value and in those cases the values in the USDA Handbook 74 should be used in determining the caloric content of the food.

24. Comments were received objecting to any declaration of the U.S. RDA greater than 100 percent in a serving. Those taking this position were concerned that consumers would be misled by such claims, and also that claims of significance on the basis of 10 percent of the U.S. RDA, e.g., 120 percent compared to 110 percent, would confuse consumers.

In naturally occurring products such nutrient levels are rare. When they do occur, the Commissioner concludes that it is unreasonable to deny those marketing the product the right truthfully to label the nutritional content.

The Commissioner is aware that the addition of vitamins or minerals to a food at levels greater than 50 percent of the U.S. RDA may in some cases be allowed. However, when such values are added to a food and not covered by a regulation permitting such levels of added nutrients, the product will be regulated as a dietary supplement under § 80.1 (38 FR 2152).

The Commissioner is of the opinion that the issue of proper food fortification cannot be appropriately dealt with under nutrition labeling. The Commissioner stated in the preamble to the regulation as published on January 19, 1973, that the agency is preparing a number of regulations regarding the addition of nutrients to foods. New Part 100, establishing nutrition guidelines, is published in this issue of the FEDERAL REGISTER. In addition to specific regulations and guidelines, the Commissioner will prepare a regulation incorporating overall agency policy on addition of nutrients to foods. Until this action is complete, the agency will review food fortification on an individual basis using the principles stated in existing and newly published regulations.

25. One manufacturer pointed out that the language stating that vitamins and minerals other than the basic five vitamins and two minerals could be listed, did not make it clear that these must be listed as a percent of the U.S. RDA. The language has been changed to make this clear. In addition, the mandatory listing of the other vitamins when they are added is also made clear by a slight modification in the regulation.

26. A few comments were received requesting that values other than those selected for the U.S. RDA be chosen for nutrition labeling in § 1.17(c)(7)(iv). As stated in the preamble to the regulation as published on January 19, 1973, this question was carefully considered, and the values selected were those considered appropriate for nutrition labeling as well as for use by the agency in establishing standards for special dietary foods and dietary supplements. While no change in the current U.S. RDA will be made at this time, the Commissioner will review the U.S. RDA values periodically to insure that they are in general agreement with the National Academy of Sciences' values for the Recommended Dietary Allowances.

27. One comment was received pointing out that the terms "thiamine" and "folic acid" have been replaced by the terms "thiamin" and "folacin" in the listing of accepted nomenclature by the International Union of Nutritional Sciences (Nutrition Abstracts and Reviews, 40, 395, 1970), the recognized international nutrition organization. The Food and Drug Administration uses the nomenclature of the United States Pharmacopeia, and their accepted spelling and names for these two products are used in the regulation.

28. One comment was received asking that choline and inositol be included in the list of essential nutrients in order to permit their inclusion in products such as infant formulas, products intended for use as the total daily diet, medical diets, and similar products. Such products are

special dietary foods, and this question is therefore properly considered under Part 125. This comment will be included with those received on that document in response to the tentative order published on January 19, 1973 (38 FR 2143). Special dietary foods are specifically exempted from nutrition labeling under § 1.17(h) (1), (3), and (4). Any change in the list of essential nutrients under Part 125 will be accompanied by reconsideration of the list of nutrients appropriate for label declaration under § 1.17(c)(7)(iv).

29. One comment was received asking that, in addition to the percent of the U.S. RDA, manufacturers be required to list the actual amounts of each nutrient present, e.g., milligrams or International Units. The Commissioner had considered this type of labeling in the early stages of development of nutrition labeling. As consumer understanding of the quantities was very limited and labeling as a percent of daily allowances offers a procedure for indicating the nutrient contribution of a food without extensive nutrition training, the use of actual values has been rejected for consumer labeling. Actual values may, under § 1.17(f) of the revised regulation, be furnished to interested professionals.

30. Several manufacturers objected to 10 percent of the U.S. RDA being established as the level where claims for significance can be made. Some suggested that 5 percent of the U.S. RDA would be more appropriate, while one comment said that a system relating caloric content and contribution of vitamins, minerals, and protein would be more appropriate.

The Commissioner recognizes that any minimum value established for claiming a significant nutrient content will be arbitrary. Since all foods will be able to indicate their nutrient content under nutrition labeling and consumers will be able to make nutrient comparisons, the point at which a product can claim to be a "significant" source of a nutrient takes on less importance than when actual nutrient contents were not available for consumer comparison. In addition, the use of 5-percent increments for protein, vitamin, and mineral levels between 10 percent and 50 percent of the U.S. RDA, and 10-percent increments at levels over 50 percent, make it unreasonable to select a value of 5 percent of the U.S. RDA or less as significant. The level of 10 percent of the U.S. RDA was selected as significant since it represents a major difference throughout most of the range at which nutrient values commonly are present in foods.

The use of a caloric-nutrient ratio for establishing the nutritional significance of a product is a concept which was considered in the early development of nutrition labeling. While the concept is one which nutritionists are currently developing, it is not readily understood by consumers, and to use such information would, in the opinion of the Commissioner, result in problems of consumer understanding and use. The Commissioner has therefore retained 10 percent of the U.S. RDA as the level for declaring significance as stated in the regulation published on January 19, 1973.

31. Information on other properties of food, e.g., fiber content, sugar content, or moisture content, is included on labels or in labeling by some manufacturers. Comments were received asking that such information be allowed to appear on labels. While such information is not nutrition information as described in this regulation, the Commissioner recognizes that some consumers want to know about these other properties of food. Such information can be included on the label or in labeling provided it is not false or misleading, and is not prohibited by other regulations. This information may not be placed on the label as part of or with nutrition labeling or other mandatory information, but it may immediately follow or otherwise be contiguous to the mandatory information on the information panel. The information on these other properties should be stated for the same serving size (portion) as used for nutrition labeling.

32. It has been suggested that the definition of a "lot," which is based solely upon the common container code or marking, may be inapplicable to methods of continuous production (e.g., bread and milk). In recognition of this possibility, § 1.17(e)(1) has been changed to state that, in such situations, a lot will be regarded as a day's production.

33. Concern was voiced by several manufacturers that official methods of analysis are not applicable to their products, and it would therefore be difficult to determine if they are in compliance. The Commissioner has stated in the regulation that the official procedures will be those of the Association of Official Analytical Chemists (AOAC) or other reliable and appropriate analytical procedures. Questions of methodology may be submited to the agency, and the suitability and applicability of alternative procedures will be resolved in that way.

34. Virtually all comments expressed concern about meeting the compliance requirements contained in the regulations within the time limits established by the uniform effective date. The Commissioner is aware that difficulties may be encountered by some firms, whereas for other firms compliance will not present any significant problem. Accordingly, the Commission has concluded to approach this matter in the following way.

First, there are some companies and industries which will find it difficult or even impossible to meet the first requirement under the uniform effective date, that labels printed after December 31, 1973, must comply with the new requirements. For those who are now gathering the data necessary for compliance, this represents insufficient time to obtain that information before such orders must be placed. For example, some companies and industries are now conducting intensive analytical surveys which must be continued over a sufficiently representative period of time in order to make them statistically valid. The Commissioner wishes to encourage this type of program which will for the first time result in a detailed data base upon which truthful and accurate nutritional labeling can rest. Accordingly, the Commissioner will entertain all reasonable requests for extension of this first part of the uniform effective date, where the petitioner shows the type of analytical data being obtained and the time schedule involved, as justification for this extension. Extensions will be granted only upon a showing of good cause therefor, based upon an on-going program. Such extensions should be requested promptly. All correspondence relating to such extensions will be placed on file for public review at the office of the hearing clerk.

Second, the Commissioner again reiterates the point made in the preamble to the regulations as published in the FEDERAL REGISTER of January 19, 1973, that temporary exemptions will be considered, upon a showing of economic hardship, for any enriched standardized food which, by reason of the standard, must include added nutrients and thereby is required by § 1.17 to bear nutrition labeling. Many manufacturers of these foods unquestionably have the quality control and analytical capabilities at this time to meet this requirement without significant difficulty. Other manufacturers, however, and particularly small manufacturers, may not presently have these capabilities. The Commissioner does not wish those manufacturers who cannot immediately meet these requirements to be forced to eliminate the nutrients from the food, or to discontinue production of the food, or to suffer an economic and competitive hardship. Accordingly, the Commissioner will entertain petitions which demonstrate such a hardship, and will grant temporary exemptions upon a showing of good cause. Such exemptions will not be granted on an across-the-board or industrywide basis, but rather upon a detailed factual showing by an individual firm justifying a temporary exemption.

The Commissioner anticipates that these exemptions will be only temporary in nature, not permanent. Adequate time will be provided for these manufacturers to obtain the necessary quality control and analytical methodology, or to contract for it, without undue hardship. It must be recognized, however, that no permanent exemption could be justified. Indeed, it would be unfair to permit any extensive exemption for one group of manufacturers and to require their competitors to achieve full compliance with § 1.17 within the uniform effective date.

Third, comments were received from several groups, including consumer advocates, suggesting that the agency establish interim compliance requirements that would allow greater variation from the stated label values for nutrients in Class II foods (i.e., foods with naturally occurring (indigenous) ingredients) after the December 31, 1974, effective date. Such an approach would allow a

substantially greater tolerance during the first year, with a more rigid requirement each year for 3 years until the requirement contained in § 1.17(e)(4)(ii) is reached. It was also suggested that a moratorium period be established during which only gross violations would result in regulatory action.

The Commissioner is in agreement with the concepts underlying these comments but believes that the matter should be approached in a different way. Many manufacturers, and particularly those who began 2 or 3 years ago to prepare for these regulations, are now fully able to meet the compliance requirements established in § 1.17. The compliance requirements adopted by the Commissioner are relatively flexible, and do not impose very difficult standards. Indeed, it is the intent of the Commissioner that they be tightened as experience develops with nutrition labeling, rather than relaxed. In any event, they present entirely realistic standards at this time for any company with sound quality control and who wishes to engage in nutrition labeling.

On the other hand, the Commissioner is well aware that this is an entirely new program, relying upon quality control requirements, analytical capabilities, and testing programs of a nature that have not previously been used. Like any new program, it will take time for both industry and Government to work out problems that are encountered. The Commissioner cannot and does not expect full and complete adherence to the exact compliance requirements established in § 1.17 on an immediate basis. It is entirely likely that, acting in the best of faith, deviations will occur.

The Commissioner concludes that the most equitable approach to this matter is to retain the compliance requirements of the regulation as published on January 19, 1973, but to exercise substantial discretion in their enforcement during the first few years in which they are in use. In exercising this discretion, the Commissioner will take into account all pertinent information. It is impossible to articulate a rigid compliance rule precisely because of the factors discussed above, i.e., in some instances full compliance is entirely feasible and should be required, whereas in other instances it cannot realistically be expected on an immediate basis. The principal factor that will guide enforcement policy will be the action taken by the manufacturer or distributor to obtain the analytical data, the quality control procedures, and the testing programs necessary to achieve compliance with the regulation. The Commissioner does not anticipate taking regulatory action during this initial period where a good faith attempt has been made to achieve compliance, even though exact compliance has not been achieved.

The Commissioner has concluded that an industrywide relaxation of the compliance requirements contained in § 1.17 or a moratorium on regulatory action based on a sliding scale of compliance during the next 3 years, is not in the public interest and is not justified by the facts. Those who have advocated this position are largely companies which chose not to prepare for this regulation by obtaining analytical data and establishing quality control procedures adequate to assure compliance with whatever regulation might issue. Several of their competitors, on the other hand, anticipated the new regulatory requirements that have now been promulgated and are fully prepared for nutrition labeling. The Commissioner concludes that, while enforcement flexibility will be retained, the failure of some manufacturers and distributors to prepare for these regulations cannot be accepted as justification for a total moratorium or a failure to require compliance where it should be achieved.

Food distributors who depend on many small- or medium-sized processors to provide a private label product have pointed out several problems which they feel are unique to this type of operation. Their suppliers are not uniformly capable of initiating programs to develop the information and controls required for nutrition labeling, and these distributors must print up their labels before they know what specific manufacturers may be among their suppliers during a given year. Distributors of private label products are therefore concerned that it may be impossible to place nutrition labeling on their products which they are sure will meet the compliance requirements as currently stated.

While the Commissioner agrees that these organizations have some problems which are unique to this form of marketing, it is not reasonable to require manufacturers of major brands to meet the compliance procedures while providing exceptions for certain of their competitors. In the discussion of comments on compliance and the use of representative values, the Commissioner has provided several alternatives to permit manufacturers to initiate nutrition labeling as soon as basic nutrient information is available without undue risk. A mechanism for obtaining sufficient time to achieve the compliance levels is provided to manufacturers, and is also available for distributors working with these manufacturers.

Fourth, some of the comments suggest that the second step of the uniform effective date, requiring that all food shipped in interstate commerce after December 31, 1974, be labeled in compliance with § 1.17, should be extended for up to 5 years, and particularly for Class II foods. This suggestion is closely tied to the problem of the compliance standard.

The Commissioner has concluded that no justification has been presented for extension of the effective date of the regulation beyond December 31, 1974. Petitions for extension of the first step of the effective date, requiring that all labels printed after December 31, 1973, shall conform to § 1.17, have already been discussed above.

Some comments noted that, for products containing Class II nutrients, it may take more than one growing season to obtain the data necessary for accurate nutrition labeling. The Commissioner is prepared to deal with these problems on an individual basis. If a manufacturer or a trade association begins immediately to establish a complete program to obtain the analytical data necessary for accurate nutrition labeling, the Commissioner will consider an extention of the December 31, 1973, label printing requirement. When those data become available a year from now, that manufacturer or trade association may then review them with the Food and Drug Administration and a determination may be reached as to whether they present sufficiently accurate and reliable information on which labels for the following year may be based. By repeating this process for the succeeding years, labels will bear the most accurate and reliable information available and consumers will have the assurance that the label represents the contents of the product as accurately and reliably as possible at the present time.

For the reasons explained at some length in the preamble to the regulation as published on January 19, 1973, the Commissioner has concluded that the industrywide or representative data presently available from such sources as USDA Handbook 8 is not sufficiently reliable or accurate to be a basis for nutrition labeling at this time. The Commissioner pointed out then, and reiterates now, that it is essential that representative data be checked by analysis of individual lots to give assurance that the label value adequately represents the product offered, at least until accurate and complete data are available on an industrywide, regional, and manufacturer basis. In time, the use of standard representative data, backed up by periodic analytical spot checks, will undoubtedly be possible in achieving compliance with § 1.17. The Commissioner encourages agricultural experiment stations, producer associations, food manufacturers, and others to expand efforts in the gathering of food nutrient composition data, in order to provide the information essential for the expansion of nutrition labeling.

35. One comment suggested that use of a standard compliance requirement of 20 percent for Class II nutrients is inequitable because it allows greater variability at a high level of the U.S. RDA and virtually no variability at a low level of the U.S. RDA. Moreover, variability at a low level of the U.S. RDA will have a far smaller effect on the overall nutrient content of the U.S. RDA. Moreover, variability at high level of the U.S. RDA.

The Commissioner recognizes the validity of this point. Nevertheless, even at levels of 10 percent of the U.S. RDA or less, the variability permitted using the 20-percent compliance standard is 2 percent or 1 percent of the U.S. RDA. In addition, the variability of the available analytical methods for determining nutrient content in Class II foods at levels

below 10 percent of the U.S. RDA in many instances exceeds this 20-percent variability permitted for compliance purposes under the regulation, and no regulatory action will be taken unless the nutrient level found in the product exceeds both the 20-percent compliance standard and whatever variability is recognized for the particular method being used. In all instances, therefore, the actual variability permitted will exceed the 20-percent standard specified under the regulation, and in many instances it will be double that standard or greater. Accordingly, the Commissioner has concluded that it is unnecessary to incorporate into the regulation any explicit provision to alleviate the narrower range of variability permitted at the low end of the U.S. RDA scale, because this is built into the regulation through the use of methodology that has its own degree of precision. The regulation has been revised to state that fact.

36. One organization requested that, for compliance purposes, minimum levels of calories, protein, carbohydrate, and fat be established below which values for these nutrients would not be subject to the compliance requirements of the regulation. The specific values suggested were 40 calories, 5 grams of protein, 10 grams of carbohydrate, and 5 grams of fat. Other comments pointed out that, for some foods, analytical problems would make it very difficult to evaluate compliance.

The Commissioner realizes that analytical problems do exist for some foods. However, for many foods the analytical procedures will permit determinations of even these low levels of protein, carbohydrate, and fat. For example, one can identify the fat content in a milk product with sufficient accuracy to distinguish between 3.2b grams and 3.5 grams of fat per 100 grams of milk product. Consumers should expect that products claiming only 1 gram of fat would have close to that amount, and not as much as 5 grams. A separate compliance position for these lower levels would actually provide no greater protection for manufacturers in terms of risk of mislabeling. Since the agency, as well as manufacturers, recognizes the analytical problems, compliance actions relating to these nutrient levels will reflect the accuracy of the available analytical methodology as well as the tolerance permitted in the regulation. Until experience with the regulation indicates that a more specific procedure is required for low levels of protein, carbohydrate, and fat content, this section of the regulation will be left unchanged. Since caloric content is actually directly related to the protein, carbohydrate, and fat content, the limits on these nutrients will provide a control on calories.

37. Many manufacturers have prepared pamphlets and charts for physicians, dietitians, and teachers providing more detailed nutrition composition information, including the actual amounts of the various nutrients. This labeling material often is not amenable to the format established in § 1.17, and in any event usually provides this information in more precise amounts rather than in the increments established in § 1.17. Several comments requested that these materials be permitted for use without requiring nutrition labeling, or at least that they not be required to be prepared in the exact format established in § 1.17.

It is not the intention of the agency to restrict the information flow from food manufacturers to professionals in the fields of health, nutrition, foods, or education generally. Thus, the Commissioner concludes that this type of labeling material should be permitted, and indeed encouraged, as long as it also contains, or has attached to it, nutrition labeling in the form in which it is provided to consumers under § 1.17. An appropriate change has been made in § 1.17(f) to reflect this.

The Commissioner also concludes that such material should not be permitted to be used without also requiring nutrition labeling for the foods involved. The nutrient values set forth in such material will be required to meet the compliance levels contained in § 1.17, and therefore the manufacturer or distributor of the food must already have sufficient information to permit nutrition labeling in order to use these other materials. Such materials, even when distributed only to professionals, are routinely redistributed to the public on a widespread basis, and often are a primary source of nutrition information for interested consumers. Thus, although such detailed materials should be encouraged as a good source of nutrition information, and should not be restricted to the label form established in § 1.17, the Commissioner concludes that no sound basis exists for exempting them from the general rule that the use of any nutrition information will require full nutrition labeling for the foods involved.

38. Nutrition labeling for infant and baby foods was included in the regulation at the request of professional organizations and individuals specifically interested in infant and child nutrition. Several comments were received requesting that the U.S. RDA contained in § 1.17(h)(1) for an infant or child under 4 years of age be further refined to include a separate U.S. RDA for infants from birth to 12 months. The Commissioner recognizes that the U.S. RDA standard that presently exists for children up to 4 years of age is not wholly adequate and should be improved. The Commissioner invites all interested persons to consider appropriate new U.S. RDA's for this age level which can be incorporated in § 1.17, and Part 125 in the future.

In the interim, the Commissioner concludes that the U.S. RDA levels established in this regulation are reasonable and will result in no harm whatever with respect to infant nutrition. None of the comments suggest that infant and baby foods should be totally exempt from nutrition labeling until better U.S. RDA's can be established. Accordingly, the regulation is not changed in this respect.

The fact that the U.S. RDA's for infants and babies may be revised should in no way deter food manufacturers from including this information on their labels, based upon the present regulation. When new U.S. RDA's are adopted, which may well take several years, the Commissioner will include an adequate effective date in order to provide manufacturers with sufficient time to achieve compliance, in order to avoid economic hardship that may result from label changes.

39. Those persons concerned with infant feeding also pointed out that no protein standard was established for labeling infant and junior foods. The Commissioner agrees that the standards of 45 grams and 65 grams to be used for nutrition labeling of protein in foods, as contained in § 1.17(c)(7)(ii)(c), are not suitable for labeling strained infant and junior foods. A standard protein value of 20 grams for protein with a protein efficiency ratio (PER) equal to or greater than casein, and a value of 28 grams of protein for other proteins with a PER less than casein, was suggested in several comments. These values appear to be reasonable for labeling baby food products, and are consistent with protein values of the National Academy of Sciences Food and Nutrition Board for this age grouping. These suggestions have been incorporated into the regulation for use by manufacturers of baby foods until a revised set of U.S. RDA for this age can be developed.

40. One comment pointed out that, although iodized salt is exempt from nutrition labeling when sold as such, any product to which it is added is not exempt from nutrition labeling. This was an oversight in the regulations, and has been corrected by a change in § 1.17(h)(5).

41. Exemptions for food packed for institutional use, for food being shipped in bulk containers, and for food shipped to a manufacturer as an ingredient for use in another processed food, were requested by many manufacturers. The basic arguments against providing nutrition labeling on these products was that the information would not be seen by the consumer. In the case of institutions such as hospitals, nursing homes, and schools, those commenting also pointed out that a dietitian was often involved in the ordering of the food, and the purchase specifications were more informative than the nutrition labeling.

The labeling regulations promulgated under the Federal Food, Drug, and Cosmetic Act, including § 1.17, apply to all food shipped in interstate commerce, unless otherwise exempted. The Commissioner recognizes that many products are manufactured only for use in institutional feeding, and that under these circumstances the ultimate consumer would not have an opportunity to see the label. In certain instances, e.g., hospitals and school lunch programs, the persons responsible for planning menus and purchasing food may have special training and be aware of the nutritional qualities of the foods, or may even establish

nutrition specifications for certain food products being purchased. However, nutrition labeling can serve to assist these individuals in identifying the most nutritious products, and provide a means for evaluating nutrition claims. The Commissioner has therefore decided that products requiring nutrition labeling because of added nutrients or label claims being shipped solely for institutional food service use shall be exempt from the requirements of the nutrition labeling regulation, if the manufacturer provides the nutrition information required by § 1.17 directly to those institutions and such information is kept current with changes in formulation or processing procedures. Manufacturers may also distribute factual information on the composition and nutritional quality of foods to professionals in the health and education fields which need not be in the format required for nutrition labeling, in addition to nutrition labeling.

The situation involving products being shipped from one manufacturer to another for use as ingredients in a processed food is different from foods manufactured and shipped to food service units which will prepare them for immediate consumption. The Commissioner concludes that bulk shipments of products such as flour, sugar, sirups, oils, and shortenings, or other products which are shipped in bulk form for use in manufacturing other foods shall be exempt from nutrition labeling. It is assumed that the manufacturer purchasing these food products will have established specifications for the product and no purpose would be served to require nutrition labeling.

43. Comments were received about the prohibition in § 1.17(i)(1) against labeling claims that the dietary properties of a food are adequate or effective in the prevention or treatment of any disease or symptom. Some comments erroneously interpreted this as prohibiting virtually any discussion of the nutritional usefulness of any food.

The Commissioner fully recognizes that all nutrients assist in preventing nutrient deficiencies and thus, in that sense, prevent disease. The prohibition contained in this provision in no way prohibits a truthful representation that a given nutrient is essential to human nutrition and good health and well-being. At the same time, it must be recognized that claims related to specific disease conditions render the product a drug under section 201(g)(1)(B) of the Act.

This prohibition was adopted because of widespread promotional practices stating or implying that a nutrient or food should be consumed in order to prevent a specific disease. For example, it has been common practice to promote nutrients by stating that a deficiency in a particular nutrient leads to a specified disease, thus implying that a consumer who fails to purchase the product in question may suffer from that disease or that the product in question will prevent that disease. The Food and Drug Administration and the Federal Trade Commission have successfully brought numerous cases to prevent such promotional practices over the years, but in spite of this success in the courts manufacturers and distributors of some products continue to use such claims.

The Commissioner concludes that reliance upon scare promotional claims, designed to panic the public into believing that nutrient fortification or supplementation is necessary to prevent the onset of severe deficiency diseases, is neither supported by the available scientific and medical facts nor fosters good nutritional practices. Promotion designed to educate the public about the need for basic nutrition planning for daily food needs, and to emphasize the importance of essential nutrients to good health, is both truthful and conducive to sound nutrition.

43. Comments were received objecting to § 1.17(i)(2), which prohibits the use of labeling claims suggesting that conventional foods in the diet, as usually prepared, are inadequate to meet nutritional needs. No data were furnished in the comments to show that a diet of conventional foods cannot satisfy all nutrition needs. To clarify this prohibition, however, the phrase "balanced diet" will be substituted for "diet," since it is obvious that an unbalanced or extremely poor diet could be deficient in nutrients. This prohibition was discussed fully in the preambles, findings of fact, and conclusions of law with respect to Part 125, published on January 19, 1973 (38 FR 2148).

44. Concern has been expressed that the labeling prohibitions contained in § 1.17(i)(3) and (4) would preclude factually supportable statements that, for example, a particular food has been handled by the producer or manufacturer in a special way in order to retain a higher nutrient content than competitive foods. This is not the intent of these prohibitions nor are they so worded.

These prohibitions are intended to prohibit unsupported generalizations about nutrient losses because of soil, transportation, and processing that have often been used in promotional literature in the past. They do not preclude a manufacturer or distributor who had adequate scientific data to show a higher nutrient retention in his product than in a competitor's product from making that claim. Nor do they prohibit a claim that a particular food has a higher nutrient content than is ordinarily true because of the soil in which it is grown, if that claim is backed up by adequate scientific data showing the differences between the soils involved and the resulting nutrient differences in the foods. Finally, a manufacturer may, without question, suggest cooking or handling methods for optimum nutrient retention. In order to clarify this, the phrase "the daily diet" is substituted for diets in § 1.17(i)(4). Any such claims will, of course, also require full nutrition labeling under § 1.17.

45. Several requests were received asking that manufacturers identify the natural and added vitamins in their products. The regulation permits, but does not require, such identification, and the statement of ingredients will in many instances reveal this information. The Commissioner concludes that there is insufficient differences between these two types of nutrients to justify requiring a special label designation of this kind.

Section 1.17(i)(6) forbids any suggestion that a natural vitamin is superior to an added vitamin, but permits any truthful designation of any nutrient as natural in origin. This prohibition was included because of widespread claims that nutrients of natural origin are superior to synthetic nutrients. At the present time, there is no valid scientific evidence to support such a claim of superiority, nor is there evidence to support any suggestion that synthetic nutrients are superior to natural nutrients.

One comment objected to § 1.17(i)(6) on the basis that it suggested that natural vitamins in a food were inferior to added vitamins. After reviewing the wording of the regulation, the Commissioner concludes that it in no way suggests that added vitamins are superior to vitamins natural to a food. Any such claim would be subject to prompt regulatory action.

46. One comment questioned the basis for delaring rutin and other bioflavonoids as unnecessary nutrients, and challenged the prohibition against combining these ingredients with nutrients already found to be essential in human nutrition. The basis for the Commissioner's conclusions on this matter was set out in detail in the preambles, findings of fact, discussion, and conclusions of law with respect to Part 125 and § 80.1, as published in the FEDERAL REGISTER for January 19, 1973 (38 FR 2143 and 2152). No data or information were submitted to show that any of these ingredients is essential in human nutrition or otherwise beneficial to health. The courts have previously upheld the Food and Drug Administration's position that inclusion of such ingredients in the product falsely represents nutritional value (United States v. Vitasafe, 226 F. Supp. 266 (D. N.J., 1964); United States v. Nuciomin, No. 71 C 585(2) (E.D. Mo., 1972)). Nevertheless, the Commissioner has concluded that it is reasonable to permit these ingredients to be marketed alone or in combination as foods, without claims for nutritional value. Any citizen who wishes to purchase and consume them will have an opportunity to do so.

47. The Commissioner has carefully reviewed and considered all comments, and to the extent that they are not encompassed within the above discussion or modifications made in the regulation they are rejected.

Accordingly, having considered the additional comments received and other relevant information, the Commissioner concludes that new § 1.17, as promulgated in the FEDERAL REGISTER of January 19, 1973 (38 FR 2125), should be repromulgated to reflect the technical modifications discussed above. Therefore, pursuant to provisions of the Federal Food, Drug, and Cosmetic Act (secs. 201, 403, 701(a), 52 Stat. 1040–1042 as amended, 1047, 1055; 21 U.S.C. 321, 343,

371(a)) and under authority delegated to him (21 CFR 2.120), the Commissioner hereby amends new § 1.17 to read as follows:

§ 1.17 Food; nutrition labeling.

(a) Nutrition information relating to food may be included on the label and in the labeling of a product: *Provided*, That it conforms to the requirements of this section. Except as provided in paragraph (b) of this section, inclusion of any added vitamin, mineral, or protein in a product or of any nutrition claim or information, other than sodium content, on a label or in advertising for a food subjects the label to the requirements of this section, and in labeling for a food subjects the label and that labeling to the requirements of this section.

(1) Solicitation of requests for nutrition information by a statement "For nutrition information write to _____" on the label or in the labeling or advertising for a food, or providing such information in a direct written reply to a solicited or unsolicited request, does not subject the label or the labeling to the requirements of this section if no other nutrition claim is made on the label or in other labeling or advertising, if the reply to the request conforms to the requirements of this section and contains no additional nutrition information, and if no vitamin, mineral, or protein is added to the food.

(2) If any vitamin and/or mineral is added to a food so that a single serving provides 50 percent or more of the U.S. Recommended Daily Allowance (U.S. RDA) for adults and children 4 years or more of age, as specified in § 125.1 of this chapter, of any one of the added vitamins and/or minerals, unless such addition is permitted or required in other regulations, e.g., a standard of identity or nutritional quality guideline, or is otherwise exempted by the Commissioner the food shall conform to the standard or identity set forth in § 80.1, of this chapter, and shall also conform to the labeling established in § 80.1 of this chapter, except that the labeling established in paragraph (c) of this section including the order for listing vitamins and minerals established in paragraph (c)(7)(iv) of this section, shall be used in lieu of the labeling established in § 80.1 (j)(1) of this chapter.

(b) All nutrient quantities (including vitamins, minerals, calories, protein, carbohydrate, and fat) shall be declared in relation to the average or usual serving or, where the food is customarily not consumed directly, in relation to the average or usual portion. Another column of figures may be used to declare the nutrient quantities in relation to the average or usual amount consumed on a daily basis, in the same format required in paragraph (c) of this section for the serving (portion), where reliable data have established that the food is customarily consumed more than once during the day and the average or usual amount so consumed.

(1) The term "serving" means that reasonable quantity of food suited for or practicable of consumption as part of a meal by an adult male engaged in light physical activity, or by an infant or child under 4 years of age when the article purports or is represented to be for consumption by an infant or child under 4 years of age. The term "portion" means the amount of a food customarily used only as an ingredient in the preparation of a meal component (e.g., ½ cup flour, ½ tablespoon cooking oil or ¼ cup tomato paste). A label statement regarding a serving (portion) shall be in terms of a convenient unit of such food or a convenient unit of measure that can be easily identified as an average or usual serving (portion) and can be readily understood by purchasers of such food (e.g., a serving (portion) may be expressed in slices, cookies, or wafers; or in terms of ounces, fluid ounces, teaspoonfuls, tablespoonfuls, or cupfuls).

(2) A teaspoonful shall be considered to mean 5 milliliters (approximately ⅙ fluid oz.) in volume; a tablespoon shall be considered to mean 15 milliliters (approximately ½ fluid oz.) in volume; and a cupful shall be considered to mean 240 milliliters (approximately 8 fluid oz.) in volume. The weight of the serving (portion) may also be expressed in grams.

(3) The declaration of nutrient quantities shall be on the basis of the food as packaged. Another column of figures may be used to declare the nutrient quantities on the basis of the food as consumed after cooking or other preparation, in the same format required in paragraph (c) of this section for the food alone: *Provided*, That the specific method of cooking or other preparation shall be disclosed in a prominent statement immediately following the information required by paragraph (c) of this section.

(c) The declaration of nutrition information on the label and in labeling shall contain the following information in the following order, using the headings specified, under the overall heading of "Nutrition Information Per Serving (Portion)." The terms "Per Serving (Portion)" are optional and may follow or be placed directly below the terms "Nutrition Information."

(1) "Serving (portion) size": A statement of the serving (portion) size.

(2) "Servings (portions) per container": The number of servings (portions) per container.

(3) "Caloric content" or "Calories": A statement of the caloric content per serving (portion), expressed to the nearest 2-calorie increment up to and including 20 calories, 5-calorie increment above 20 calories and up to and including 50 calories, and 10-calorie increment above 50 calories. Caloric content shall be determined by the Atwater method as described in A. L. Merrill and B. K. Watt, "Energy Value of Foods—Basis and Derivation," USDA Handbook 74 (1955).[1] Caloric content may be calculated on the basis of 4, 4, and 9 calories per gram for protein, carbohydrate, and fat respectively unless the use of these values gives a caloric value more than 20 percent greater than the caloric value obtained when using the more accurate values determined by use of the Atwater method as found in USDA Handbook 74 (1955).

(4) "Protein content" or "Protein": A statement of the number of grams of protein in a serving (portion), expressed to the nearest gram. Protein content may be calculated on the basis of the factor of 6.25 times the nitrogen content of the food as determined by the appropriate method of analysis of the Association of Official Analytical Chemists, 11th edition, 1970, except when the official procedure for a specific food requires another factor.

(5) "Carbohydrate content" or "Carbohydrate": A statement of the number of grams of carbohydrate in a serving (portion) expressed to the nearest gram.

(6) "Fat content" or "Fat": A statement of the number of grams of fat in a serving (portion) expressed to the nearest gram. Fatty acid composition, cholesterol content, and sodium content may also be declared in compliance with §§ 1.18 and 125.9 of this chapter.

(i) When fatty acid composition is declared, the information on fatty acids required by § 1.18(c) shall be placed on the label immediately following the statement of fat content. The declaratory information statement required by § 1.18 (c) shall be placed either immediately following the statement on fat and fatty acids or shall be appropriately referenced by symbol and placed immediately following the completed nutrition information statement.

(ii) When cholesterol content is declared, the information on cholesterol required by § 1.18(b) shall immediately follow the statement on fat content (and fatty acids, is stated). The declaratory information statement required by § 1.18 (d) shall be placed either immediately following the statement on cholesterol or shall be appropriately referenced by symbol and placed immediately following the completed nutrition information statement.

(iii) When both fatty acid and cholesterol information are provided, the declaratory information statement may be combined as permitted by § 1.18(d).

(iv) When sodium is declared, the information on sodium required by § 125.9 of this chapter shall be placed on the label immediately following the statement on fat content (and fatty acid and/or cholesterol, if stated).

(7) "Percentage of U.S. Recommended Daily Allowances (U.S. RDA)": A statement of the amount per serving (portion) of the protein, vitamins, and minerals, as described in this subparagraph, expressed in percentage of the U.S. Recommended Daily Allowance (U.S. RDA).

(i) The percentages shall be expressed in 2-percent increments up to and in-

[1] Copies may be obtained from: Division of Nutrition (BF-124), Bureau of Foods, Food and Drug Administration, 200 C Street SW., Washington, DC 20204.

cluding the 10-percent level, 5-percent increments above 10 percent and up to [illegible] including the 50-percent level, and [illegible]ercent increments above the 50-per[illegible] level. Nutrients present in amounts less than 2 percent of the U.S. RDA may be indicated by a zero, or by an asterisk referring to another asterisk placed at the bottom of the table and followed by the statement "contains less than 2 percent of the U.S. RDA of this (these) nutrient (nutrients)." However, when a product contains less than 2 percent of the U.S. RDA for each of five or more of the eight nutrients specified in subdivision (iii) of this subparagraph, the manufacturer or distributor may choose to declare no more than three of those nutrients and none of the remainder listed in subdivision (iv) of this subparagraph. The statement "contains less than 2 percent of the U.S. RDA of _____", listing whichever of the eight nutrients are present at less than 2 percent of the U.S. RDA and have not been declared, shall directly follow the declared nutrient in the same type size. Any nutrient declared shall always appear in the order established in subdivision (iv) of this subparagraph.

(ii) The declaration of protein, which shall come first, shall be a statement of the amount per serving (portion) of protein, expressed as a percentage of the U.S. RDA.

(a) The U.S. RDA of the protein in a food product is 46 grams if the protein efficiency ratio (PER) of the total pro[illegible] in the product is equal to or greater [illegible] that of casein, and 65 grams if [illegible] PER of the total protein in the product is less than that of casein. The percentage of the U.S. RDA shall be declared as described in subdivision (i) of this subparagraph.

(b) Total protein with a PER less than 20 percent of the PER of casein may not be stated on the label in terms of percentage U.S. RDA, and the statement of protein content in grams per serving (portion) under subparagraph (4) of this paragraph shall be modified by the statement "not a significant source of protein" immediately adjacent to the protein content statement regardless of the actual amount of protein present.

(iii) The declaration of vitamins and minerals as a percent of the U.S. RDA which shall follow the protein declaration, shall include vitamin A, vitamin C, thiamine, riboflavin, niacin, calcium, and iron, in that order, and shall include any of the other vitamins and minerals listed in subdivision (iv) of this subparagraph when they are added and may list any of the other vitamins and minerals listed in subdivision (iv) of this subparagraph when they are naturally occurring in the order listed therein.

(iv) The following U.S. Recommended Daily Allowances (U.S. RDA) and nomenclature are established for these vitamins and minerals, essential in human [illegible]trition:

[illegible]min A, 5,000 International Units.
[illegible]min C, 60 milligrams.[2]
[illegible]iamine, 1.5 milligrams.[2]
Riboflavin, 1.7 milligrams.[2]
Niacin, 20 milligrams.
Calcium, 1.0 gram.
Iron, 18 milligrams.
Vitamin D, 400 International Units.
Vitamin E, 30 International Units.
Vitamin B_6, 2.0 milligrams.
Folic acid, 0.4 milligram.[2]
Vitamin B_{12}, 5 micrograms.
Phosphorus, 1.0 gram.
Iodine, 150 micrograms.
Magnesium, 400 milligrams.
Zinc, 15 milligrams.
Copper, 2 milligrams.
Biotin, 0.3 milligrams.
Pantothenic acid, 10 milligrams.

These nutrients and levels have been derived by the Food and Drug Administration from the "Recommended Dietary Allowances," published by the Food and Nutrition Board, National Academy of Sciences-National Research Council, and are subject to amendment from time to time as more information on human nutrition becomes available.

(v) No claim may be made that a food is a significant source of a nutrient unless that nutrient is present in the food at a level equal to or in excess of 10 percent of the U.S. RDA in a serving (portion). No claim may be made that a food is nutritionally superior to another food unless it contains at least 10 percent more of the U.S. RDA of the claimed nutrient per serving (portion).

(d) Products with separately packaged ingredients or to which other ingredients are added by the user may be labeled as follows:

(1) If a product is comprised of two or more separately packaged ingredients enclosed in an outer container, nutrition labeling of the total product shall be located on the outer container to provide information for the consumer at the point of purchase. However, when two or more food products are simply combined together in such a manner that no outer container is used, or no outer label is available, each product shall have its own nutrition information, e.g., two boxes taped together or two cans combined in a clear plastic overwrap.

(2) If a food is commonly combined with another ingredient(s) before eating and directions for such combination are provided, another column of figures may be used to provide a list of the nutrient contents for the final combination in the same format required in paragraph (c) of this section for the the food alone (e.g., a dry ready-to-eat cereal may be described with one set of percentage U.S. RDA values for the cereal as sold (per ounce), and another set for the cereal and milk as suggested in the label (per ounce of cereal and one-half cup of vitamin D fortified whole milk); and a cake mix may be labeled with one set of percentage U.S. RDA values for the dry mix (per serving), and another set for a serving of the final cake when prepared). The type and quantity of the other ingredient(s), to be added by the user to the product shall be specified.

(e) Compliance with this section shall be determined as follows:

(1) A collection of primary containers or units of the same size, type, and style produced under conditions as nearly uniform as possible, designated by a common container code or marking, or in the absence of any common container code or marking a day's production, constitutes a "lot."

(2) The sample for nutrient analysis shall consist of a composite of 12 subsamples (consumer units), taken one from each of 12 different randomly chosen shipping cases, to be representative of a lot. Composites shall be analyzed by Association of Official Analytical Chemists (AOAC) methods where available or, if no AOAC method is available, by reliable and appropriate analytical procedures. Alternative methods of analysis may be submitted to the Food and Drug Administration to determine their acceptability.

(3) Two classes of nutrients are defined for purposes of compliance:

Class I. Added nutrients in fortified or fabricated foods.
Class II. Naturally occurring (indigenous) nutrients.

If any ingredient which contains a naturally occurring (indigenous) nutrient is added to a food the total amount of such nutrient in the final food product is subject to Class II requirements unless the same nutrient is also added.

(4) A food with a label declaration of a vitamin, mineral, or protein shall be deemed to be misbranded under section 403(a) of the act unless it meets the following requirements:

(i) *Class I vitamin, mineral, or protein.* The nutrient content of the composite is at least equal to the value for that nutrient declared on the label.

(ii) Class II vitamin, mineral, or protein. The nutrient content of the composite is at least equal to 80 percent of the value for that nutrient declared on the label.

Provided, That no regulatory action will be based on a determination of a nutrient value which falls below this level by a factor less than the variability generally recognized for the analytical method used in that food at the level involved.

(5) A food with a label declaration of calories, carbohydrates, or fat shall be deemed to be misbranded under section 403(a) of the act unless the nutrient content of the composite is no greater than 20 percent in excess of the value for that nutrient declared on the label.

(6) Reasonable excesses of a vitamin, mineral, or protein over labeled amounts are acceptable within good manufacturing practices. Reasonable deficiencies of calories or fat under labeled amounts are acceptable within good manufacturing practices.

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[2] The following synonyms may be added in parentheses immediately following the Biotin, 0.3 milligram.

Vitamin C	Ascorbic acid
Folic acid	Folacin
Riboflavin	Vitamin B_2
Thiamine	Vitamin B_1

(f) Nutrition information provided by a manufacturer or distributor directly to professionals (e.g., physicians, dietitians, educators) may vary from the requirements of this section but shall also contain or have attached to it the nutrition information exactly as required by this section.

(g) The location of nutrition information on a label shall be in compliance with § 1.8d.

(h) The following foods are exempt from this section or are subject to special labeling requirements:

(1) Except where expressly covered by § 125.5 of this chapter, infant, baby, and junior-type foods marketed and promoted for children under 4 years of age shall include nutrition information on the label and in labeling in compliance with this section except that the term "serving" shall mean that reasonable quantity of food suited for or practicable of consumption by an infant or child under 4 years of age and that the U.S. RDA levels for infants and children under 4 years of age contained in § 125.1(h) of this chapter shall be used in lieu of the U.S. RDA levels contained in paragraph (c)(7)(iv) of this section. For the purposes of labeling these foods with a percent of the U.S. RDA for protein, a value of 20 grams of protein shall be the U.S. RDA value for protein with a protein efficiency ratio (PER) equal to or greater than casein, and 28 grams if the PER of the protein is less than the PER of casein but greater than 20 percent of casein.

(2) Dietary supplements, the nutrients of which consist solely of vitamins and/or minerals, shall be labeled in compliance with §§ 80.1 and 125.3 of this chapter, except that the labeling of a dietary supplement in food form, e.g., a breakfast cereal, shall conform to the labeling established in paragraph (c) of this section, including the order for listing vitamins and minerals established in paragraph (c)(7)(iv) of this section, in lieu of the labeling established in § 80.1 (i)(1) of this chapter.

(3) Any food represented for use as the sole item of the diet shall be labeled in compliance with Part 125 of this chapter.

(4) Foods represented for use solely under medical supervision in the dietary management of specific diseases and disorders shall be labeled in compliance with Part 125 of this chapter.

(5) Iodized salt shall be labeled in compliance with § 3.87 of this chapter and when used in a food does not subject that food to labeling under this section if it is declared in the ingredient statement by its name (iodized salt) and neither iodine nor iodized salt is otherwise referred to on the label or in labeling or advertising.

(6) A nutrient(s) included in food solely for technological purposes may be declared solely in the ingredient statement, without complying with this section, if the nutrient(s) is otherwise not referred to on the label or in labeling or in advertising.

(7) A standardized food containing an added nutrient(s), e.g., enriched flour, and included in another food as a component may be declared in the ingredient statement by its standardized name, without compliance with this section, if neither the nutrient(s) nor the component is otherwise referred to on the label or in labeling or in advertising.

(8) Food products shipped in bulk form for use solely in the manufacture of other foods and not for distribution to consumers in such bulk form or container.

(9) Food products containing an added vitamin, mineral, or protein, or for which a nutritional claim is made on the label or in labeling or in advertising, which are supplied for institutional food service use only: *Provided*, That the manufacturer or distributor provides the nutrition information required by this section directly to those institutions on a current basis.

(i) A food labeled under the provisions of this section shall be deemed to be misbranded under sections 201(n) and 403(a) of the act if its labeling represents, suggests, or implies:

(1) That the food because of the presence or absence of certain dietary properties, is adequate or effective in the prevention, cure, mitigation, or treatment of any disease or symptom.

(2) That a balanced diet of ordinary foods cannot supply adequate amounts of nutrients.

(3) That the lack of optimum nutritive quality of a food, by reason of the soil on which that food was grown, is or may be responsible for an inadequacy or deficiency in the quality of the daily diet.

(4) That the storage, transportation, processing, or cooking of a food is or may be responsible for an inadequacy or deficiency in the quality of the daily diet.

(5) That the food has dietary properties when such properties are of no significant value or need in human nutrition. Ingredients or products such as rutin, other bioflavonoids, para-aminobenzoic acid, inositol, and similar substances which have in the past been represented as having nutritional properties but which have not been shown to be essential in human nutrition may not be combined with vitamins and/or minerals, added to food label in accordance with this section, or otherwise used or represented in any way which states or implies nutritional benefit. Ingredients or products of this type may be marketed as individual products or mixtures thereof: *Provided*, That the possibility of nutritional, dietary, or therapeutic value is not stated or implied (e.g., their labeling does not state that their usefulness in human nutrition has not been established and does not otherwise disclaim nutritional, dietary, or therapeutic value).

(6) That a natural vitamin in a food is superior to an added or synthetic vitamin, or to differentiate in any way between vitamins naturally present from those added.

Effective date. All labeling ordered after December 31, 1973, unless extended by the Commissioner on petition for good cause shown, and all labeling used for products shipped in interstate commerce after December 31, 1974, shall comply with this regulation. (Secs. 201, 403, 701(a), 52 Stat. 1040–1042 as amended, 1047, 1055; 21 U.S.C. 321, 343, 371(a).)

Dated: March 7, 1973.

CHARLES C. EDWARDS,
Commissioner of Food and Drugs.

NOTE: Incorporation by reference provisions approved by the Director of the Federal Register, January 15, 1973.

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The Nature of Vitamin D Resistance of Patients With Chronic Renal Disease

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Chronic renal disease is characteristically attended by derangements in calcium homeostasis and bone metabolism.[1-3] Many investigators have documented an impaired intestinal absorption of calcium in patients with chronic renal failure using either classical metabolic balance or isotopic marking techniques.[3-5] This defect has also been noted in patients without manifest azotemia or acidosis[3,6] and has been confirmed in chronic experimental renal insufficiency by Kessner and Epstein.[7] Liu and Chu initially considered a "resistance" to vitamin D action in the uremic individual as a cause for defective intestinal calcium absorption.[8] Stanbury and Lumb[3] and Dent and co-workers[9] have also suggested that the "acquired vitamin D resistance" results primarily in the defective absorption of calcium from the gastrointestinal tract. The subsequent studies of Fletcher et al made during the therapeutic trials of alkali, aluminum hydroxide, vitamin D, and calcium supplements in uremic osteodystrophic patients appear confirmatory in this regard.[10] Although the intestinal resistance to vitamin D has been repeatedly confirmed in uremic subjects, the relationship between the defective in situ bone mineralization observed in azotemic renal failure and the resistance to vitamin D therapy is presently still unknown. Since the development of renal osteodystrophy does not result in any further compromise in the intestinal absorption of calcium in patients already in renal failure,[4] it has been suggested that the relentless progression of the bone lesions in the chronic uremic state results primarily from a redistribution of calcium within the body.[3,4] Should this hypothesis prove correct, a resistance to vitamin D limited to the intestinal mucosa and leading sequentially to a state of chronic calcium deprivation, hypocalcemia, secondary hyperparathyroidism, osteitis fibrosa, and osteomalacia may suffice to explain the observed insensitivity to vitamin D in patients with renal osteodystrophy.

Whereas hypocalcemia, hyperphosphatemia, and disturbances in dietary intake, acid base balance, and parathyroid hormone secretion can be explained by the progressive loss of functioning renal mass,[2] the exact nature of the proposed vitamin D resistance of azotemic man still remains to be ascertained. Vitamin D_3 and its biologically active 25-hydroxycholecalciferol metabolite act primarily by affecting the remodeling of bone and by stimulating the intestinal transport of calcium. In this regard, 25-hydroxycholecalciferol is reportedly 1.4 times

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more active per unit weight than vitamin D_3 in curing rickets in rats and chicks and stimulates both in vivo and in vitro intestinal calcium and bone resorption more rapidly than vitamin D_3.[11,12] The so-called resistance to vitamin D therapy observed in the chronic renal state could represent either a defective metabolism of vitamin D; the expression of a circulating vitamin D antagonist; a failure in end organ (bone and intestine) responsiveness to vitamin D or 25-hydroxycholecalciferol or both; or a combination thereof. A failure in end organ (intestinal) responsiveness has been suggested by the clinical observation that raising plasma assayable vitamin D activity to ten times the normal value fails to influence the intestinal malabsorption of calcium in uremic subjects.[1] Since circulating vitamin D_3 and 25-hydroxycholecalciferol are normally bound to albumin, lipoproteins, and α-globulins,[13] the low molecular weight proteinuria[14] and lipoproteinuria[15] reported of patients with chronic renal disease could also result in significant wasting of these protein bound sterols.

To test the above hypothesis citing defective vitamin D_3 metabolism, the absorption and metabolism of a vitamin D_3 preparation labeled with tritium was recently evaluated in eight young adult patients with chronic renal failure in a controlled clinical research center environment. The chronic uremic state was identified by inulin clearances ranging from 2.3 to 11.4 ml/min, hyperphosphatasia, hyperphosphatemia, proteinuria, and roentgenographic evidence of severe bone demineralization.[16] The results of these studies revealed that although the intestinal absorption of vitamin D_3 was normal in the chronic uremic state, its metabolic fate was abnormal and characterized by a twofold increment in plasma fractional turnover rate, an abnormal accumulation of biologically inactive lipid-soluble vitamin D_3 metabolites in the plasma and a decrease in the circulating levels of 25-hydroxycholecalciferol. Whereas no qualitative difference in the circulating protein bound moieties of vitamin D_3 and 25-hydroxycholecalciferol were noted, the urinary excretion of both substances was appreciable, averaging 29.4% and 48.2% of the urinary lipid soluble material, respectively. These later observations were striking since, with the exception of small amounts of glucuronide and acid vitamin D_3 conjugates, vitamin D_3 and 25-hydroxycholecalciferol are normally not excreted by the kidney.[16] The studies also revealed that the alterations in the metabolism and excretion of vitamin D_3 and 25-hydroxycholecalciferol observed in the chronic uremic state were completely reversed following renal homotransplantation, whereas long-term intermittent dialysis was ineffectual in this regard.[16] Many of the abnormalities present in patients with chronic renal disease such as anemia, hypertension, glucose intolerance, thrombocytopenia, encephalopathy, and polyneuropathy are often improved by dialysis.[17-22] In contrast, the decreased intestinal absorption of calcium is usually unaffected by dialysis. Progressive renal osteodystrophy, and skeletal demineralization are not infrequently observed despite the reversal of hypocalcemia, hyperphosphatemia, and acidosis to normal.[18,23] These observations are consistent with the aforementioned findings that hemodialysis, in contrast to homotransplantation, was ineffectual in altering the derangement of the metabolism of vitamin D_3.

The results of these limited investigations in humans with chronic renal disease suggests that renal failure and the attendant chronic uremic state imposes a defect in the metabolic (enzymatic) conversion of vitamin D_3 to its active metabolite, 25-hydroxycholecalciferol, as well as an accelerated destruction of vitamin D_3 itself. It could be postulated that these abnormalities, combined with the unrelenting renal wastage of vitamin D_3 and 25-hydroxycholecalciferol, lead to a decrease in their circulating and tissue concentrations which in turn results in the intestinal malabsorption of calcium and a distortion of the normal sequence governing both the remineralization and remodeling of bone. A modification of this hypothesis, to include the accelerated enzymatic destruction of 25-hydroxycholecalciferol must also be considered since the results of the vitamin D_3 studies with radioactive tracers obtained in uremic subjects are not inconsistent with this possibility.

Since enzyme systems regulating protein synthesis, anerobic glycolysis, transamination, oxidative phosphorylation, and glutamic acid decarboxylation have all been reportedly inhibited in the uremic state,[24-26] a hypothesis incriminating acquired abnormalities in enzymatic formation and/or degradation of vitamin D_3 and its metabolites in uremia is not unique. Animal evidence indicates that the liver is the site of the maximum concentration of vitamin D_3 labeled with tritium following either oral or intravenous administration.[27] Other studies in humans reveal that an orally administered dose of vitamin D_3 labeled with tritium is rapidly incorporated by the liver and excreted into bile.[28] Recent reports documenting the

epatic conversion of vitamin D_3 to 25-hydroxycholecalciferol in the rat (DeLuca, H.F., personal communication) and abnormal vitamin D_3 metabolism in human cirrhosis[28] establish the liver as an essential organ for the metabolic transformation of vitamin D_3. Since derangements in hepatic enzyme function are not infrequently caused by the chronic uremic state,[29] hepatic dysfunction may be contributing significantly to the disturbances of vitamin D_3 metabolism noted in the patients with chronic renal disease. Schachter and co-workers have also demonstrated that, in the rat, vitamin D acts directly on the small intestine without previous inactivation in another organ.[30] Since, after intravenous administration of vitamin D_3 labeled with tritium, more than 80% of the intestinal radioactivity represents 25-hydroxycholecalciferol, the metabolism and subsequent transformation of vitamin D_3 by the intestine may also be significant. Preliminary observations in our laboratory concerning the fate of orally administered vitamin D_3 labeled with tritium in humans with thoracic duct cannulation also suggest that the intestine plays an essential role in the conversion of vitamin D_3 to 25-hydroxycholecalciferol.[31] The effect of chronic uremia on the intestinal metabolism of vitamin D_3 is, however, presently purely conjectural.

In order to further elucidate the nature of the abnormal metabolism of vitamin D_3 as observed in chronically uremic subjects, the chronic uremic state was experimentally induced in adult rats as previously described by unilateral nephrectomy (on the right) and ligation of most of the primary and secondary divisions of the main left renal artery.[29] Ninety to 100 days following nephrectomy and segmental infarction of the contralateral kidney, the uremic as well as pair fed control animals were injected with a preparation of vitamin D_3 labeled with tritium and killed 72 hours later. Blood, urine, and feces of the uremic and control animals were subjected to combustion, and thin layer and silicic acid column chromatographic analysis as previously described.[28]

When compared to the control animals, the metabolism of vitamin D_3 was significantly deranged in the rats with chronic uremia. Alterations in circulating and excreted vitamin D_3 and 25-hydroxycholecalciferol in the uremic animals were remarkably similar to those defined earlier for uremic man[16] with an increase in plasma turnover rate, a decrease in circulating 25-hydroxycholecalciferol, and an abnormal urinary excretion of protein bound vitamin D_3 and 25-hydroxycholecalciferol. In addition, and of utmost significance, the intestinal mucosa and bone of uremic animals contained appreciably less vitamin D_3 and 25-hydroxycholecalciferol than that of the control groups.

The results of the radioactive vitamin D_3 studies in uremic animals currently support the conclusion that the resistance to therapeutic doses of vitamin D_3 often seen in uremic subjects stems from an acquired defect in vitamin D_3 metabolism. It is tentatively postulated that this metabolic defect leads to an alteration, both in the concentration and physiological expression of the vitamin and its active metabolite in intestine and bone resulting ultimately in diminished intestinal absorption of calcium and defective mineralization of bone. There is mounting evidence that vitamin D_3 normally exerts its effects on intestinal calcium transport by mediating the synthesis of a specific calcium binding protein.[32-34] To date vitamin D-dependent calcium binding proteins have been detected in the intestinal mucosa of the rat, dog, and monkey.[34] The nature of the interaction of vitamin D with the mucosal cell that leads to the formation of the calcium binding protein is still in doubt. Recent studies by Zull and co-workers[35] and Norman[36] suggest that this interaction represents either a direct 25-hydroxycholecalciferol effect on the deoxyribonucleic acid-messenger ribonucleic acid (DNA-mRNA) complex of the mucosal cell or an indirect alteration of the permeability characteristics of the nuclear membrane. Although the product of this nuclear or membrane interaction has been proposed to be a translocase or transport enzyme,[35] the possibility that the substance formed is identical to the vitamin D dependent calcium-binding protein recently isolated from the chick mucosa by Taylor and Wasserman[32-34] must be entertained. Should this interpretation prove correct, an alteration in the physiological expression of vitamin D_3 and 25-hydroxycholecalciferol in uremia could be attributed to both decreased synthesis and concentration of the intestinal calcium binding protein. This proposal is presently hypothetical and awaits direct experimental confirmation.

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Effect of Vitamin D on ^{65}Zn Absorption, Distribution and Turnover in Rats [1]

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ABSTRACT Experiments were designed to study the effect of dietary vitamin D on the absorption, distribution and turnover of ^{65}Zn in growing rats. In the first experiment, whole-animal retention of oral ^{65}Zn by rats fed zinc-supplemented, but not zinc-deficient, diets was slightly ($0.1 > P > 0.05$) increased by continuous supplementation with vitamin D. Retention of injected ^{65}Zn was not similarly enhanced, suggesting a possible effect on ^{65}Zn absorption. In the second experiment ^{65}Zn absorption and turnover were determined by more detailed analysis of whole-animal ^{65}Zn retention patterns, and the distribution of oral and injected ^{65}Zn at 60 hours postadministration was examined. The effect of vitamin D on uptake of oral ^{65}Zn was most pronounced when the vitamin was suddenly added to the diet of previously vitamin D-deficient rats. At 60 hours skeletal, but not soft tissue, uptake of either oral or injected ^{65}Zn was significantly increased by the supplementation of vitamin D to vitamin D-deficient rats. However, skeletal specific activity (% ^{65}Zn per g) was increased by vitamin D only with the orally administered ^{65}Zn. An explanation consistent with all data is that the increased absorption of dietary zinc attributed to vitamin D probably results, not from a direct effect of the vitamin, but from a homeostatic response to the increased need for zinc which accompanies enhanced skeletal growth and calcification.

Zinc absorption and metabolism are affected by a number of dietary variables (1). The antagonistic effect of calcium on zinc utilization noted particularly in swine has been shown in the rat with both practical (2) and semipurified (3) diets to be manifest primarily at the site of zinc absorption. Effects of dietary phosphorus on zinc nutrition have also been studied (1, 3, 4).

The pronounced effect of calcium on zinc nutrition and the role of vitamin D in calcium absorption and metabolism suggest a possible effect of dietary vitamin D on zinc utilization. Published results on such an effect, however, are not in agreement. Whiting and Bezeau (5), through use of conventional balance studies, were unable to show any effect of vitamin D on zinc metabolism in swine when the ration contained 140 ppm zinc, but vitamin D was reported to decrease zinc absorption from a practical ration containing no supplemental zinc. They suggested a possible precipitation of zinc deficiency owing to extensive use of vitamin D supplements. Martin and Patrick (6) reported that addition of vitamin D to the diet of chicks dosed orally with ^{65}Zn increased the amount of ^{65}Zn per gram of tibia when 1% calcium was fed, but produced the opposite effect when the calcium level was raised to 1.6%. A positive effect of vitamin D on zinc metabolism in chickens was indicated further by the work of Kienholz et al. (7) and of Worker and Migicovsky (8). The latter workers fed a single oral dose of vitamin D_3 to rachitic chicks and 36 hours later administered ^{65}Zn either orally or by injection. Chicks dosed orally with vitamin D and later with ^{65}Zn had significantly more ^{65}Zn in the tibiae at 24 hours postadministration than did vitamin D-depleted chicks not dosed with vitamin D. Because the same response was not apparent when ^{65}Zn was injected subcutaneously, it was concluded that vitamin D increased the absorption of zinc from the intestinal tract of the chicken and that vitamin D promoted absorption of many cationic elements in addition to calcium. In contrast, Wassenman (9) reported that duodenal absorption of ^{65}Zn in the chick was not enhanced by vitamin D_3 under the same conditions used to demonstrate significant

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increases due to vitamin D_3 in the absorption of calcium and other alkaline earth cations.

Studies on rats in our laboratory have failed to support the idea that vitamin D exerts a direct effect on zinc utilization. The experiments to be reported indicate that any enhancement of ^{65}Zn absorption by rats supplemented with vitamin D is correlated closely with increased skeletal ^{65}Zn uptake and probably results, not from a direct effect of vitamin D, but from a homeostatic response to the increased need for zinc which accompanies stimulated skeletal calcification and growth. A preliminary report of this work has been published.[2]

MATERIALS AND METHODS

Weanling male rats of the Holtzman strain were used in these experiments. They were housed individually in either stainless steel or galvanized metal cages and were given distilled water ad libitum in glass water bottles. The composition of the basal semipurified diets, containing neither zinc nor vitamin D, is shown in table 1. These diets were supplemented as required with zinc as zinc oxide and vitamin D as calciferol. Rations were assayed for zinc content by atomic absorption spectrophotometry[3] after dry-ashing for 24 hours at 550°.

TABLE 1

Composition of basal diet

	Exp. 1	Exp. 2
	g/kg	*g/kg*
Acid-hydrolyzed casein [1]	180.0	
Alcohol-extracted casein [2]		200.0
Glucose monohydrate [3]	712.7	632.7
Cellulose [4]		60.0
Corn oil	55.0	55.0
Tryptophan	2.2	2.2
Mineral mixture [5]	46.2	46.2
Vitamin mixture [6]	3.9	3.9

[1] Salt-free, General Biochemicals, Inc., Chagrin Falls, Ohio.
[2] Laboratory-prepared from crude casein.
[3] Cerelose, Corn Products Company, Argo, Illinois.
[4] Solka Floc, Brown Company, New York.
[5] Provided in g/kg of diet: $CaCO_3$, 12.1; $CaHPO_4$, 0.27; KH_2PO_4, 17.06; NaCl, 13.5; $MgSO_4$, 2.43; $Fe(C_6H_5O_7)\cdot 6H_2O$, 0.31; $MnSO_4\cdot H_2O$, 0.058; $CuSO_4$, 0.058; KI, 0.00025; and $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 0.000175.
[6] Provided in mg/kg of diet; thiamine-HCl, 5; riboflavin, 5; niacin, 25; Ca pantothenate, 20; pyridoxine-HCl, 2.5; folic acid, 0.2; menadione, 0.5; biotin, 0.1; vitamin B_{12}, 0.02; ascorbic acid, 50; inositol, 100; choline chloride, 1400; and (in IU/kg) vitamin A, 5000; α-tocopherol, 110.

High specific activity ^{65}Zn[4] was administered as the zinc-glycine complex at pH 7.4 either orally in 5 g of feed following a 5 to 10 hour fast or by intramuscular injection. To determine the administered dose, a whole-animal gamma-scintillation detector[5] was used to assay each rat for ^{65}Zn content exactly 4 hours after the feed containing ^{65}Zn was first offered or ^{65}Zn was injected (losses were negligible to that point and diet consumption was complete). The rats were assayed for ^{65}Zn at numerous time intervals thereafter to determine whole-animal ^{65}Zn retention. The data, corrected for coincidence loss, background radiation, radioactive decay of the isotope and minor fluctuations in counting efficiency, were expressed as a percentage of the initially administered dose and plotted on a logarithmic scale as a function of time. For experiment 2, the percentage absorption of the orally administered ^{65}Zn and biological half-life of 3 resolved components of the ^{65}Zn retention curves were determined as outlined by Heth and Hoekstra (2). A CDC 1604 computer was programmed to perform all necessary calculations.

Organ and tissue samples obtained by dissection of some of the rats after ^{65}Zn administration in experiment 2 were assayed for ^{65}Zn either in the whole-animal detector or in a well-type crystal scintillation counter.[6] The carcasses which remained after dissection were also assayed for ^{65}Zn and were then fed to a colony of *Dermestes lardarius*, insects which feed on dead animal tissue. The insect colony consumed all of the soft tissue and reduced the rat carcasses to the skeletal systems within 3 to 4 days. The bones were then rinsed in acetone to remove any remaining insects, crushed and assayed for ^{65}Zn in the crystal scintillation detector. By subtracting the ^{65}Zn content of the skeletal system from that of the carcass before it was fed to the *Dermestes* colony, the ^{65}Zn

[2] A preliminary report of this research was presented at the 1963 annual meeting of the American Society of Animal Science at East Lansing, Michigan.
[3] Model 214 atomic absorption spectrophotometer, manufactured by the Perkin-Elmer Company, Norwalk, Connecticut.
[4] The radiozinc was obtained as $^{65}ZnCl_2$ in HCl from Oak Ridge National Laboratory, Oak Ridge, Tennessee.
[5] Armac scintillation detector model 440 manufactured by Packard Instrument Company, Inc., La Grange, Illinois.
[6] Well-type crystal scintillation detector manufactured by Nuclear-Chicago, Inc., Chicago.

content of the soft tissue was determined. All ^{65}Zn measurements were corrected for coincidence loss, background, radioactive decay and difference in counting efficiency between the 2 instruments. The data were then expressed as a percentage of the total initial ^{65}Zn dose.

All statistical comparisons of treatment means were by Student's t test and Duncan's new multiple range test (10). An inverse sine transformation was routinely applied to all percentage data, as suggested by Steel and Torrie (10).

RESULTS

Experiment 1: Effect of vitamin D on ^{65}Zn retention. The purpose of this experiment was to compare the effect of vitamin D deficiency and continuous supplementation of vitamin D on whole-animal retention of oral and injected ^{65}Zn by rats receiving either a zinc-deficient or a zinc-supplemented diet. Sixteen rats received the basal diet shown in table 1 with no source of vitamin D, and 16 additional rats received the same diet supplemented with 8000 IU vitamin D_2/kg. Within each level of dietary vitamin D, 8 rats were fed the zinc-deficient (2.0 ppm) diet and the remaining 8 received the zinc-supplemented (20.7 ppm) diet. Rats were pair-fed within a given level of dietary zinc to eliminate any differences in size or growth rate from consideration of the effects of vitamin D on ^{65}Zn retention.

After 3 weeks on experiment, 1 μCi ^{65}Zn was administered by diet after a 5-hour fast to 4 of the rats on each dietary regimen and by intramuscular injection to the remaining 4 rats. Whole-animal ^{65}Zn measurements were made as described in the Methods section at 12 to 30-hour intervals until 170 hours (injected ^{65}Zn) or 260 hours (oral ^{65}Zn) postadministration.

Whole-animal retention of the ^{65}Zn is plotted as a function of time for the oral dose in figure 1 and for the injected dose in figure 2. The extremely tenacious retention of ^{65}Zn by zinc-deficient rats has been pointed out previously (3); this effect was noted whether the ^{65}Zn was fed or injected, indicating that zinc-deficient rats exhibit both a remarkably high absorption and a very slow rate of excretion of body zinc.

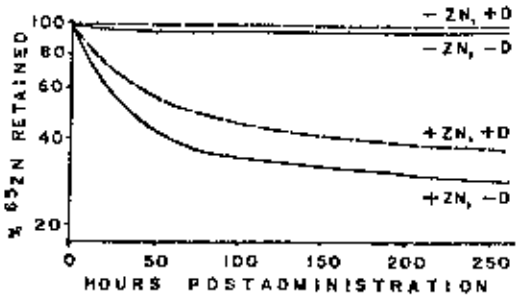

Fig. 1 Exp. 1. Effect of dietary zinc and vitamin D on whole-body retention of ^{65}Zn-glycine administered by diet. Four rats/treatment group were fed the respective diets for 3 weeks prior to and 11 days after a single ^{65}Zn administration. The glucose-casein hydrolysate diets contained: −Zn, 2 ppm Zn; +Zn, 21 ppm; −D, no vitamin D added to diet; +D, 8,000 IU/kg. Rats were pair-fed within each level of dietary zinc but not between zinc levels.

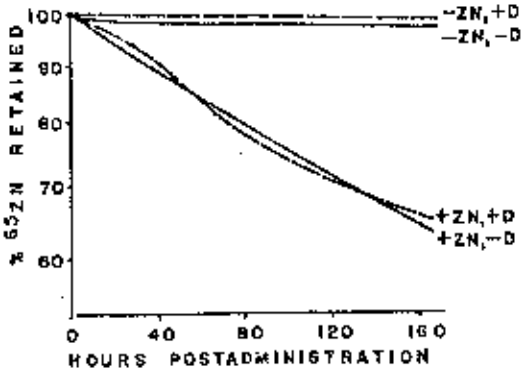

Fig. 2 Exp. 1. Effect of dietary zinc and vitamin D on whole-body retention of ^{65}Zn-glycine injected intramuscularly. Conditions were as described for figure 1.

The presence of vitamin D resulted in a slightly increased retention of oral ^{65}Zn at both levels of dietary zinc. This effect was not statistically significant in either case, although it approached significance ($P < 0.1$) for the adequate zinc diet. Since the small difference in ^{65}Zn retention between the 2 levels of dietary vitamin D appeared by 50 hours postadministration and remained essentially constant thereafter, it was probably the result of a difference in the amount of the original dose initially absorbed. This conclusion is supported by the lack of effect of vitamin D on the rate of turnover of injected ^{65}Zn (fig. 2) at either level of dietary zinc. This experi-

ment has been repeated both with and without pair-feeding; in each case, a slight increase in retention of the orally administered ^{65}Zn dose by the vitamin D- supplemented rats was noted, but in no case was the difference statistically significant.

Experiment 2: Effect of vitamin D supplementation on the absorption, distribution and turnover of ^{65}Zn. This experiment was designed to study the effects of sudden supplementation of vitamin D to vitamin D-deficient rats and to obtain data for the rat more directly comparable to those reported for chicks by Worker and Migicovsky (8). Studies on vitamin A were included for comparison to the effects of vitamin D.

One hundred and sixty weanling rats were assigned at random to 5 treatment groups of 32 animals each. The composition of the control diet fed to treatment group 5 is that shown in table 1, but it contained in addition 10,000 IU vitamin D_2/kg by calculation and 26.3 ppm zinc by analysis. For the other treatment groups, vitamin D was omitted in treatments 1 and 2 and vitamin A was omitted in treatments 3 and 4. Feed was supplied ad libitum.

After the animals had been fed the experimental diets for 21 days, rats in treatment 2 (—D) received 2000 IU vitamin D_2 and those in treatment 4 (—A) received 1000 IU vitamin A. In each case the vitamin was administered orally in 5 g of feed following a 10-hour fast. Thereafter, treatment groups 2 and 4 were fed the nondeficient control diet ad libitum. Rats in treatment groups 1 and 3 were fed the original deficient diets (—D and —A, respectively) thoughout the experiment. Thirty-six hours after vitamin supplementation to groups 2 and 4, 1 μCi of ^{65}Zn was administered to each animal on experiment. Of the 32 rats in each treatment group, 20 received the ^{65}Zn orally in 5 g of feed following a 10-hour fast and the remaining 12 rats received the ^{65}Zn by intramuscular injection. More rats were given the isotope orally than by injection because of the greater variation which is encountered in retention of ^{65}Zn administered by the oral route. The initial administered dose was measured as described in the Methods section.

All rats were assayed for ^{65}Zn content 5 times during the 60 hours after the initial measurement. At 60 hours, 10 of the 20 rats in each group which had received ^{65}Zn orally and 6 of the 12 that had received ^{65}Zn by injection were killed and dissected. The liver, kidneys, pelt, gastrointestinal tract and contents and remaining carcasses were weighed and assayed for ^{65}Zn content, after which the carcasses were fed to the *Dermestes* colony to obtain the skeletal systems, which were similarly weighed and analyzed for ^{65}Zn. Rats not killed at 60 hours were assayed for ^{65}Zn at 18 separate time-intervals between 60 and 612 hours postadministration.

As shown in figure 3, the response to vitamin supplementation was rapid; within several days the rats given the supplements (treatment groups 2 and 4) weighed almost as much as the controls (group 5) and were growing at a significantly faster rate than the deficient animals (groups 1 and 3). The vitamin D-deficient rats continued to grow slowly throughout the course of the experiment, but those deficient in vitamin A reached a growth plateau and began losing weight at about 4 weeks with the experimental diets. Most of the rats in treatment group 3 died of vitamin A deficiency before 612 hours postadministration of ^{65}Zn.

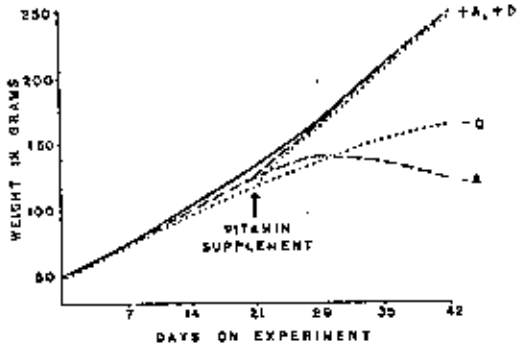

Fig. 3 Exp. 2. Growth of rats (5 treatment groups of 32 rats each) fed a glucose-casein diet with and without vitamins A and D. Treatments were: —A, vitamin A omitted from diet throughout experiment; —D, vitamin D omitted from diet throughout experiment; +A+D, corresponding groups with respective deficiency corrected at 21 days and a group fed vitamin A (5,000 IU/kg diet) and vitamin D (10,000 IU/kg diet) throughout experiment.

Data obtained by resolving the whole-animal ^{65}Zn retention curves and determining the percentage of ^{65}Zn absorption as described previously (2) are shown in table 2. Mean absorption of dietary ^{65}Zn was lower in this experiment than values reported previously for a similar semipurified diet (3); this difference was shown to result primarily from the replacement in the diet of casein hydrolysate with alcohol-extracted casein and to the inclusion of 6% cellulose[7] as a source of dietary bulk. Within the present experiment, however, none of the treatment means for ^{65}Zn absorption differed significantly from the others. The slightly increased absorption attributed in experiment 1 to continual supplementation with vitamin D was not noted in the corresponding comparison in the present data (treatment groups 5 vs. 1). However, rats in treatment groups 3 and 4, which received vitamin D continuously in the diet but were vitamin A-deficient for all or part of the experiment did absorb somewhat more ^{65}Zn than the vitamin D-deficient rats in group 1. In this respect, the absorption data for treatments 3 and 4 may be more representative of the type of response usually observed in a comparison of long-term vitamin D supplementation and deficiency than the corresponding value for treatment 5. The vitamin D-deficient rats in group 2, which had been given the vitamin D supplement 36 hours before ^{65}Zn administration, absorbed somewhat more ^{65}Zn than the vitamin D-deficient rats in group 1 that received no supplement. However, like the effect of continuous vitamin D supplementation observed in experiment 1, this difference only approached significance ($P < 0.1$).

Supplementation of vitamin A to rats deficient in that vitamin did not enhance ^{65}Zn absorption. If anything, the effect of the vitamin A was to lower ^{65}Zn absorption, although the difference was not significant ($P > 0.1$).

Table 2 also shows the mean biological half-life for the first components of the resolved ^{65}Zn retention curves; these values reflect the rate of passage of ingested ^{65}Zn through the gastrointestinal tract. None of these means was significantly different from the others. The biological half-life of 2 further segments of the ^{65}Zn retention curves (table 2) represents turnover of absorbed or injected ^{65}Zn. No significant difference in biological half-life of the 108- to 324-hour component was noted between any of the groups for a given route of ^{65}Zn administration. This is in agreement with the results shown in figure 2. For the 372- to 612-hour component, the biological half-life of injected ^{65}Zn was significantly ($P < 0.05$) shorter

[7] Solka Floc, Brown Company, New York.

TABLE 2

Effects of vitamins A and D on the absorption and turnover of ^{65}Zn

Treatment [1]	Method of ^{65}Zn administration	^{65}Zn absorption	Biological half-life of ^{65}Zn: First component (0–60 hr)	Second component (108–324 hr)	Third component (372–612 hr)
		%	hours	hours	hours
1 (−D)	oral	13.4 ± 1.2 [2]	12.5 ± 0.9 [2]	435 ± 23 [2]	826 ± 44 [2]
	injection			502 ± 16	914 ± 36
2 (−D, then +D)	oral	19.8 ± 3.1	11.6 ± 1.0	466 ± 16	1153 ± 42
	injection			453 ± 21	1019 ± 49
3 (−A)	oral	19.0 ± 1.8	10.1 ± 1.0	437 ± 17	undetermined [3]
	injection			541 ± 24	undetermined [3]
4 (−A, then +A)	oral	17.2 ± 1.8	13.5 ± 1.8	447 ± 30	undetermined [3]
	injection			541 ± 24	undetermined [3]
5 (+D, +A)	oral	13.4 ± 2.2	9.9 ± 0.8	480 ± 27	1063 ± 31
	injection			484 ± 26	989 ± 17

[1] (−D) and (+D) indicate without and with vitamin D; (−A) and (+A) indicate without and with vitamin A.

[2] Treatment means ± SE.

[3] Data for 372–612 hour component of whole-animal retention curve for treatments 3 and 4 not determined due to death of most of the vitamin A-deficient animals before 600 hours postadministration.

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than that of the absorbed isotope for rats given the vitamin D supplement, but not for vitamin D-deficient rats. In addition, rats that received the vitamin D supplement had a longer ^{65}Zn biological half-life than vitamin D-deficient rats for the oral dose ($P < 0.01$), but for the injected dose, the difference was not significant. These observations suggest that more of the ^{65}Zn absorbed by vitamin D-supplemented rats was turning over less rapidly than that absorbed by vitamin D-deficient rats and may therefore have been in bone.

The distribution of ^{65}Zn in rats at 60 hours postadministration is presented in table 3. Supplementation of vitamin A to rats deficient in that vitamin had no significant effect on the amount of either oral or injected ^{65}Zn present in any of the body components analyzed.

Although the effect of vitamin D on the absorption of ^{65}Zn as determined by analysis of whole-animal retention curves (table 2) was not significant, the data in table 3 indicate that, at 60 hours postadministration, the vitamin D-supplemented

TABLE 3

Effects of vitamins A and D on the distribution of ^{65}Zn at 60 hours postadministration

Sample		Treatment [1]	Portion of total initial ^{65}Zn present in sample at 60 hours postadministration: Oral ^{65}Zn	Injected ^{65}Zn
			%	%
Whole animal	1	(−D)	19.2 ±2.8 [2]	83.0 ±0.5 [2]
	2	(−D, then +D)	16.2 ±1.5	78.0 ±2.1
	3	(−A)	16.3 ±1.6	79.4 ±1.2
	4	(−A, then +A)	18.0 ±1.5	81.7 ±1.0
	5	(+D, +A)	17.5 ±1.6	81.0 ±1.3
Gastrointestinal tract and contents	1	(−D)	9.1 ±1.8	12.2 ±0.8
	2	(−D, then +D)	3.7 ±0.5	11.7 ±0.7
	3	(−A)	5.0 ±0.9	10.4 ±0.5
	4	(−A, then +A)	6.3 ±0.9	11.2 ±0.7
	5	(+D, +A)	4.8 ±0.6	9.8 ±0.3
Liver	1	(−D)	1.2 ±0.2	6.5 ±0.3
	2	(−D, then +D)	1.2 ±0.2	5.8 ±0.2
	3	(−A)	1.2 ±0.2	5.7 ±0.2
	4	(−A, then +A)	1.4 ±0.1	6.3 ±0.3
	5	(+D, +A)	1.5 ±0.2	6.6 ±0.2
Kidneys	1	(−D)	0.22±0.02	0.97±0.04
	2	(−D, then +D)	0.22±0.02	0.90±0.05
	3	(−A)	0.25±0.03	1.11±0.04
	4	(−A, then +A)	0.21±0.03	1.15±0.06
	5	(+D, +A)	0.26±0.03	1.20±0.03
Pelt (skin and hair)	1	(−D)	1.6 ±0.2	12.3 ±1.7
	2	(−D, then +D)	1.7 ±0.1	8.4 ±0.4
	3	(−A)	1.8 ±0.2	9.9 ±0.8
	4	(−A, then +A)	1.6 ±0.2	10.3 ±0.6
	5	(+D, +A)	1.9 ±0.3	9.8 ±0.6
Other soft tissues	1	(−D)	4.8 ±0.1	33.4 ±0.7
	2	(−D, then +D)	4.6 ±0.4	27.6 ±0.8
	3	(−A)	4.6 ±0.6	31.6 ±1.3
	4	(−A, then +A)	4.8 ±0.4	32.2 ±1.5
	5	(+D, +A)	5.7 ±0.7	34.9 ±1.1
Skeleton	1	(−D)	1.9 ±0.2	14.8 ±0.7
	2	(−D, then +D)	3.9 ±0.3	19.8 ±0.6
	3	(−A)	3.1 ±0.3	16.6 ±0.6
	4	(−A, then +A)	3.0 ±0.2	16.1 ±0.9
	5	(+D, +A)	3.0 ±0.3	15.7 ±0.4

[1] (−D) and (+D) indicate without and with vitamin D; (−A) and (+A) indicate without and with vitamin A.
[2] Treatment means ± SE.

rats had a significantly smaller fraction of the initial oral ^{65}Zn dose remaining in the digestive tract than the unsupplemented vitamin D-deficient rats of group 1. This effect was greatest ($P < 0.01$) when the vitamin D supplement was suddenly given to previously vitamin D-deficient rats (group 2), but was also significant ($P < 0.05$) for the rats which had been continuously fed vitamin D (groups 3, 4 and 5).

Vitamin D supplementation also resulted in an increased incorporation of oral ^{65}Zn into the skeleton. Again, the effect was most marked ($P < 0.001$) following supplementation of vitamin D to previously deficient rats, but was also noted ($P < 0.01$) in the case of continuous vitamin D supplementation. The extent of increased deposition of oral ^{65}Zn in the skeleton appeared to be correlated with the removal of ^{65}Zn from the digestive tract.

Vitamin D had no significant effect on the amount of oral ^{65}Zn found at 60 hours postingestion in liver, kidneys, pelt or other soft tissues. Also, it was found earlier [5] that vitamin D had no significant effect on soft-tissue zinc concentrations in rats fed the same type of semipurified diet and receiving various levels of dietary zinc.

The effects of vitamin D on zinc metabolism may relate entirely to the stimulation by vitamin D of bone growth and calcification. This effect is readily observed by comparing treatment groups 1 and 2 in table 4. The average weight of the skeletal system (rats given ^{65}Zn orally and by injection combined) was 4.4 g for the vitamin D-supplemented vs. 3.6 g for the vitamin D-deficient rats ($P < 0.01$), indicating that about 20% of the total weight of the skeletal system was deposited by the vitamin D-supplemented rats during the 96 hours from vitamin supplementation to killing. Enhanced skeletal uptake of ^{65}Zn was measured during the final 60 hours of this 96-hour period. In view of the relatively high zinc content of the skeleton, it is probably not unexpected that animals responding to vitamin D supplementation absorbed more of an orally administered dose of ^{65}Zn than did D-deficient rats. Supplementation with vitamin A did not stimulate skeletal growth during the interval studied (5.3 g for treatment group 3 vs. 4.9 g for treatment group 4; $P > 0.1$) and, as noted above, had no significant effect on uptake of dietary ^{65}Zn.

Unlike the results reported for chicks by Worker and Migicovsky (8), an effect ($P < 0.001$) of supplemental vitamin D on skeletal ^{65}Zn was also observed (table 3) when the isotope was injected intramuscularly, although the relative difference was less than in the case of the orally administered ^{65}Zn. The vitamin D-supplemented rats had a correspondingly smaller fraction of the injected dose present in the pelt ($P < 0.05$) and in the soft tissue ($P < 0.001$) than unsupplemented vitamin D-deficient rats. Liver and kidney showed a similar effect to other soft tissues, but the differences were not statistically significant. A likely explanation for the difference between the present results and those reported by Worker and Migicovsky (8) is that in the latter case only 24 hours were allowed between ^{65}Zn injection and killing

[5] Becker, W. M., and W. G. Hockstra, unpublished data.

TABLE 4

Effects of vitamins A and D on skeletal weight and skeletal ^{65}Zn

Treatment [1]	Oral ^{65}Zn		Intramuscular ^{65}Zn	
	Wt of skeleton [2]	Specific activity [3]	Wt of skeleton [2]	Specific activity [3]
	g	*%/g*	*g*	*%/g*
1 (−D)	3.7 ± 0.2 [4]	0.53 ± 0.05 [4]	3.5 ± 0.2 [4]	4.3 ± 0.2 [4]
2 (−D, then +D)	4.4 ± 0.1	0.69 ± 0.08	4.6 ± 0.2	4.4 ± 0.3
3 (−A)	5.5 ± 0.2	0.57 ± 0.05	4.9 ± 0.2	3.4 ± 0.1
4 (−A, then +A)	4.8 ± 0.2	0.63 ± 0.04	5.0 ± 0.2	3.2 ± 0.1
5 (+D, +A)	4.7 ± 0.1	0.65 ± 0.06	4.8 ± 0.2	3.2 ± 0.1

[1] (−D) and (+D) indicate without and with vitamin D; (−A) and (+A) indicate without and with vitamin A.
[2] Separated from soft tissues by *Dermestes lardarius* (see text).
[3] Percentage of total initial ^{65}Zn/g of skeleton at 60 hours postadministration.
[4] Treatment means ± SE.

and that this was insufficient time for significant skeletal growth compared with 60 hours in the present study. When skeletal ^{65}Zn content was expressed as specific activity (percentage of total initial ^{65}Zn/g skeleton, table 4) to correct for differences in total skeletal size, there was no effect on injected ^{65}Zn of supplementing vitamin D to vitamin D-deficient rats. For the orally administered ^{65}Zn, however, increased skeletal uptake by the vitamin D-supplemented rats far exceeded the difference in skeletal size, such that a large difference ($P < 0.001$) also existed in specific activity of the bone between the vitamin D-deficient and vitamin D-supplemented rats. This is further verification that an increased ^{65}Zn absorption occurred following vitamin D supplementation of vitamin D-deficient rats. When the label was in the small pool of zinc in the gut, an increased absorption would substantially increase the specific activity of the circulating zinc pool supplying zinc for bone growth and calcification. In contrast, when the much larger body zinc pools were labeled (as by injection) such increased absorption of stable zinc would have little effect on the specific activities of the circulating zinc pool and thus of bone zinc. In fact, the increased absorption of stable zinc would have decreased somewhat the specific activity of the circulating zinc pool, such that despite the stimulation of bone growth due to the added vitamin D, no difference was observed in the deposition of ^{65}Zn per unit weight of bone in the presence or absence of vitamin D.

DISCUSSION

The results of these experiments indicate that the uptake of oral ^{65}Zn by rats is enhanced slightly in the presence of adequate vitamin D and that this effect is more noticeable when the vitamin D supplement is suddenly given to rats previously deficient in vitamin D. Even in the latter case, however, there was no significant increase in ^{65}Zn content of soft tissues, and in other experiments, dietary vitamin D, whether deficient, adequate or excessive, had little or no effect in alleviating or aggravating the zinc-deficiency syndrome in rats[9] or swine.[10]

The effect of vitamin D on ^{65}Zn absorption in the experiments reported here was closely correlated with an increased deposition of ^{65}Zn in the skeleton. The role of zinc in bone is not clearly defined, but bone is known to contain relatively high amounts of zinc. Work by Haumont (11) and by Haumont and Vincent (12) suggests that zinc is localized at the boundary between mineralized bone and preosseous substance and is deposited exactly at the time when calcification begins. When supplemental vitamin D stimulates bone calcification and thereby increases the need for zinc in bone, it seems logical that the additional zinc required is drawn from circulating body pools and that these in turn are replenished by an enhanced absorption of dietary zinc from the intestinal tract.

Such an indirect effect of vitamin D on zinc absorption is supported by the observed correlation between skeletal growth, skeletal uptake of ^{65}Zn and absorption of dietary ^{65}Zn. Rats continually supplemented with vitamin D (in exp. 1 and in treatments 3, 4 and 5 of exp. 2) retained slightly more oral ^{65}Zn than vitamin D-deficient rats and had deposited a larger proportion of ^{65}Zn in the skeletal system by 60 hours postingestion. A more pronounced effect of vitamin D on zinc absorption was noted when the vitamin was administered to previously deficient animals (in exp. 2) and was closely correlated with a sudden increase in skeletal growth and a much greater uptake of ^{65}Zn by bone than was observed for either the vitamin D-deficient or continuously supplemented rats.

It appears reasonable, therefore, to conclude that the increase in uptake of ^{65}Zn by skeletal tissue when vitamin D-deficient animals are treated with vitamin D is due to stimulation of bone growth and calcification by the vitamin and that the increased absorption of dietary zinc attributed to the vitamin is a homeostatic response to the increased need for zinc which results. Such an explanation would not be in disagreement with the results of Worker and Migicovsky (8) nor with the results of Wasserman (9). Moreover,

[9] See footnote 8.

[10] Roberts, H. F., W. G. Hoekstra and R. H. Grummer 1961 Vitamin D, zinc and calcium in swine nutrition. J. Animal Sci., 20: 950 (abstract).

Cotzias et al. (13) have demonstrated that a homeostatic mechanism controlling zinc absorption does indeed exist.

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German Translation

Vitamin D Hypercalcemic Cardiovascular Disease

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A combination of supravalvular aortic stenosis, mental retardation and characteristic facies was reported in 1961 by Williams et al. (21) and in 1962 by Beuren et al. (2) were then able to demonstrate that multiple peripheral pulmonary stenoses and dental malformations constitute a further component of this combination of malformations. Black and Bonham-Carter (3) and Hooft et al. (12) were the first to draw attention to the similarity of the facies of patients with supravalvular aortic stenosis and of those with "idiopathic" hypercalcemia. Bonham-Carter and Sutcliffe (4) checked the autopsy findings of 6 patients who had died from "idiopathic" hypercalcemia, and found in 2 cases a supravalvular aortic stenosis, and in all chilfren multiple peripheral arterial stenoses. At about the same time, Garcia et al. (10) were able to diagnose intravitam (during life) a hypercalcemia and a supravalvular aortic stenosis in an infant with a typical facies.

These authors discuss the possibility that the hypercalcemia is

either a component of a genetically determined anomaly affecting multiple body systems, or that the entire syndrome is caused by an abnormal vitamin D metabolism. Bonham-Carter and Sutcliffe (4) assumed that hypercalcemia causes a vascular growth inhibition at definite "stress points" and that the mothers of such children have a sensitivity to vitamin D at least during pregnancy.

Reports are also available on supravalvular aortic stenoses without mental retardation and with a normal facial expression, both in isolated cases and in cases with a family occurrence (6, 22). It remained unclear whether a connection with hypercalcemia was also present in these cases.

Reports on "idiopathic" hypercalcemia were published for the first time by Lightwood (14) and Franconi et al. Since that time, numerous authors have devoted their attention to this syndrome. The occurrence of heart murmurs in hypercalcemic children was explained by the presence of calcifications in the valvular apparatus of the heart, or was related to the frequent hypertonia observed in these patients. In the majority of articles published on this subject, the opinion is expressed that there is a connection between "idiopathic" hypercalcemia and vitamin D metabolism, but that the precise relationship is still unclear. Manios and Antener (16) have found that in two children with "idiopathic" hypercalcemia the vitamin D activity of blood serum is 4-5 times as high as that of normal test subjects, and noted a considerable increase of vitamin D activity after a vitamin D loading of these patients with physiological doses. The different sensitivity towards vitamin D and a possible hypersensitivity has been repeatedly pointed out in the literature (8, 11). Hooft et al. (11) have also reported on a family occurrence of vitamin D hypersensitivity.

According to their report, a vitamin D intoxication can arise from an acute or chronic overdosage or through an endogenous sensitivity already with "normal" vitamin D doses.

The results obtained so far can be summarized as follows:

1. A relationship between "idiopathic" hypercalcemia and a disturbance of vitamin D metabolism has been established with certainty.

2. The characteristic facies of patients with hypercalcemia is the same as that of children with supravalvular aortic stenosis.

3. When patients suffering from both sicknesses have a typical facies, mental retardation is also present.

4. In some cases, hypercalcemia and a combination of supravalvular aortic stenosis and peripheral pulmonary stenoses have been noted in the same patients.

5. In both syndromes, a family occurrence has been described; however, in cases of supravalvular aortic stenoses, only in patients without a typical facies.

Personal Observations

Up to the end of the past century, practically all congenital cardiac defects have been precisely described from a pathological-anatomic viewpoint. On the other hand, supravalvular aortic stenosis was found for the first time in 1930 by Mencarelli (17) during an autopsy. This observation was thus made at a time when vitamin D prophylaxis was already in common use. Although since that time a relatively large number of studies have dealt with this cardiac defect, a total of only 68 reported cases is listed in an American review article (18). On the other hand, we were struck,

already as early as 1964, by our large numbers of patients with supravalvular aortic stenosis (10 patients), as compared to the number of such patients at large American heart centers (2, 20). So far, we have been able to detect supravalvular aortic stenoses and multiple peripheral pulmonary stenoses in a total of 37 patients; these include 22 children with a typical facies and mental retardation. In 9 patients, the facial expression is normal, and there is no mental retardation. In addition, we found in two families 6 cases, which have already been reported elsewhere (13). The case histories of these patients disclose repeated administration of massive (stoss) vitamin D doses, sometimes in amounts of 600,000 I.U. (international units) each, and data on current intake of vitamin D preparations by the mothers during pregnancy.

The great differences in the degree of severity of the deformation, and also of cardiac defects, are generally known. The degree of severity of multiple stenoses in both vessels differs widely in our patients. Sometimes, stenoses in the aorta predominate, while in other cases multiple stenoses of the pulmonary artery are again noted more frequently. In many cases of supravalvular aortic stenosis, a waist-shaped constriction of the aorta is observed, which begins at the upper edge of Valsalva's sinus. However, a review of a large group of patients with this type of deformation shows that a generally hypoplastic aorta is found in many cases. Also in cases of multiple, peripheral pulmonary stenoses, the disease corresponds, in severe cases, to a generalized hypoplasia of the pulmonary artery.

Combined aortic and pulmonary stenoses have been very seldom reported and then only in isolated cases. In recent years, however, we have observed rather frequently a combination of aortic and pulmonary stenoses,

sometimes in isolated cases and sometimes with a family occurrence. In contrast to other valvular aortic and pulmonary stenoses, this type of deformation is characterized by a general hypoplasia of both large vessels and valve rings; in such patients, mental retardation is also frequently noted. Since in some patients with a typical facies of supravalvular aortic stenoses and "idiopathic" hypercalcemia no supravalvular pressure gradients could be detected, but, on the other hand, a general hypoplasia of both large vessels with considerable pressure increases in both ventricles was noted, it was suspected that these patients also were children sensitive to vitamin D. From a pathological-anatomic standpoint, the large vessels of these patients, as in the case of supravalvular aortic stenoses, are characterized by a considerable thickening of the media and a corresponding constriction of the arterial lumen, as well as by a hypoplasia affecting the circumference of the vessels. The clinical picture and the identical pathological-anatomic vascular changes leave no doubt that the same genesis lies at the base of both groups of diseases. If we exclude all cases which so far are still uncertain, then we have a total of 11 patients with a generalized hypoplasia of the aortic and pulmonary base and a hypoplasia of both large vessels in their overall course; 8 of these patients were observed in one family. This gives us a total of 48 patients with "vitamin D-hypercalcemic cardiovascular disease". In one of these patients, a vitamin D and dihydrotachysterol (AT-10) treatment was carried elsewhere during the first 6 months of life, since a tetany was suspected. This patient was the child of a family in which 8 patients with hypoplastic large vessels were found. In two members of this family autopsy findings are available, which are identical with those observed in supravalvular

aortic stenoses; for this reason, a family sensitivity to vitamin D is likely.

In one of our patients with the typical facies of a supravalvular aortic stenosis and mental retardation, a hypercalcemia had been diagnosed earlier (15); this child had a nephrocalcinosis. Another child with the overall combination of deformities mentioned above had a renal diabetes insipidus with lime incrustations in both kidneys, which could be detected by radiology.

In different series of tests with young rats and rabbits of several generations, which were treated with different doses of vitamin D and in part with dihydrotachysterol, we were able to produce supravalvular aortic stenoses. Similar and yet unpublished results were reported to us by Friedman (9).

Selye (19) coined the concept of topical and systemic calciphylaxis, a hypersensitive state induced by a calcifying product with subsequent administration, after a definite time interval, of a so-called challenging agent. Dihydrotachysterol and vitamin D are primarily sensitizing calcification products, whereas iron is an effective challenging agent. We can assume that Selye's animal tests (1962) will find numerous clinical parallel cases. Thus, Creutzfeldt (5) has described a case of calciphylaxis-pancreatitis.

Vitamin D and iron are freely administered to children and infants, and in Germany vitamin D is mainly given in the form of Vigantol single massive (stoss=shock) doses. During the first year of life, infants receive up to 5 Vigantol stoss doses, sometimes in doses of up to 600,000 I.U. (international units) per stoss. During pregnancy, one Vigantol stoss dose in the sixth and ninth month of pregnancy is recommended; this dose far

exceeds the actual requirement for the prevention of rickets. In the U.S.A., a daily dose of 400 I.U. vitamin D is recommended for rickets prevention, which corresponds to the actual needs and is a more physiological dose. According to our experience, the danger lies in preventive stoss therapy, but once vitamin D sensitivity has been induced, the danger probably lies already in the presence of physiological amounts of vitamin D.

Summary

The relationship between hypercalcemia, vitamin D sensitivity or intoxication and supravalvular aortic stenosis is certain. Vascular lesions can be reproduced in animal tests. A vitamin D sensitivity is present not only in cases of supravalvular aortic stenosis, but also in cases of generalized hypoplasia of both large vessels. The number of patients suffering from hypercalcemia or vitamin D hypercalcemic cardiovascular diseases is relatively high and is larger in Germany than in countries where a more physiological prevention of rickets is practiced. The findings cast a new light on the teratogenic action of drugs which are now administered rather freely. An overdosage of vitamin D, in case of a genetically determined hypersensitivity, makes it possible for the disease to materialize. There is an urgent need to carry out a reorganization of rickets prophylaxis in Germany, and it is the purpose of this article to prompt such a reorganization program. We shall give a detailed report of our studies elsewhere.

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Vitamin-D-hypercalcämische Herz- und Gefäßerkrankung

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Über die Kombination von supravalvulärer Aortenstenose, geistiger Retardierung und charakteristischer Facies wurde 1961 von Williams und Mitarbeitern (21) und 1962 von Beuren und Mitarbeitern (1) berichtet. Diese Autoren sprachen die Vermutung aus, daß es sich um ein neues Syndrom handelt. Beuren und Mitarbeiter (2) konnten dann zeigen, daß multiple periphere Pulmonalstenosen und identische Zahnmißbildungen ein weiterer Bestandteil dieser Kombination von Mißbildungen sind. Black und Bonham-Carter (3) sowie Hooft und Mitarbeiter (12) wiesen erstmals auf die Ähnlichkeit der Facies der Patienten mit supravalvulärer Aortenstenose und der mit „idiopathischer" Hypercalcämie hin. Bonham-Carter und Sutcliffe (4) kontrollierten die autoptischen Befunde von sechs Patienten, die an „idiopathischer" Hypercalcämie gestorben waren, und fanden in zwei Fällen eine supravalvuläre Aortenstenose und bei allen Kindern multiple periphere arterielle Stenosen. Etwa gleichzeitig konnten Garcia und Mitarbeiter (10) bei einem Kleinkind mit der typischen Facies eine Hypercalcämie und eine supravalvuläre Aortenstenose intra vitam diagnostizieren.

Diese Autoren diskutierten die Möglichkeit, daß die Hypercalcämie entweder eine Komponente einer genetisch determinierten und multiple Körpersysteme betreffenden Anomalie ist oder daß das gesamte Syndrom durch einen anormalen Vitamin-D-Stoffwechsel verursacht wird. Bonham-Carter und Sutcliffe (4) vertraten die Hypothese, daß die Hypercalcämie eine Wachstumshemmung der Gefäße an bestimmten „Stresspunkten" verursacht und daß die Mütter solcher Kinder zumindest während der Schwangerschaft eine Vitamin-D-Sensibilität haben.

Es wurde auch über supravalvuläre Aortenstenosen ohne geistige Retardierung und mit normalem Gesichtsausdruck, sowohl in Einzelfällen als auch mit familiärem Vorkommen, berichtet (6, 22). Ungeklärt blieb, ob auch bei diesen Fällen ein Zusammenhang mit der Hypercalcämie besteht.

Über „idiopathische" Hypercalcämie wurde erstmals von Lightwood (14) und von Fanconi und Mitarbeitern (7) berichtet. Seitdem hat sich eine große Zahl von Autoren mit diesem Krankheitsbild beschäftigt. Das Vorkommen systolischer Herzgeräusche bei hypercalcämischen Kindern wurde mit Verkalkungen des Klappenapparates des Herzens erklärt oder auf die bei diesen Patienten häufige Hypertonie bezogen. In der Mehrzahl der bisher zu diesem Thema erschienenen Arbeiten kommt zum Ausdruck, daß eine Verbindung zwischen der „idiopathischen" Hypercalcämie und dem Vitamin-D-Stoffwechsel besteht, daß die exakten Zusammenhänge jedoch unklar sind. Manios und Antener (16) haben bei zwei Kindern mit „idiopathischer" Hypercalcämie eine vier- bis fünfmal so hohe Vitamin-D-Aktivität des Blutserums gegenüber normalen Versuchspersonen festgestellt und nach einer Vitamin-D-Belastung dieser Patienten mit physiologischen Dosen ein erhebliches Ansteigen der Vitamin-D-Aktivität beobachtet. Wiederholt ist in der Literatur auf eine unterschiedliche Sensibilität gegenüber Vitamin D und auf eine mögliche Überempfindlichkeit hingewiesen worden (8, 11). Hooft und Mitarbeiter (11) haben auch über familiäres Auftreten einer Vitamin-D-Überempfindlichkeit berichtet. Demnach kann eine Vitamin-D-Intoxikation durch eine akute oder chronische Überdosierung oder durch eine endogene Empfindlichkeit bereits bei „normalen" Vitamin-D-Dosen entstehen.

Bisher kann also wie folgt zusammengefaßt werden:

1. Ein Zusammenhang zwischen der „idiopathischen" Hypercalcämie und einer Störung des Vitamin-D-Stoffwechsels gilt als gesichert.
2. Die charakteristische Facies der Patienten mit Hy-

percalcämie ist die gleiche wie die der Kinder mit supravalvulärer Aortenstenose.

3. Wenn die Patienten beider Krankheiten die typische Facies haben, ist auch eine geistige Retardierung vorhanden.
4. Hypercalcämie und die Kombination von supravalvulärer Aortenstenose und peripheren Pulmonalstenosen sind in einigen Fällen bei den gleichen Patienten nachgewiesen worden.
5. Bei beiden Krankheitsbildern ist ein familiäres Vorkommen beschrieben worden, bei den supravalvulären Aortenstenosen allerdings nur bei Patienten ohne typische Facies.

Eigene Beobachtungen

Praktisch alle kongenitalen Herzfehler waren bis zum Ende des vergangenen Jahrhunderts pathologisch-anatomisch exakt beschrieben worden. Die supravalvuläre Aortenstenose dagegen wurde erstmals im Jahre 1930 von Moncarelli (17) bei einer Autopsie gefunden. Diese Beobachtung fällt also in eine Zeit, in der die Vitamin-D-Prophylaxe bereits üblich war. Obwohl sich seitdem eine relativ große Zahl von Arbeiten mit diesem Herzfehler befaßt hat, stellt ein Übersichtsartikel in der amerikanischen Literatur nur 68 insgesamt mitgeteilte Fälle zusammen (18). Dagegen war bereits im Jahre 1964 unsere große Zahl von Patienten mit supravalvulärer Aortenstenose (zehn Patienten) im Vergleich zu großen amerikanischen Herzzentren aufgefallen (2, 20). Wir haben bis heute bei insgesamt 37 Patienten supravalvuläre Aortenstenosen und multiple periphere Pulmonalstenosen nachweisen können. Es handelt sich dabei um 22 Kinder mit der typischen Facies und mit geistiger Retardierung. Bei neun Patienten ist der Gesichtsausdruck normal und eine geistige Retardierung nicht vorhanden. Darüber hinaus fanden wir in zwei Familien sechs Fälle, über die wir bereits an anderer Stelle berichtet haben (13). In den Anamnesen dieser Patienten finden sich wiederholte Vitamin-D-Stöße zum Teil mit je 600000 I. E. und Angaben über die laufende Einnahme von Vitamin-D-Präparaten der Mutter während der Gravidität.

Die großen Unterschiede im Schweregrad jeder Mißbildung, auch der Herzfehler, sind allgemein bekannt. Der Schweregrad der multiplen Stenosierungen in beiden Gefäßen ist bei unseren Patienten sehr unterschiedlich. Einmal stehen die Stenosen in der Aorta im Vordergrund, bei anderen überwiegen wieder die multiplen Stenosen der Arteria pulmonalis. Bei der supravalvulären Aortenstenose handelt es sich in vielen Fällen um eine taillenförmige Einengung der Aorta, die am oberen Rand des Sinus von Valsalva beginnt. Wenn man jedoch ein großes Krankengut mit dieser Mißbildung übersieht, fällt auf, daß es sich in vielen Fällen um eine generell hypoplastische Aorta handelt. Auch bei den multiplen peripheren Pulmonalstenosen entspricht die Erkrankung bei den schweren Fällen einer generalisierten Hypoplasie der Arteria pulmonalis.

Kombinierte Aorten- und Pulmonalstenosen sind sehr selten und nur vereinzelt mitgeteilt worden. Dagegen haben wir in den letzten Jahren relativ häufig eine Kombination von Aorten- und Pulmonalstenose zum Teil in Einzelfällen, zum Teil mit familiärem Vorkommen beobachtet. Im Gegensatz zu anderen valvulären Aorten- und Pulmonalstenosen ist diese Mißbildung durch eine generelle Hypoplasie beider großen Gefäße und der Klappenringe gekennzeichnet. Auch bei diesen Patienten ist häufig eine geistige Retardierung vorhanden. Da wir bei einigen Patienten mit der typischen Facies der supravalvulären Aortenstenosen und der der „idiopathischen" Hypercalcämie keine supravalvulären Druckgradienten feststellen konnten, dagegen aber eine generelle Hypoplasie beider großen Gefäße mit erheblichen Druckerhöhungen in beiden Ventrikeln, lag der Verdacht nahe, daß es sich auch bei diesen Patienten um Vitamin-D-empfindliche Kinder handelt. Pathologisch-anatomisch sind die großen Gefäße dieser Patienten, wie bei den supravalvulären Aortenstenosen, durch eine erhebliche Verdickung der Media und entsprechende Einengung des Lumens der Arterien sowie durch eine die Zirkumferenz der Gefäße betreffende Hypoplasie gekennzeichnet. Das klinische Bild und die gleichartigen pathologisch-anatomischen Veränderungen an den Gefäßen lassen keinen Zweifel, daß beiden Gruppen die gleiche Genese zugrunde liegt. Wenn wir alle bisher noch unsicheren Fälle ausscheiden, so bleiben uns insgesamt elf Patienten mit einer generalisierten Hypoplasie der Aorten- und Pulmonalbasis und Hypoplasie beider großen Gefäße in ihrem gesamten Verlauf. Davon wurden acht Patienten in einer Familie beobachtet. Dies bringt die Gesamtzahl unseres Krankengutes mit „Vitamin-D-hypercalcämischer Herz- und Gefäßerkrankung" auf 48. Bei einem dieser Patienten wurde eine Vitamin-D- und Dihydrotachysterol- (AT 10®-)Behandlung während der ersten sechs Lebensmonate anderenorts durchgeführt, da eine Tetanie vermutet wurde. Es handelt sich um das Kind einer Familie, in der acht Patienten mit hypoplastischen großen Gefäßen gefunden wurden. Bei zwei dieser Familienmitglieder liegen autoptische Befunde vor, die identisch sind mit denen supravalvulärer Aortenstenosen. Eine familiäre Vitamin-D-Sensibilität liegt daher nahe.

Bei einem unserer Patienten mit der typischen Facies der supravalvulären Aortenstenose und mit geistiger Retardierung war früher eine Hypercalcämie nachgewiesen worden (15). Dieses Kind hat eine Nephrocalcinose. Ein weiteres Kind mit der gesamten Kombination der genannten Mißbildungen hat einen renalen Diabetes insipidus mit röntgenologisch nachweisbaren Kalkeinlagerungen in beiden Nieren.

in verschiedenen Versuchsreihen mit jungen Ratten und jungen Kaninchen mehrerer Generationen, die mit unterschiedlichen Dosen Vitamin D und zum Teil mit Dihydrotachysterol behandelt wurden, ist es uns gelungen, supravalvuläre Aortenstenosen zu produzieren. Ähnliche noch unveröffentlichte Ergebnisse wurden uns von Friedman (9) mitgeteilt.

Selye (19) prägte den Begriff der lokalen und der System-Calciphylaxie, eines Zustandes von Hypersensibilität, der durch einen Verkalkungsstoff hervorgerufen werden kann und dem nach einem bestimmten Intervall ein sogenannter Provokator folgt. Sensibilisierende Verkalkungsstoffe sind vor allem das Dihydrotachysterol und das Vitamin D, während Eisen als ein wirkungsvoller Provokator gilt. Es ist anzunehmen, daß die Tierexperimente von Selye (1962) mannigfaltige klinische Parallelen finden werden. So wurde von Creutzfeldt (5) eine Calciphylaxie-Pankreatitis beschrieben.

Vitamin D und Eisen werden im Kindesalter freizügig verabreicht, in Deutschland Vitamin D vor allem in Form von Vigantol®-Stößen. Es werden bis zu fünf Vigantol-Stöße im ersten Lebensjahr, zum Teil mit Dosen bis zu 600000 I.E. je Stoß, verabreicht. Während der Gravidität werden je ein Vigantol-Stoß im sechsten und neunten Schwangerschaftsmonat empfohlen. Dies ist eine den tatsächlichen Bedarf weit überschreitende Rachitisprophylaxe. In den USA werden täglich 400 I.E. Vitamin D zur Rachitisprophylaxe empfohlen. Dies entspricht dem tatsächlichen Bedarf und ist physiologischer. Nach unseren Erfahrungen liegt die Gefahr in der Stoßprophylaxe, bei einmal ausgelöster Vitamin-D-Sensibilität wahrscheinlich aber bereits in physiologischen Mengen von Vitamin D.

Zusammenfassung

Der Zusammenhang zwischen Hypercalcämie, Vitamin-D-Sensibilität oder -Intoxikation und supravalvulärer Aortenstenose ist sicher. Im Tierversuch sind die Gefäßläsionen reproduzierbar. Eine Vitamin-D-Sensibilität liegt nicht nur bei den supravalvulären Aortenstenosen vor, sondern auch bei generalisierten Hypoplasien beider großen Gefäße. Die Zahl der an Hypercalcämie oder Vitamin-D-hypercalcämischer Herz- und Gefäßerkrankung leidenden ... ist relativ hoch und in Deutschland größer als in Ländern, in denen eine mehr physiologische Rachitisprophylaxe getrieben wird. Die Befunde werfen ein neues Licht auf die teratogene Wirkung heute relativ freizügig verabreichter Pharmaka. Durch die Vitamin-D-Überdosierung kommt es bei der genetisch determinierten Überempfindlichkeit zur Krankheitsrealisierung. Es besteht die dringende Veranlassung, die Durchführung der Rachitisprophylaxe in Deutschland zu reorganisieren. Es ist der Zweck dieser Mitteilung, dazu den Anstoß zu geben. Wir werden an anderer Stelle ausführlich über unsere Untersuchungen berichten.

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LONG TIME FEEDING EXPERIMENTS WITH ACTIVATED ERGOSTEROL. I.

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In the earliest reference to the action of large doses of irradiated ergosterol, Rosenheim and Webster (1) reported that 10,000 times the minimum antiricketic dose was not lethal to rats. Kreitmair and Moll (2), using "enormous doses," described pathological findings for several species, and observed a species difference in susceptibility. Collazo, Rubino, and Varela (3) observed that rats died within 6 weeks when fed the McCollum high calcium, rickets-producing Diet 3143 plus 50,000 times overdosage of activated ergosterol. The important work of Harris and Moore (4) demonstrated that 1000 times overdosage of vitamin D was harmless to rats, whereas 100,000 times overdosage was rapidly fatal. The agent which produced the characteristic lesions was apparently the activated ergosterol *per se*, and not unchanged ergosterol or decomposition products. Light, Miller, and Frey (5) reported that while 100,000 times overdosage was fatal, 10,000 times had no effect on growth during 6 months. Numerous other papers, mostly quite uncritical, have dealt with the effects of extraordinary doses. In nearly every case the authors failed to determine the potency of the product which they employed. They stated only the quantity of irradiated ergosterol in mg.

It must be accepted that remarkably large doses of activated ergosterol are not immediately toxic, although sufficiently enormous doses do produce serious effects. Up to the present, published experiments have been based on short time tests, covering at the most a few months of life in the rat. It is our purpose to describe the results of tests continued from infancy to old age.

The ergosterol was activated by exposing a solution in arachis

oil to the rays of a mercury vapor quartz lamp. Preliminary tests (thirty-four rats) indicated that there was no great difference in the toxicity of ergosterol irradiated in the oil, in ether under a reflux, or as crystals in air, provided the antiricketic potency of the preparations was the same. These tests also showed that the composition of the basal ration is a factor in the action of ergosterol. In all experiments the ergosterol solution was triturated with the ration, fresh mixtures being made weekly.

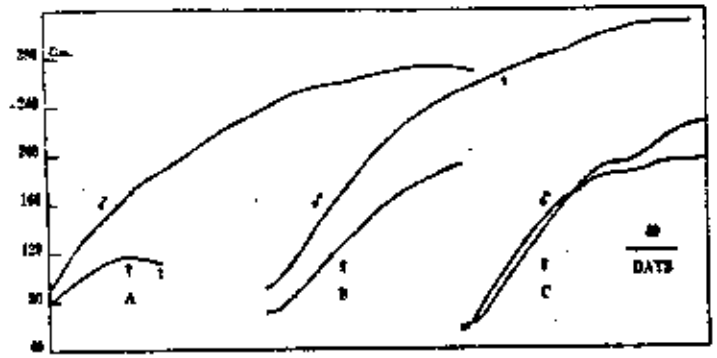

FIG. 1. Composite growth curves of rats on (Curves A) Diet 3143 plus minimum antiricketic dose of activated ergosterol, (Curves B) Diet 3143 plus 100 × overdose of activated ergosterol, and (Curves C) Diet 3143 plus minimum antiricketic dose (0.25 per cent) of cod liver oil.

Experiments with Diet 3143.

In our first long time series the ergosterol solution was standardized to contain 100 times the vitamin D content of average cod liver oil. The basal ration was the rickets-producing Diet 3143 (6). On this diet, without vitamin D, young rats ordinarily die of rickets after several weeks.

Nine rats, 24 days old, were put on Diet 3143. After 19 days, when the rats were rickety, we added to their diet 0.0025 per cent of the ergosterol solution—the very minimum which, in separate tests, would bring about partial healing of rickets. Fig. 1, Curves A, reveals that with this supplement the animals have survived many months. Growth was poor in comparison with that of rats on a normal stock ration (Fig. 2, Curves A). The males are still living after 12 months. One female died after

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3 months, and the remainder, being in poor condition, were killed. Autopsy showed rickets.

In a similar series ten rickety rats were given 0.25 per cent of the ergosterol solution; *i.e.*, 100 times the minimum antiricketic dose. After 12 months all are living except one male which developed a respiratory infection and died after 7 months. The growth rate for both sexes (Fig. 1, Curves B) was better than with the minimum dose, but it was decidedly inferior to the growth rate of

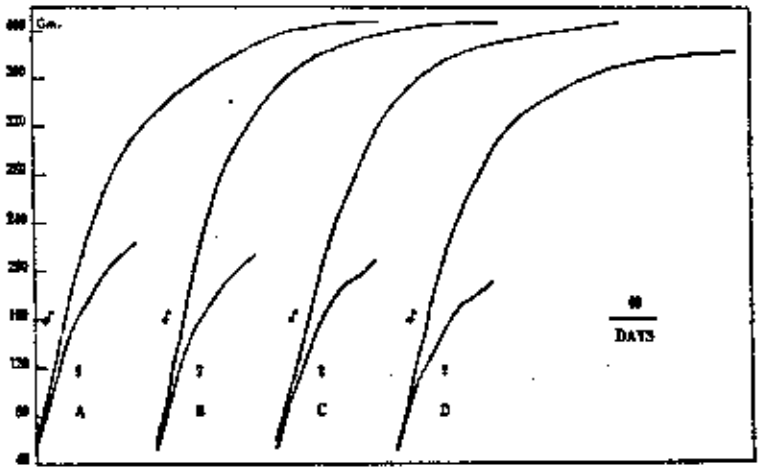

FIG. 2. Composite growth curves of rats on (Curves A) stock diet, (Curves B) stock diet plus minimum antiricketic dose of activated ergosterol, (Curves C) stock diet plus 100 × overdose of activated ergosterol, and (Curves D) stock diet plus non-irradiated ergosterol equal in amount to the activated ergosterol in C.

normal animals. Reproduction was faulty, the young frequently being eaten at birth and in no instance surviving longer than 5 weeks.

In addition to these series, we have made tests (fourteen rats) with concentrated solutions, the animals receiving in Diet 3143 doses from 10,000 to 60,000 times the minimum. The rats showed very wide individual differences in susceptibility to these great doses. During the 1st week some of them lost weight but later developed a partial tolerance. They survived for from 1 to 14 months.

Seven normal rats, 24 days old, weighing from 50 to 60 gm., were put on Diet 3143 supplemented with 0.25 per cent of cod liver oil. Assays had shown this to be the minimum antiricketic level, equivalent to 0.0025 per cent of the standard activated ergosterol solution. Fig. 1, Curves C, indicates that the growth curves of this series were again subnormal and not essentially different from the curves obtained with supplements of activated ergosterol. It follows, therefore, that the depressed growth rate was due to some inherent fault in Diet 3143, and not to the ergosterol or to the oil. The fault was evidently not a lack of vitamin A, since cod liver oil did not correct it.

Experiments with Stock Diet.

As it was manifestly impossible to obtain normal growth and reproduction on Diet 3143, even with added vitamins A and D, we began extensive tests, employing stock diet as the basal ration. For 4 years our breeding colony has been reared on a modified Steenbock stock diet, compounded as follows:

Yellow maize	57
Whole milk powder	25
Linseed oil meal	12
Crude casein	3.7
Alfalfa leaf meal	1.5
"Iodized" table salt	0.4
Calcium carbonate	0.4

To this ration the various additions of ergosterol were made. For each series ten rats were taken from their mothers when 24 days old, and selected to weigh between 50 and 60 gm. Considerable selection was necessary to obtain the requisite uniformity with these superior animals.

Fig. 2 shows the growth curves of rats for 10 months on (Curves A) the normal stock ration, (Curves B) the same plus the minimum antiricketic dose of activated ergosterol, (Curves C) the same plus 100 times overdose of activated ergosterol, and (Curves D) the same plus a quantity of non-irradiated ergosterol equal in amount to the activated ergosterol in Curves C. It is meaningless to state, as is often done in the medical literature, that a certain number of mg. of activated ergosterol were administered. The potency of irradiated ergosterol varies enormously with the condi-

tions under which activation is performed, values from zero to a million times the potency of average cod liver oil being obtainable. The material used in most of the present work had a potency about 400,000 × prior to dilution. The ergosterol intake of the rats receiving the minimum antiricketic dose may therefore be estimated as 1/160,000,000 gm. per day when the food intake was 10 gm.; i.e., the modified diet then contained 1 part of ergosterol to 160,000,000 parts of ration.

It is evident from Fig. 2 that after 10 months the three groups of rats which received ergosterol preparations show growth curves in no way inferior to the normal growth curve with stock ration. Curve D may seem inferior, but one should note that these animals were comparatively small for their age when started. Up to now the general health of the animals has been good, with the following exceptions: On the unsupplemented stock ration one female got a severely infected tail, which was successfully treated with hexylresorcinol. Another female exhibited a transient mammary infection. Of the animals receiving the minimum antiricketic dose of activated ergosterol, and the 100 times overdose, one male in each group has a respiratory infection. One male receiving the untreated ergosterol has shown a lack of equilibrium since the 5th month, and several males have shown minor respiratory infections.

All four groups reproduced normally. The growth curves and general well being of the offspring have been normal. Representative numbers of the second generation have in turn been bred, and their offspring, still receiving the test diets, also appear normal. This phase of our study involved 459 animals. In conformity with our usual practice we reduced all litters to eight.

In another series much larger doses were administered. With the same standard solution of activated ergosterol (100 × the potency of cod liver oil) we ran experiments on two more groups of ten rats each. The first group (Fig. 3, Curves A) received 2.5 per cent of this solution in stock diet (= 1000 × overdosage), and the second group (Fig. 3, Curves B) 10 per cent (= 4000 × overdosage). Ten more rats were used in an arachis oil control (Fig. 3, Curves C), these receiving 10 per cent of oil but no ergosterol.

been mated three times (with males of the same group). One of these females became pregnant twice, bearing eleven and nine young. Two females failed to become pregnant the first time, but bore young the second, with litters of seven and five. Two females bore twelve and three young the first time, but failed the second. The average number in the litter was eight and the average weight of the young at 24 days of age was 28 gm.—about 40 per cent below normal. All the females failed to reproduce following the third mating.

The control rats on 10 per cent arachis oil had come from the same litters originally as the rats on the 4000 × overdose. Reproduction was better. The average number in the litter was nine, and the average weight of the young at 24 days of age was 42 gm. —only slightly below normal.

This phase of our study involved 231 animals, and it shows that *the effects of overdosage with activated ergosterol become distinct when about 4000 times the minimum antiricketic dose is administered over an extended period.*

Fig. 3, Curves D, illustrates the effect of an overdosage of 40,000 times. The ten rats comprising this test were given the same basal ration, *i.e.* stock diet, as were those in the preceding series. Since it was impossible to administer a quantity of the activated ergosterol solution of standard strength (100 × cod liver oil) sufficient to obtain 40,000 × overdosage, we used a special arachis oil solution that was 1000 times more potent than cod liver oil. 10 per cent of this extremely potent preparation was employed. It is evident from Fig. 3, Curves D, that decidedly toxic effects were produced. After about 2 months the animals began to die, and none of them survived longer than 6 months. They presented the picture of hypervitaminosis described by Collazo, Rubino, and Varela, and others.

Experiments on the Effect of Added Ca and P.

It is particularly interesting to compare the effects of this enormous dose on rats receiving stock diet, with the effects of the same dose given in conjunction with an excess of calcium or phosphorus. Fig. 4, Curves A, illustrates that normal growth obtains with stock ration plus 3 per cent additional calcium carbonate.

Fig. 3 shows in all cases a depressed growth rate over 9 months. Between the 1000 × and 4000 × overdoses there is, strangely, little difference, and the depression is but slightly more than in the case of the arachis oil controls. One must conclude, therefore, that the depression is due partly to the activated ergosterol and partly to excessive fat.

In appearance the rats receiving the 1000 × overdose are normal. One male, however, has had a disturbed equilibrium since

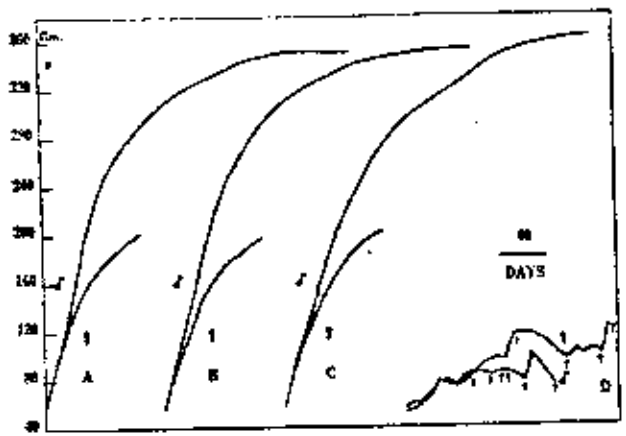

Fig. 3. Composite growth curves of rats on (Curves A) stock diet plus 1000 × overdose of activated ergosterol, (Curves B) stock diet plus 4000 × overdose of activated ergosterol, (Curves C) stock diet plus 10 per cent of arachis oil, and (Curves D) stock diet plus 40,000 × overdose of activated ergosterol.

the 3rd month. One female receiving the 4000 × overdose developed respiratory trouble and diarrhea after 8 months. One male on the arachis oil control developed respiratory trouble after 7 months. All the arachis oil controls and the males on 4000 × overdosage are normal in appearance after 9 months, but the females which received 4000 × overdosage are disheveled and weak, and are losing weight.

The rats on 1000 × overdosage have reproduced normally, and their young have developed at a normal rate. Those on 4000 × overdosage have not done so well. Each of the five females has

carbonate. Again it is evident that the salt addition itself had no effect on growth, and that the 10 per cent addition of arachis oil slightly depressed the growth. When both phosphate and the 40,000 × overdose of activated ergosterol were used, results no different from the earlier experiment without phosphate (Fig. 3, Curves D) were obtained in the case of the females. The males, however, appeared to be slightly protected. Thus we see that while the toxic effect of enormous doses of activated ergosterol is

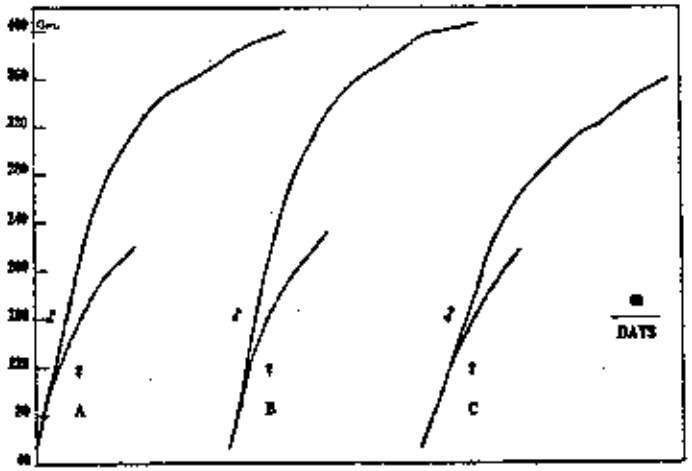

Fig. 5. Composite growth curves of rats on (Curves A) stock diet plus minimum antirickettic dose (0.25 per cent) of cod liver oil, (Curves B) stock diet plus 10 × overdose (2.5 per cent) of cod liver oil, and (Curves C) stock diet plus 40 × overdose (10 per cent) of cod liver oil.

accentuated by additional calcium, an addition of phosphate results in no potentiation, and may possibly lessen the effect of the drug.

Experiments with Stock Diet and Cod Liver Oil.

For purposes of comparison, we have made three sets of tests with stock ration supplemented with cod liver oil. The oil was a medicinal Newfoundland product of average potency, which was carefully assayed, and had served as the standard of potency for preparing the activated ergosterol solutions. Three groups of ten

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Fig. 4, Curves B, shows that with stock ration plus 3 per cent calcium carbonate plus 10 per cent arachis oil the growth rate is depressed slightly, just as it was in the case of stock ration with 10 per cent of this oil. Fig. 4, Curves C, shows that when 10 per cent of the highly potent 1000 × solution (40,000 × overdosage) was added to the stock ration containing the extra 3 per cent of $CaCO_3$, the toxic effect of the activated ergosterol was very marked. The

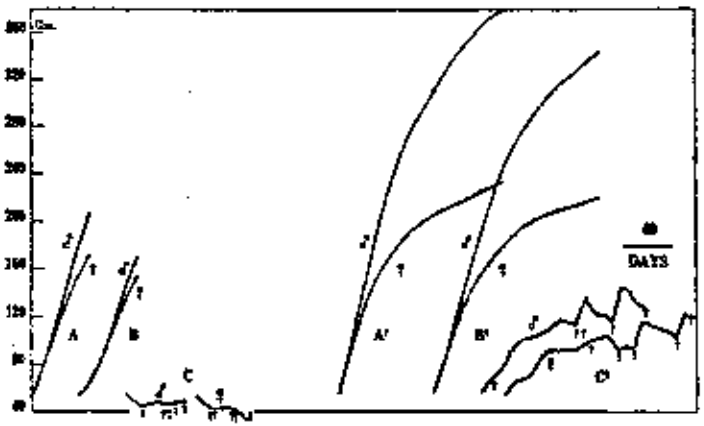

FIG. 4. Composite growth curves of rats on (Curves A) stock diet plus 3 per cent of calcium carbonate, (Curves B) stock diet plus 3 per cent of calcium carbonate plus 10 per cent of arachis oil, (Curves C) stock diet plus 3 per cent of calcium carbonate plus 40,000 × overdose of activated ergosterol, (Curves A') stock diet plus 3 per cent of disodium phosphate, (Curves B') stock diet plus 3 per cent of disodium phosphate plus 10 per cent of arachis oil, and (Curves C') stock diet plus 3 per cent of disodium phosphate plus 40,000 × overdose of activated ergosterol.

rats lost weight from the 1st day, and all died within 7 weeks. They presented Collazo's picture in its most wretched form. In a repetition of this experiment, a female near death was returned to normal stock ration. It is most interesting to note that this rat recovered with great rapidity, and has borne an excellent litter of twelve young.

In Fig. 4, Curves A', B', and C' correspond to Curves A, B, and C, the experiment being conducted exactly as before, except that disodium phosphate, $Na_2HPO_4 \cdot 12H_2O$, was used instead of calcium

rats each were given 0.25, 2.5, and 10 per cent of this oil in their diet. The growth curves are shown in Fig. 5. It would seem that the curves for the minimum antirickctic dose of cod liver oil (0.25 per cent), and for 10 × overdosage (2.5 per cent), are similar to those for the unsupplemented stock ration and for the lower levels of activated ergosterol (Fig. 2). However, a 40 × excess (10 per cent) of cod liver oil depressed the growth rate distinctly—to the same degree as did 10 per cent of arachis oil, and slightly less than did 4000 times excess of activated ergosterol (Fig. 3). To explain the growth-depressing action of cod liver oil and arachis oil it is not necessary to postulate a specific toxic principle, inasmuch as the effect can well be associated with the unusual fattiness of the ration.

After 7 months the general appearance of the rats receiving cod liver oil is good. Two males on 0.25 per cent have recently developed respiratory infections. Except for one female on 10 per cent oil, which died in labor, all three groups reproduced normally. The offspring of the rats receiving 0.25 and 2.5 per cent oil have grown at the normal rate. The offspring of the 10 per cent group were inferior, their average weight at 24 days being 37 gm.—about 18 per cent below normal. These observations on reproduction involved 241 young.

Throughout our experiments group consumption records were kept. They revealed no fact of significance except that less food was consumed by the animals receiving strongly toxic preparations and 10 per cent of cod liver oil.

Experiments on Protection Against Rickets.

With so many second and third generation rats at our disposal, we made use of the opportunity to study the influence of activated ergosterol in the mother's diet on resistance to rickets in the young. In the following experiments 200 rats were used. Methods of investigation included the line test (200 rats), ash determinations on alcohol-extracted femurs (56 rats), and blood analyses (80 rats). Parallel determinations showed that there is little choice in the three methods, the simple line test giving results just as significant as the more complicated analytical procedures. Group averages showing rickets by the line test on tibias gave 33 per

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cent ash in femurs, 14 mg. per cent Ca in serum, and 2 mg. per cent P in serum.

Omitting a description of the somewhat intricate details of the investigation, we have summarized the results as follows:

Group 1.—Parents on normal stock diet. Young nursed by their own mothers. Young put on rickets-producing diet. Young developed rickets.

Group 2.—Parents on normal stock diet. Young nursed by foster-mother from birth to weaning, the foster-mother receiving minimum antiricketic dose of activated ergosterol in stock diet. Young put on rickets-producing diet. Young developed rickets.

Group 3.—Parents on stock diet plus 100 × overdose of activated ergosterol. Young nursed by their own mother for 3 days, then by foster-mother receiving normal stock diet. Young put on rickets-producing diet. Young developed rickets.

Group 4.—Parents on stock diet plus 1000 × overdose of activated ergosterol. Young nursed by foster-mother from birth to weaning, the foster-mother receiving normal stock diet. Young put on rickets-producing diet. Young developed rickets.

Group 5.—Parents on stock diet plus 1000 × overdose of activated ergosterol. Young nursed by their own mother for 3 days, then by foster-mother receiving normal stock diet. Young put on rickets-producing diet. Young developed only slight rickets.

Group 6.—Parents on normal stock diet. Young nursed by foster-mother during first 4 days, the foster-mother receiving 1000 × overdose of activated ergosterol in stock diet. From 4th day to weaning young returned to their own mother on normal stock diet. Young put on rickets-producing diet. Young developed only slight rickets.

Group 7.—Parents on stock diet plus 100 × overdose of activated ergosterol. Young nursed by their own mother for 16 days, then by foster-mother receiving normal stock diet. Young put on rickets-producing diet. Young developed only slight rickets.

One of us has previously given evidence (7) that the Greenland seal, living on a strongly antiricketic diet of fish, does not transfer vitamin D to the young *in utero*. The present experiment is another example, *a fortiori*, of the same phenomenon. It would appear also that no large amount of activated ergosterol is transmitted through the milk, although nursing rats did obtain sufficient ergosterol for partial protection when their mothers were given great overdoses.

Interesting in this connection is the observation by Hess and Weinstock (8) that vitamin D from cod liver oil was not transferred through the placenta or milk in sufficient amount to protect the

young. Apparently it is a general rule that vitamin D is not transmitted through the placenta, and not through the milk unless very large amounts are administered—by mouth as in our experiments, or by irradiating the mother (9–11).

The investigations which we have reported involved some 1200 rats, including second and third generations. The work covered the period from infancy to late maturity. It is our intention to continue the study on the same original rats, keeping them under observation until they die. We shall later report on the blood chemistry and tissues changes.

SUMMARY.

1. In long time experiments activated ergosterol administered to rats in doses 100 times greater than the minimum antiricketic level showed no effect on general appearance, growth, reproduction, or resistance to respiratory infections. 1000 times overdosage was just perceptibly harmful, 4000 times overdosage definitely injurious, and 40,000 times overdosage strongly toxic.

2. The toxic action was potentiated by calcium carbonate; unaffected or slightly lessened by disodium phosphate.

3. No significant quantity of activated ergosterol was received by rats *in utero*. Nursing rats received in their milk a small amount of vitamin D when the mothers were given enormous overdoses.

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IDIOPATHIC HYPERCALCEMIA OF INFANCY, WITH FAILURE TO THRIVE*

Report of Three Cases, with a Consideration of the Possible Etiology

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PHILADELPHIA

THE syndrome of "idiopathic hypercalcemia of infancy, with failure to thrive" has been reported with some frequency in the United Kingdom during the past few years. It appears to be relatively uncommon in North America, only 2 cases having been recorded in some detail[1,2] and 2 others briefly noted.[3] The reasons for this discrepancy in incidence among two populations, similar in many ways, are not understood.

Three infants with this condition, which was severe in 1 and mild in 2, have been observed at the Children's Hospital of Philadelphia within a period of two years. This communication is presented to focus attention on this disorder of infancy, lest it be more common, but unrecognized, in the United States, and to relate the observations concerning these 3 cases to the published reports from England, with particular consideration of the possible etiology.

CASE REPORTS

CASE 1. K.S., an 11-month-old female infant, was first seen for the diagnosis and treatment of "dwarfism" and poor weight gain (Fig. 1). She had weighed 2.3 kg. (5 pounds, 1 ounce) at birth and had not been fed at the breast. The estimated daily intake of vitamin D before the initial examination was 1400 international units daily. She consistently refused solid foods and had a poor appetite. She gained weight and grew satisfactorily until 3 or 4 months of age, after which she failed to thrive (Fig. 2).

Physical examination revealed a malnourished infant who was irritable and possessed a peculiar elfike facies, with sparse hair on the head. There were prominent epicanthic folds, as well as an overhanging upper lip, and the bridge of the nose appeared to be underdeveloped for the age, as did the mandible. There were no teeth. There was a paradoxical combination of apparent disinterest in her environment with a rather alert countenance, as well as a degree of muscular activity that was greater than would be expected in the face of a generalized moderate hypotonia. She could support her head and was able, with some difficulty, to sit and to pull herself to a standing position. She had generalized pruritus and was covered with scratch marks. She weighed 5 kg. (11 pounds) and was 65 cm. tall; the upper-to-lower-body ratio was 1.55, and the circumference of the head was 41 cm., that of the chest 35 cm., and that of the abdomen 30 cm. A loud systolic murmur was audible over the entire precordium and was transmitted to the left posterior aspect of the thorax.

The blood pressure was 130/90.

Examination of the blood showed a red-cell count of 2,270,000, with a hemoglobin of 7.4 gm. per 100 ml., and a white-cell count of 9450, with 49 per cent neutrophils, 23 per cent lymphocytes, 6 per cent monocytes and 2 per cent eosinophils. Urinalysis revealed a specific gravity of 1.010, a pH of 7.0 and a trace of albumin and a + + test for acetone; the sediment contained 4 or 5 white cells per high-power field. A culture of a specimen of urine obtained by catheterization indicated the presence of *Escherichia coli* and *Proteus vulgaris*. The chemical examinations of the serum are shown in Table 1.

Trypsin was present in the stool. Subdural taps were negative. Radiologic examination of the long bones revealed an abnormal density throughout, which was especially marked in the zones of provisional calcification, resembling osteopetrosis (Fig. 3), and increased density at the base of the skull (Fig. 4). An intravenous pyelogram showed poor dye concentration but the silhouette was adequate to rule out any abnormality; there was stippling in both renal shadows suggestive of renal calcinosis.

All supplemental vitamin D was discontinued, and feeding with unfortified whole cow's milk containing Karo syrup was instituted. The infant was then discharged on 500 mg. of chloramphenicol daily.

At 12 months of age the serum calcium rose to 15.0 mg., and the nonprotein nitrogen to 108 mg. per 100 ml. Treatment with 20 mg. of cortisone acetate daily, in divided doses by mouth, was instituted.

At 15 months of age, the weight had risen to 5.5 kg. (12 pounds, 3 ounces), and the patient was 66 cm. tall. The serum calcium was 14.0 mg., and the nonprotein nitrogen 85 mg. per 100 ml.

She weighed 5.8 kg. (12 pounds, 14 ounces) at 16 months of age and had progressed more in the previous 8 weeks than in any similar period since the onset. The cortisone treatment had been continued. She was less irritable, and the pruritus had diminished. The blood pressure was 130/90 to 160/120. The serum calcium was 9.6 mg. and 11.8 mg. on 2 successive days, and the nonprotein nitrogen 83 mg. per 100 ml. The serum phosphorus was 6.1 mg. per 100 ml. Owing to difficulties in obtaining unfortified cow's milk, the formula was changed to the following: strained beef, 312 gm.; Dextri-Maltose, 71 gm.; olive oil, 100 ml.; and water, 1080 ml. The dose of cortisone was diminished to 10 mg. daily.

At 22 months of age, the patient weighed 6.6 kg. (14 pounds, 8 ounces). The serum calcium was 10.5 mg., and the nonprotein nitrogen 54 mg. per 100 ml.

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Two months later she weighed 7 kg. (15 pounds, 8 ounces) and was 61 cm. tall. The cardiac murmur persisted. X-ray study of the chest revealed a normal cardiac silhouette, and there had been no other clinical evidence of organic heart disease. She had become playful and happier (Fig. 5) and spoke three or four words. The serum calcium was 10.8 mg., and the phosphorus 4.1 mg. per 100 ml.

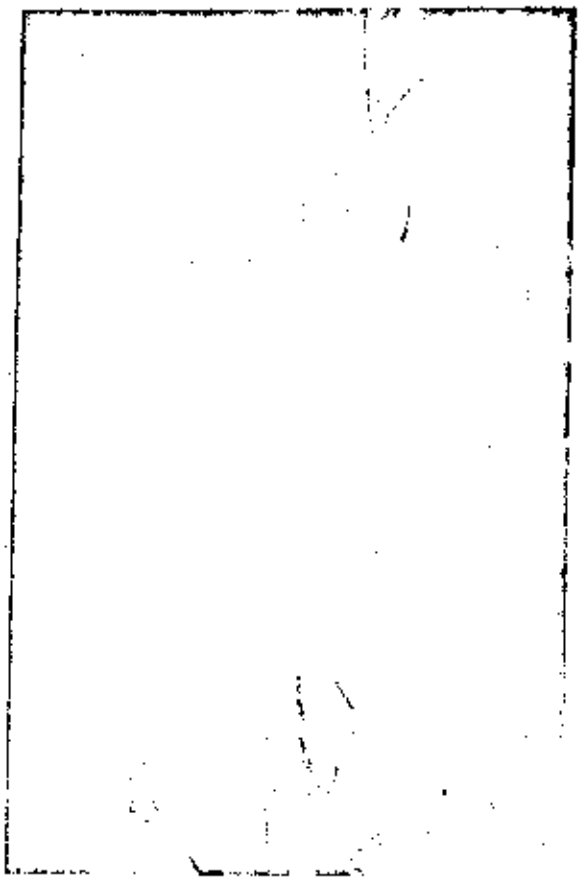

FIGURE 1. *Appearance of Case 1 at Eleven Months of Age.*

At 27 months of age she was readmitted to the hospital for detailed examination. The blood pressure had fallen to 126/60. The murmur persisted. The serum bicarbonate was 29 milliequiv. per liter, and the calcium was 11.6 mg., the phosphorus 5.5 mg., and the cholesterol 256 mg. per 100 ml. There was a total of 20.7 mg. of calcium in a 24-hour specimen of urine — this was determined after 11 months on a low-calcium diet. Radiologic examination indicated a diminution of the osteosclerosis, with no evidence of rickets; the calcification of the falx cerebri was again noted, and the sagittal sutures of the skull was completely united. The bone age determined at multiple centers was 2 to 2½ years. The patient was discharged on a smaller dose of cortisone (5 mg. daily), with continuation of the antibiotics.

At 29 months of age she weighed 7.7 kg. (17 pounds) and was 75 cm. tall. The blood pressure was 120/70. There was a great change in her general appearance, with a subsidence of the facial features noted earlier and an increase in subcutaneous fat. She was walking fairly well, but with support. At 33 months of age there was continued improvement in her general appearance, but there appeared to be moderate retardation of intellectual development. The serum calcium was 11.3 mg., and the phosphorus 5.6 mg. per 100 ml.

CASE 2. L.L., an 18-month-old male infant, was first seen for consideration of "growth failure." An older female sibling had always been healthy. The patient had been born 1 month prematurely and weighed 2.2 kg. (4 pounds, 12 ounces) but appeared healthy. He was not fed at the breast. The total daily intake of vitamin D was calculated to approximate 2500 international units. Between the ages of 4 and 8 weeks projectile emesis occurred once a day, ceasing after a time, but since then he had gained weight and height poorly. The parents described marked polyuria and polydipsia. He had received an unknown dose of desiccated thyroid between the ages of 12 to 14 months, with no improvement. He had always shown a disinclination toward solid foods.

Physical examination demonstrated the peculiar cushing facies noted in Case 1; the similarity was at once striking (Fig. 6). A soft systolic murmur was distinctly audible at the apex but was not transmitted.

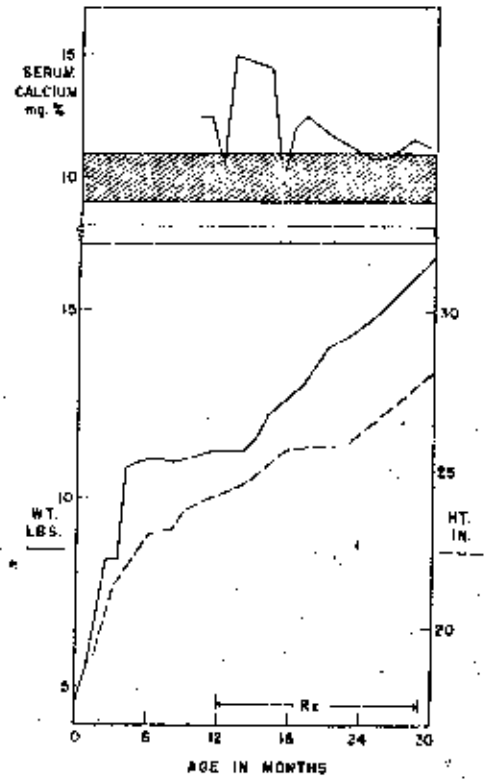

FIGURE 2. *Progress of Case 1 since Birth, Showing Growth Failure at the Time of Onset and Serum Calcium Levels. Treatment consisted of a diet low in calcium and vitamin D, with cortisone.*

The patient was admitted to the hospital at 20 months of age, when he weighed 7.5 kg. (16 pounds, 9 ounces) and was 74 cm. tall. The blood pressure was 120/70. Generalized hypotonia was present. The laboratory data are summarized in Table 1. The protein-bound iodine was 8.6 microgm. per 100 ml. The bone age was 13 months; there were no abnormalities of the long bones or skull, and calcification in the renal area was not noted.

The patient was discharged on a low-calcium diet utilizing a beef-base formula as in Case 1. All vitamin D supplements were discontinued.

Ten weeks later he was walking alone, and his appetite had improved. The serum calcium was 12.3 mg. per 100 ml.

At 2 years of age, the serum calcium was 11.9 mg. per 100 ml. There was marked improvement in muscular strength and appetite. He weighed 7.8 kg. (17 pounds, 4 ounces) and was 75 cm. tall.

CASE 3. M.S., a 13½-month-old male infant, was first seen because of poor growth. A male sibling, 4 years of age, had always been well. The patient weighed 3.7 kg. (8 pounds, 5 ounces) at delivery. He was not breast fed. At 9 months of age he weighed 8.2 kg. (18 pounds). He then became listless, mild anorexia developed, and he gained very little weight during the subsequent 4½ months. There was no history of excessive intake of vitamin D, and it was estimated that he received about 1200 international units daily. He sat alone at 6 months, crawled at 10 months and was able to stand without support at the time of the 1st examination.

On physical examination at 13½ months he weighed 8.6 kg. (19 pounds) and was 75 cm. tall; the circumference of the head was 48 cm., that of the chest 46 cm., and that of the abdomen 42 cm. The facies was vaguely suggestive of that described in Cases 1 and 2. He was active in his crib but unable to move around to any extent because of a distinct generalized hypotonia. The heart, lungs and abdomen were normal.

Examination of the blood showed a red-cell count of 3,550,000, with a hemoglobin of 13.1 gm. per 100 ml., and a white-cell count of 8500, with 38 per cent neutrophils, 56 per cent lymphocytes and 6 per cent monocytes. Urinalysis indicated a specific gravity of 1.027 to 1.030, a pH of 4.5 to 7.0, a + + to + + + test for acetone, a + + Sulkowitch test and no phenylpyruvic acid; there were no white cells in the sediment. Radiologic study revealed bones of normal density and a normal bone age. The protein-bound iodine was 5.8 microgm. per 100 ml. Certain laboratory data are shown in Table 1. The urinary calcium was 30.2 mg. per day, and the phosphorus 273 mg. per day. The spinal fluid was normal. The Kolmer test was negative. The patient was discharged on a diet without additional vitamin D and with no attempt to restrict calcium intake. Two months later the serum calcium was again 12.0 mg., and the phosphorus 3.1 mg. per 100 ml.

DISCUSSION

In 1932 Lightwood[4] probably first described what has since been termed "idiopathic hypercalcemia of infants, with failure to thrive."[16] The child discussed at this relatively early date presented most of the clinical features associated with the severe form of the syndrome. The findings were not associated with a high intake of vitamin D. The patient died, and postmortem study showed renal calcinosis and osteopetrosis.

The problem was recalled in 1951, when Fanconi[6] and Butler[7] simultaneously presented their observations on 1 infant each, remarking on the coincidence of growth failure, hypercalcemia and certain other clinical features detailed below. Subsequently, these authors and their co-workers presented a detailed joint report.[8] In 1952 Lightwood and Payne[5] described 10 infants with hypercalcemia and failure to thrive, whom they delineated from the syndrome of "renal rickets." Since then, numerous cases have been reported in the British literature but only 4 in the American.[1-3]

The symptoms and clinical features of idiopathic hypercalcemia of infancy with failure to thrive present a constant pattern in the various reports cited. There is no sex difference. The onset of the clinical manifestations, particularly the cessation of growth, is between six weeks and nine months of age. Case 2 is said to have been thriving poorly since birth. There may be marasmus in the severe form of the disorder. Gastrointestinal complaints are common and include emesis (often projectile), anorexia and constipation. Polyuria and polydipsia (reported only in Case 2) occur in about 50 per cent. There is generalized muscular hypotonia in almost all patients, and this frequently misleads the examiner into believing that the child is mentally retarded. Paradoxically, most children are alert and not sluggish in spite of the hypotonia. The facies has been described as "elflike," with prominent epicanthic folds, overhanging upper lip and underdeveloped bridge of the nose and mandible; these features were distinctive in Cases 1 and 2. However, the characteristic facies may be absent.[9] In about 40 per cent there is hypertension. A harsh systolic cardiac murmur is audible in many and has not been attributable to cardiac disease -- Lowe et al.[10] found extensive metastatic calcification, including the heart and lungs, in 1 case. Transient facial palsies may occur, and nonparalytic squint is sometimes present. The head may fail to grow during the active stage of the disease.

The chemical findings described in 10 infants by Lightwood and Payne[5] have been noted regularly. The serum calcium is elevated, and the levels above 11.5 mg. per 100 ml. are significant[11] when persistent and accompanied by the other features. The blood urea nitrogen is sometimes elevated, but the serum phosphorus, phosphatase, bicarbonate and chloride are usually normal. The serum alpha$_2$ globulins and gamma globulins are often elevated. Other biochemical findings[11] include an elevated serum cholesterol in 60 per cent at some time during the stage of the disease; this was true in Case 1 only. The serum phosphorus may rise with continued progress of the disease in the severe form. The serum alkaline phosphatase is almost never elevated and may be low in 40 per cent.

Nephrocalcinosis was detected in approximately 20 per cent of the reported cases. Intravenous pyelograms reveal no abnormality, other than poor concentration of the dye.[2] There is increased density of the long bones and the base of the skull, resembling true osteosclerosis (Albers-Schönberg disease), in 20 per cent, and in a larger number there are only transverse lines of increased density in the long bones. Craniostenosis may occur in 10 to 20 per cent of cases with the severe form of the disorder. Evidence of renal infection is present in about 50 per cent of cases.

A distinction has been drawn between a benign and a severe form of idiopathic hypercalcemia of infancy, and this has been sharply defined by Lowe et al.[10] The benign form is said to be transient, self-limited and subject to relatively early remission with no permanent consequences; the severe form is more protracted and may be fatal, or with remission there is mental and physical retardation. The characteristic facies is absent in the benign form. Hypertension, heart murmur

Etiology

The cause of idiopathic hypercalcemia of infancy, with failure to thrive, is not established. Several factors have been incriminated, and, at present, sensitivity to vitamin D has been assigned a prominent role by many authors.

Table 1 *(Concluded).*

Age at Onset	Serum Chloride	Serum Bicarbonate	Alkaline Phosphatase	Total Protein	Albumin	Alpha Globulin	Gamma Globulin	pH of Urine	Specific Gravity of Urine
mo.	*milliequiv./liter*	*milliequiv./liter*	*units*	*gm./100 ml.*	*gm./100 ml.*	*gm./100 ml.*	*gm./100 ml.*		
4	—	21	6.9	8.1	3.3	—	—	5.0-7.0	1.010-1.020
1	103	21	3.5	7.2	5.0	0.8	0.7	6.0	1.014
8-9	103	23	3.5	6.9	3.5	1.1	0.7	7.0	1.022

*No infants breast fed.

Fanconi[6] attributed the disease to a collection of separate congenital defects. Butler[8] believed the condition to result from an abnormal sensitivity to ingested calcium in the face of primary renal damage.

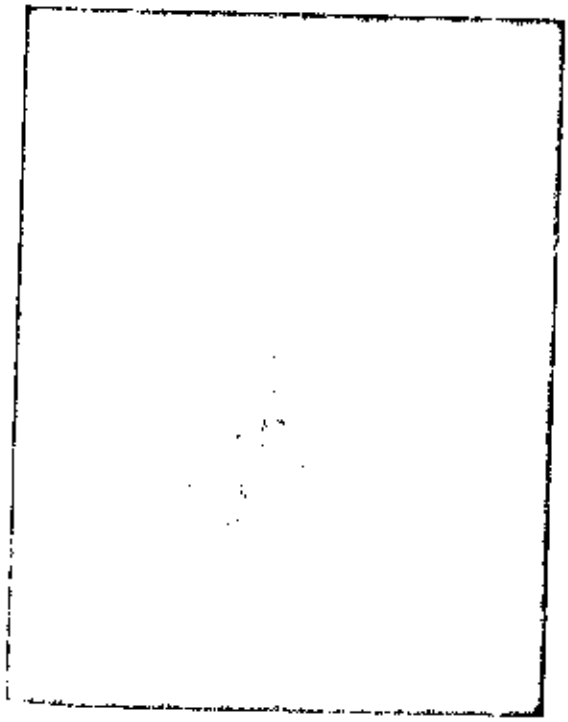

Figure 4. *X-Ray Film of the Skull in Case 1.*

It is improbable that parathyroid dysfunction is primarily responsible for this syndrome. As discussed above, the biochemical findings are not consistent with disease of the parathyroid glands; furthermore, examination of the glands[16,17] has shown them to be normal. It is possible that renal tubular immaturity, with unresponsiveness to parathormone, could produce hypercalcemia; however, it is possible to increase the urinary phosphate excretion in an infant with the syndrome by the administration of parathormone.[9,18]

The similarity of this disease to the "milk-alkali" syndrome in adults has led to consideration of the role of alkaline medications in infancy. Creery[19] pointed out that a high percentage of infants in his series had received alkaline laxatives or other remedies. In 1 American case[1] the frequent use of alkali was also reported. However, many patients received no such treatment. It is improbable that the ingestion of alkali is the sole cause of the disorder, although it cannot be denied that in occasional infants such therapy may aggravate an otherwise subclinical form. Creery and Neill[19] have suggested that renal infection per se may lead to hypercalcemia in certain children. There are many cases, generally milder, in which there has been no evidence of urinary infection, but it is possible that an earlier infection has subsided before the infant is brought under close scrutiny.

Forfar et al.[10] have described an increased calcium retention in 3 cases — 31, 48 and 69 per cent (normal, 32 per cent); others[5,10,14] have reported similar findings. Phosphorus retention is also increased.[12] Mild hypercalciuria is present in some cases.[7] The urinary amino acids are normal.[20]

Impaired renal function is evident from the frequent elevation of serum urea nitrogen or nonprotein nitrogen. This tends to subside with improvement in all but the severe cases. Fanconi[6] observed diminished renal clearance in a severe case. Sissman and Kline[7] conclude that dehydration may be the cause of the elevation of the nonprotein nitrogen; they also reported a low-normal tubular reabsorption of phosphate. In fatal cases renal calcinosis has been observed.[4,5,21,22]

Although definitive proof that vitamin D is a major cause of idiopathic hypercalcemia in infancy is lacking, there is much evidence to warrant serious attention to such a possibility. In most cases reported, the intake of vitamin D is not generally considered to be excessive; indeed, 3 of the 5 patients described by Forfar et al.[12] had received 400 to 550 international units daily, which is less than the recommended amount in the United Kingdom. Aside from the dis-

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mur and osteosclerosis are supposedly confined to the severe type. There may be a more marked elevation of the serum calcium, blood urea nitrogen and cholesterol in the severe type. In the 3 cases described above, it was not possible to classify sharply each child on the basis of this group of findings. On the basis of the biochemical findings, Rhaney and Mitchell[9] take exception to the formulation of definite criteria for such a distinction: 4 of their patients with the "benign" form died. A loud heart murmur may occur in the "benign" form, and osteosclerosis may be absent in the "severe" form. There appear to be all gradations of severity, and it is difficult to assign patients to one group or another. On a clinical basis, only Case 1 may be termed "severe."

Each of the patients described above was referred to the Children's Hospital of Philadelphia because of growth failure. The differential diagnosis merits some discussion. Although the presence of an elevated serum calcium will focus attention on the diagnosis of idiopathic hypercalcemia, other causes of growth failure should be sought.

Certain other conditions more closely related to the syndrome of idiopathic hypercalcemia require special attention.[10,11] Hyperparathyroidism may be considered, but in this condition there is a diminution in serum phosphorus (at least in the early stages), a marked rise in urinary calcium above the levels reported in the syndrome under discussion and an increase of serum alkaline phosphatase; furthermore, the bone lesions of hyperparathyroidism generally differ from those described in idiopathic hypercalcemia. In idiopathic hypercalcemia the bone lesions may suggest Albers-Schönberg disease, but in that condition the serum reveals no biochemical changes and there is often a myelophthisic anemia and a family history of the disorder. True vitamin D intoxication may be recognized by the history of the ingestion of large amounts of this vitamin and by the decalcification of bone, as a rule, rather than osteosclerosis. Renal acidosis may closely resemble idiopathic hypercalcemia but can be readily differentiated: hypotonia does not occur in renal acidosis; acidosis is absent in idiopathic hypercalcemia but present in renal acidosis — the urine is always alkaline in the former, and usually acid or neutral in the latter; and the serum calcium may rarely be high in renal acidosis, but falls with alkaline therapy — it does not fall with such therapy in idiopathic hypercalcemia. Osteosclerosis may occur in both syndromes.

Idiopathic hypercalcemia of infancy is a self-limited disease, and spontaneous recovery is the rule. In the more severe forms death from renal insufficiency may occur, or if the patient survives, physical retardation may persist for some years and mental retardation, possibly owing to premature cranial synosteosis, may become apparent.

TABLE 1. *Data in 3 Cases of Idiopathic Hypercalcemia of Infancy.**

Age at Onset	Effluent Facies	Vitamin D Dosage	Cardiac Murmur	Blood Pressure	Serum Calcium	Serum Phosphorus	Serum Cholesterol	Serum Nonprotein Nitrogen	Serum Sodium
mo.		*internat. units*			*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*milliequivalents*
4	+ + +	1400	+ + +	130/90-160/120	12.5-15.0	7.0-7.9	231	102-108	-
1	+ +	2500	+	120/70	11.7-12.5	5.5	198	30	142
4-9	±	1700	0	108/70	12.0-13.0	3.5-4.9	138	25	--

*No infants breast fed.

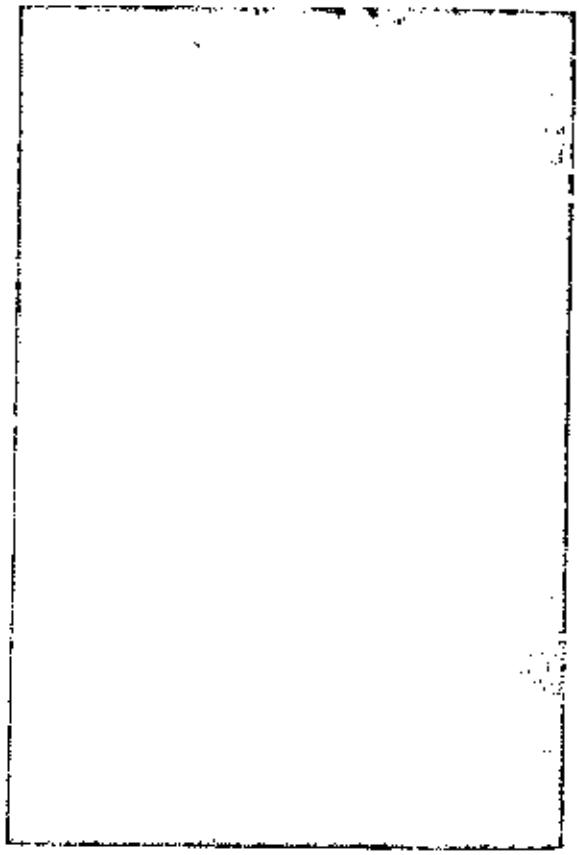

FIGURE 3. *X-Ray Film of the Long Bones in Case 1.*

similarities between idiopathic hypercalcemia and classic vitamin D intoxication discussed above, the serum citrate levels have been shown to be depressed,[19] a finding inconsistent with true vitamin D intoxication.

Is there a hypersensitivity to ordinary quantities of vitamin D in these infants? What constitutes an excessive intake? The former question has been raised

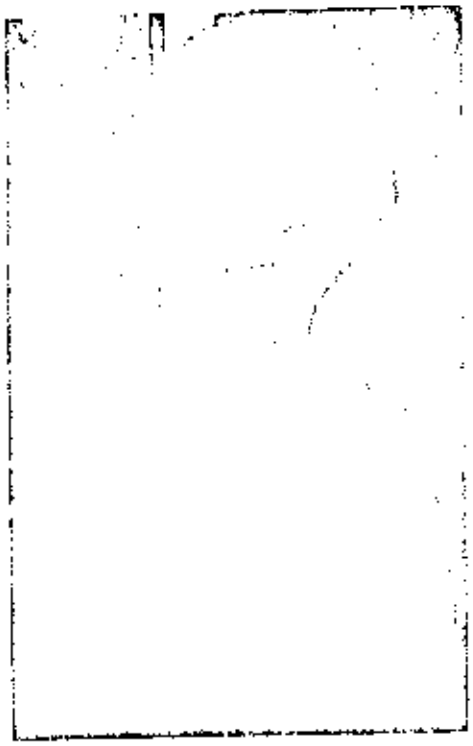

FIGURE 5. *Case 1 at Two Years of Age.*

in view of the failure to elicit by history an ingestion of what may be considered a large amount of the vitamin. Fyfe[21] has shown that the blood levels after oral administration are much higher in infants with idiopathic hypercalcemia than in normal infants. Might the same phenomenon occur in certain infants after "ordinary" doses of vitamin D? Perhaps the concepts of ordinary or extraordinary amounts of vitamin D require better definition. In a review, Jeans[22] pointed out that 200 international units of vitamin D daily produced a degree of calcium retention in infants no greater than that obtained when 800 units or more was used — at the higher level anorexia, diminished intake of food and failure to thrive developed.

Before 1943 the conventional dosage of vitamin D in England was 400 international units daily; more recently, coincident with the rise in frequency of idiopathic hypercalcemia in England, the official recommendation has been 700 units per day[14]; in addition, during this period, milk in the United Kingdom has been fortified with 1400 international units of vitamin D per quart whereas in the United States the amount is 400 units per quart. Might this seemingly inconsequential change account for the apparent difference in incidence between the two countries? Creery and Neill[20] state that no case of idiopathic hypercalcemia has occurred in "completely" breast-fed infants. None of our 3 patients were breast fed. However, some infants have been breast fed for variable, but perhaps inadequate, periods.[17] If vitamin D bears some responsibility for the condition, the fortification of artificial formulas and cow's milk requires careful scrutiny. It has been suggested[23] that the total vitamin D intake in infancy be limited to 400 to 800 international units per day — certainly this is adequate.[22] The fortification of milk, bread, virtually all infant cereals and artificially prepared

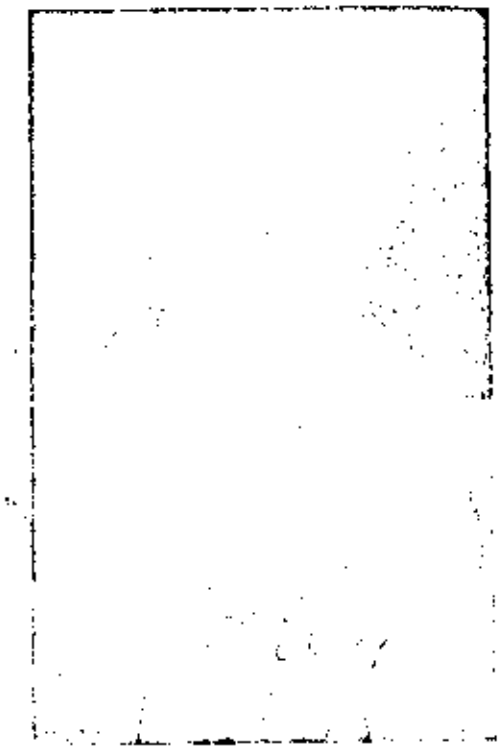

FIGURE 6. *Appearance of Case 2 at Eighteen Months of Age.*

commercial formulas in the United States is enthusiastically practiced. The practice seems to have got out of hand, and the quantities of added vitamins are frequently the focus of competitive advertisement. This fact was forcibly impressed upon us when we searched for foodstuffs containing little or no vitamin D for the treatment of the cases reported above. In the Philadelphia area it was almost impossible to obtain milk free of vitamin D.

Forfar et al.[12] have proposed an abnormality in the metabolism of cholesterol, the production of abnormal toxic sterols with vitamin D activity, as the basic cause of the disorder. Certain observations suggest that vitamin D may not be the major etiologic agent in

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idiopathic hypercalcemia. Hubble[18] administered large doses (550,000 and 1,000,000 international units) to 2 infants in remission, without producing a relapse; perhaps a temporary period of vitamin D hypersensitivity had already elapsed. The same authors[22] gave calciferol to infants during the active phase of the disease, without exaggerating the clinical features or the calcium retention. One infant in remission[20] received 2000 international units of vitamin D daily; within two weeks the serum calcium and blood urea nitrogen rose slightly but fell thereafter. In a similar experiment during remission,[24] an exacerbation was produced.

Treatment

Creery and Neill[20] obtained good results by employing the usual diet containing unfortified milk and without added vitamin D. The more thorough removal of vitamin D from the diet has been recommended by others.[10] In addition, the employment of a calcium-free diet has produced a depression of the serum calcium level.[24] Stapleton, Macdonald and Lightwood[14] believe that the use of a calcium-free diet and removal of all sources of vitamin D accelerate the rate of recovery.

It has been stated that cortisone ordinarily causes an increased excretion of calcium and phosphorus.[25] Striking improvement has been described in 1 infant with severe idiopathic hypercalcemia after the administration of 12.5 mg. of cortisone daily.[20] An increase in the excretion of calcium and phosphorus has been reported to follow the use of cortisone for idiopathic hypercalcemia.[15]

As would be expected, various agents that "bind" calcium, and thus increase renal excretion or interfere with intestinal absorption, have been tried. Lowe et al.[10] observed no beneficial effect on the serum calcium level after the oral administration of sodium ethylenediaminetetracetate; another patient died after the intramuscular administration of this compound. It is not recommended for the treatment of idiopathic hypercalcemia.

Sodium phytate (a salt of inositol hexaphosphoric acid) forms insoluble salts with calcium and has been employed to block the intestinal absorption of calcium in vitamin D poisoning and sarcoidosis by Henneman and Carroll.[26] Carter et al.[24] have attempted to employ "all-bran" as a source of phytic acid in infants with idiopathic hypercalcemia. This agent appears to be relatively free of undesirable effects. We are currently administering it to one baby (Case 2) in an attempt to hasten recovery.

The employment of sulfonamides for the concomitant urinary infection is probably contraindicated. Harris[27] has shown that they may aggravate renal damage and osteosclerosis. Although it is advisable to employ other suitable treatment for the urinary infection, Lightwood and Payne[6] do not believe that such therapy hastens the remission of the hypercalcemia.

Summary and Conclusions

Three cases of "idiopathic hypercalcemia of infancy, with failure to thrive," occurring within a two-year period in a single children's hospital in the United States, are described. The condition has been seen much more commonly in the United Kingdom, although it is possible that it is not always recognized in North America. The role of vitamin D is discussed. The more moderate use of vitamin D in infant nutrition is advisable. When the condition occurs, it is best treated with a diet low in calcium and vitamin D. If the condition is severe, small doses of cortisone may be employed.

Idiopathic hypercalcemia of infancy, with failure to thrive, is a distinct clinical entity of unknown cause, although it appears highly probable that vitamin D has an important role in the etiology. This syndrome is not synonymous with true vitamin D intoxication, but it may represent an expression of hypersensitivity to vitamin D in certain infants.

Whether or not idiopathic hypercalcemia of infancy with failure to thrive is truly uncommon in the United States, it behooves the American physician to take warning and to avoid the excessive use of vitamin D. Moderation should be practiced in the commercial houses entrusted with the preparation of infant foods. It is likely that 400 or 500 international units of vitamin D per day is adequate for almost all infants. It would be desirable to obtain better control over the intake of infants exposed to so many sources in a "vitamin-hysterical" environment. The physician and parent, unfortunately, are not always aware of an infant's total vitamin D intake. It is virtually impossible under present conditions to determine the contribution from various "hidden" sources.

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RALEIGH, N.C. 27612

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Humphrey F. Sassoon, Ph. D.
352 W. Deer Park Drive
Gaithersburg, Md. 20760

Dear Dr. Sassoon:

A search through my files yielded only a Special Bulletin of the American Institute of Baking, dated July, 1954, and a 1952 advertisement of the Ward Baking Co., proclaiming the virtues of calcium-supplemented Tip-Top bread. I am sending you the Ward item because it was not long after the calcium publicity appeared that General Baking, A.& P. and Ward and others decided to add vitamin D as well as calcium.

As I told you during our conversation in Mr. Boehne's office, the inclusion of vitamin D led to a great deal of trouble. However, we eventually succeeded in producing a stable enrichment wafer. I must confess that we (i.e., Merck) and other manufacturers were relieved when the bakers decided, about two years later, to abandon what they had come to regard as a "gimmick."

I mentioned the brief history of the optional enrichment ingredients in a review I presented at the ACS Meeting in Chicago in 1967. I enclose a reprint of my review.

I believe It was Tisdall in Toronto who deplored the deletion of vitamin D from the bread enrichment formula. At that time liquid milk fortified with vitamin D commanded a 1¢ premium over unfortified, both in Canada and in the United States, and Tisdall and others contended that the urban poor could not afford to pay the extra penny.

I recall also that John Wayt, vice-president of a large baking company in Atlanta, protested for the same reason, and continued to fortify his company's white bread as long as suitable wafers or mixtures were available.

Sincerely yours,

Clinton L. Brooke

Clinton L. Brooke

PLD

61306

Capitol Milk Producers Cooperative, inc., 428 East Patrick St., Frederick, Md. 21701

February 28, 1974 **0903**

Dr. Humphrey F. Sassoon
Tracor Jitco
1300 East Gude Drive
Rockville, Maryland 20851

Dear Dr. Sassoon:

This letter is in response to your inquiry as concerns Vitamin D fortification of milk.

We have gone to our records and are quoting some statistics which should serve your purpose well.

	% of Total Gallons		
	November 1973	November 1972	November 1971
1. Homogenized Vit. D.Milk	11.02%	15.15%	21.80%
2. Homogenized Milk 3.5% B.F.	59.24%	55.06%	61.53%
3. 2% Low Fat Milk	2.95%	2.79%	4.26%
4. 1% Low Fat Milk	18.62%	18.05%	2.83%
5. Weight Watchers (1M) Skim Milk	1.58%	1.49%	–
6. Skim Milk	4.14%	3.84%	5.27%
All other Fluid Milk & Cream	2.45%	3.62%	4.31%
Total Gallons Represented	1,029,117	902,568	824,503

1. Vitamin D fortification with 400 USP units irradiated egosterol per qt.

2. No vitamin fortification.

3. Vitamin D fortification with 400 USP units irradiated egosterol per qt. Vitamin A fortification with 2000 USP units palmitate per qt. Fortified 1.5% (by weight) Non Fat dry milk solids added.

4. No vitamin fortification. 2% (by weight) non fat dry milk solids added.

5. Vitamin D2 fortification with 400 USP units calciferol per qt. Vitamin A fortification 200 USP units of Palmitate per qt. Plus 10 mg. Ferric ammonium citrate per qt.

6. Vitamin D fortification 400 USP units irradiated egosterol per qt.

TEL MO 3-5177

We market our milk products through 206 High's convenience stores located in D.C., Md., Va., W.Va., and Penn. and try to offer the customer a choice of products.

If there is any questions that you might have about this information please do not hesitate to call.

Best Regards,

J. Paul Langley

J. Paul Langley
Assistant General Manager

JPL/vmf

Am. J. M. Sc. 221; 369-378, (1951)

VITAMIN D INTOXICATION*

By Hugh Chaplin, Jr., M.D.
Resident in Medicine

Lincoln D. Clark, M.D.
Resident in Psychiatry

Marian W. Ropes, M.D.
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Boston, Massachusetts

(From the Medical Services of the Massachusetts General Hospital and the Department of Medicine, Harvard Medical School)

During the past 20 years, vitamin D and the irradiated sterols have come into wide and, at time, indiscriminate usage. In addition to the administration of vitamin D in specific deficiency states, massive doses (50,000 to 1,000,000 units daily for periods of days to as long as 3 to 4 years) have been used in attempted treatment of such conditions as pulmonary and cutaneous tuberculosis, scleroderma, various allergic states, and especially rheumatoid arthritis[1,31,32,37].

Accordingly, as early as 1930, there began to appear in the medical literature reports of varying degrees of toxicity from massive administration of vitamin D. Observers undoubtedly failed to recognize many cases of this syndrome; and the paucity of adequate chemical and Roentgen-ray data in the majority of cases described makes even extensive review of the literature but an approximate indication of the incidence and severity of hypervitaminosis-D. Within the past 20 years, there have occurred at least 5 deaths[23,24] attributed to vitamin D intoxication and several more in which this substance was implicated as a contributing factor[5]. Data available from a review of 111 cases described in the literature are summarized in Table I.

It is the purpose of this paper to review and reëmphasize the clinical, chemical, tissue, and skeletal changes that occur in the syndrome of vitamin D intoxication; to report in detail 7 cases studied and treated within the past 3 years at this hospital; and to stress again the serious import of vitamin D as a toxic agent.

Occurrence. As is suggested above, the exact incidence of vitamin D intoxication is not known. There is obviously a wide variation in individual susceptibility; some patients display toxic symptoms on as little as 30,000 units per day for a few weeks, whereas others may tolerate 10 times that dosage for 6 to 12 months before manifesting toxic effects of the medication. High dietary calcium[17] and probably pre-existing renal disease predispose to early appearance and enhanced severity of intoxication.

Review of the multi-vitamin preparations available without prescription reveals that there are at least 2 brands containing as much as 50,000 units of vitamin D per capsule† and there is a greater number of high-concentrate

* This is publication No. 118 of the Robert W. Lovett Memorial Foundation for the study of Crippling Disease, Harvard Medical School. The expenses of this investigation have been defrayed in part by a grant from the Commonwealth Fund.

† Darthronol (Roerig); Dalsol (U. S. Vitamins).

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TABLE I.—FINDINGS IN 111 CASES OF HYPERVITAMINOSIS-D REPORTED IN THE LITERATURE

				General								Urine					Chemical					
Name	No. of Cases	Sex	Age	Arthritis	Nausea, Vomiting	Weight Loss	G. U. Symptoms	Headache	Weakness	Amount of Vit. D × 1000 u/day	Duration of Treatment	Spec. Gravity	Albumin	Casts	RBC	WBC	Hemoglobin gm./100 cc.	Non-protein Nitrogen mg./100 cc.	Calcium mg./100 cc.	Phosphorus mg./100 cc.	Alk. Phosphatase Bodansky Units %	Phenolsulfonphthalein Excretion
Tumulty	2	M	22	0	+	0	0	0		750	12 da.	1006		+	–		8.5	52	15.0	3.8		D
		M	22	0	+					750	17 da.	1005						45	15	2.0		D
Thatcher	1	M	1.5	0		+			+	(10)*	4 mo.		+	0	0	+			N	I		
Mulligan	1	M	44	0					+	?	?	1022	+	+	+	–						
Danowski	2	F	60	+	+					200	6 yr.	1013	0	D			8.5	67	15.3	4.5		D
		F	56	+	+	+				500	5 mo.	1005	+	+			9.0	55	14.5	5.2		D
Reed	43	M + F	7–72	+	+	+		+	–	300–1000	10 da.–3 yr.											
Vrtiak	20	M + F	23–66	+	–		+			150–200	1 mo.–12 mo.											
Hess	7	M + F	1	+	+				+	2–5 mg.**	1 mo.		+	+					I	I		
Jelke	1	F	1.5	0	–	+			+	900	7 wk.											
Fulschar	1	M	5	0	+	+							+									
Kern	1	M	1	0	+	+				?	sev. mo.											
Paul	5	F	50	0	+	+	+			200								N	N	N		
		M	58	+	+	+	+			130		1006							11	2.7		
		F	49	+	+	+	+	+		300	-	1017	+	·			11.6		12	3.1		
		F	65	+	+					400		1012	+				11	N	N	N		
		F	88	+	+	0	0	0		150		1002	+	+			8		13.5	5.7	5.0	
Farley	6	M + F		+	+					150–600									N	N	N	
Thatcher	1	M	1.5	0	+	+							+	+	+	+		I				
Bauer, J.	1	F	29	+	+	+	+	+	+	100–500	1 yr.			+	+	+	8.0					
Covey	5	F	66	+		+		+		200	2 wk.						11.4		12.7			
		F	54	+	+	+		+	+	330	2 mo.		+	+			12.0	I	11.5			
		F	45	+			+			50–500	1 yr.	1010					10.0		13.0			
		F	68	+	+	+		+		200	6 mo.	1051	+	+		+		I				
		M	29	0	+	+				200	4 mo.	1011	+			+			12.5			D
Donegan	2	F	59	+	+	+	+	+	+	50–150	3 yr.	1009	+				12.0	I	13.4	4.4	3	D
		F	56	+	+			+	+	150–300	6 mo.	1000	+				10.0	I	13.0	2.2	4	D
Bauer, W.	12	M + F	26–65	+	10+		2+	1+		20–300	5 wk.–1 yr.								11.1–18	5.1–5.5	N	
Freyberg	1	F	32							100–500	1 yr.											
Total	111																					

* Cod Liver Oil
** Ergosterol
D Decreased
N Normal
I Increased

pure vitamin D preparations in both liquid and capsule form containing over 50,000 units per usual dose.** Accordingly, it is not unlikely that considerable numbers of vitamin-habituated individuals may be taking toxic doses of vitamin D without benefit of medical surveillance.

However, it must be said that the chief occurrence of vitamin D intoxication is in those patients who are receiving massive doses prescribed by their physicians in attempted treatment of some particular disease condition. It is not the purpose of this paper to discuss whether such treatment should be discontinued, but rather to indicate that physicians who employ these potent vitamin D preparations should be alerted to the early signs of impending intoxication and aware of the clinical and chemical abnormalities associated with overdosage.

The Syndrome. The complete syndrome of hypervitaminosis-D has been repeatedly described and confirmed in the literature as consisting of the following:

1. *General:* weakness, fatigue, weight loss.
2. *Gastrointestinal:* nausea, vomiting, diarrhea, abdominal cramps.
3. *Neurological:* headache, paresthesias, vertigo.
4. *Psychiatric:* variable mental symptoms—depression, mild psychosis, stupor.
5. *Hematological:* normocytic, normochromic anemia.
6. *Urological:* albumin, red cells, casts in the urine, with progressive impairment in concentrating ability; frequency and nocturia.
7. *Ophthalmological:* band keratitis.
8. *Chemical:* elevated serum calcium and phosphorus, with normal or slightly elevated alkaline phosphatase. Progressive nitrogen retention.
9. *Roentgenological:* diffuse demineralization of the bones in advanced cases; periarticular soft tissue calcifications.
10. *Pathological:* Scattered calcium deposition in kidney, blood vessels, periarticular soft tissues, heart, stomach, lung, thyroid and pancreas.

Depending on the severity of intoxication, all or only a few of the above-described findings will be present, as indicated by the following case reports. Table 2 summarizes the specific laboratory and chemical data which are mentioned only generally in the case reports.

Case Reports. Case 1. (*Unit No.* 610097. *Adm.* 3-26-48. *Disch.* 5-14-48.)

This was the first admission of a 65-year-old man. For 2 years prior to admission he had suffered from arthritis of the knees. During the greater part of this time, he had ingested 200,000 to 300,000 units of vitamin D per day in treatment of his arthritis, in addition to drinking 1 to 2 quarts of milk daily. Three months before admission, his arthritis became generalized, with most marked involvement of his shoulders, wrists, hands and cervical spine. In addition, during this interval he developed anorexia, marked weight loss, weakness, fatigualbility, nocturia, and noted the appearance of non-tender fluctuant, periarticular masses in relation to his elbows, wrists, cervicothoracic spine, and several metacarpophalangeal joints.

There was no known history of previous renal disease.

Significant Physical Findings: Apathy, pallor, weakness and emaciation. Band keratitis. Senile pulmonary emphysema. Grade 2 apical systolic murmur without other significant cardiac abnormality. Blood pressure 145/60. Generalized moderately severe rheumatoid arthritis. Multiple 1.5 to 2.0 cm. soft, non-tender masses over the second metacarpophalangeal joints and ulnar and radial aspects of the wrists bilaterally. A similar 3 cm. mass was longitudinally situated over the lower cervical and upper thoracic spine. Benign prostatic hypertrophy.

** Actephen (Biochemical Research); D Vitamin Capsules (Squibb); D-Vatine (Dorsey); Davitin (I.V.C.); Decaps (Cole); Decrike (Lakeside); Dee-Ron (Warren-Teed); Dee-Osterol (Bevon); Dee Ronal (Warren-Teed); Deltalin (Lilly); Deratol (Brewer); Hi Deratol (Brewer); Diaetal (Plessner); Drisdol (Winthrop-Stearns); Ertron (Whittier); Infron Pediatric (Whittier); Super-Seal Vitamin D (Drug Products); Vidonol Capsules (Parke, Davis); Vio-D (Rowell); Viosterol Concentrated (Merrell); Vitamin D Capsules (Abbott, Bristol, Lederle, Miller, Schieffelin, Sharp and Dohme, Sherman, Ulmer, Upjohn, U. S. Vitamin, Walker Vitamin, Wyeth).

Laboratory Data: Admission hemoglobin 8.0 gm. Urine specific gravity fixed at 1.012, moderate albuminuria, markedly impaired phenolsulfonphthalein excretion. Elevated serum calcium, normal to slightly elevated serum phosphorus and alkaline phosphatase, elevated serum non-protein nitrogen, increased 24 hour urine calcium excretion. Congo Red test negative. Roentgen rays revealed moderate generalized demineralization of bones, arthritic changes in joints, and opaque calcium-containing masses in the sites described under *Physical Findings.*

Course: During the early phases of the patient's admission, his condition deteriorated. Serum calcium and non-protein nitrogen rose for a time; he became weaker and more apathetic. One important obstacle to treatment was the patient's refusal to take an adequate fluid and calorie intake or to be mobilized from bed. He was given 3 transfusions of 500 cc. whole blood, and his oral intake was supplemented by Levine tube feedings. In addition, he received 5 mg. stilbestrol per day. Approximately 1 month after admission his clinical and blood chemical status began to improve; he became alert, stronger, and regained full ambulation. The periarticular masses slowly disappeared. No significant improvement in renal function occurred other than that secondary to improved hydration. Despite transfusions and ferrous sulfate, his hemoglobin was only 9.0 gm. at discharge.

Unfortunately, no follow up is available as to his course after leaving the hospital.

Case 2.* (*Unit No.* 617407. *Adm.* 5-4-48. *Disch.* 5-21-48.)

This was the first admission of a 55-year-old woman. She presented a 30 year history of progressive spondylitis and peripheral rheumatoid arthritis with resultant bed-chair invalidism. Three months prior to admission, after taking 300,000 units of vitamin D daily for 3 months, coincident with a diet high in calcium content, she had the onset of weakness, anorexia, progressive weight loss, and noted the appearance of soft, tender masses over her low back and in relation to her knees, wrists, shoulders, and scapulae. No history of previous renal disease was obtained.

Significant Physical Findings: Pallor, weakness, and emaciation. Band keratitis. Advanced, generalized rheumatoid joint changes. Soft, slightly tender, fluctuant 1.5-8.0 cm. masses at the base of the right thumb, over the right radial tuberosity, right scapulohumeral joint, inferior border of the left scapula, left sacroiliac joint, and medial aspect of the left knee. Blood pressure: 100/65.

Laboratory Data: Admission hemoglobin 7.5 gm. Urinary specific gravity fixed at 1.010, moderate albuminuria, markedly impaired phenolsulfonphthalein excretion. Elevated serum non-protein nitrogen, calcium, phosphorus, and slightly elevated alkaline

TABLE 2.—CHEMICAL VALUES IN THE 7 CASES HERE REPORTED

Patient	Non-protein Nitrogen mg./100 cc.	Total Protein gm./100 cc.	Albumin/Globulin	Serum Calcium mg./100 cc.	Serum Phosphorus mg./100 cc.	Alkaline Phosphatase Bodansky U.	Phenolsulfonphthalein Excretion	Maximum 24 hr. Urine Calcium Excretion mg./24 hr.
Case 1	48–28	7.32	1.3	15.2–11.0	5.8–2.8	6.0–3.9	D*	445
Case 2	65–49	6.24	2.2	13.4–12.6	5.7–4.7	7.1–5.5	D	253
Case 3	70–60	7.00	1.2	14.4–10.2	6.3–4.8	7.4–3.3	D	110
Case 4	84–41	7.48	1.1	13.0–11.2	5.5–2.8	7.0–4.0	D	160
Case 5	22–17	6.72	1.0	13.7–9.4	4.8–2.7	4.0–2.6	N**	527
Case 6	50–43	7.50		15.6–14.8	3.2–3.0	1.9	D	530
Case 7	46–37	6.69	1.0	12.8	3.7	3.3	D	

Where more than one value is known, the range of values is given
*D—Decreased
**N—Normal.

* This case is included in the "Case Records of the Massachusetts General Hospital" in the New England J. Med., 1950, in press.

phosphatase [illegible] al to moderately increased 24 hour uri[illegible] calcium excretion. Roentgen rays revealed moderate generalized demineralization of bones, arthritic joint changes, and the cystic masses of calcium described above.

Course: With mobilization, low calcium diet, and forced fluids the patient's non-protein nitrogen, calcium, and phosphorus decreased, but did not reach a normal range. Alkaline phosphatase remained essentially unchanged. Her urinary calcium increased from a normal to a moderately elevated level. With 3 transfusions of 500 cc. whole blood her hemoglobin rose to 12.5 gm. She failed to tolerate stilbestrol because of nausea. The periarticular cysts decreased in size and became less fluctuant. No significant improvement in her renal status was apparent. She left the hospital against advice, much improved, but before full therapeutic results had been obtained.

Follow-up: Despite attempts at follow-up, contact with her was lost until almost 3 years later, when she was admitted terminally ill with jaundice, peritonitis, and uremia. Her serum calcium was below normal on this admission, and the previously described soft-tissue cysts had largely disappeared, though calcification in the soft tissues was clearly seen by Roentgen ray. She died after a brief period in the hospital.

Autopsy revealed widespread, generalized calcification. Her diagnoses included active disseminated pulmonary, renal, bladder and endometrial tuberculosis, Boeck's sarcoid in the lungs, nephrosclerosis and probable chronic pyelonephritis, chronic cholecystitis with multiple liver abscesses, perforation of the gall bladder, and generalized peritonitis. Calcium was found in the soft tissues, periarticular tissues, lungs, blood vessels, kidneys, liver, adrenals, mesentery and spleen. The parathyroids were small. It was the opinion of the pathologist that the calcification within the pulmonary alveoli, in the soft tissues and in the periarticular regions, was almost surely the result of hypervitaminosis-D, whereas the calcium deposits elsewhere could conceivably have resulted from her other coexisting pathological states.

Case 3. (*Unit No.* 24992. *Adm.* 6-19-47. *Disch.* 7-31-47.)

This was the second admission of a 59 year old woman, who entered for study and treatment of her rheumatoid arthritis. At age 12 she suffered an illness consistent with Sydenham's chorea but without other coincident or subsequent symptoms of rheumatic fever. She presented a 23-year history of progressive spondylitis and peripheral rheumatoid arthritis, resulting within 10 years of onset in varying degrees of deformity in all joints. During the 5 years before admission (1942-1947) she took an average of 100,000 units of vitamin D per day. Her diet was not high in calcium content. During the last year of this interval she developed anorexia, weight loss, nausea and vomiting, nocturia, and very distressing paresthesias in the legs.

Significant Physical Findings: Pallor and emaciation. Advanced peripheral joint changes and spondylitis. Hepatomegaly* (liver edge palpable 3 fingers below the inferior costal margin in midclavicular line). Rheumatic heart disease, with moderate cardiac enlargement, mitral and aortic systolic and diastolic murmurs, good cardiac compensation without treatment. Blood pressure: 190/88. Psoriasis. Band keratitis.

Laboratory Data: Admission hemoglobin 8.0 gm. Urine specific gravity fixed at 1.012, marked albuminuria. Increased non-protein nitrogen, calcium and phosphorus; normal to increased alkaline phosphatase; normal urinary calcium excretion. Congo Red test positive. *Note:* The increased phosphorus reflects the particularly severe degree of renal involvement in this case (secondary probably to both vitamin D intoxication and amyloidosis). Roentgen rays revealed moderate, generalized demineralization of bones, metastatic periarticular and extra-articular calcification, and arthritic changes in joints.

Course: Because of the presence of an active duodenal ulcer, the patient was placed on a 6 meal bland diet. She was given aluminum hydroxide in an attempt to decrease phosphorus absorption. No significant change occurred in the level of her increased non-protein nitrogen or serum phosphorus. Her serum calcium actually increased. She did not receive stilbestrol. The hemoglobin level showed no persistent increase despite 2 transfusions of 500 cc. whole blood.

Follow-up: The patient had a subsequent admission (9-1-48 to 9-4-48) for treatment of an exacerbation of her duodenal ulcer. In addition she has been seen regularly at the M. G. H. Arthritis Clinic up to the present. By 11-13-49 her serum calcium had fallen to within normal limits and has remained such. However, her serum phosphorus and non-protein nitrogen remained elevated until 10-20-49, when these determinations were found to be within normal limits. Her hemoglobin has slowly risen to a level of 12 gm. Roentgen ray studies have revealed a de-

* Secondary amyloidosis.

crease in the amount of her periarticular calcification.

Case 4. (*Unit No.* 8997. *Adm.* 12-8-47. *Disch.* 1-19-48.)

This was the fourth admission of a 70-year-old woman. She gave a 15 year history of generalized rheumatoid arthritis. Shortly prior to this admission, she took 150,000 units of vitamin D daily for 6 months. The calcium content of her diet during this time is not known. Toward the end of this period she suffered the onset of nausea, vomiting, diarrhea, anorexia, weight loss, weakness, mental depression, and nocturia. A diagnosis of vitamin D intoxication was made, and hospital admission for study advised. She was known to have had a chronic urinary tract infection since 1938, without, however, apparent impairment of renal function prior to this admission.

Significant Physical Findings: Pallor, senility, weakness, signs of recent weight loss. Arteriosclerotic heart disease with slight cardiac enlargement. An apical systolic murmur with thrill. Soft, aortic systolic murmur. Blood pressure 140/70. Senile emphysema. Advanced, generalized, peripheral rheumatoid arthritis. Rheumatoid nodule over the styloid process of the right radius. Band keratitis.

Laboratory Data: Admission hemoglobin was 9.0 gm. Maximum urinary specific gravity 1.014, moderate albuminuria and pyuria, markedly impaired phenolsulfonphthalein excretion. Increased serum non-protein nitrogen and calcium; slightly increased phosphorus and alkaline phosphatase; normal urinary calcium excretion (1 determination). Roentgen rays revealed moderate, generalized demineralization of bones and severe arthritic changes.

Course: A urinary tract infection (probably a B. coli chronic pyelonephritis) was controlled with sulfadiazine. Her non-protein nitrogen fell to within normal limits, and her levels of serum calcium and phosphorus showed a downward trend. No essential change in her renal function occurred. The hemoglobin level showed no change.

Follow-up: Following discharge, the patient was seen regularly in the clinic until July, 1949. The non-protein nitrogen remained moderately elevated, and albuminuria and pyuria, as well as nocturia and polyuria persisted. Otherwise, except for arthritis, she remained in fair health—ambulatory and alert. The patient was readmitted on 2-2-50 and died on 2-8-50. The cause of death was probably progressive cerebral thrombosis. At the time of admission her non-protein nitrogen was 37 mg., serum calcium 10.8, phosphorus 3.4, and phosphatase [illegible] units. A severe B. coli urinary tract infection was present.

Autopsy revealed no definite visceral or other extra-skeletal calcification other than that compatible with her age.

Case 5. (*Unit No.* 631593. *Adm.* 9-14-48. *Disch.* 3-12-49.)

This was the first admission of a 38-year-old woman. Three years before admission she had onset of pain in her thoracic spine. Eight months later she developed fatigue and arthritis involving multiple peripheral joints. This exacerbation persisted up to the time of admission. Seven months before entry she took 300,000 units of vitamin D daily for 3 weeks. During the subsequent period, she continued to take vitamin preparations of unknown composition in unknown quantity. Her diet during this period did not contain excessive amounts of calcium. The toxic symptoms, consisting of anorexia, nausea, vomiting, diarrhea, and weight loss, ensued 3 weeks before admission.

Significant Physical Findings: Pallor and weakness. Impaired hearing. Grade 2 apical systolic murmur without other cardiac abnormality. Blood pressure 120/70. Moderately severe spondylitis and peripheral rheumatoid arthritis. Band keratitis.

Laboratory Data: Admission hemoglobin 10.0 gm. Maximum urine specific gravity 1.012, slight albuminuria, moderate pyuria, B. coli and B. pyocyaneus bacilluria, normal phenolsulfonphthalein excretion. Serum non-protein nitrogen normal; increased serum calcium, normal to slightly elevated phosphorus, normal alkaline phosphatase; increased urinary calcium excretion. Congo Red test normal. Roentgen rays revealed slight, generalized demineralization of bones and joint changes consistent with rheumatoid arthritis.

Course: By 10-20-48 the symptoms of vitamin D intoxication had subsided under treatment with forced fluids and low calcium diet. Serum calcium, phosphorus, and urinary calcium excretion fell to within normal range. The urinary tract infection cleared on sulfadiazine.

Follow-up: The patient was followed regularly in the clinic. Except for her arthritis, she remained well until the occurrence of a gastric ulcer in December, 1949. She was admitted to the hospital (2-2-50 to 3-8-50) for treatment of her ulcer, which healed uneventfully on a medical program. At the time of this admission, the hemoglobin [illegible] 11.0 gm., urinalysis normal, and serum [illegible]cium, phosphorus, and alkaline phospha[illegible] within normal limits.

CASE 6* (*Unit. No.* 693324. *Adm.* 3-1-50. *Disch.* 3-10-50.)

This 40-year-old man was admitted with a provisional diagnosis of nephritis. The past history was not remarkable save for 2 episodes (at ages 14 and 20 years) of apparent acute nephritis lasting approximately 1 year on each occasion, and exhibiting albuminuria, cylindruria, and blood pressure elevation. During the 20 years prior to admission, however, repeated physical examinations and urinalyses failed to reveal any evidences of nephritis or hypertension.

For a period of 8 mos. prior to admission, he took 200,000 units of vitamin D daily in treatment for chronic bursitis of his right shoulder. His diet during this time was not high in calcium content. Three months before admission he experienced progressive weakness, fatigue, severe polydypsia and polyuria, without other manifestations of vitamin D intoxication.

Significant Physical Findings: Pallor and undernutrition. Blood pressure 160/92. No evidence of intrinsic cardiac disease. Bilateral, slight costovertebral angle tenderness. Band keratitis.

Laboratory Data: Admission hemoglobin 9.0 gm. Slight albuminuria, moderate pyuria without urinary tract infection, impaired concentrating ability (maximum 1.010). Moderately elevated non-protein nitrogen, markedly elevated serum calcium, normal phosphorus and alkaline phosphatase. Increased urinary calcium excretion. Roentgen rays of the skeleton were not remarkable except for calcification in the area of the right subacromial bursa.

Course: Despite the brevity of the patient's hospitalization, definite improvement was observed without treatment other than cessation of vitamin D ingestion, ambulation, and forced fluids. At discharge his fluid intake and output were slightly less than half the excessive values he demonstrated on admission. The non-protein nitrogen and serum calcium showed a downward trend.

No follow-up is available at the present time.

CASE 7. (*Unit No.* 696311. *Adm.* 4-17-50. *Disch.* 4-26-50).

This 48-year-old woman was admitted to the Medical Service in transfer from the Neurological Service, where she had been admitted for investigation of undiagnosed headache. She presented a 10 year history of mild, generalized, peripheral rheumatoid arthritis. For a 6 year period, terminating 4 months before admission, she had taken 50,000, or more, units of vitamin D per day. During the 10 months prior to admission she had suffered progressive weakness, fatigue, anorexia, post-prandial nausea and vomiting, weight loss of 14 lbs., severe frontal headaches, and nocturia 3 times nightly without other genito-urinary symptoms. It is of interest that early in her intoxication the nature of her symptoms was not recognized, and in an effort to relieve them, her physician switched to another, equally potent, preparation of vitamin D.

Significant Physical Findings: Emaciation. Minimal arthritic changes in multiple peripheral joints. Two small nodules (probably rheumatoid) over the extensor surface of the right elbow and one over the distal end of the right humerus. Liver enlarged 2 finger breadths below the right costal margin. Blood pressure 180/106.

Laboratory Data: Admission hemoglobin 10.0 gm. Urine specific gravity fixed at 1.014, minimal albuminuria, occasional granular and hyaline casts, and markedly impaired phenolsulfonphthalein excretion. Elevated serum calcium, with normal phosphorus and alkaline phosphatase, elevated non-protein nitrogen, increased 24-hour urinary calcium excretion, elevated bromsulfonphthalein retention, thymol turbidity 6.0 units, and cephalin flocculation 3 plus at 24 and 48 hours. Roentgen rays revealed minimal arthritic changes in elbows, knees, and hands, without generalized osteoporosis or metastatic calcification.

Course: The patient was treated with low calcium diet, forced fluids, transfusions, ambulation, and supportive measures for her arthritis. On this program she showed some symptomatic improvement and her non-protein nitrogen fell to within normal limits. Unfortunately, further follow-up is not available because of failure of the patient to keep her return appointments.

Comments. No correlation can be established relating the duration or severity of the arthritic disease in these patients to the development of vitamin D intoxication. Furthermore, although 1 patient had extensive secondary amyloidosis and 2 had coincident urinary tract infections, one cannot substantiate from the case data available the reasonable speculation that pre-existing renal disease might

* This patient, sent in consultation to Dr. F. Demetto Adams because of the constitutional and genitourinary symptoms, is to be reported in more detail by Dr. Adams at a later date.

predispose to the renal complications of vitamin D intoxication.

It is interesting that the 2 patients with a definite history of unusually high calcium intake during the period of vitamin D therapy exhibited the most severe intoxication, and were the only cases in our series that developed calcium-containing cysts. This aggravation of vitamin D intoxication by high calcium intake substantiates the work done in experimental animals by Harris[17]. Unfortunately, in none of our cases was the diet studied for its ash content, for in accordance with Gough's[13] work in animals, it might be anticipated that vitamin D intoxication would be aggravated by any alkaline ash diet.

It will be noted that 6 of the patients exhibited so-called "band keratitis", a lesion which is commonly associated with disturbed calcium metabolism in a variety of disease conditions[6,38]. Its regular occurrence in vitamin D intoxication is almost certainly due to the existence of hypercalcemia, and is in no way a specific finding related to the vitamin D compound itself.

Both of the most serious cases of vitamin D intoxication reported in this paper were remarkable for the demonstration of calcium-containing cysts. With rare exceptions (Paul[24]—1 case, Danowski[8]—1 case), this phenomenon has not been reported previously as part of the syndrome of vitamin D poisoning. It is difficult to know whether to classify it as a specific manifestation of vitamin D overdosage or to rank it along with band keratitis as a manifestation of disturbed calcium metabolism. In both of these patients the cysts were localized largely in the periarticular areas and in the vicinity of bursae. The cysts were not fixed to the skin, but seemed firmly attached to the underlying tissues. They were [illegible]ctuant, did not transilluminate, and varied from 1.5 to 8.0 cm. in diameter.

Biopsies of several of these masses were made; the following is a brief summary of a representative pathological report:*

"A portion of the tissue seems to represent the lining of a cyst, bursa, or joint cavity with an inner layer of cells resembling hyperplastic and hypertrophied synovial cells and an outer layer of dense, partially hyalinized connective tissue. This membrane is thrown into irregular folds and is involved by a diffuse granulomatous inflammatory process. The granulomatous reaction is of the 'foreign body' type with many giant cells which often appear to be centered about irregular granules which measure up to 20 microns in diameter. These granules stain like calcium with hematoxylin and like fibrin with Mallory's aniline blue, Mallory's phosphotungstic acid hematoxylin, and eosin methylene blue. They are present in all portions of the membrane, as well as in the cavity of the cyst."

The mechanism of formation of these cysts remains obscure. Although in our cases they were associated with underlying joint disease, similar cysts have been described in both primary and secondary hyperparathyroidism without significant coincident joint disease. Accordingly, it seems likely that these cysts are merely a manifestation, like band keratitis, of profoundly disturbed calcium metabolism and are not pathognomonic of vitamin D intoxication *per se*.

The material contained within the cysts could be readily aspirated through an 18-gauge needle and presented a milky, somewhat viscous appearance. The chemical nature of this material is of some interest. In 2 of our cases, calcium, phosphorus, and alkaline phosphatase determinations were made upon the fluid itself and upon the sediment centrifuged from the fluid. The calcium/phosphorus ratio

* Obtained through the kind cooperation of Dr. Peter Kulka, M.D., of the Department of Pathology, Harvard Medical School.

of the sediments in these cases was more than twice that present in bone, a finding at variance with Paul's[27] case, in which the calcium/phosphorus ratio was reported as identical with that of bone.

Differential Diagnosis. The problem of differential diagnosis is generally not a difficult one. The only conditions with which vitamin D intoxication would be likely to be confused are primary and secondary hyperparathyroidism. In these latter, the serum phosphorus will be either low (primary hyperparathyroidism) or considerably elevated (end-stage renal disease with accompanying secondary hyperparathyroidism) and the alkaline phosphatase will be considerably elevated. In vitamin D intoxication, on the other hand, the serum phosphorus and alkaline phosphatase are normal or only slightly elevated.

Prognosis. The prognosis in cases of vitamin D intoxication, although difficult to judge before treatment is instituted, may generally be considered favorable following prompt cessation of administration of the drug and early institution of the therapeutic measures indicated below. As previously noted, if intoxication has been severe it may result in death. If fatal outcome does not occur, some degree of irreversible renal damage may remain, usually manifested by impaired renal concentrating capacity and diminished phenolsulfonphthalein excretion. In most cases, the metastatic cysts, band keratitis, azotemia, hypercalcemia, anemia, and symptomatology will disappear over a period of weeks or months following institution of treatment.

Prevention. Physicians should employ this potentially dangerous medication only in disease conditions in which existing evidence indicates a reasonable rationale for usage. Fortunately, the syndrome of hypervitaminosis D is slow in its development and affords opportunity for the physician to detect intoxication early, before significant damage has occurred. It is the belief of the authors that the incidence of severe intoxication can be entirely eliminated if the following simple precautions are taken in all patients being given large doses of vitamin D.

1. The patient should be informed sufficiently of the nature of the toxic symptoms to permit voluntary cessation of treatment if these symptoms appear.

2. Routine urinalysis and hemoglobin determinations should be checked at least every two weeks during treatment.

3. Blood calcium determinations should be followed at monthly intervals.

4. If significant abnormalities are found in the course of the above measures, treatment should be discontinued, and more complete laboratory studies should be obtained in order to clarify the nature of these changes.

Treatment. The treatment of the syndrome of hypervitaminosis D is directed towards: *a*, cessation of vitamin D intake; *b*, reduction of serum calcium level by restriction of calcium intake and encouragement of maximum urinary excretion.

Accordingly, in this group of 6 patients the following program of treatment was evolved: 1, cessation of vitamin D intake; 2, strict low calcium diet; 3, avoidance of undue exposure to sunlight; 4, avoidance of alkali; 5, maximum mobilization of the patient; 6, sufficient fluids to insure a daily urinary output of at least 2000 cc.

Some of our cases received stilbestrol, 5 to 10 mg. daily, on the theoretical assumptions that: *a*, it might increase calcium deposition in bone by favoring osteoid formation[2]; and, *b*, by increasing urinary citric acid excretion, there would be increased calcium excretion as calcium citrate.

Summary. 1. The characteristics of the syndrome of hypervitaminosis D have been described and data available on over 100 cases from the literature have been summarized.

2. Seven additional cases seen at the Massachusetts General Hospital within the past 8 years have been described in some detail, with emphasis upon the findings of band keratitis and metastatic calcium cysts. The histology of these cysts has been described as well as the nature of the Ca/P ratio of their contents.

3. Current methods for treatment of the syndrome are described.

4. In addition to the already available evidence regarding the inefficacy of non-specific therapeutic use of vitamin D, as in most of the conditions listed earlier in this paper, special stress is laid upon the hazards of such therapy. If vitamin D is used in massive doses, it is pointed out that careful follow-up is obligatory for the early detection of the clinical and laboratory manifestations of intoxication.

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Studies on The Influence of Vitamin D on The Female Sexual Function

(II) Histological Changes of Ovary and Uterus in Vitamin D Deficient and Vitamin D Overdosed Albino Rats

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I reported before about the general condition, sexual cycle and conceivability of vitamin D deficient and vitamin D overdosed albino rats.

In this paper I report about histological changes of the ovaries and uteri of those albino rats.

Methods of Experimentation

(1) Dyeing

Mainly hemotoxylin-eosin double staining and van Gieson staining according to the necessity.

(2) Calculation of the numbers of follicles and luteins.

Taking the reports of Matsumoto, Tarui and Koga as references, I first dissected a whole ovary into a series of pieces and calculated (Table 1).

Next I compared this calculation with that which I did previously, choosing a series of twenty pieces from the middle of the whole series of the dissected ovary.

As a consequence, I observed that there was no significant difference between both calculations. So I chose thirty pieces from three parts of the middle of the whole ovary. And I got the average calculation of both the right and the left of those three. With the distinction of sizes of follicles as follows: those over 500 μ as major, those from 200 μ to 500 μ

as medial, those below 200 μ as minor. Also I classified luteins as follows: those over 1000 μ as major, those from 200 μ to 500 μ as medial, those below 200 μ as minor.

Results of The Experimentation

(1) Vitamin D deficiency experimentation

1) Changes in non-sterilized infant albino rats

In five samples of infant albino rats which had been feed with a vitamin D-free diet for ninety days, each of the ovaries of those albino rats (No. 57,58,59), whose sexual cycles lost their order, weighed 34.5 mg. on an average. This figure had very little difference from the 36.4 mg. of the recovery examination group. Each ovary of those albino rats (No. 56,60) whose sexual cycles were not influenced by the vitamin D-free diet was pale as seen from the outside and weighed 16 mg. on an average.

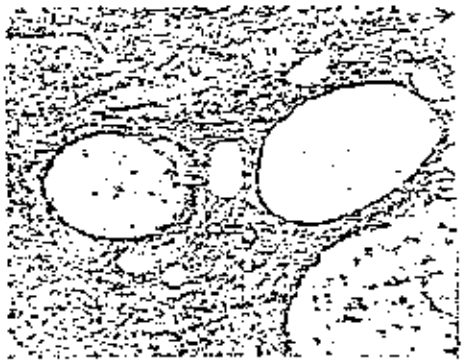
Histological changes in the latter, (albino rats whose sexual cycles were not influenced by the vitamin D-free diet) show that those albino rats had no luteins or major follicles and that most of the medial follicles were not healthy. In those unhealthy medial follicles the cells of granular membrane were lacking in uniformity and out of order, and an invasion of connective tissue and white blood corpuscles into them was also recognized. Among the minor follicles some were healthy, but most were not. In those unhealthy minor follicles, egg cells and glanular layer disappeared. Most of them looked slightly vesicular (Pic. 1). The initial follicles showed no histological change. In the interstitial tissue the cells were random in density and an increase of closed follicles was significant. The luteins were small in number and were invaded by connective tissue and were lacking

Table 1

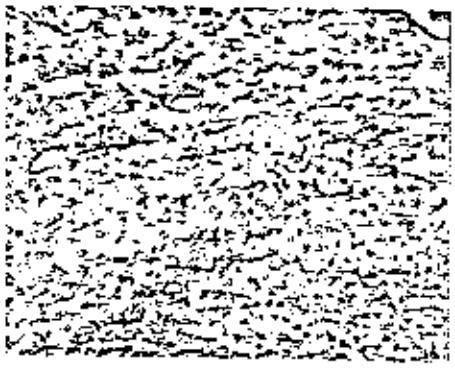
The Number of Follicles and Lutein Bodies of The Rats' Ovary by The Normal Foods

No. of Experimental Animal	Sexual Cycle	Initial Follicle	Major Follicle		Medial Follicle		Minor Follicle		Lutein
			N	R	N	R	N	R	
11	D	++	0	0	7.5	3.5	10.5	5	12.0
12	D	++	0	0.5	6	4.5	9	5	13.5
13	P	+++	3.5	1.5	4.5	3	8.5	3.5	12
14	P	++	2.5	2	5	2.5	8	4	10.5
15	P	+++	2	1	4.5	2	9	3.5	12
16	O	++	2	1	4.5	2.5	10	4.5	11.5
17	O	+++	2.5	0.5	5	3.5	9	3	10
18	M	++	0	1	8.5	4	11	4.5	14.5
19	M	++	0	0	6	3.5	9.5	5.5	12.5
20	M	++	0	0.5	5.5	3.5	8.5	2	13.5

D = Dioestrus O = Oestrus N = Normal
P = Praeoestrus M = Metooestrus R = Retrograde



Pic. 1. The deterioration of follicle granule membrane cells (D-deficiency immature rats).



Pic. 2. Unequal distribution of lutein cells (D-deficiency immature rats).

in uniformity of color. In the dark colored parts, the nuclei were dense, and the lutein cells were small and manysided. In the light colored parts the lutein cells were large and manysided and had vacuoles. (Pic. 2, Table 2).

The weight of each uterus decreased a little, and histologically in the upper skin of the mucous membranes a slight atrophy was recognized and boundaries among cells became indistinct. Nuclei were out of order. Cells were lacking in protoplasm. The growth of the uterus gland was inhibited. Gland cells contained vacuoles and were mostly out of order. The interstitium and muscle layer became atrophied, and dilatation and congestion of blood vessels was not observed (Pic. 3).

In the recovery examination, the retrograde symptom of follicles remained a little (Pic. 4). But the rest showed normal views and the growth of the uteri did not differ from examples of the normal.

2) Changes in non-sterilized mature albino rats

With the exception of two out of the ten samples which had been dosed with the vitamin D-free diet for ninety days, all showed a continuous stillness period on the 64th to 88th day. Except for two samples which died on the 76th and on the 81st day, the ovaries were pale and flat. An average weight of each of the ovaries of these albino rats was 25.2 mg., while that of the 50 I.U. of vitamin D treated group was 36.4 mg.

Histologically there were no major follicles and most of the medial follicles retrograded. There were very few healthy minor follicles. Many of the minor follicles showed retrograde metamorphosis of the granular layer and a significant invasion of connective tissue into them. Some of them resembled cysts. (Pic. 5). The luteins were few in number and were noticeably invaded by connective tissue. Boundaries among the cells were not distinctive. In the interstitial tissue were seen dense nuclei (Table 3).

Table 2

The Ovary of Non-Sterilized Immature Rats Under D-Deficiency

	No. of Experimental Animal	Sexual Cycle	Weight	Blood Vessel	Inter-stitium	Initial Follicles	Major Follicles		Medial Follicles		Minor Follicles		Closed Follicles	Luteins
							N	R	N	R	N	R		
The re-	51	O	35.5	C	N	++	2	1	3.5	2.5	7.5	6	average	12.5
covery	52	P	38	C	N	++	1	2	4.5	3	4	5.5	not so many	11
examina-	53	D	34.5	N	N	++	0	0	5	5.5	7	5	average	13
tion	54	D	38	N	N	++	0	0	2.5	4	5.5	5	not so many	11
group	55	M	36	N	N	+	0	1	3	3.5	6	4.5	not so many	10.5
The vit-	56	D	18.5	C	dense	++	0	0	0.5	2.5	2.5	0	many	0
amin D	57	M	33.5	N	N	+	0	0	1.5	2	3	4.5	many	8.5
defici-	58	D	37	N	N	++	0.5	1	1.5	2.5	3.5	4	not so many	11.5
ency	59	D	33	C	dense	+	0	0	2	2.5	4	8	many	9
group	60	D	13.5	C	dense	+	0	0	0	2.5	3	8	many	0

N = normal R = retrograde C = congested

Table 3

表3 D欠乏状態における非去勢成熟シロネズミの卵巣

動物番号	性周期	重量	間質	血管	原始卵胞	大卵胞 健N	大卵胞 退	中卵胞 健	中卵胞 退	小卵胞 健	小卵胞 退	閉鎖卵胞	黄体
61	D	29.5	正N	正N	+	0	0	0	2.5	3.5	5.5	多数	10
63	55日持続	27	正N	充血	++	0	0	0	4	5	12.5	多数	9.5
64	69日持続	26.5	やや疎	充血	+	0	0	0.5	3	3	9	多数	8
66	M	31	正N	正N	++	0	0	3	2.5	4.5	4	中多数	11
67	78日持続	22.5	疎	充血	++	0	0	0	2.5	2	4.5	多数	8
68	61日持続	21	やや疎	充血	+	0	0	0	2	3	9.5	多数	7.5
69	73日持続	25	疎	充血	+	0	0	0	3	3	11	多数	9
70	71日持続	19.5	疎	充血	+	0	0	0	2	4	8.5	多数	8.5

1 -- The Ovary of Non-Sterilized Mature Rats by D-Deficiency
2 -- No. of experimental animals
3 -- Sexual cycle
4 -- Weight
5 -- Interstitium
6 -- Blood vessel
7 -- Initial follicles
8 -- Major follicles
9 -- Medial follicles
10 -- Minor follicles
11 -- Closed follicles
12 -- Lutein
13 -- days stable
14 -- a little scarce
15 -- not so many

C = congestion N = normal R = retrograde S = scarce

Pic. 3. The uterus: Slightly atrophied vitamin D deficient infant albino rat.

Pic. 4. An follicle in the recovery exam. An infant albino rat.

Pic. 5. The ovary of the mature albino rat. (vitamin D deficiency).

Pic. 6. The uterus of a mature albino rat. (endosed with 1,000 I.U. of vitamin D for 9 days).

Pic. 7. The ovary: The deformation of follicles and granule-layer, and the invasion of connective tissue (endosed with 10,000 I.U. of vitamin D for 90 days).

Pic. 8. The ovary: Medial and small ovary cell show retrograde metamorphosis. The border of lutein cell is unclear. The concentration of interstitium cell. (endosed with 50,000 I.U. of vit. D continuously).

The weight of two samples of uteri, which did not show any continuous stillness was 256 mg, each, and in the control group 384 mg. on an average.

Those two samples showed no change in the mucous membrane, epithelium showed slight atrophy both in the interstitium and in the muscle layer. The other samples weighed 239.2 mg. on the average, and the epithelium of their mucous membranes were atrophied and out of order, with vacuoles and indistinct boundaries among cells in them. Uterus glands were undergrown and the gland cells were lightly dyed and the protoplasm had vacuoles. But the gland cells were in order, and in the interstitium and muscle layer were atrophied parts. The nuclei were heavily dense. Gland cells were not invaded by eosinophilic cells.

(2) Vitamin D overdose experimentation

I) Changes in non-sterilized mature albino rats

(i) Group dosed with 1,000 I.U. of vitamin D (dosed for 90 days once a day)

The average weight of the each ovary was 44.8 mg. Many of the major and medial follicles were normal. Abnormality was not observed in the initial follicles. Many luteins were seen. Boundaries among the cells were distinct. Generally the tendency of congestion was seen. The interstitial tissue was dense and contained heavily dyed spindle-shaped and round-shaped nuclei. Many of the uteri were hypertrophied like dropsy and the walls of the uteri became thick. The uteri weighed 808 mg. on an average. Folding of the uterine endometriums was obviously crooked. The epitheliums were very thick. The nuclei were located in the lower parts and lightly dyed (Pic. 6). The uterus glands and interstitium were well grown. The muscle layer became obviously thick. And in the interstitium and muscle layer was

seen an invasion of eosinophilic cells.

(ii) Group dosed with 5,000 I.U. of vitamin D (dosed for 90 days once a day)

The average weight of each ovary was 38.8 mg. Some of the major, medial and minor follicles were healthy. But most of them were rather retrograded. And many big closed follicles were recognized. No abnormality in the initial follicles was observed. Luteins were many and became heavily congested. Some of the lutein cells had vacuoles. In the connective tissue dilatation and congestion of blood vessels was seen. Each uterus weighed 480.6 mg. on an average and histologically this figure showed little difference from the former group (i). But many epithelial cells of the mucous membrane contained vacuoles and nuclei in them were out of order. The thickness of the interstitium and muscle layer of this group was not so obvious as that of the former group.

(iii) Group dosed with 10,000 I.U. of vitamin D (dosed for 90 days once a day)

The average weight of each ovary was 28.5 mg. Most of the major follicles showed retrograde metamorphosis of the egg cells and granular layer. A heavy invasion of connective tissue was observed in them. And also many big closed follicles were observed (Pic. 7). Many minor and medial follicles showed retrograde symptoms but the initial follicles showed no abnormality. Many luteins were found and were conspicuously invaded by connective tissue and were congested. And boundaries among the cells were indistinct. The lutein cells contained vacuoles.

The average weight of uteri was 367.8 mg. and uterus cavities were small. The epithelial cells of the mucous membrane contained vacuoles and

the nuclei in them were out of order. The uterus glands were undergrown. The interstitium and muscle layer were more atrophied than those of the group dosed with 5,000 I.U. of vitamin D. (Table 4, 5).

(iv) Group dosed with 50,000 I.U. of vitamin D and 100,000 I.U. of vitamin D (continuously dosed once a day until the point of death)

Ovaries were pale and flat. There were no healthy major follicles. Most of the medial and minor follicles showed retrograde metamorphosis, but the initial follicles did not show a significant change. Many of the luteins were medial and minor and were very much invaded by connective tissue. Boundaries among the cells were completely indistinct. In the interstitium the nuclei were heavily dense and very much congested (Pic. 8).

The lumens of the uteri were small. The epithelium of the mucous membrane looked like dropsical swelling and in part exfoliate. The epithelial cells were lightly dyed and had indistinct boundaries and lightly dyed nuclei, which were out of order. The interstitial cells were lightly dyed and had hypertrophied nuclei (Pic. 9). The muscle layer was atrophied and was congested and invaded to a small degree by eosinophilic cells (Table 6, 7).

2) Changes in the sterilized albino rats

In the six samples out of ten which had been dosed once a day with a total amount of 10,000 I.U. of vitamin D could be seen the appearance of the symptoms of the estrus in the (CHITSUSHIKOTSU) (a part of vagina bones) between the 6th and 16th days. Referring to those uteri histologically, the uterus walls were obviously crooked. The epithelium of the mucous membrane was very thick and formed vacuoles and was invaded by white blood corpuscles. Uteri glands were well-grown and gland vessels were winding and

Table 4

表4 D過剰投与における非去勢成熟シロネズミの卵巣

	実験動物番号	性周期	体重	間質	血管	成熟卵胞	大卵胞 健康	大卵胞 退化	中卵胞 健康	中卵胞 退化	小卵胞 健康	小卵胞 退化	閉鎖卵胞	黄体
D千単位群	101	P	46	正常	充血	＋＋	3.5	1.5	5.5	2.5	11	5	普通	13
	102	M	48.5	正常	充血	＋＋	0	1	5.5	2	12	2.5	小数	10.5
	103	D	42.5	正常	充血	＋＋	0	0	5	4.5	11.5	5	小数	13
	104	D	46	正常	充血	＋＋	0	0	4.5	3.5	13	3.5	普通	12
	105	O	39	正常	充血	＋＋	3	2	4.5	3	12.5	4	小数	13.5
D三千単位群	111	D	42.5	正常	充血	＋＋	0.5	0	3	6.5	5.5	5	やゝ多数	18
	112	O	36	正常	充血	＋＋	3.5	1	2.5	3	6.5	7.5	普通	11.5
	113	O	39	正常	充血	＋＋	3	1.5	2.5	4	3.5	6	普通	12
	114	M	36.5	正常	充血	＋＋	0	0.5	3.5	5.5	3	7.5	小数	12.5
	115	P	40	正常	充血	＋＋	2.5	2.5	2.5	4.5	4	9.5	小数	14
D一万単位群	116	O	31.5	疎	充血	＋＋	2.5	2.5	3.5	4.5	1.5	6.5	普通	12.5
	117	P	30	密	充血	＋＋	2.0	3.5	2.5	3.5	2	9	やゝ多数	15.5
	118	D	25.5	疎	充血	＋＋	0	0.5	1	2.5	3.5	8.5	普通	14
	119	D	26.5	正常	充血	＋	1	1.5	1.5	4.5	4	12.5	普通	13
	120	M	29	正常	充血	＋＋	0	2.5	0.5	4	1.5	9.5	やゝ多数	15.5

A = average, L = little, R = retrograde, N = normal, D = dense, S = scarce, C = congestion

1 -- The Ovary of Non-Sterilized Mature Rats by D-Overdose
2 -- No. of experimental animal
3 -- Sexual cycle
4 -- Weight
5 -- Interstitium
6 -- Blood vessel
7 -- Initial follicle
8 -- Major follicles
9 -- Medial follicles
10 -- Minor follicles
11 -- Closed follicles
12 -- Lutein
13 -- 1,000s I.U. of vit. D
14 -- 3,000s I.U. of vit. D
15 -- 10,000s I.U. of vit. D
16 -- not so many

Table 5

表5　D過剰状態における非去勢成熟シロネズミの子宮

	重量	子宮壁	粘膜上皮					子宮腺			間質	筋層	好酸性細胞	血管
			発育	核界	染色	空胞	核	発育	空胞	重列				
D 1,000 I.U.群	803	屈曲	きわめて良	明瞭	淡染	+	低在	良	+	正	H 肥・大	薄明に肥大 H	++	やゝ充血
D 5,000 I.U.群	480.6	屈曲	良	不鮮明	良染	+	低在一部乱	良	+	正	H 肥・大	H 肥・大	++	充血
D 10,000 I.U.群	357.8	平滑	やゝ良	不鮮明	淡染	+	乱雑	不良	+	やゝ不正	やゝ薄	正常	++	充血

H = hypertrophied

1 -- The Uteri of Non-Sterilized Mature Rats by D-Overdose
2 -- Weight
3 -- Uterus wall
4 -- Epithelium of the mucous membrane
5 -- Uterus gland
6 -- growth
7 -- boundary
8 -- dying
9 -- vacuolus
10 -- nucleus
11 -- order
12 -- Interstitium
13 -- muscle layer
14 -- eosinophilic cells
15 -- blood vessel
16 -- 1,000 I.U. of vit. D
17 -- 5,000 I.U. of vit. D
18 -- 10,000 I.U. of vit. D
19 -- lower part but in part out of order

Table 6

① 表6 D過剰状態における非去勢成熟ラットの卵巣

	性周期	重量	血管	間質	閉鎖卵胞	大卵胞 健N	大卵胞 退R	中卵胞 健N	中卵胞 退C	小卵胞 健N	小卵胞 退S	
D 50,000 I.U. 群	持続	25.1	充血	疎	+	0	0.3	0.3	5.3	1.5	6.8	
D 100,000 I.U. 群	持続	22.7	充血	疎	+	0	0	0.5	4.2	1.2	6.2	

N = normal, R = retrograde, S = scarce, C = congested

1 -- The Ovary of Non-Sterilized Mature Rats by Ultra-Overdose of Vitamin D
2 -- Sexual cycle
3 -- Weight
4 -- Blood vessel
5 -- Interstitium
6 -- Initial follicle
7 -- Major follicle
8 -- Medial follicle
9 -- Minor follicle
10 -- Closed follicle
11 -- Lutein
12 -- The group dosed with 50,000 I.U. of Vit. D.
13 -- The group dosed with 100,000 I.U. to Vit. D.

Table 7

表7　D[illegible]における非去勢成熟シロネズミの子宮

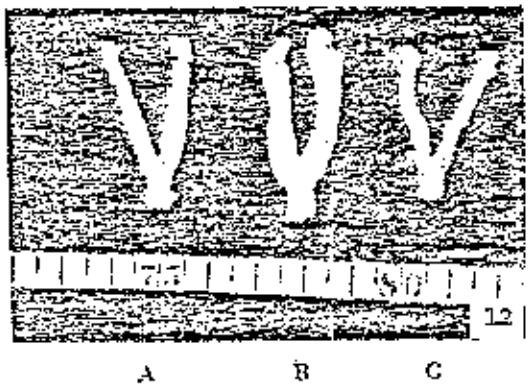
	重量	宮壁	粘膜上皮					子宮腺			間質	筋層	コメント	
			発育	境界	染色	空胞	核	発育	空胞	配列	質	筋		
D 50,000 I.U. 群	382	平滑	不良	不鮮明	正常	+	乱雑	不良	+	やゝ不正	やゝ疎	疎	+	
D 100,000 I.U. 群	275.6	平滑	不良	不鮮明	正常	+	乱雑	不良	+	やゝ不正	疎	疎	—	

1 -- The Uteri of Non-Sterilized Mature Rats by D-Overdose
2 -- Weight
3 -- Uterus wall
4 -- The epithelium of the mucous membrane
5 -- Uterus gland
6 -- Interstitium
7 -- Muscle layer
8 -- Eosinic
9 -- Blood vessel
10 -- growth
11 -- boundary
12 -- dying
13 -- vacuolus
14 -- nucleus
15 -- order
16 -- The group dosed with 50,000 I.U. of vit. D.
17 -- The group of 100,000 I.U. of vit. D

Pic. 9. The uterus: The border of epithelial cells become unclear, the nucleus disorder, and easy to dye.

Pic. 10. The uterus: The epithelium swells, vacuoles found, white blood cell saturates, uterus gland grows well, interstitium swells and hyperemis, eosinophilic cell saturates significantly (sterilized rats, dosed with 10,000 I.U. of vitamin D)

Pic. 11. Vagina: The keratinizations of epithelial cell are significant (dosed with 10,000 I.U. of vitamin D to sterilized rats).



A: uterus dosed with 5,000 I.U. of vitamin D
B: uterus dosed with 1,000 I.U. of vitamin D
C: uterus dosed with 10,000 I.U. of vitamin D

Pic. 12. The ovary of the sample of the dosed group with vitamin D.

nuclei of the gland epithelial cells were disposed in good order. The interstitium muscle layer was thick and obviously congested and was invaded by eosinophilic cells (Fic.10).

Considerations

(1) Vitamin D deficiency experimentation

Most of the follicles both in the infant groups and in the mature groups showed retrograde metamorphosis and an increase of the number of closed follicles. The degree of retrograde metamorphosis almost matched the change of the sexual cycle. As a whole, most of the mature follicles could not be recognized. The most obvious change was that the follicles showed retrograde metamorphosis in the process of growth. In the infant group many follicles showed cyst-like forms and in the mature group an invasion of connective tissue into the follicles was seen as retrogradation of the cells of granular membrane and many follicle walls were blockaded by the connective tissue. In the recovery experimentation which aimed to examine whether these changes resulted from D-deficiency or not, these changes were restored to the extent that was not so different from those of normal samples.

Therefore, D-deficiency resulted in female sexual ability troubles and the atrophy of genitals. Here referring to the reports of some authorities, Sekiguchi said that the follicles of albino rats being raised with the rachitis diet become atrophied and become smaller in number and that especially the large follicles were affected by that diet very much and retrograde metamorphosis was seen in epithelial cells. Imura reported that the rachitic albino rats lacking vitamin A showed that obvious changes

were not seen in the connective tissue of the ovaries but the follicles degenerated, and the cells became atrophied and no new lutein was seen. These reports indicated almost the same results as the results of my experimentation, and the number of luteins he reported was slightly large. Instead large amounts of vitamin D caused often estruses and the increase of numbers of luteins. But those luteins showed obvious retrograded metamorphosis already. Considering the fact that there was seen a tendency of atrophy in the uteri, I think I may observe that at first as a passing phenomenon the stimulus effect was seen but according to the length of the dose period, the sexual function was gradually adversely affected. Therefore in the 1000 I.U. of vitamin D group the ovaries were obviously atrophied and congested and the growth of follicles became active. Those changes showed the apparent accelerating effect. Through these changes, it was certified that proper amounts of vitamin D dosage accelerate female sexual functions apparently histologically too.

But while Watanabe said that the sexual cycle stopped proceeding to the growth stop of the animals, according to my experimentation, some samples indicated that in the infant group I also recognized that before the growth stopped, the resumption of the sexual cycle was obstructed already, but in the mature group I got results opposite of his: that after the decrease of weight effected by vitamin D deficiency, the sexual cycle stopped.

Totsu also reported that he reached the same results as mine; that after the decrease of weight of the animals, their sexual cycles stopped. However he did not think that this was caused by vitamin D deficiency but thought that this resulted from the rachitic diet. He reoprted that even

though he added vitamin D to the rachitic diet, the sexual cycle was in the same way obstructed. In my vitamin D dosage recovery experimentation however, no sexual cycle was observed, and I recognized changes which were not very different from those seen histologically in normal samples. Also, Totsu's experimentation period was 60 days, but in my experimentation the seven sample animals out of eight showed that after 60 days of the vitamin D deficient diet, their sexual cycles stopped. This result seems to mean that diet composition was one of the reasons why the results of the experimentation were different between us.

(2) Vitamin D overdosage experimentation

The weight of ovaries of 1000 I.U. of vitamin D and 5000 I.U. of vitamin D groups was bigger than that of the control groups. The growth condition of the follicles of the D 1000 group exceeded that of the control samples and most of them did not show the symptom of retrogradation. Both in the 5000 I.U. and in the 1000 I.U. vitamin D groups, most of the major follicles showed retrograde metamorphosis and quite a few luteins were seen but were invaded by connective tissue, and the boundaries of the cells were indistinct. In the protoplasm vacuoles were observed. The connective tissue was rough. Uteri in the 1000 I.U. and the 5000 I.U. groups were hypertrophied (Pic. 12) and many lumens expanded as in dropsy. But the uteri in the 10,000 I.U. vitamin D group were not different from those in the control group, but rather showed a tendency to be atrophied.

Watanabe reported that small amounts of (BIGANTOR) dosage caused frequent estruses and affected the female genitals acceleratedly and that medial amounts of dosage accelerated the symptoms of retrogradation and a large amounts of dosage caused only the symptom of retrogradation.

Totsu reported that small amounts of BIGANTOR dosage gave no trouble to the ovaries but gave rather good effects. And medial amounts caused histological disturbances and large amounts of it gave most of the major and medial follicles retrograde metamorphosis and a great degree of trouble. In my experimentation, too, a small doses resulted in good change. But I could not see often estruses as Watanabe had reported. But in the group receiving large doses the symptoms of retrogradation appeared in proportion to the amount of it and the whole body was affected and the sexual function stopped completely. Adler Boltink dosed albino rats with vitamin D and caused them to have the symptom of frequent estruses. Dodds dosed sterilized albino rats with large amounts of vitamin D and found the appearance of the symptom of estruses and hypertrophy of uteri and reported that vitamin D had the Estrogenic Effect. Freedman dosed women in the menopause with vitamin D and reported the same thing. But Totsu said that the reason why vitamin D dosage changed an abnormal sexual cycle into a normal one was due to the increase of weight and the improvement of the general condition. Ueno reported that every vitamin did not cause the symptom of frequent estruses. Therefore I, too, injected a total amount of 10,000 I.U. of vitamin D once a day into sterilized albino rats and found that the symptom of estruses in the (CHITSUSHIKOTSU) (a part of vagina bones) and saw the very same changes in uteri and vaginas as those caused by Estrogen dosages.

Conclusions

Vitamin D deficient albino rats showed that their follicle systems had trouble. Retrograde changes of growing follicles were especially

obvious and ovulations were impeded.

On the contrary, a small amount of vitamin D stimulated the growth of follicles and caused the uterus to thicken and accelerated the sexual function. But a large, long-term dose caused at first a stimulus effect as a passing phenomenon and eventually caused the genitals to atrophy and stopped the sexual function completely.

Hence I would like to express my thanks to my honored teacher for his guidance and revision.

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原　　著

ビタミンDの雌性性機能におよぼす影響に関する実験的研究

Vitamin 19(3):321-5, (1960)

(II) ビタミンD欠乏および過剰シロネズミの卵巣と子宮の組織学的所見

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STUDIES ON THE INFLUENCE OF VITAMIN D ON THE FEMALE SEXUAL FUNCTION

(II) HISTOLOGICAL CHANGES OF OVARY AND UTERUS IN VITAMIN D DEFICIENT AND VITAMIN D OVERDOSED ALBINO RATS

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The author has studied, histologically, the effect of vitamin D on the ovary and the uterus of albino rat, and reached to the following conclusions. Deficiency of vitamin D affects the follicular tissues of ovaries and especially the regressive degeneration of the growing follicles, which prohibits the ovulation, is very remarkable. Along with these changes in the ovaries, the uterus becomes atrophic. But these changes are recovered by an administration of vitamin D almost to normal condition. On the other hand, the animals, which has been given small amount of vitamin D, reveal the acceleration of the follicular development and the enlargement of uterus. These facts show that vitamin D has enhanced the functions of the sexual organs. If the animals are given a large amount of vitamin D over a long period of time, it will enhance the sexual function temporarily, but then the ovaries and the uteri will become atrophic and will finally lose their function completely.

わたくしはさきにD欠乏および過剰シロネズミの一般状態，性周期，妊娠能力について報告した[1]．本報ではそれらの卵巣および子宮の組織学的所見について報告する．

実　験　方　法

(1) 染　色

ヘマトキシリン・エオジン重染色を主とし必要に応じてvan Gieson染色を行なった．

(2) 卵胞および黄体数の計算

Mikami, Sasaki, 吉沢[2]の報告を参考にして，まず一応卵巣全体の連続切片を作成して計算し（表1），ついで中央部の連続20切片について計算したものとを比較した．その結果両者間の計算値に大差ないことを知ったので，中央部3ヵ所より30片を作つて計算し，左右両卵の平均をとつた．

卵胞および黄体の大小の区別は吉沢の分類にしたがつて卵胞は500μ以上を大，500～200μを中，200μ以下を小とし黄体は1000μ以上を大，1000～500μを中，500μ以下を小とした．

実　験　成　績

表1　基本食餌者シロネズミ卵巣の連続切片による卵胞および黄体数

動物番号	性周期	原始卵胞	大卵胞 健	大卵胞 退	中卵胞 健	中卵胞 退	小卵胞 健	小卵胞 退	黄体
11	D	＋	0	0	7.5	3.5	10.5	5	12.0
12	D	＋	0	0.5	6	4.5	9	5	13.5
13	P	＋	3.5	1.5	1.5	3	8.5	3.5	12
14	P	＋	2.5	2	5	2.5	8	4	10.5
15	P	＋	2	1	4.5	2	9	3.5	12
16	O	＋	2	1	4.5	2.5	10	4.5	11.5
17	O	＋	3.5	0.5	5	3.5	9	3	10
18	M	＋	0	1	8.5	4	11	4.5	11.5
19	M	＋	0	0	6	3.5	9.5	5.5	12.5
20	M	＋	0	0.5	5.5	3.5	8.5	2	13.5

D＝性休止期　　O：発情期
P＝発情前期　　M：発情後期

図1　卵胞刺激濾胞細胞の段状消失（D欠乏幼シロネズミ）

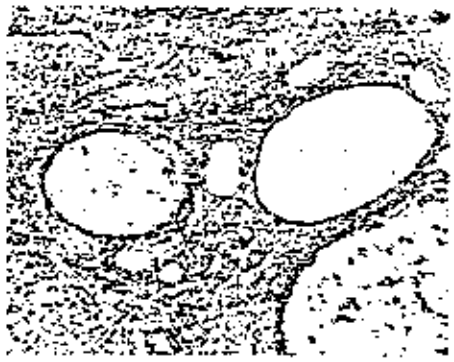


図2　黄体細胞の不均一性（D欠乏幼シロネズミ）

（1）　D欠乏実験

1）　非去勢幼シロネズミにおける所見

D欠乏食90日間投与5例のうち不妊な性周期が発表した動物(No. 57, 58, 59)の卵巣は平均重量31.5mgで同復試験群の36.4mgと差がなく性周期の徴候をみないもの(No. 55, 60)では外観位内で平均重量16mgであつた。

組織学的所見では後者には異常大卵胞をみとめず，中卵胞にもほとんど健全なものがなく顆粒膜細胞に大小不同で配列が乱れ結合織の侵入，白血球の浸潤がみられた。小卵胞には一部健全なものもあつたが大多数は退行像を示し卵細胞および透明帯が消失し，小嚢胞状を呈するのが多かつた(図1)。原始卵胞は組織学的変化をみなかつたが一般に少数であつた。間質細胞は極胞が密接きまざまに配列し，また卵巣卵胞が顕明であつた。黄体は数が少なく結合織の侵入色の不均一性を示し浸潤部では核が萎縮してまたは小多角形を呈し浸潤部では大多角形で空胞をものがあつた(図2，表2)

表2　D欠乏試験における非去勢幼シロネズミの卵巣

	動物番号	性周期	重量	卵巣	間質	原始卵胞	大卵胞 健	大卵胞 退	中卵胞 健	中卵胞 退	小卵胞 健	小卵胞 退	黄体
同復試験群	51	O	35.5	充血	正	＋	2	1	3.5	2.5	7.5	5	普通
	52	P	38	充血	正	＋	1	2	4.5	3	4	5.5	やゝ多数
	53	D	31.5	正	正	＋	0	0	5	5.5	7	5	普通
	54	D	38	正	正	＋	0	0	2.5	4	5.5	5	やゝ多数
	55	M	36	正	正	＋	0	1	3	3.5	6	4.5	やゝ多数
D欠乏群	56	D	18.5	充血	密	＋	0	0	0.5	2.5	2.5	0	多数
	57	M	33.5	正	正	＋	0	0	1.5	2	3	4.5	多数
	58	D	37	正	正	＋	0.5	1	1.5	2.5	3.5	4	やゝ多数
	59	D	33	充血	密	＋	0	0	2	2.5	4	8	多数
	60	D	13.5	充血	密	＋	0	0	0	2.5	3	8	多数

図3　子宮：軽度の萎縮像をみる
（D欠乏幼シロネズミ）

図4　回復試験例の卵胞
（幼シロネズミ）

子宮の重量はいずれもやゝ減少し，組織学的には粘膜上皮に軽度の萎縮を認め細胞の境界不鮮明となり核は配列不整，原形質にとぼしかつた．子宮腺の発育はやゝ不良で腺細胞は空胞を有し，おおくは配列不整で間質，筋層も萎縮し血管の拡張，充血は認められなかつた（図3）．

回復試験では卵胞の退行像がやゝ残つていたが（図4）他は正常所見を示し子宮の発育も正常例と大差がなかつた．

図5　成熟シロネズミの卵巣　（D欠乏）

2）　非去勢成熟シロネズミにおける所見

D欠乏食90日間投与10例中2例を除き，他は64～88日目で持続的静止期を示した．

76日目と81日目に死亡した2例を除き卵巣は蒼白，平滑で平均重量は対照のD50 i.c. 投与群の36.4mgにたいし25.2mgであつた．

組織学的には大卵胞なく中卵胞はほとんど退行し小卵胞にも健全なものは少なかつた．

その多くは顆粒層の退行変性，結合線の進入が著明で（図5）一部に嚢胞状をなすものもあつた．黄体は少なく結合線の進入が著明で細胞間の境界不鮮明となり間質組織では核濃縮を認めた（表3）．

子宮は対照群の平均重量389.4mgにたいして持続的静止期を示さなかつた2例では256mgで粘膜上皮に変化をみないが間質および筋層に軽度の萎縮像を認めた．その他の例は平均重量239.2mgで粘膜上皮は萎縮し配列不整，空胞を認め細胞境界不鮮明であつた．子宮腺は発育不良で腺細胞の核はやゝ淡染し原形質に空胞

表3　D欠乏状態における非去勢成熟シロネズミの卵巣

動物番号	性周期	重量	間質	血管	原始卵胞	大卵胞		中卵胞		小卵胞		閉鎖卵胞	黄体
						健	退	健	退	健	退		
61	D	29.5	正	正	＋	0	0	0	2.5	3.5	5.5	多数	10
63	55日持静	27	正	充血	＋＋	0	0	0	4	5	12.5	多数	9.5
64	69日持静	26.5	やゝ疎	充血	＋	0	0	0.5	3	3	9	多数	8
66	M	31	正	正	＋＋	0	0	3	2.5	4.5	4	やゝ多数	11
67	78日持静	22.5	疎	充血	＋＋	0	0	0	2.5	2	4.5	多数	8
68	61日持静	21	やゝ疎	充血	＋	0	0	0	2	3	9.5	多数	7.5
69	73日持静	25	疎	充血	＋	0	0	0	3	3	11	多数	9
70	71日持静	19.5	疎	充血	＋	0	0	0	2	4	6.5	多数	8.5

を認めたが配列は雑で間質，筋層にも萎縮像を認め核は濃縮しエオジン好性細胞の浸潤はなかつた．

（2） D過剰投与実験

1）卵去勢成熟シロネズミにおける所見

(i) D 1,000 r. u. 投与群（1日1回90日間投与）

卵巣の平均重量は44.8mgで大中・卵胞ともに正常なものがおゝく原始卵胞に異状を認めなかつた．黄体も多数認められ細胞境界は明瞭で一般に充血の傾向がみられた．間質組織は密で濃染する紡錘ないし円形の核を有していた．

図6　成熟シロネズミ子宮（D）
（1,000 r. u. 9日間投与例）

子宮は水腫様に肥大したものがおおく子宮粘膜肥厚し平均重量は808mgであつた．子宮粘膜被覆は組織若明で，上皮はつよく肥厚し核は低在性に配列し淡染した（図6）．子宮腺，間質の発育は良好で筋層は著明に肥厚していた．なお間質，筋層にエオジン好性細胞の浸潤を認めた．

(ii) D 5,000 r. u. 投与群（1日1回90日間投与）

卵巣の平均重量は38.8mgで大・中・小各卵胞とも健全なものも認められたが，むしろ退行像を示すものの方がおおく，かつ大閉鎖卵胞多数を認めた．しかし原始卵胞には異状がなかつた．黄体は多数存し充血が強く一部の黄体細胞には変性がみられた．間質組織では血管の拡張，充血があつた．

子宮は平均重量480.6mgで組織学的には前群と大差がなかつたが粘膜上皮細胞に変性を認めるものがおおく核の配列が不整であつた．間質，筋層の肥厚は前群ほど著明ではなかつた．

(iii) D 10,000 r. u. 投与群（1日1回90日間投与）

卵巣の平均重量は28.5mgで大閉鎖は大部分卵胞，顆粒層の退行変性をきたし結合織の進入が強く，かつ

図7　卵巣：卵細胞，顆粒層の変性，結合
の進入（D10,000 r. u. 90日間投与例

図8　卵巣：中小卵巣の退行変性，黄体細
の境界不鮮明，間質細胞核濃縮．
（D50,000 r. u. 連続投与例）

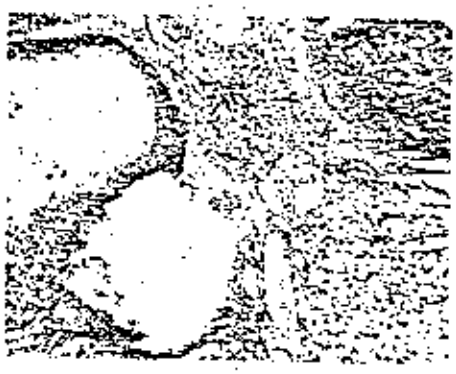


図9　子宮：粘膜上皮細胞の境界不鮮明，
配列不整，淡染
（D50,000 r. u. 連続投与例）

大閉鎖卵胞の多数を認めた（図7）．小中卵胞は
体を示すものが多かつたが原始卵胞には異状を
かつた．黄体も多数認められたが結合織の進入
血を示し細胞境界不鮮明となり黄体細胞に変
みられた．

子宮は平均重量307.2mgで子宮腺がせまく

表4　D過剰状態における非去勢成熟シロネズミの卵巣

	動物番号	性周期	重量	間質	血管	原始卵胞	大卵胞 健	大卵胞 退	中卵胞 健	中卵胞 退	小卵胞 健	小卵胞 退	間質卵胞	黄体
D千単位群	101	P	46	正	充血	＋	3.5	1.5	5.5	2.5	11	5	普通	13
	102	M	48.5	正	充血	＋	0	1	5.5	2	12	2.5	小数	16.5
	103	D	42.5	正	充血	＋	0	0	5	4.5	11.5	5	小数	13
	104	D	48	正	充血	＋	0	0	4.5	3.5	13	3.5	普通	12
	105	O	39	正	充血	＋	3	2	4.5	3	12.5	4	小数	13.5
D三千単位群	111	D	42.5	正	充血	＋	0.5	0	3	6.5	5.5	5	やゝ多数	15
	112	O	36	正	充血	＋	3.5	1	2.5	3	6.5	7.5	普通	11.5
	113	O	39	正	充血	＋	3	1.5	2.5	4	3.5	6	普通	12
	114	M	36.5	正	充血	＋	0	0.5	3.5	5.5	3	7.5	小数	12.5
	115	P	40	正	充血	＋	2.5	2.5	2.5	4.5	4	9.5	小数	14
D一万単位群	116	O	31.5	疎	充血	＋	2.5	2.5	3.5	4.5	1.5	6.5	普通	12.5
	117	P	30	密	充血	＋	2.0	3.5	2.5	3.5	2	9	やゝ多数	16.5
	118	D	25.5	疎	充血	＋	0	0.5	1	2.5	3.5	8.5	普通	14
	119	D	26.5	正	充血	＋	1	1.5	1.5	4.5	4	12.5	普通	13
	120	M	29	正	充血	＋	0	2.5	0.5	4	1.5	9.5	やゝ多数	15.5

表5　D過剰状態における非去勢成熟シロネズミの子宮

	重量	子宮腔	粘膜上皮 発育	粘膜上皮 境界	粘膜上皮 染色	粘膜上皮 空胞	粘膜上皮 核	子宮腺 発育	子宮腺 空胞	子宮腺 配列	間質	筋層	好性細胞 エオジン	血管
D 1,000 I.U.群	803	屈曲	きわめて良	明瞭	淡染	＋	低在	良	＋	正	肥大	著明に肥大	＋	やゝ充血
D 5,000 I.U.群	480.6	屈曲	良	不鮮明	良染	＋	低在一部乱雑	良	＋	正	肥大	肥大	＋	充血
D 10,000 I.U.群	367.8	平滑	やゝ良	不鮮明	淡染	＋	乱雑	不良	＋	やゝ不正	やゝ著	正常	＋	充血

細胞には空胞形成があり核の配列は不整であつたが子宮腺の発育は不良で間質，筋層は5,000 I.U.群よりも萎縮が強かつた(表4, 5)．

(iv)　D 50,000 I.U., 100,000 I.U.投与群(1日1回鏡死の状態になるまで連続投与)　卵巣蒼白，平滑で健全な大卵胞なく中・小卵胞も大部分退行変性を示したが原始卵胞には有変がなく，黄体のおおくは中・小黄体で結合組の進入強く細胞の境界はきつたく不鮮明となり間質組織は核が萎縮し一般に充血が強かつた(図8)．

子宮は内腔せまく粘膜上皮は浮腫状を呈し一部には変性が認められた．上皮細胞は淡染し，境界不鮮明となり配列不整の淡染する核を認めた．間質細胞も淡染性で肥大した核を認め(図9)，筋層は萎縮し，しかも充血がありエオジン好性細胞の浸潤がわずかにみとめられた(表6, 7)．

2)　去勢シロネズミにおける所見

1日1回ずつD 10,000 I.U.を投与したものでは10例中6例に，6～16日で腟脂膜に発情様像の出現を認めた．それらの子宮を組織学的に検索すると子宮腺腔は屈曲著明で粘膜上皮細胞は肥厚し空胞形成，白血球の浸潤を認めた．子宮腺の発育は良好で腺管は迂曲し腺上皮細胞の核は整然と配列していた．間質筋層の肥厚，充血しエオジン好性細胞の浸潤が著明であつた(図10)．

表6　D超過刺状態における非去勢成熟シロネズミの卵巣

	性周期	重量	血管	間質	感染卵胞	大卵胞		中卵胞		小卵胞	
						健	退	健	退	健	退
D 50,000 i.u. 群	持続	25.4	充血	疎	+	0	0.3	0.3	3.3	1.6	6.8
D 100,000 i.u. 群	持続	22.7	充血	疎	+	0	0	0.5	4.2	1.2	6.2

表7　D超過刺状態における非去勢成熟シロネズミの子宮

	重量	子宮腺	粘膜上皮					子宮腺			間質	筋層
			発育	増殖	変色	変性	核	発育	増殖	配列	浮腫	
D 50,000 i.u. 群	382	平滑	不良	不鮮明	正常	+	乱雑	不良	+	やや不正	やや縮	緊縮
D 100,000 i.u. 群	275.6	平滑	不良	不鮮明	正常	+	乱雑	不良	+	やや不正	緊縮	緊縮

図10　子宮：粘膜上皮変形，変性形成，間質球細胞，腺発育良好，間質浮腫，エオジン好色性変性顕著（去勢シロネズミ，D 10,000 i.u. 連続投与例）

図11　腟：上皮角化亢進（去勢シロネズミ D10,000 i.u.）（連続投与例）

これらの所見はエストロゲン投与時の所ており腟の所見もまた正常発情期ならびにン投与時の所見とまったく同様であった(D

考　　察

(1) D欠乏実験

幼若期，成熟期ともに卵巣の大半は退行卵胞の増加を認め退行変性の程度と性周期は一致する。全般に成熟卵胞をほとんどがその発育過程において退行変性をきたすとも顕著な変化で，幼若期では変性を生おおく成熟期では顆粒膜細胞の退行につれ進入がみられ卵胞腔を閉塞する状態となるい。これらの変化がはたしてD欠乏に起因検するために行なつた回復試験では，これ正常例と大差ない程度に回復していた。

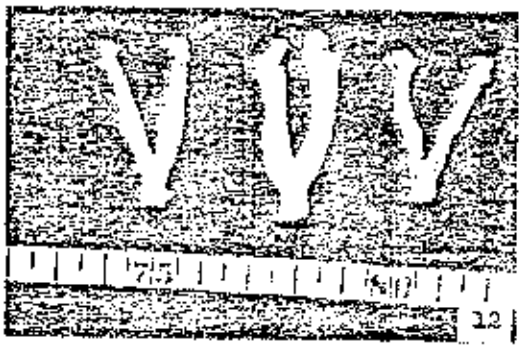
したがつてD欠乏は卵巣性機能の障害とをきたすことをしる。いま卵巣の損害をみ卵はシロネズミをクル病食で飼育すると，多数をきたし，とくに大きい卵胞に変化が顆粒細胞に退行変性をみている。片方はA欠うクル病シロネズミでは卵巣の間質組織にいが，卵胞に変性し卵胞は発育をきたし黄みないといい，黄体は一般に大・中卵胞認めるが卵胞欠乏群にもなお健全なものをいつている。これらの成績にわたくしの実

は同様である．ただ渡辺は動物の発育停止に先んじて性周期が停止するとのべているが，わたくしの実験では幼若群には発育停止前すでに性周期の発現が阻止された例もあるが，成熟群ではDの欠乏により体重が減少した後になつてはじめて性周期の停止をみ，氏と相反する成績をえた．しかして都筑[4]もわたくしと同様に動物の体重減少後に性周期の停止をみたと報告しているが，これをDの影響とは考えずクル病食によるものでクル病食にDを添加しても同じく性周期が障害されたと述べている．ところがわたくしのD投与回復試験群では性周期がみられず，組織学的にも正常例と大差ない所見をえた．また都筑の実験期間は60日であるが，わたくしの実験では動物8例中7例までがD欠乏食投与60日以降になつてはじめて性周期の停止をみているので，食餌組成の違いも実験成績が相異する一因と考えられる．

(2) D過剰実験

卵巣の重量はD1,000 i.u., 5,000 i.u. 群では対照より大であり，1,000 i.u. 群における卵胞の発育状態は対照例をしのぐ，多数の黄体を認め退行像を示すものはほとんどみられない．5,000 i.u. および 1,000 i.u. 群では大卵胞の大部分が退行変性し，きわめて多数の黄体をみるが黄体の大小不揃いで境界が不明瞭となり卵形質内に結合を認め間質組織は一般に細である．

図12　D投与例子宮の肉眼所見



A　　B　　C

A：D5,000 i.u. 投与子宮
A：D1,000 i.u. 投与子宮
C：D10,000 i.u. 投与子宮（対照と大差なし）

子宮はD1,000 i.u., 5,000 i.u. 群では肥大明瞭で（図12）内腔が狭小に拡大するものが多いが10,000 i.u. 群では対照例と大差なく，きわめてわずかの黄体の組成をしめした．

渡辺[5]は少量のビガントール投与は発情期の頻発をきたし，卵巣性性器に刺激的に作用し中等量では退行現象が増進し大量ではもつぱら退行現象のみを認めたといい，都筑[4]は少量のビガントール投与は卵巣になんら障害を与えず，むしろ好影響を与えるが，中等量ではすでに組織の障害をみ，大量では大・中卵胞のことごとくが退行変性し高度の障害をみとめたと述べている．わたくしの実験でも少量投与群に良好な成績をえたが，渡辺の報告のごとき発情期の頻発はなく黄体がやゝおおい程度で大量でかえつて発情期の頻発，黄体数の増加がみられた．しかしそのさいの黄体はすでに退行変性が顕著で，子宮には萎縮の傾向がみられることから，さいしょ一時的には刺激作用をみるが投与期間がながくなるとともに性機能がしだいに障害されるものと思われる．しかるに1,000 i.u. 群では卵巣，子宮ともに著明に肥大，充血し卵胞の発育がさかんとなり明らかに促進的効果を示した．これらの所見からみて適量のD投与は明らかに性腺性機能を亢進することが組織学的にも確認されたといいうる．しかし大量投与群においては投与量に比例して退行像が出現し，かつ全身状態のおかされるとともに性機能は完全に停止する．

またAdler・Böltink[6]はシロネズミにDを投与して頻回の発情期像を出現させ，Dodds[7]は去勢シロネズミに大量のDを投与して発情期像の出現と子宮の肥大をみとめて，Dにはエストロゲン様作用があるとのべ，さらにFreedman[8]は更年期婦人にDを使用してやはりエストロゲン様作用を認めたといつている．ところが都筑はD投与により不整な性周期が正常となるのは性腺の増加，一般状態の好転による現象であるといい，上野はいずれのビタミンを用いても発情期像は頻発しなかつたとのべている．そこでわたくしも去勢シロネズミに1日1回ずつD10,000 i.u. を注射したところ，数日後に発情期像があらわれ，かつ組織学的にも子宮および膣にエストロゲン投与による所見とまつたく類似の所見をみとめることができた．

む　す　び

D欠乏シロネズミでは卵胞系統が障害され，ことに発育過程の卵胞の退行性変化が顕著で，排卵が阻害される．

これに反してDの少量投与は卵胞の発育を促進し子宮の肥大を招来し性機能を亢進させる．ところが大量投与は，さいしょ一時的に刺激作用を示すが，ついには性器を萎縮せしめ性機能をまつたく停止せしめる．

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THE AMELIORATION OF HYPERVITAMINOSIS D IN RATS WITH VITAMIN A*

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PLATES 16 TO 19

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Two outstanding characteristics of hypervitaminosis D in experimental animals are skeletal abnormalities and soft tissue calcification, both of which occur in growing and adult animals (1-7). Although hypervitaminosis A causes rarefaction and fragility of the bones of growing animals (8, 9), soft tissue calcification is never found. On the contrary, vitamin A deficiency may cause renal calcification (10). Isotopic studies have shown that a single massive dose of vitamin D causes a marked increase in urinary radiocalcium derived from bone, whereas a single toxic dose of vitamin A increases urinary radiophosphorus (11). Since vitamins A and D appear to affect different constituents of bone, the following experiments were designed to investigate the effects of combined hypervitaminosis A and D in rats. Unexpectedly, it was found that the severity of the symptoms of hypervitaminosis D was greatly decreased and, in some cases, no toxicity was observed if sufficiently large amounts of vitamin A were administered simultaneously with vitamin D. A partial report of these observations has been published (12).

Experimental Procedure

General.—Albino male rats of the Holtzman strain were used in all experiments. The stock laboratory diet contained adequate amounts of vitamins A and D for normal growth and maintenance. The vitamin A palmitate (Merck) used in these experiments was generously supplied by the Merck, Sharp and Dohme Research Laboratories. Whenever necessary, it was diluted with purified sesame oil. The crystalline vitamin D_2 (calciferol) was kindly donated by Roxan-Philips Company. It was dissolved in a minimum of alcohol before diluting with purified sesame oil. The vitamins were administered daily by stomach tube on a body weight basis unless otherwise noted.

Specimens for histologic study were fixed in buffered neutral formalin. After suitable fixation, bones were decalcified with formic acid—sodium formate solution. The decalcified

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bones and soft tissues were embedded in paraffin and sections were stained with hematoxylin and eosin. In addition, the soft tissues were stained with alizarin red S to detect calcium deposits.

Specific.—

Experiment A-1: Five groups, each consisting of eight 120 to 150 gm rats, were used. Group I, control rats; Group II, 60,000 international units of vitamin D; Group III, 30,000 international units of vitamin A; Group IV, 60,000 units of vitamin D plus 15,000 units of vitamin A; Group V, 60,000 units of vitamin D plus 30,000 units of vitamin A. These animals were maintained for 5 weeks on this regimen until death or the time of sacrifice.

Experiment Q-2: Four groups, each consisting of fifteen 100 to 150 gm rats, were used. Group I, control rats; Group II, 60,000 units of vitamin D; Group III, 30,000 units of vitamin A; Group IV, 60,000 units of vitamin D plus 30,000 units of vitamin A. The surviving animals were sacrificed at the end of 15 days. Vitamins were not administered to the rats over 2 week-ends.

Experiment C-3: Five groups, each consisting of six 85 to 100 gm rats, were used. Group I, control rats; Group II, 18,000 units of vitamin D; Group III, 18,000 units of vitamin D plus 300 units of vitamin A; Group IV, 18,000 units of vitamin D plus 3,000 units of vitamin A; Group V, 18,000 units of vitamin D plus 30,000 units of vitamin A. The experiment continued for 60 days at which time the animals were sacrificed.

RESULTS

The Effect of 60,000 Units of Vitamin D with and without Vitamin A on Weight Gain, Mortality, and Histology.—It may be seen in Table I that with the exception of one rat all animals which had received 60,000 units of vitamin D per 100 gm of body weight were dead within 3 weeks. The animals which had been given 15,000 units of vitamin A along with 60,000 units of D faired slightly better, only three were dead at the end of 3 weeks and at the end of 4 weeks seven were dead. The life span, however, of the rats which had received 60,000 units of vitamin D plus 30,000 units of vitamin A was significantly prolonged; only four animals out of eight were dead at the end of 5 weeks. Similar findings were observed in experiment Q-2 (Table II). At the time the animals were sacrificed (after 11 days of vitamin administration) eight of fifteen rats which had received vitamin D alone had died, whereas all rats that had been given 30,000 units of vitamin A along with 60,000 units of vitamin D were alive. Although the concomitant administration of vitamin A increased the survival time of the hypervitaminotic D rats, it did not counteract the loss in body weight. Rats which had been given 30,000 units of vitamin A alone did not gain as well as the controls.

The changes found in the skeletal system of hypervitaminotic D rats were the same as those described by earlier investigators (2, 6, 13). The tables of the skull were much thinner than the controls (Fig. 1) and showed many resorption cavities containing vascular elements. Subperiosteal new bone formation was seen on the tables. Medullary areas were filled with basophilic, reactive new bone. There were few multinucleated osteoclasts in the skull. Supplementation

of 60,000 units of vitamin D with 30,000 units of vitamin A altered this picture considerably (Fig. 2). The tables of this membranous bone were largely intact. Medullary areas contained only occasional resorption cavities which had fewer vascular elements than similar areas in the skulls of the animals which had received only vitamin D. Thin seams of basophilic osteoid were found on a few trabeculae, but none were seen in the subperiosteal areas. Skulls of the heaviest animals in this group appeared almost indistinguishable from those of the controls.

A similar beneficial of vitamin A was observed in the tibiae of these animals. The widths of the epiphyses of the tibiae of the animals which received only

TABLE I

Effect of 60,000 Units of Vitamin D with and without Vitamin A on Body Weight and Rat Mortality

Treatment	No. of animals	Weeks on treatment						No. dead at 5 weeks
		0	1	2	3	4	5	
		gm	gm	gm	gm	gm	gm	
Control	8	137 (0)*	177 (0)	217 (0)	254 (0)	275 (0)	303‡ (0)	0
60,000 D	8	138 (0)	107 (1)	98 (3)	90 (3)	90‡		7
30,000 A	8	137 (0)	166 (0)	194 (0)	219 (0)	252 (1)	286‡ (0)	1
60,000 D + 15,000 A	8	138 (0)	118 (0)	100 (0)	90 (3)	90‡ (4)		7
60,000 D + 30,000 A	8	138 (0)	121 (0)	101 (0)	91 (0)	83 (1)	81* (3)	4

* The numbers in parentheses refer to the number of rats which died between weighing periods; the other numbers refer to the average body weight.

‡ Sacrificed for histology.

vitamin D were narrowed approximately 50 per cent as compared with those of the control animals (Fig. 3). Columns of hypertrophic cartilage cells frequently were disrupted and were staggered horizontally in a random fashion. Numerous sinusoids filled the metaphyseal area adjacent to the zone of provisional calcification. More distally in the metaphyses, the medullary areas and subperiosteal regions contained masses of basophilic osteoid. The diaphyseal cortex was honeycombed with resorption cavities which contained many multinucleated osteoclasts.

The organization and widths of the epiphyses of the tibiae of the rats that received 30,000 units of vitamin A simultaneously with vitamin D (Fig. 4) appeared almost normal, especially in the heavier animals. In this group, less basophilic osteoid was found in the medullary regions of the metaphyses. There was a marked reduction in the amount of subperiosteal new bone formation and in the number of resorption cavities in the diaphyseal cortex.

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Changes in the kidneys of hypervitaminotic D rats in certain respects were similar to those reported by others (14). There was extensive cortical calcification (Fig. 5). Both convoluted and collecting tubules contained intraluminal casts. Little or no inflammatory reaction to the interstitial calcific deposits was found. In the medulla, occasional collecting tubules contained intraluminal calcific casts. Large arteries in hilar areas showed heavy deposits of calcium in the intima and in the deep layers of the media.

The administration of 30,000 units of vitamin A improved considerably the histologic picture. There was remarkably little calcification in cortical areas and in the hilar vessels of all animals (Fig. 6). Calcific casts were found in the medulla of some specimens. The kidneys of one of the heaviest animals in this group contained no calcific deposits.

TABLE II

Effect of 60,000 Units of Vitamin D with and without 30,000 Units of Vitamin A on Body Weight and Rat Mortality

Treatment	No. of animals	Days on treatment				
		0	4	7	11	15
		gm	gm	gm	gm	gm
Controls	15	133	142	158	179	191
60,000 D	15	129	111	109 (3)*	98 (2)*	96 (3)*
30,000 A	15	131	139	152	162	171
60,000 D + 30,000 A	15	133	110	110	101	101

* The numbers in parentheses refer to the number of rats which died between weighing periods; the other numbers refer to the average body weight.

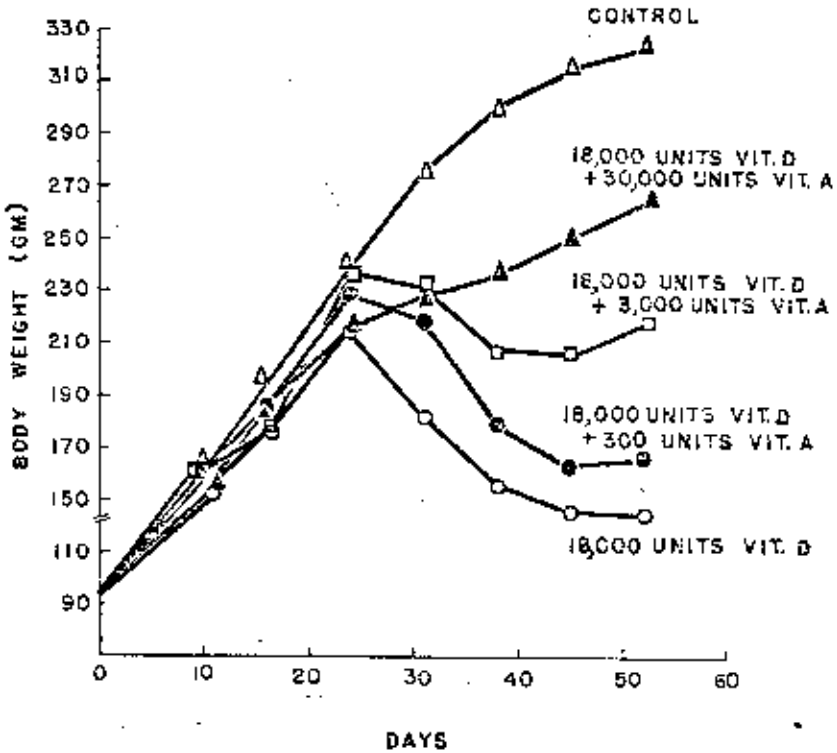
In the heart, vitamin D alone caused heavy calcification of some degenerating muscle fibers and of the intima of all vessels larger than small arterioles (Fig. 7). There was little or no evidence of an inflammatory response in areas where calcium was deposited. In none of the rats that received the vitamin A supplement was there significant myocardial damage (Fig. 8). Some specimens appeared normal while others had moderate calcification of the larger arterial walls. In this organ too the relative extent of arterial calcification paralleled the animal's weight at the time of sacrifice; the heavier the animal, the less the damage.

The bladders of all rats appeared normal, despite the severity of lesions elsewhere. Arterioles and even larger vessels of this organ showed no calcification.

The histologic picture seen in the animals receiving 15,000 units of vitamin A was intermediate between the one seen when vitamin D was used alone and th[illegible]en 30,000 units of vitamin A was administered concomitantly with the [illegible] D).

The rats which received only 30,000 units of vitamin A did not gain as much weight as the control rats (Tables I and II). Cement lines in the skull and tibial cortex seemed to be more prominent than they were in the normal controls. In some areas, the cement lines appeared in a mosaic pattern.

The Effect of Prolonged Administration of 18,000 Units of Vitamin D, with and without Varying Amounts of Vitamin A, on Weight Gain, Mortality, and Histology.—All groups gained weight for about 2 weeks. At this time the rats



TEXT-FIG. 1. Growth curves of rats which survived the 6 week period.

which received vitamin D alone or vitamin D supplemented with either 300 or 3,000 units of vitamin A began to lose weight rapidly (Text-fig. 1). The greater the supplement of vitamin A, the less was the weight loss. The rats which received 30,000 units of vitamin A together with the D gained weight throughout the experimental period, although not as well as the control animals. In this experiment also, the fewest pathologic changes were found in the heaviest animals.

Changes in the skulls and tibiae of the rats which had recei[illegible]0,000 units

of vitamin D alone were essentially similar in pattern and degree to those seen in the animals which received 60,000 units of vitamin D alone (Figs. 1 and 3). Administration of 30,000 units of vitamin A daily simultaneously with the vitamin D almost completely prevented the appearance of pathologic changes in the skulls and tibiae of half the animals. In the other half, minimal amounts of porosity and basophilic osteoid were observed. Addition of 300 units of vitamin A did not alter significantly the changes seen when 18,000 units of vitamin D were given alone. The histologic appearance of the skulls and tibiae of the animals which had received 3,000 units of vitamin A was intermediate between that of the animals given vitamin D alone and that when 30,000 units of vitamin A was combined with the D.

In this experiment, calcification of the kidneys was confined largely to the medullary region. Few intraluminal casts were found in the cortex, even in the lightest animals. In half of the rats which had received 30,000 units of vitamin A in addition to vitamin D, kidneys were nearly normal; in the other half, only minimal amounts of calcification were observed. 300 units of vitamin A did not prevent the renal lesions, while the histologic picture of those rats given 3,000 units of vitamin A was intermediate between that seen with vitamin D alone and vitamin D plus 30,000 units of vitamin A.

DISCUSSION

The data presented in this report were derived from three of many experiments. In general, the findings have been consistent in young and adult rats. In these studies, a good correlation existed between the final body weight and the severity of bone, arterial, and renal lesions. The lighter the animal, the greater the damage. This was observed in the animals which had received vitamin D alone and also in those which had received vitamin A together with vitamin D. It is interesting to note that some tissues calcify heavily (*i.e.*, arterial walls) while others show no evidence of calcification (*i.e.*, bladder wall).

The pathologic findings in the kidneys of rats which had been acutely poisoned with massive doses of vitamin D (Experiments A-1 and Q-2) differed considerably from those of the animals chronically poisoned with smaller doses of the vitamin (Experiment C). In the former group, heavy calcification was found in the renal cortex and to a lesser extent in the medulla; while in the latter group, calcification was limited largely to the medulla. In acute experiments, there was rapid osteolysis which resulted in the release of large quantities of calcium from bone into the blood stream. Since the proximal convoluted tubules are believed to be the site of calcium reabsorption, it is not surprising that this added burden would cause extensive calcification in this area. On the other hand, in the chronic experiment where only 18,000 units of vitamin D were administered, the rate of bone resorption must have been less and, consequently, the proximal tubules were not affected as severely.

However, in this experiment there was still an appreciable amount of medullary calcification. Since the collecting tubules are responsible for reabsorbing water, it is not unreasonable to expect that the solubility products of calcium-phosphate salts might be exceeded and, consequently, these structures would become calcified in the more chronic form of hypervitaminosis D.

The skeletal changes in these studies are similar to those reported by Selye (6), Harris and Innes (2), and Follis (13). Similar changes also have been found in the rabbit (14). Fewer pathologic changes were seen in both long and flat bones when 30,000 units of vitamin A were given together with either 60,000 or 18,000 units of vitamin D. This amelioration of toxicity was more dramatic in the acute experiments (Experiments A-1 and Q-2). Severity of lesions seemed dependent on the amount of vitamin A administered with vitamin D. The more vitamin A given, the less was the damage to the tissues. Although the pathologic changes in the skeletons of hypervitaminotic D rats were greatly reduced by simultaneous administration of vitamin A, rarely were they abolished completely.

Following administration of excessive amounts of vitamin D, hypercalcemia and hypercalciuria are seen before histologic evidence of renal damage (2). Moreover, they occur on a calcium-free diet (2), suggesting that the first changes of hypervitaminosis D may be of skeletal origin and that the soft tissue changes are secondary to these. If this postulate is true, the beneficial effects of vitamin A in diminishing the soft tissue calcification of hypervitaminosis D could readily result from its action on skeletal tissues. Although the mechanism of the action of vitamin A in decreasing the toxicity of massive doses of vitamin D on bone is not known, at least two possibilities exist. First, vitamin A, when given in large amounts, may interfere with the osteolytic action of vitamin D. Evidence for this may be seen in Fig. 2, in which the combination of vitamin A with D resulted in a decreased number of resorption cavities in the osseous cortex. Another explanation for vitamin A action possibly may be found in its effect on normal bone. It is known that large amounts of this vitamin hasten both osseous maturation and remodeling (15). In hypervitaminosis D, large amounts of basophilic osteoid are produced. If vitamin A increases the rate of maturation of this osteoid, more mineral would be utilized and less spilled into the extracellular fluids. Either of these two possibilities or a combination might explain the beneficial action of this vitamin on hypervitaminosis D.

An interesting question raised by this investigation is whether the physiological or biochemical function of vitamin D is dependent on the tissue level of vitamin A or its metabolites. In these studies, it was noted that as the amount of vitamin A was increased in hypervitaminotic D animals, there were fewer pathologic changes, better weight gain and longer life span. Gross-Selbeck (16) in 1935 reported that large amounts of vitamin A concentrates decreased the

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lethal toxicity of massive amounts of vitamin D. Thoenes (17) in an interesting paper speculated that vitamin A was an essential factor for the action of vitamin D and that administration of large amounts of vitamin D created a "relative avitaminosis A." This present study tends to support the concept that some of the damage of hypervitaminosis D may be in part due to a relative avitaminosis A.

SUMMARY

The administration of relatively large amounts of vitamin A to hypervitaminotic D rats decreases the toxicity of this condition as evidenced by increased longevity, better weight gain, decreased soft tissue calcification and by considerable improvement in the histologic appearance of osseous structures.

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EXPLANATION OF PLATES

Plate 16

Fig. 1. Skull, 60,000 units of vitamin D per 100 gm daily for 11 days. Hematoxylin and eosin. × 168. Cross-section just lateral to sagittal suture. Note rarefaction, vascularity, osteoid tissue in subperiosteal and medullary regions.

Fig. 2. Skull, 60,000 units of vitamin D per 100 gm and 30,000 units of vitamin A per 100 gm daily for 11 days. Hematoxylin and eosin. × 168. Cross-section just lateral to sagittal suture. This picture does not differ significantly from that seen in the normal control and should be compared with Fig. 1.

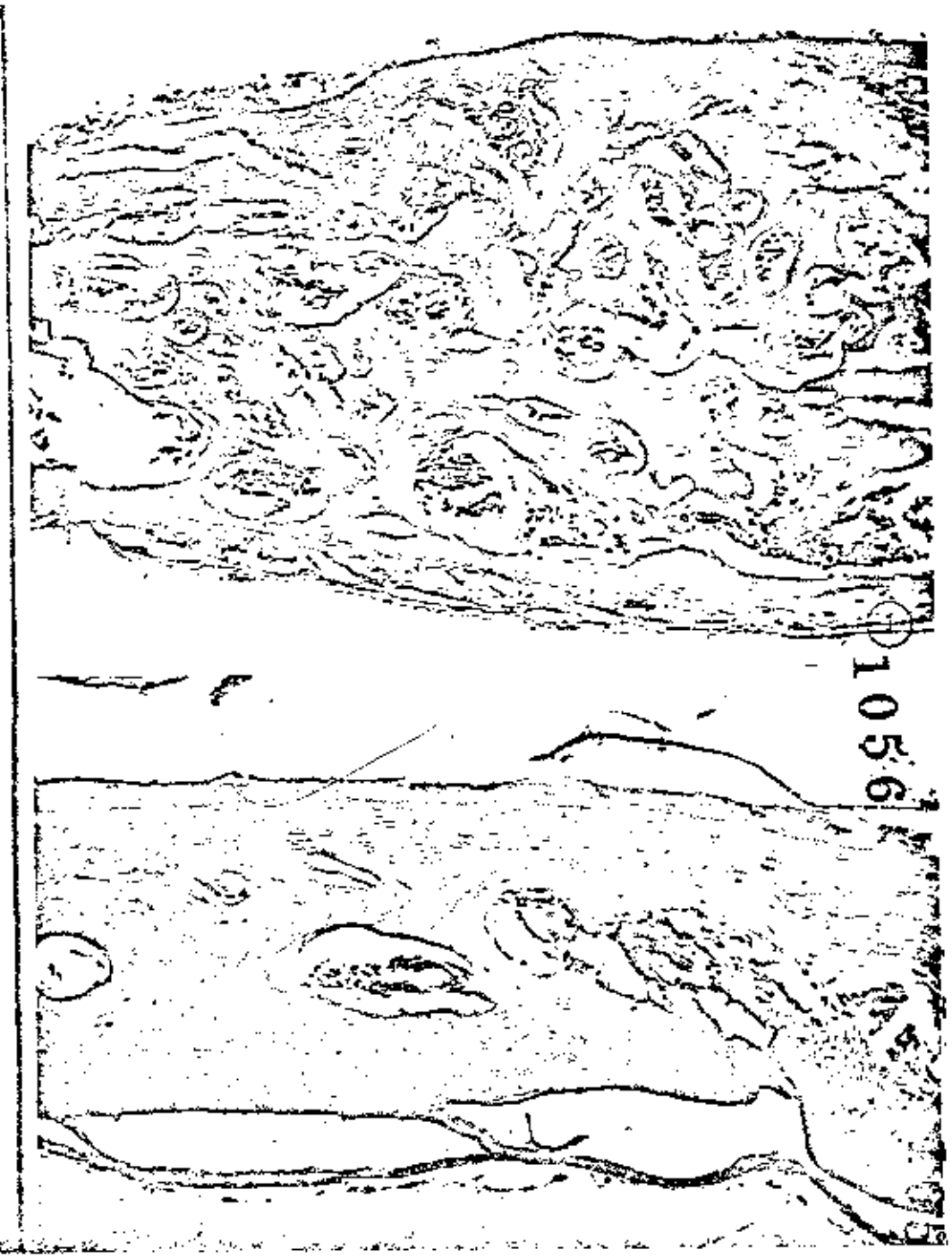

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PLATE 17

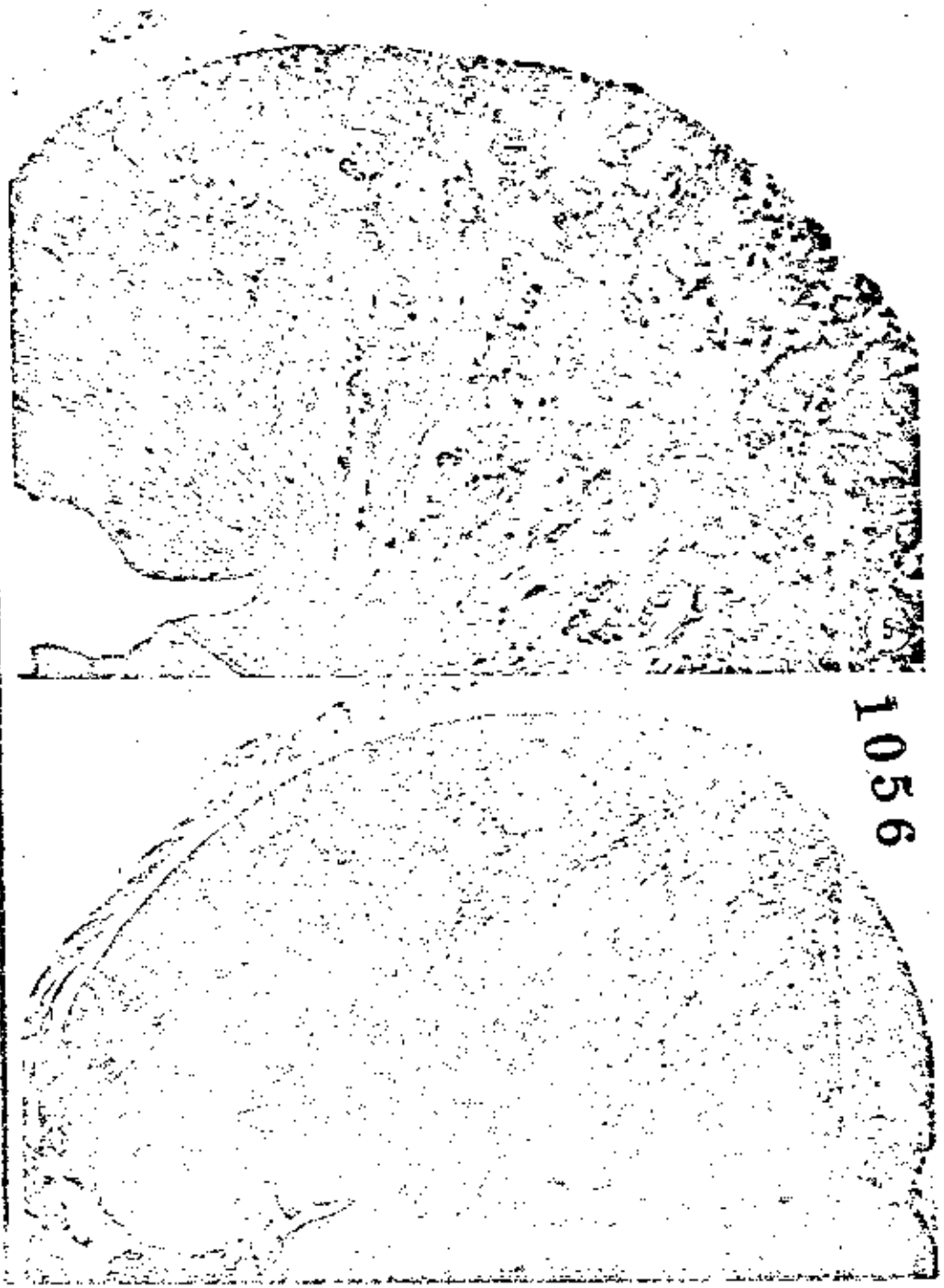
FIG. 3. Upper tibia, same treatment as Fig. 1. Hematoxylin and eosin. × 65. Longitudinal section. Note thin epiphyseal plate, masses of endosteal and subperiosteal osteoid tissue.

FIG. 4. Tibia, same treatment as Fig. 2. Hematoxylin and eosin. × 65. Longitudinal section. Note normal appearance of epiphyseal plate, lack of subperiosteal new bone and relatively little osteoid in medullary areas. Compare with Fig. 3.

PLATE 18

FIG. 5. Kidney, same treatment as Fig. 1. Alizarin red S. × 19. Sagittal section. Areas of calcification appear black. Note relative difference in the extent of cortical and medullary involvement.

FIG. 6. Kidney, same treatment as Fig. 2. Alizarin red S. × 19, sagittal section. No calcification is seen. Compare with Fig. 5.



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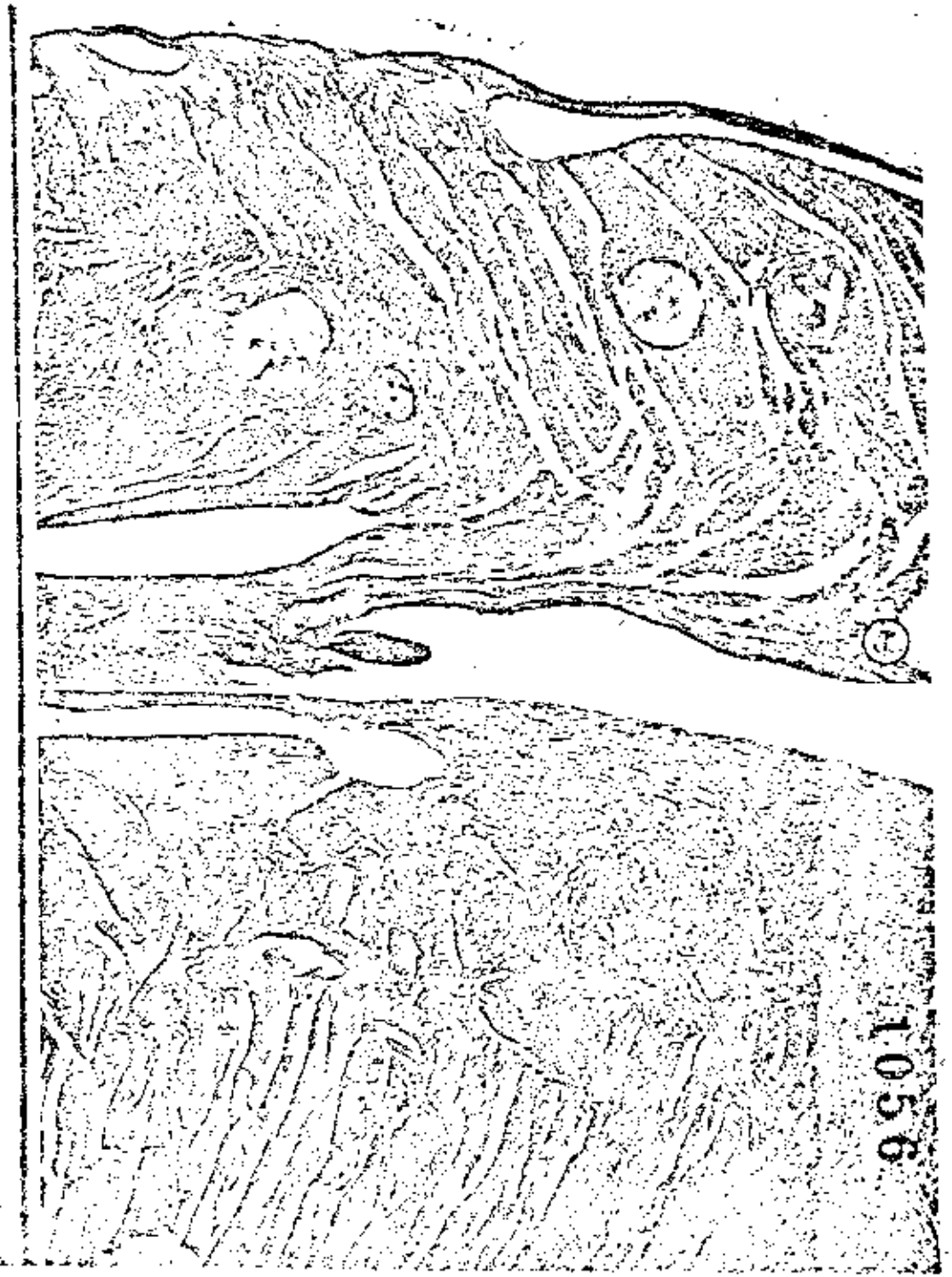

PLATE 19

FIG. 7. Heart, same treatment as Fig. 1. Hematoxylin and eosin. × 83. Note black areas in intimal regions of vessels.

FIG. 8. Heart, same treatment as Fig. 2. Hematoxylin and eosin. × 83. No evidence of calcification of vessels. Compare with Fig. 7.

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Ophthalmol. [illegible] 658, (1948)

[illegible]PERCALCEMIA AND BAND KERATOPATHY

Report of Nineteen Cases

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ABNORMALITIES in serum calcium values have only recently been recognized to have ocular manifestations. The first reported observation is that of Meesmann (1938).[1] In his study on hypocalcemia Meesmann included a case in which band-shaped opacities of the cornea and opacities of the conjunctiva developed while the blood calcium was high. The cause of the hypercalcemia was overdosage with AT 10® (dihydrotachysterol), which the patient had been taking for hypoparathyroidism following thyroidectomy. The patient also had glaucoma, and Meesmann stated the belief that the primary cause of the opacities was glaucomatous dystrophy of Bowman's membrane, but he suggested that the hypercalcemia might have been a contributing cause and pointed out significantly that the opacities became less as the blood calcium was reduced to normal. Haldimann (1941)[2] described band-shaped opacities of the cornea in 2 patients with sarcoid and hypercalcemia. In view of the fact that the opacities developed in 1 patient before other evidence of sarcoidosis of the eye and in the other patient without ocular sarcoidosis at any time, Haldimann suggested that the hypercalcemia alone might be the responsible factor.[3] Walsh and Howard (1947)[4] reported a series of 16 cases, including a preliminary report of 7 of our series, in which hypercalcemia from a variety of causes was associated with opacities of the cornea and conjunctiva in otherwise normal eyes. These authors pointed out

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3. The systemic aspects presented by these 2 patients have been discussed elsewhere (Schupbach, A., and Wernly, M.: Hyperkalcämie und Organverkalkungen bei Boeckschen Krankheit, Acta. Med. Scandinav. **115**:401, 1943).

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that conjunctival opacities also occur characteristically in [illegible]lation with hypercalcemia but that there was no correlation of the blood calcium level with either the corneal or the conjunctival opacities. Frost, Sunderman and Leopold[5] reported an additional case of calcific deposits in the cornea and conjunctiva resulting from prolonged overdosage with vitamin D. It is the purpose of the present report to describe our 19 cases (including the 7 previously incorporated in the report of Walsh and Howard[4]). This will make a total of 32 cases which have been reported to date. The importance of the subject is not great from the point of view of local functional disturbances produced by the opacities, since these rarely encroach on the pupillary area, but the findings do have correlative value in the diagnosis of systemic disturbances of calcium metabolism and may indicate what is taking place in the kidneys. Moreover, the fact that in hypercalcemia, calcium precipitates in the eye and has a characteristic distribution in the eye has interesting physiologic implications.

The ocular findings to be reported consist of subconjunctival opacities, variously described as white flecks or as glasslike crystals, and corneal changes, consisting of a diffuse superficial opacity in the palpebral fissure with a limbal margin that is usually abrupt and concentric with the limbus but is separated from it by a clear interval. The axial margin, on the other hand, fades away gradually a few millimeters centralward. The corneal changes are those generally called band keratitis, but, for obvious reasons, are more properly designated as band keratopathy. While the conjunctival opacities might be confused with calcification of the conjunctiva which occurs commonly without definite local or systemic disease, the band opacities in the cornea can be readily distinguished from the white limbal girdle and other opacities which occur "normally."

REPORT OF CASES

The following cases are grouped according to the etiologic factors in hypercalcemia.

HYPERPARATHYROIDISM

CASE 1.—M. C., a housewife aged 41, who complained of multiple tumors and fractures of bones of three years' duration, was referred to the hospital with the diagnosis of hyperparathyroidism. Because of increased serum calcium, low serum inorganic phosphorus and characteristic changes in bone, she had undergone four operations, with removal of parathyroid tissue each time. Nevertheless, her symptoms continued, and she sustained her last fracture while en route to the hospital! There were no ocular symptoms. Roentgenograms showed a staghorn renal calculus on the right, generalized skeletal decalcification, bone cysts, absence of the lamina dura and pathologic fractures.

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...ory analyses showed the following serum values: calcium, 15.4 mg.; inorga... phosphorus, 3.6 mg.; alkaline phosphatase, 20.6 to 26.5 Bodansky units; nonprotein nitrogen, 37 mg., and total protein, 6.1 Gm., per hundred cubic centimeters, with an albumin-globulin ratio of 2.6; carbon dioxide content, 22.5 to 24.4 milliequivalents. Urinalysis showed a negative to 3 plus reaction for albumin, 3 to 15 leukocytes and 0 to 40 red blood cells per cubic millimeter, abundant Escherichia coli, ability to concentrate to 1.016, 15 per cent excretion of phenolsulfonphthalein in fifteen minutes and excretion of 359 to 445 mg. of calcium in twenty-four hours.

Examination of the eyes showed a superficial corneal opacity concentric with the limbus in the palpebral fissure. It was separated from the limbus by a clear interval and extended axialward, to fade out approximately 3 mm. from the limbus. Examination of the conjunctivas was inadequate, since the patient could not be studied with the slit lamp biomicroscope, but there was a reflex from the surface of the conjunctiva resembling that from massed fish eggs.

At a fifth operation, a carcinoma of the parathyroid with metastases to the lymph nodes was partially removed.

Case 2.—P. G., a housewife aged 59, complained of generalized weakness, dyspnea, polyuria, polydipsia and swelling of the ankles. There were no ocular symptoms. Roentgenograms showed a ureteral stone, a duodenal ulcer and normal bony texture of the ribs and spine. Laboratory analyses showed the following serum values: calcium, 11.8 to 12.7 mg.; inorganic phosphorus 2.4 to 3.1 mg.; alkaline phosphatase, 1.6 Bodansky units, and nonprotein nitrogen, 23 mg., per hundred cubic centimeters; carbon dioxide content 23.8 to 28.1 volumes per cent. Urinalysis showed no albumin, an occasional leukocyte, inability to concentrate beyond 1.014 and 15 per cent excretion of phenolsulfonphthalein in fifteen minutes.

Examination of the eyes showed, in addition to unusually thin scleras, a superficial opacity on the nasal side of the cornea in the palpebral fissure, extending approximately 3 mm. from the limbus and separated from it by a thin, clear interval.

Adenoma of the parathyroid gland was removed at operation.

Case 3.—X. T., a farmer aged 62, complained of polyuria, polydipsia and painless swelling of the left side of the maxilla. There were no ocular symptoms. Roentgenograms showed nephrocalcinosis, generalized skeletal decalcification and several bone cysts. Laboratory analyses showed the following serum values: calcium, 15.2 to 17.3 mg.; inorganic phosphorus, 2.4 to 3.7 mg.; alkaline phosphatase, 8 to 14 Bodansky units; nonprotein nitrogen, 31 to 45 mg., and total protein, 5.9 to 7.7 Gm., per hundred cubic centimeters; carbon dioxide content, 16.8 to 27.9 milliequivalents. The urine showed a negative to a 3 plus reaction for albumin, numerous leukocytes, abundant Escherichia coli, inability to concentrate beyond 1.015, no excretion of phenolsulfonphthalein in fifteen minutes and excretion of 560 mg. of calcium in twenty-four hours.

The eyes were examined for the first time ten weeks after the operation. At that time there were numerous white opacities in the superficial conjunctivas, most of which were less than 1 mm. in size and resembled a flocculent precipitate. They could be seen only with a slit lamp. There was no band keratopathy, and the eyes were otherwise normal.

Biopsy of the tumor of the maxilla revealed epulis. At a second operation an adenoma of the parathyroid was removed.

Case 4.—E. K., a masseur aged 51, complained of tightness, stiffness and pain in the left calf. There were no ocular symptoms. A diagnosis of arteriosclerosis obliterans was made.

Twelve years previously he had been admitted with a diagno... hyperparathyroidism. Roentgenograms showed nephrocalcinosis and skeletal decalcification with bone cysts, two of which showed pathologic fractures. Laboratory analyses showed the following serum values: calcium, 16.8 mg.; inorganic phosphorus, 2.4 mg.; nonprotein nitrogen 32 mg., and total protein 5.7 Gm., per hundred cubic centimeters. Urinalysis showed 1 to 3 plus reaction for albumin, many leukocytes, an occasional red blood cell, Esch. coli, inability to concentrate beyond 1.018 and 5 to 15 per cent excretion of phenolsulfonphthalein in thirty minutes. An adenoma of the parathyroid was removed. The eyes were not examined at that time.

At the last admission laboratory analyses showed the following serum values: calcium, 11 mg.; inorganic phosphorus, 3.9 mg.; alkaline phosphatase, 3.9 Bodansky units; nonprotein nitrogen, 29 mg.; total protein, 6.7 Gm., per hundred cubic centimeters, with an albumin-globulin ratio of 2; carbon dioxide content, 29.7 milliequivalents. The urine showed no albumin, 3 to 20 leukocytes per cubic millimeter and Esch. coli.

Table 1.—*Data on Patients with Hyperparathyroidism*

			Laboratory Data, Mg. or Units/100 Cc.				Urinary Calcium				
Sex	Age	Present Complaint	Calcium	Phosphorus	Phosphatase	Nonprotein Nitrogen	Amount, Mg./24 hr.	Stone	Roentgenographic Findings	Duodenal Ulcer	Comment
F	41	Multiple fractures	15.4	3.6	20.6-26.5	37	359-445	+	Marked decalcification; bone cysts; fractures	—	Carcinoma of parathyroid
F	59	Weakness and polyuria	11.8-12.7	2.4-3.1	1.6	23	.....	+	No decalcification (ribs and spine)	+	Adenoma of parathyroid
M	62	Epulis	15.2-17.3	2.4-3.7	8-14	31-45	560	+	Marked decalcification; epulis	..	Adenoma of parathyroid
M	39	Multiple fractures	16.8	2.4	4.6-16.5	32	.....	+	Marked decalcification; bone cysts	—	Adenoma of parathyroid

* Data in table are those obtained prior to removal of the adenoma of the parathyroid, that is, twelve years prior to the present examination.

Examination of the eyes showed an arcuate gray opacity at the nasal periphery of the cornea. This was superficial, was concentric with the limbus, from which it was separated by a clear interval, and faded out gradually 1 to 2 mm. from the limbus. The changes in the eyes were slight but otherwise typical of band keratopathy. There was only one opacity of the conjunctiva, over the region of the external rectus muscle.

Of this series of 4 cases in which the cause of the hypercalcemia was hyperparathyroidism, band keratopathy with conjunctival deposits was present in 2, band keratopathy alone in 1 and conjunctival deposits alone in 1. One of the patients had carcinoma of the parathyroid with extension to the lymph nodes. The other patients had adenomas of the parathyroid, removed at operation.

The pertinent data in these 4 cases are summarized in table 1. From the diagnostic point of view the important findings were as follows: increased serum calcium, depressed or normal serum

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inor phosphorus, elevation of phosphatase and characteristic decalcification of the bones, with cyst formation in 3 of the cases. But most interesting is the occurrence in all of either nephrocalcinosis or nephrolithiasis, for, as will be discussed subsequently in consideration of the pathogenesis of band keratopathy, the mechanism by which calcification occurs in the cornea may be similar to that by which it occurs in the renal tubules; indeed, one of the most important practical consequences of band keratopathy may be its implications regarding the presence of similar changes in the kidneys. Also noted in table 1 is the occurrence in some of the patients of duodenal ulcer, for, as will be indicated in some types of hypercalcemia, the coexistence of ulcer with the high calcium, high alkali diet appears to be of significance.

VITAMIN D POISONING

Case 5.—F. A., an engineer aged 27, complained of polydipsia and polyuria. For six years he had undergone extensive irradiation for generalized Hodgkin's disease. For four years he had taken 500,000 units of vitamin D a week for pruritus. The only ocular complaints were slight photophobia and burning of the eyes. Roentgenograms showed areas of increased density in the skull, spine and pelvis and miliary tuberculosis of the lungs. Laboratory analyses revealed the following serum values: calcium, 12.6 mg.; inorganic phosphorus, 4.3 mg.; alkaline phosphatase, 1.9 Bodansky units; nonprotein nitrogen, 52 mg.; total protein, 5.6 Gm. per hundred cubic centimeters, with an albumin-globulin ratio of 1.5; carbon dioxide content, 30.5 milliequivalents. The urine showed a negative to 1 plus reaction for albumin, 5 to 40 leukocytes and 0 to 4 red blood cells per cubic millimeter, cocci, ability to concentrate to 1.016, 15 per cent excretion of phenolsulfonphthalein in thirty minutes and excretion of 1,290 mg. of calcium in twenty-four hours.

Examination of the eyes showed a typical band keratopathy, symmetric in the two eyes, which extended approximately 3 mm. from the limbus on both the nasal and the temporal side. It was visible to the unaided eye.

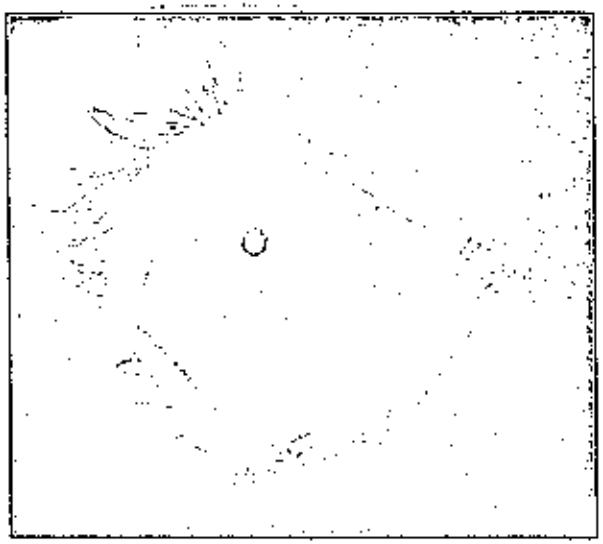
The patient died, and autopsy revealed nephrocalcinosis, miliary tuberculosis and Hodgkin's lymphoma.

Case 6.—E. B., a housewife aged 39, complained of abdominal pain, nausea, vomiting and swelling of the joints. For five years she had taken 100,000 units of vitamin D daily for rheumatoid arthritis. Roentgenograms showed a duodenal ulcer, extensive decalcification of the bones about the joints, periarticular calcification of soft tissues and no excretion of radiopaque dye in one hundred and five minutes. Laboratory analyses showed the following serum values: calcium, 10.2 to 14.4 mg.; inorganic phosphorus, 4.8 to 6.3 mg.; alkaline phosphatase, 3.3 to 7.4 Bodansky units; nonprotein nitrogen, 50 to 70 mg.; total protein, 7.0 Gm., per hundred cubic centimeters, with an albumin-globulin ratio of 1.15; carbon dioxide content, 21.4 to 24.9 milliequivalents. The urine showed a 4 plus reaction for albumin, 2 to 15 leukocytes per cubic millimeter, no organisms, inability to concentrate above 1.016, no excretion of phenolsulfonphthalein in fifteen minutes and excretion of 110 mg. of calcium in twenty-four hours.

Examination of the eyes showed superficial corneal opacities concentric with, but separated from, the limbus in the palpebral fissures, extending axialward about 3 mm. The conjunctivas also showed numerous fine opacities (presumably calcific) beneath the epithelium.

Case 7.—A. M., a woman aged 70, complained of nausea, vomi arrhea and depression. From eight to two months prior to admission she d taken 150,000 units of vitamin D daily for rheumatoid arthritis, from which she had suffered for fifteen years. She had consumed large amounts of milk. There were no ocular symptoms. Laboratory analyses revealed the following serum values: calcium, 11.7 to 13.9 mg.; inorganic phosphorus, 4.3 to 5.3 mg.; alkaline phosphatase, 4.0 Bodansky units; nonprotein nitrogen, 41 to 66 mg.; total protein, 7.5 to 8.2 Gm., per hundred cubic centimeters, with an albumin-globulin ratio of 1.1; carbon dioxide content, 21.4 milliequivalents. The urine showed a 1 to 3 plus reaction for albumin, 0 to many leukocytes and 0 to 30 red blood cells per cubic millimeter, no organisms, ability to concentrate to 1.017, 1 per cent excretion of phenolsulfonphthalein in fifteen minutes and excretion of 160 mg. of calcium in twenty-four hours.

Examination of the eyes without the aid of a slit-lamp showed a definite band type of opacity in the palpebral fissure extending to 2 or 3 mm. from the limbus. No abnormality of the conjunctiva was to be seen grossly.



Photograph of the right eye of the patient in case 8. The paralimbal opacities can be seen as densest in the palpebral fissure and are characterized by an abrupt peripheral margin, bordering on a clear zone which separates it from the limbus and an axial margin fading out centrally 2 to 3 mm. from the limbus.

Case 8.—T. C., a postal clerk aged 65, complained of vomiting and nocturia. For the past two to three years he had taken up to 300,000 units of vitamin D daily for rheumatoid arthritis. He consumed large quantities of milk. Recently several painless lumps had appeared in the skin of the neck, hands and wrists. The only ocular complaint was a mild foreign body sensation. Roentgenograms showed dense bones; calcification of soft tissues about the elbows, wrists and back, and rheumatoid arthritis. Laboratory analyses showed the following serum values: calcium, 11.6 to 15.2 mg.; inorganic phosphorus, 4.3 to 5.2 mg.; alkaline phosphatase, 4.4 Bodansky units; nonprotein nitrogen, 45 mg.; total protein, 7.32 Gm., per hundred cubic centimeters, with an albumin-globulin ratio of 1.25. The urine showed a negative to a 3 plus reaction for albumin, 0 to 30 leukocytes and 0 to 3 red blood cells per cubic millimeter, Staphylococcus albus, inability

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to concentrate above 1.012, 5 per cent excretion of phenolsulfonphthalein in fifteen minutes and excretion of 445 mg. of calcium in twenty-four hours.

Examination of the eyes showed a typical band type of superficial opacities in the palpebral fissure of both corneas, the band being separated from the limbus by a narrow, clear interval and extending axialward 3 to 4 mm. (figure). While the opacities were densest between 8 and 10 o'clock and 5 and 2 o'clock, some of the opacity extended down to 6 o'clock but none up to 12 o'clock. The peripheral portions of the opacity had many clear lacunas, presumably in areas corresponding to portions of the opacity which had sloughed off. The conjunctivas showed a follicular reflex from their surface temporal to the limbus, and with the biomicroscope there were several subepithelial crystalloid opacities.

Case 9.—P. A., a woman aged 55, complained of "lumps" about various joints. There were no ocular symptoms. From four to one and a half months prior

Table 2.—*Data on Patients with Vitamin D Poisoning*

Patient No., Sex, Age	Present Complaint	Laboratory Data, Mg. or Units/100 Cc.				Urinary Calcium		Roentgenographic Findings	Duodenal Ulcer	Dose of Vitamin D, Units/Day	Comment
		Calcium	Phosphorus	Phosphatase	Nonprotein Nitrogen	Amount, Mg./24 Hr.	Stone				
5 M 27	Polydipsia; polyuria; pruritus	13.8	4.3	1.9	52	1,290	+	Increased density of spine, pelvis and skull	..	600,000 for 4 yr.	Tuberculosis; Hodgkin's disease
6 F 39	Epigastric pain	10.2-14.4	4.3-6.1	3.2	36-70	...	—	Metastatic calcification	+	100,000 for 5 yr.	Rheumatoid arthritis
7 F 70	Nausea; diarrhea; depression	11.7-13.9	4.3-5.3	4.0	80	100	—	Slight decalcification	..	150,000 for 6 mo.	Rheumatoid arthritis
8 M 63	Vomiting; nocturia	11.6-15.2	4.8-5.3	4.4	45	445	..	Excessive calcification in bone; metastatic calcification in skin	..	300,000 for 2-3 yr.	Rheumatoid arthritis
9 F 54	"Lumps" in soft tissues	13.4	5.7	5.9	62	125	—	Metastatic calcification in soft tissues	—	300,000 for 2½ mo.	Rheumatoid arthritis

to admission she had taken 300,000 units of vitamin D daily for rheumatoid arthritis, from which she had suffered for fourteen years. She had drunk 1 to 2 quarts (1 to 2 liters) of milk daily for several years. Painless lumps had appeared over one shoulder, both wrists and several fingers soon after she had started the vitamin D therapy. Roentgenograms showed normal bony texture; calcification of soft tissues about the hands, shoulders, back and knees, and rheumatoid arthritis. Laboratory analyses showed the following serum values: calcium, 13.4 mg.; inorganic phosphorus, 5.7 mg.; alkaline phosphatase, 5.9 Bodansky units; nonprotein nitrogen 62 mg.; total protein, 6.24 Gm., per hundred cubic centimeters, with an albumin-globulin ratio of 2.22; carbon dioxide content, 26.6 milliequivalents. The urine showed a 2 plus reaction for albumin, 0 to 3 leukocytes and 0 to 12 red blood cells per cubic millimeter, inability to concentrate above 1.010, less than 5 per cent excretion of phenolsulfonphthalein in fifteen minutes and excretion of 125 mg. of calcium in twenty-four hours.

Examination of the eyes showed bilateral and symmetric opacification of the nasal and temporal portions of the paralimbal cornea in the palpebral fissure.

These opacities were superficial, were separated from the limbus by a relatively clear zone and did not extend more than 2 to 3 mm. axialward.

In this series of 5 patients with hypercalcemia and band keratopathy, 4 had taken excessive vitamin D on account of rheumatoid arthritis and 1 had taken it for pruritus. The dose of vitamin D varied from 100,000 to 500,000 units daily, and the time over which the vitamin was taken varied from two and a half months to five years. All the patients had renal insufficiency, as indicated by the nonprotein nitrogen and inorganic phosphorus levels of the blood, but it is noteworthy that the diagnosis of nephrocalcinosis was not evident roentgenographically, but was made only at autopsy on the 1 patient who died. It is entirely possible, therefore, that nephrocalcinosis was present in the other patients.

In contrast to the series of patients with hyperparathyroidism, those with excessive vitamin D intake showed no characteristic decalcification of the bones and no significant elevation in the serum phosphatase level.

SARCOID

Case 10.—F. L., an engineer aged 25, complained of shortness of breath, loss of weight and productive cough. The only ocular symptoms were mild redness of the eyes and a feeling of dryness for the preceding several weeks. The patient's occupation had entailed exposure to beryllium silicate powder. Roentgenograms showed sarcoidosis of the lungs and increased density of the spine. Laboratory analyses showed the following serum values: calcium, 11.3 to 15.6 mg.; inorganic phosphorus, 3.0 to 3.6 mg.; alkaline phosphatase, 6.7 to 8.4 Bodansky units; nonprotein nitrogen, 26 mg., and total protein, 6.7 to 7.1 Gm., per hundred cubic centimeters. The urine showed a negative to a 1 plus reaction for albumin, 0 to 3 leukocytes per cubic millimeter, ability to concentrate to 1.020 and 40 per cent excretion of phenolsulfonphthalein in fifteen minutes.

Examination of the eyes showed slight ciliary and conjunctival redness. The corneas showed grossly an arcuate opacity of the cornea concentric with the limbus but separated from it by approximately 1 mm. The opacity was predominantly in the palpebral fissure but involved the lower portion of the cornea as well. With the slit lamp this opacity appeared entirely superficial and faded out axialward approximately 3 mm. from the limbus. On the scleral side of the limbus numerous superficial white lines radiated from the limbus but did not extend more than 1 to 2 mm.

Case 11.—A. M., a housewife aged 36, complained of shortness of breath and cachexia. There were no ocular symptoms. Roentgenograms showed sarcoidosis of the lungs, renal stones, slight skeletal decalcifications and a fusiform area of calcification in the left buttock. Laboratory analyses showed the following serum values: calcium, 13.0 to 15.5 mg.; inorganic phosphorus, 2.9 to 3.8 Bodansky units; nonprotein nitrogen 26.5 to 75 mg.; total protein, 8.7 to 9.5 Gm., per hundred cubic centimeters, with an albumin-globulin ratio of 0.7; carbon dioxide content, 28.9 milliequivalents. The urine showed a 2 to 3 plus reaction for albumin, 5 to many leukocytes and 1 to 10 red blood cells per cubic millimeter, no organisms, ability to concentrate to 1.020, 10 per cent excretion of phenolsulfonphthalein in fifteen minutes and excretion of 433 mg. of calcium in twenty-four hours.

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amination of the eyes showed band keratopathy in both eyes, more pronounced on the nasal than on the temporal side. In addition to the diffuse granular opacities, the band contained several tiny opaque loops, simulating the configuration produced by obliterated vessels.

Biopsy of a lymph node, removed at operation, showed Boeck's sarcoid.

The patient returned seven years later. Laboratory analyses of the serum revealed calcium, 10.0 mg; inorganic phosphorus, 3.0 mg., and nonprotein nitrogen, 24 mg., per hundred cubic centimeters.

Examination of the eyes showed considerable reduction in the band keratopathy, with practically no residuum nasally and only a 1 mm. band temporally. There were, however, numerous subepithelial opacities of the conjunctivas adjacent to the temporal limbus.

The association of sarcoid with beryllium poisoning has been noted several times in the literature, and the first of these 2 cases has been previously included in a series illustrating this association.[6]

TABLE 3.—*Data on Patients with Sarcoidosis*

Patient No., Sex, Age	Present Complaint	Laboratory Data, Mg. or Units/100 Cc.: Calcium	Phosphorus	Phosphatase	Nonprotein Nitrogen	Protein	A/G	Urinary Calcium: Amount, Mg./24 Hr.	Stone	Roentgenographic Findings	Duodenal Ulcer	Comment
10 M 25	Shortness of breath and loss of weight	11.8-15.6	3.0-3.6	6.7-8.4	26	6.7-7.1	...	...	—	Increased density of spine	—	Sarcoidosis secondary to beryl. poisoning
11 F 36	Cachexia	13.0-15.5	2.9-3.8	2.9-8.7	26.5-75	8.7-9.6	0.7	432	+	Mild decalcification	—	Metastatic calcifications in both eyes

One patient in this group showed impairment of renal function, as judged by the elevation of the nonprotein nitrogen and inorganic phosphorus levels of the blood, and at least 1 had nephrolithiasis. The changes in the kidneys and eyes, therefore, are similar to those resulting from excessive vitamin D intake.

UREMIA

CASE 12.—C. M., a clerk aged 49, complained of epigastric pain and shortness of breath. Five years previously he had had a sympathectomy for Buerger's disease (thromboangiitis obliterans). For twenty-five years he had taken large amounts of milk and alkali for relief of a duodenal ulcer. Roentgenograms showed a duodenal ulcer and normal bony texture. Laboratory analyses showed the following serum values: calcium, 11.4 to 12.8 mg.; inorganic phosphorus, 3.5 to 6.6 mg.; alkaline phosphatase, 2.2 to 3.1 Bodansky units; nonprotein nitrogen, 53 to 95 mg; total protein 6.81 Gm., per hundred cubic centimeters, with an

6. Hardy, H. L.: New Clinical Syndrome: Delayed Chemical Pneumonitis Occurring in Workers Exposed to Beryllium Compounds, Bull. New England M. Center 9:16-24, 1947.

albumin-globulin ratio of 1.8; carbon dioxide content, 35.3 milli... ents. The urine showed a 2 plus reaction for albumin, a few leukocytes, rare red blood cells, hyaline casts, inability to concentrate beyond 1.012, 7 per cent excretion of phenolsulfonphthalein in fifteen minutes and excretion of 109 mg. of calcium in twenty-four hours.

Examination of the eyes showed superficial opacities on the nasal and temporal sides of both corneas, of symmetric distribution in the two eyes. The opacity was chalk white and was densest toward the limbus, where it was slightly elevated and contained clear lacunas. It was separated from the limbus by a narrow, clear zone and faded off gradually axialward about 3 mm. from the limbus. It was more pronounced nasally than temporally. There were a few crystalloid, superficial opacities in the perilimbal conjunctiva and a follicular reflex from the conjunctiva in the palpebral fissure resembling that from massed fish eggs. Examination sixteen months later, showed that the ocular condition was unchanged.

CASE 13.—A. C., a student aged 25, complained of protracted vomiting and emaciation. He had noted mild burning of the eyes. Roentgenograms showed periosteal formation of new bone; metastatic calcification in the tentorium, falx and bronchi, and small kidneys. There was no evidence of duodenal ulcer. Laboratory analyses showed the following serum values: calcium, 11.2 to 12.5 mg.; inorganic phosphorus, 4.4 to 4.8 mg.; alkaline phosphatase, 1.1 to 6.8 Bodansky units; nonprotein nitrogen 40 to 85 mg.; total protein 7.2 Gm., per hundred cubic centimeters; carbon dioxide-combining power 24.1 to 30.2 milliequivalents. The urine showed a 2 to 3 plus reaction for albumin, 0 to 6 leukocytes per cubic millimeter, inability to concentrate beyond 1.012 and 1 per cent excretion of phenolsulfonphthalein in twenty-four hours.

Examination of the eyes showed an abortive band type of corneal opacity concentric with the temporal limbus in the palpebral fissure. The adjacent conjunctiva was somewhat elevated and red and contained several subepithelial opacities.

The patient died three years later, without presenting further evidence of the fundamental cause of his disease.

CASE 14.—V. Z., a housewife aged 23, complained of anorexia, nausea, vomiting, polydipsia and polyuria. She had avoided use of milk and alkalis. There were no ocular symptoms. The blood pressure was 190 systolic and 100 diastolic. Roentgenograms showed two calculi in the left ureter, slight decalcification of the spine, a normal lamina dura and poor renal excretion of radiopaque dye on the right and none on the left. Laboratory analyses showed the following serum values: calcium 13.2 to 13.4 mg.; inorganic phosphorus, 3.8 to 4.6 mg.; alkaline phosphatase, 1.8 to 2.6 Bodansky units, and nonprotein nitrogen, 40 to 45 mg., per hundred cubic centimeters; carbon dioxide content, 30 milliequivalents. The urine showed no albumin, a few leukocytes and red blood cells, a few alpha hemolytic streptococci, inability to concentrate above 1.012, excretion of 15 per cent of phenolsulfonphthalein in fifteen minutes and excretion of 187 to 413 mg. of calcium in twenty-four hours.

Examination of the eyes showed narrow bands of a superficial white opacity of irregular lacelike character situated on both the nasal and the temporal portion of the cornea of both eyes in the palpebral fissure. There were also many superficial irregular white opacities in the conjunctiva beneath the epithelium, but overlying the blood vessels.

Biopsy of two parathyroid glands showed secondary hyperplasia.

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CASE 15.—A. M., a gardner aged 45, complained of weakness, nocturia and polydipsia. He had taken large amounts of milk and alkali for relief of a duodenal ulcer, from which he had suffered for many years. Five years previously he had passed renal calculi. For the past three years he had noted mild redness of the eyes. Roentgenograms showed renal stones, a duodenal ulcer and normal bony texture. Laboratory analyses showed the following serum values: calcium, 14.6 to 7.7 mg.; inorganic phosphorus, 4.0 to 9.4 mg.: alkaline phosphatase, 1.7 to 5.3 Bodansky units; nonprotein nitrogen 58 to 83 mg.; total protein 9.1 to 9.8 Gm., per hundred cubic centimeters, with an albumin-globulin ratio of 1.8; carbon dioxide content, 27.4 to 31.1 milliequivalents. The urine showed 2 to 40 leukocytes and 0 to 3 red blood cells per cubic millimeter, no organisms, inability to concentrate beyond 1.010, and 5 per cent excretion of phenolsulfonphthalein in fifteen minutes.

Examination of the eyes showed symmetric changes in the palpebral fissures in the corneas, consisting of slightly elevated white opacities concentric with the limbus, from which they were separated only by relative thinning, and extending axialward 2 to 3 mm., where they became nonelevated and apparently subepithelial. The opacities were situated on both the nasal and the temporal side of the corneas and were thought to be typical of band keratopathy. There were also yellowish and irregular plaques, measuring 4 to 5 mm. in diameter, situated, one each, in the nasal and temporal episcleras of both eyes. An attempt was made to remove one of these plaques for biopsy, but it was too firmly attached to the sclera and was left in situ.

The patient was kept under observation until he died in uremia six years later, during which time no evident change took place in his eyes.

Biopsy of two parathyroid glands, removed at operation, revealed a normal structure.

Autopsy showed nephrocalcinosis and calcification of the dura mater, respiratory diaphragm, costal cartilages, tendons and blood vessels.

CASE 16.—E. F., a stenographer aged 48, complained of pruritus and weakness. There were no ocular symptoms. Roentgenograms showed skeletal decalcification with bone cysts, osteoarthritis, a calcified deposit near the right elbow and contracted kidneys. Laboratory analyses showed the following serum values: calcium 8.8 mg.; inorganic phosphorus, 8.9 to 10.1 mg.; nonprotein nitrogen 140 to 155 mg.; total protein, 6.6 to 6.7 Gm., per hundred cubic centimeters, with an albumin-globulin ratio of 1.35; carbon dioxide content, 21.7 milliequivalents. The urine showed a 1 to 2 plus reaction for albumin, few leukocytes and red blood cells, no organisms, inability to concentrate beyond 1.012 and 5 per cent excretion of phenolsulfonphthalein in fifteen minutes.

Examination of the eyes showed a superficial opacity of each cornea on the nasal and temporal sides in the palpebral fissure, extending from the limbus approximately 2 mm. axialward, where it gradually faded out. No definite clear zone separated it from the limbus. Numerous tiny opacities also were situated just beneath the epithelium of the conjunctiva in the palpebral zone. These were placed superficial to the blood vessels and moved with blinking of the lids.

The patient died a few months later, without further examination.

CASE 17.—J. L., a fireman aged 52, complained of hematemesis. For many years he had taken large amounts of milk and alkali for relief of a duodenal ulcer.

Three years previously he had been admitted to the hospital with the complaint of a painful right eye. He stated he had had a "cataract operation" on the right eye as a child and that the vision had subsequently been poor. On this admission the following note was made: "Right eye was red, and practically the

entire cornea was infiltrated with calcium deposits. There was moderate ectasia. The chamber was deep. The pupil was small and immobile. The fundus reflex was absent. Tension was 3 plus. The left eye showed moderate but diffuse conjunctival redness and a marginal corneal opacity temporally and nasally from 2 to 5 o'clock and from 10 to 7 o'clock. The chamber, pupil and tension were normal." Because the glaucoma in the right eye was not relieved by miotics and the pain persisted, the eye was enucleated. Histologic examination of the cornea showed that the stroma and the posterior surface were not abnormal. Bowman's membrane was intact and appeared normal in its axial portion for an extent of 4 mm., but became increasingly calcified toward the periphery. At its limbal extremity the calcification of Bowman's membrane was densest and extended into the superficial stroma. Anterior to Bowman's membrane, and separated from it by a clear interval (an artefact), there was a new-formed membrane of lamellar stratification having a thickness about one-third that of the entire stroma. This new-formed membrane was composed for the most part of connective tissue with a variable amount of collagen and variable amount of hyalinization. At its periphery the membrane contained epithelium, which in the sections was not connected with the surface epithelium. In the anterior part of this new-formed membrane were calcific plaques, some of which were erupting through to the surface. These, like the calcification in Bowman's membrane, were more conspicuous toward the periphery of the cornea, where they could be seen extending through the whole thickness of the membrane. Also noteworthy were areas of calcification of the superficial half of the sclera in the region overlying the ciliary body. This was about 2 mm. in length on one side and involved one-half the thickness of the sclera and an area about 1 mm. in length on the other side and involved one-fourth the thickness of the sclera.

At the time of the present examination, roentgenograms revealed a duodenal ulcer, normal bony texture and no excretion of the radiopaque dye in forty-five minutes. Laboratory analyses showed the following serum values: calcium, 11.4 mg.; inorganic phosphorus, 4.7 mg.; alkaline phosphatase, 5.3 Bodansky units; nonprotein nitrogen, 80 to 105 mg.; total protein 6.8 Gm., per hundred cubic centimeters; carbon dioxide content, 21.8 milliequivalents. The urine showed a negative to a 1 plus reaction for albumin and inability to concentrate beyond 1.010.

Examination of the left eye showed a typical band-shaped opacity of the cornea in the palpebral fissure extending on the nasal and temporal sides from the limbal margin approximately 3 mm. axialward. The limbal edge of the opacity was irregularly scalloped and slightly raised, showing clear lacunas corresponding to portions of the opacity which had presumably been sloughed off. The opacity was entirely superficial. The conjunctiva showed grossly a follicular type of reflex in a 3 mm. zone temporal to the limbus and examination with the slit lamp biomicroscope revealed several subepithelial crystalloid opacities.

CASE 18.—E. M.,[7] a carpenter aged 55, complained of nocturia, pruritus and swelling of the legs. For twenty one months he had taken daily 4 to 5 quarts (4 to 5 liters) of milk and considerable alkali for relief of a duodenal ulcer. Physical examination showed movable subcutaneous nodules of the forearms. Roentgenograms showed calcification of the soft tissues of the extremities, and clubbing of the calyces was evident in retrograde pyelograms. Laboratory analysis showed the following serum values: calcium, 9.6 to 13.0 mg.; inorganic phosphorus, 3.7 to 5.9 mg.; alkaline phosphatase, 3.2 to 4.5 Bodansky units; nonprotein nitrogen,

7. Dr. Charles Burnett, of the Massachusetts Memorial Hospital, 65 East Newton Street, Boston 18, furnished the data on this patient.

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80 mg.; total protein, 6.8 to 8.2 Gm., per hundred cubic centimeters; carbon dioxide-combining power, 24.0 milliequivalents. The urine showed a 1 to 2 plus reaction for albumin, occasional leukocytes and red blood cells, inability to concentrate beyond 1.011, less than 1 per cent excretion of phenolsulfonphthalein in fifteen minutes and excretion of 78 mg. of calcium in twenty-four hours.

Examination of the eyes showed a superficial opacity of the cornea in the paralimbal region of the palpebral fissure. It was present on both the nasal

TABLE 4.—*Data on Patients with Uremia*

Patient No.	Sex	Age	Present Complaint	Laboratory Data, Mg. or Units/100 Cc.: Calcium	Phosphorus	Phosphatase	Nonprotein Nitrogen	Urinary Calcium: Amount, Mg./24 Hr.	Stone	Roentgenographic Findings	Duodenal Ulcer	Comment
12	M	49	Epigastric pain	11.4-12.8	3.5-6.0	2.2-3.1	28-35	109	..	No decalcification	+	Intermittent glandular [illegible] and milk in diet
13	M	25	Vomiting 2½ yr.	11.2-12.5	4.4-4.8	1.1-6.8	40-86	.....	..	Calcification in bronchi and ureters; periostical new bone	—	Patient [illegible] hyperpituitarism [illegible]
14	F	23	Vomiting; anorexia; polydipsia; polyuria	12.2-13.4	3.8-4.6	1.8-2.8	40-45	187-418	+	Mild decalcification	—	Two operations on parathyroid region showed secondary hyperplasia only
15	M	43	Weakness; nocturia; polydipsia	14.0-7.7	4.0-9.4	1.7-6.3	58-171	.....	+	No decalcification	+	Much milk and milk in diet; no parathyroid adenoma found [illegible] gically [illegible] post [illegible]
16	F	48	Pruritus; weakness	8.8	8.9-10.1	.....	140-155	.....	—	Marked decalcification	..	Patient [illegible] a few [illegible] later
17	M	52	Hematemesis	8.3-11.4	3.9-4.7	4.4-5.3	65-109	.....	—	No decalcification	+	Much milk and milk in diet
18	M	56	Nocturia; edema	9.6-13.0	3.7-5.9	2.2-4.5	80-100	78	—	Metastatic calcification in soft tissues	+	Much milk and milk in diet
19	M	75	Urinary frequency	11.6-13.4	4.1-5.4	11.7-14.1	35-27	250-495	—	Ostitis deformans (Paget's disease)	..	Much milk in diet; hyperplasia thyroid [illegible] not exc[illegible]

and the temporal side, was about 4 mm. in length, was separated from the limbus by a narrow, clear interval and extended axialward about 2 mm. There were also scattered translucent bodies superficial in the conjunctiva extending 6 to 7 mm. temporal to the limbus and a few similar bodies extending nasalward.

CASE 19.—B. S., a fireman aged 75, complained of urinary frequency. For over a year he had taken daily 3 capsules containing a white powder. For several months he had taken 2 quarts (2 liters) of milk a day. There were no ocular symptoms. Roentgenograms showed generalized Paget's disease (ostitis deformans) and good renal excretion of radiopaque dye. Laboratory analyses showed the following serum values: calcium, 11.5 to 13.4 mg.; inorganic phosphorus, 4.1 to 5.4 mg.; alkaline phosphatase, 11.7 to 14.1 Bodansky units; nonprotein nitrogen, 35 to 57 mg.; total protein, 6.1 Gm., per hundred cubic centimeters; carbon dioxide content, 30.6 milliequivalents. The urine showed a negative to a 2 plus reaction for albumin, 0 to 8 leukocytes and 0 to 3 red blood cells per cubic millimeter, no organisms, inability to concentrate beyond 1.010, 15 per cent excretion of phenolsulfonphthalein in fifteen minutes and excretion of 250 to 495 mg. of calcium in twenty-four hours.

Examination of the eyes showed a typical band of keratopathy extending 2 to 3 mm. onto the cornea, without any definite clear zone separating it from the periphery. It was more pronounced on the temporal than on the nasal side. It was accompanied with three to four translucent crystals in the adjacent conjunctiva.

The cause of this patient's hypercalcemia and hyperphosphatemia with impaired renal function has not been determined. Differential diagnosis includes hyperparathyroidism, rapidly advancing osteitis deformans and the syndrome of Burnett and associates.[8]

From several points of view this group of cases is most interesting. They have in common uremia, band keratopathy and, with 1 exception, hypercalcemia. The etiologic factor was not apparent, but it is noteworthy that in at least 5 of them (cases 12, 15, 17, 18 and 19) high milk and high alkali diets had been taken for some time and the cases fall under the syndrome described by Burnett and associates,[8] in which renal damage (nephrocalcinosis?) is believed to result from high calcium and alkali intake. In 2 other cases in the series (cases 13 and 14) there was prolonged vomiting, and a nephrocalcinosis secondary to the chronic alkalosis may similarly have developed. The pathogenesis of the band keratopathy would presumably be the same as that of the calcification of the kidneys.

With the development of sufficient renal damage, the serum nonprotein nitrogen and inorganic phosphorus increase. But since the phosphorus and calcium levels of the blood tend to maintain reciprocal levels, the calcium which was originally abnormally high may fall to abnormally low levels as uremia supervenes. Thus, in case 15, when there was only moderate uremia, as indicated by serum nonprotein nitrogen of 80 mg., the calcium was 14.6 mg. and the inorganic phosphorus 4.7 mg. per hundred cubic centimeters, but when, toward the terminal stage, the serum nonprotein nitrogen had risen to 171 mg. and the inorganic phosphorus to 9.4 mg. per hundred cubic centimeters the calcium had fallen to the abnormally low level of 4.7 mg. per hundred cubic centimeters. We are not prepared to state to what extent band keratopathy may occur with renal damage and rising serum

8. Burnett, C. H.; Commons, R. R.; Albright, F., and Howard, J. E.: Hypercalcemia Without Hypercalciuria or Hypophosphatemia, Calcinosis and Renal Insufficiency: A Syndrome Following Prolonged Intake of Milk and Alkali, New England J. Med. 240:787-794 (May 19) 1949.

inorganic phosphorus in cases in which there is not a preexisting hypercalcemia. Whereas the data on case 16 suggest that this change in the cornea may occur, the patient was first seen in the terminal stage of her disease, at a time when the serum nonprotein nitrogen and inorganic phosphorus levels were very high. The normal serum calcium may or may not have been preceded by a high level.

SUMMARY

Band keratopathy and calcification of the conjunctiva is reported in 18 cases of hypercalcemia and in another case in which the calcium level of the blood may be presumed to have been elevated previously. The hypercalcemia was due to hyperparathyroidism in 4 cases, to vitamin D poisoning in 5 cases and to sarcoidosis in 2 cases. In the remaining 8 cases it was associated with severe renal damage, owing, in some of the cases, to a high calcium and high alkali intake. The corneal opacity consisted of paralimbal opacification of the cornea extending 2 to 3 mm. axialward in the palpebral fissure. Many of the patients had nephrocalcinosis or nephrolithiasis, and the process occurring in the kidneys was thought to be analogous to that occurring in the cornea.

243 Charles Street (14).

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Dis. Childh., 1965, 40, 535.

INFANTILE HYPERCALCAEMIA AND CARDIOVASCULAR LESIONS

EVIDENCE, HYPOTHESIS, AND SPECULATION

BY

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Since the recognition of the benign and severe forms of infantile hypercalcaemia (Lightwood, 1952; Fanconi, Girardet, Schlesinger, Butler, and Black, 1952) circumstantial evidence has assigned a causal role to vitamin D ingested by infants peculiarly sensitive to that substance. The disease failed to disappear, though its incidence declined, after the vitamin D_2 content of infant foods and vitamin concentrates had been reduced (Stewart, Mitchell, Morgan, Lowe, and Thomson, 1964; British Paediatric Association Report, 1964). It accounted for the admission of 39 infants to the Royal Hospital for Sick Children, Glasgow, during 1956-57 at the height of its incidence, but of only 13 during 1962-63. All but one of these 13 patients lived outside the city (10) or on its periphery (2), a situation contrasting with the recent incidence of infantile rickets in the area (Arneil and Crosbie, 1963), which has been largely confined to the central districts of the city.

Children poisoned with a massive dose of vitamin D may sustain damage to the cardiovascular system (Hjelt, Tähkä, and Hallman, 1956), and it is quite logical to expect similar effects in a disease acknowledged as qualitatively identical. Cardiovascular lesions have indeed been reported in infantile hypercalcaemia, and while their inconstancy and variety are notable they represent a serious aspect of the disease deserving close attention.

Electrocardiographic and Clinical Findings

An abnormal contour of the ST-T complex in the bipolar limb leads was reported in 14 of 24 patients suffering from infantile hypercalcaemia (Coleman, 1959), but further experience and the use of praecordial leads have shown the incidence to be higher. Indeed 12 of the 13 patients admitted to hospital during 1962-63 demonstrated the abnormality; no electrocardiographic examination was made of the remaining patient. The abnormal pattern bears little resemblance to that usually attributed to hypercalcaemia and may persist longer than the elevation of serum calcium. Instead of the Q-T interval (QT) being short and reflecting a short S-T interval, it is of average duration because the shortened S-T interval is masked by a broad T wave: this has a flattened or notched summit and is usually tall. The abnormal T wave is typically present not only in bipolar leads I and II, but also in unipolar leads from V4 to V6 (Fig. 1). Furthermore even in bipolar leads with apparently normal T wave summits the waves can be shown to be of significantly greater length than in healthy infants (Coleman, 1961).

Of the 13 patients with infantile hypercalcaemia during 1962-63, 4 gave transient electrocardiographic evidence of left ventricular hypertrophy. Three

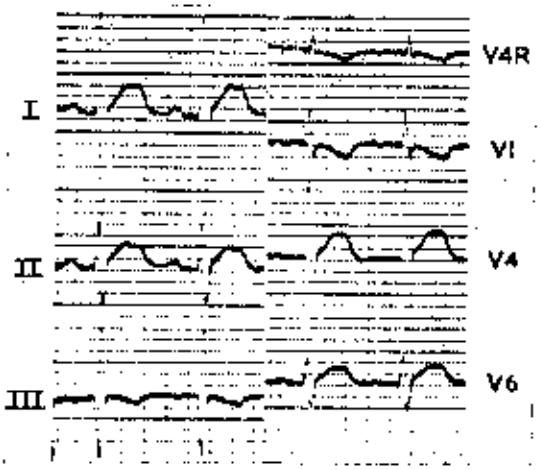


FIG. 1.—Infantile hypercalcaemia. Broad plateau T waves in leads I, II, V4, and V6.

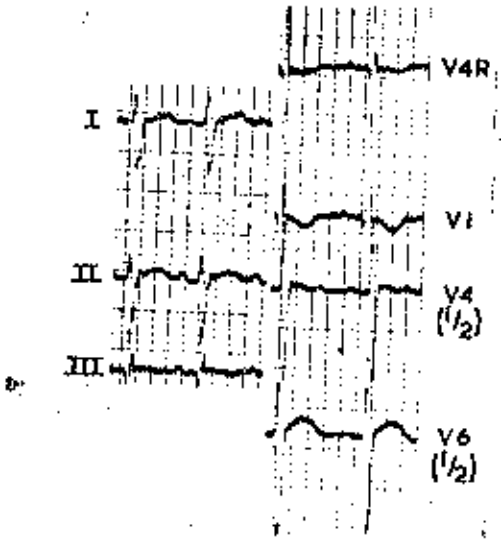


FIG. 2.—Infantile hypercalcaemia and pulmonary stenosis. V4 and V6 recorded at half-standard amplitude. Right ventricular hypertrophy and abnormal T waves (II and V6).

others had systemic hypertension which was most pronounced in the single patient who suffered from the disease in its severe form. This patient, from his first attendance when 5 months old, had a systolic murmur at the pulmonary area and by 18 months physical signs characteristic of pulmonary valve stenosis, with electrocardiographic evidence of right ventricular hypertrophy (Fig. 2). Cardiac catheterization confirmed the diagnosis; the right ventricular pressure was 45/0 mm. Hg, but the systolic pressure gradient of 30 mm. Hg over the pulmonary valve was regarded as insufficient to justify surgical treatment. None of these patients died.

Experimental Findings

Because the electrocardiographic changes were so specific and might result from a myocardial lesion, the possibility of a specific lesion caused by vitamin D was examined after its administration in large doses to young rabbits.

Vitamin D_3 in arachis oil was given by mouth to 9 rabbits, vitamin D_2 in arachis oil was given to 9 rabbits, and there were 9 controls. The rabbits belonged to 6 litters, and the administration of vitamin D was carried out within 10 days and completed by the age of 6 weeks. The dosage per g. body weight was from 100 to 770 international units of vitamin D_3 and from 40 to 420 units of vitamin D_2. Where possible they were allowed to survive to 4, 8, or 40 weeks after the beginning of the experiment, but 7 that had received vitamin D died, and another had to be killed earlier than planned.

An aortic lesion was present in every rabbit given vitamin D, but in none of the controls. In most the lesions were widespread, but the thoracic aorta was most severely affected (Fig. 3). The inner half of the media was affected and in some instances there was intimal thickening. The least severe lesions were the most cellular, with many fibroblasts and macrophages. The elastic fibres were distorted or even disrupted and between the elastic laminae

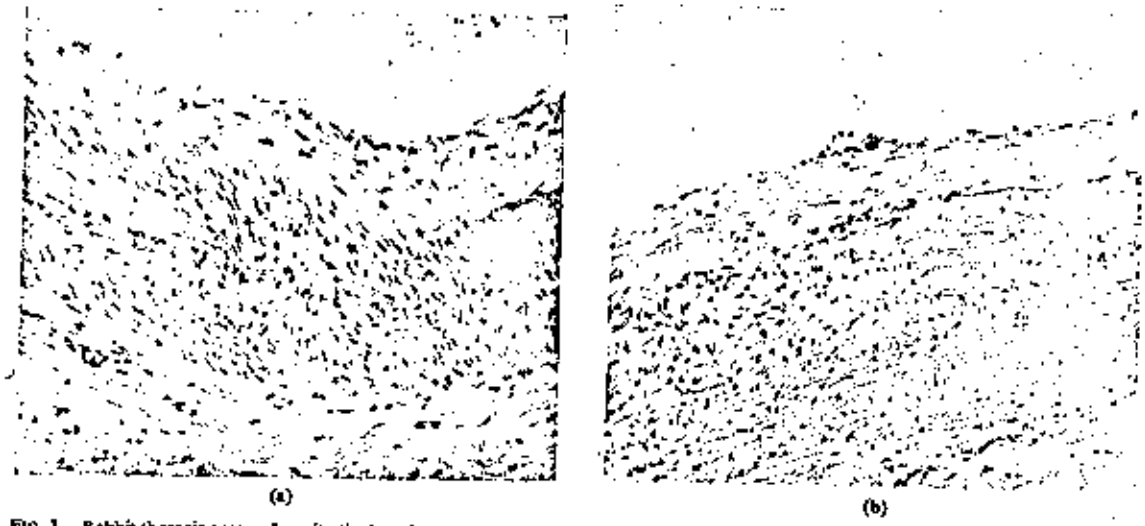


FIG. 3.—Rabbit thoracic aorta. Longitudinal sections. (H. and E. × 150.) (a) Early lesion. Thickened intima. Elastic laminae straightened, thickened, and disrupted. Fibroblast proliferation. (b) Severe lesion showing in addition cartilage formation and proliferation of fibroblasts and macrophages.

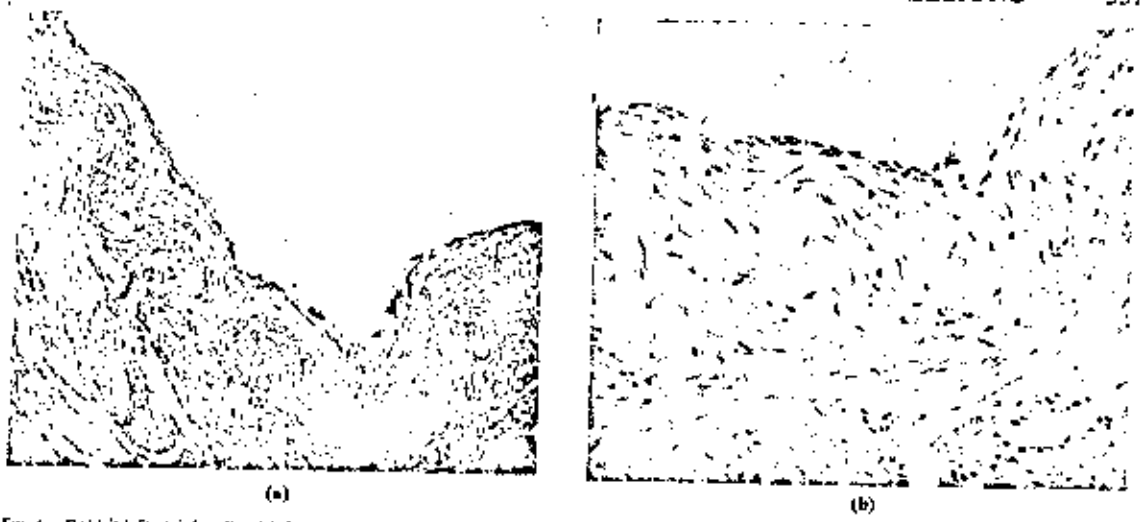


FIG. 4.—Rabbit left atrial wall. (a) Subendocardial fibrous tissue proliferation; collagenous layer and deeper fibroelastic layer. (Verhoeff-van Gieson. × 67.) (b) Another example. Fibrous tissue proliferation overlying normal muscle. (H. and E. × 275.)

muscle cells were destroyed. Here calcium deposition was often suggested by staining reactions; cartilage had formed in 2 instances and bone in one instance.

Two died in congestive heart failure (D_2 347 units/g.; D_3 103 units/g.); histology of the heart revealed no focal lesion but hypertrophy of muscle fibres and severe aortic wall lesions. Four others (D_2 230 units/g., 40 units/g.; D_3 770 units/g., 660 units/g.) had focal myocardial lesions which in 3 consisted of periarterial muscle fibre necrosis with staining reactions suggesting the presence of calcium, and, in relation to dead muscle fibres, a cellular reaction consisting of macrophages, fibroblasts, and multinucleate giant cells. In the fourth rabbit (D_2 40 units/g.) focal lesions were present at some distance from the arterial branches and consisted of a patchy loss of muscle fibres with replacement by granulation tissue in which were macrophages, eosinophils, and occasional giant cells, but without evidence of calcium deposition. The rabbit that became ill and had to be killed 6 weeks after the administration of vitamin D (D_2 40 units/g.) was the only one in which coronary artery lesions were detected; there was distortion and disruption of the internal elastic lamina, thickening of the intima, and narrowing of the media.

In 10 rabbits that had received vitamin D there were lesions immediately subjacent to the mural endocardium of the left ventricle, left atrium, or right ventricle (D_2 40–420 units/g.; D_3 100–770 units/g.). Immediately beneath the endocardium there was a collagenous layer containing only a few fibroblasts; in some there was a deeper and substantially thicker layer of fibroelastic tissue which sometimes surrounded islands of muscle cells (Fig. 4).

Serum calcium levels in excess of those of the control animals (10-11·5 mg./100 ml.) were found as long as 32-38 weeks after the administration of vitamin D_2 or D_3 had ceased (12·9-14·1 mg./100 ml.).

Discussion

The wide but inconstant array of cardiovascular lesions reported in a relatively few cases of infantile hypercalcaemia has included left ventricular or biventricular myocardial hypertrophy, myocardial fibrosis, or calcification, valvular endocardial lesions including fibrosis and calcification, lesions of the intima and inner media of arteries including the coronaries, and peripheral systemic and pulmonary arterial stenosis (Schlesinger, Butler, and Black, 1956; Joseph and Parrott, 1958; Lamy, Nezelof, Fauré, Jammet, and Aussannaire, 1958; Rashkind, Golinko, and Arcasoy, 1961; Bonham Carter and Sutcliffe, 1964).

Aortic Lesions. Black and Bonham Carter (1963) have described aortic supravalvar stenosis in a child thought to have suffered from severe infantile hypercalcaemia. Histological features in the aorta were thickening of the media with fibrosis and some deficiency of both muscle and elastic tissue, a circular ridge of fibrous tissue, and, distal to this stricture, intimal thickening with atheromatous change. There was fibrous thickening of the aortic valve.

The lesions in rabbits reported here indicate the susceptibility of the aortic media and intima in one

mammalian species to damage by vitamin D. Extensive examination of the controls failed to reveal any vestige of the spontaneous aortic lesion known to occur in some laboratory rabbits (Kesten, 1935). The histological pattern of the experimental lesion seems to resemble that of the human lesion described by Black and Bonham Carter (1963), and therefore lends support to the concept of a causal relation between infantile hypercalcaemia and aortic supravalvar stenosis on the assumption that the former disease is causally linked with vitamin D activity.

Endocardial Lesions. The experimental lesion immediately subjacent to the mural endocardium has no exact counterpart in infantile hypercalcaemia. The only endocardial lesion detected during 1962-63 in the hospital in association with infantile hypercalcaemia was pulmonary stenosis confirmed by cardiac catheterization. The same association occurred in a male infant of 6 months who died of the severe type of infantile hypercalcaemia in 1954. In addition to advanced renal medullary calcinosis necropsy revealed pale, thickened, rigid, but discrete, pulmonary valve cusps in a valve ring of average size, these appearances being due to the presence of oedematous fibrillary collagen within the cusps (Fig. 5).

This pulmonary valve lesion may have its parallel in the aortic valve lesion mentioned by Black and Bonham Carter (1963).

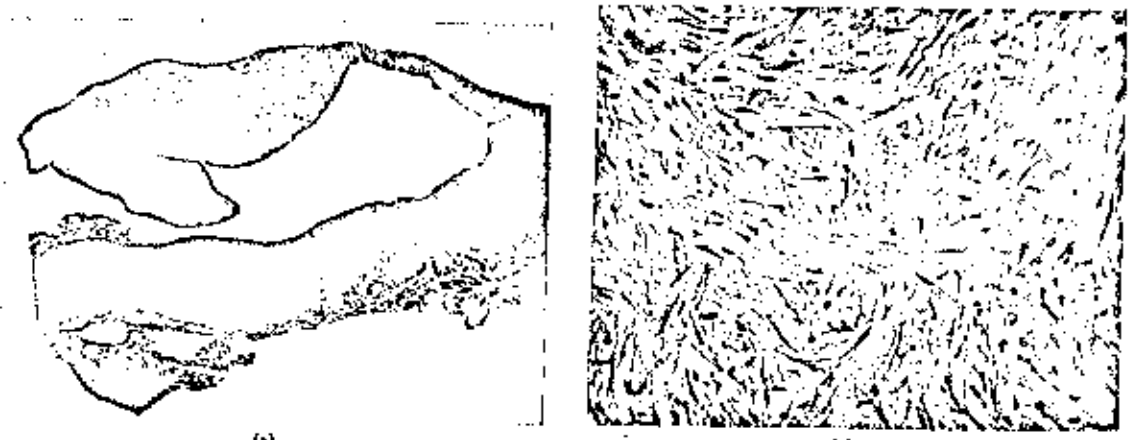
The fibroelastic form of the experimental lesion is reminiscent of congenital endocardial fibroelastosis, and like that disease affects principally the left heart chambers. Endocardial fibroelastosis remains of uncertain aetiology. It has no recognized connexion with infantile hypercalcaemia or vitamin D, but its known association with congenital cardiovascular malformations is so inconstant (Forfar, Miller, Bain, and Macleod, 1964) that other or additional causal factors are likely. The experimental lesion indicates decisively that a form of endocardial fibroelastosis may be caused by a toxic substance, but since the human disease usually presents in the earliest weeks of life (Forfar *et al.*, 1964) its occurrence then seems to require the transplacental passage of the toxic substance. Not only has transmission through the placenta been claimed for vitamin D (Decio and Alamanni, 1956), but the early appearance of the striking clinical features of severe infantile hypercalcaemia suggests the likelihood of prenatal intoxication in some instances. The lesion of congenital endocardial fibroelastosis may not be confined to the endocardium, but may, as in the case of infantile hypercalcaemia, occasionally include myocardial fibrosis or calcification and coronary artery lesions (Blumberg and Lyon, 1952; Thomas, Lee, McGavran, and Rabin, 1956). Curiously, the hospital incidence of endocardial fibroelastosis proven at necropsy, as of infantile hypercalcaemia diagnosed clinically, has declined since 1958 when measures had been taken and the reasons publicized for reducing the vitamin D_2 intake of infants (Table).

TABLE

NUMBER OF CASES DURING FIRST YEAR OF LIFE

Disease	1954–58	1959–63
Infantile hypercalcaemia	61	24
Endocardial fibroelastosis	19	10

Outside the cardiovascular system, however, severe infantile hypercalcaemia and endocardial



(a) (b)

FIG. 5.—Infantile hypercalcaemia and pulmonary stenosis. (a) Irregular bulbous enlargement of free extremity of pulmonary valve cusp. (H. and E. × 22·5.) (b) This enlargement caused by proliferation of oedematous fibrillary collagen. (H. and E. × 183.)

fibroelastosis are quite dissimilar, and an attempt to reconcile these as effects of a single intrauterine toxic agent relies on the concept that the quality of teratogenic effect depends on the dose of the agent and the timing of its application during embryogenesis.

Myocardial Lesions. The infrequency of myocardial and coronary artery lesions in the experimental material would not suggest the probability of a high incidence in infantile hypercalcaemia, though in that disease similar lesions have been found at necropsy (Rashkind *et al.*, 1961). It is evident, however, that no conclusion is possible as to the relative amounts of vitamin D required in different species to produce similar lesions and especially in relation to a disease dependent for its existence on hypersensitivity.

The electrocardiographic features of infantile hypercalcaemia, which are of such constancy as to be of diagnostic value, are caused neither by hypercalcaemia nor by any generalized electrolyte disturbance. Quinidine intoxication may temporarily produce prominent and broad T waves, and a lengthened Q-T interval (Sagall, Horn, and Riseman, 1943), indicating the increased refractory period of the ventricular myocardium. In infantile hypercalcaemia the abnormal shape of the ST-T complex in leads I, II, and V4 to V6 outlasts the hypercalcaemic phase of the disease, and it is tempting to suggest that it signifies left ventricular myocardial damage. In children poisoned with massive doses of vitamin D_2 a closely similar ST-T pattern has been described (Jelke, 1946; Skatvedt, 1947; Hjelt *et al.*, 1956), and the possibility exists that a specific toxic effect is exerted on myocardial metabolism by vitamin D or some related toxic sterol, or that a specific pattern of myocardial damage is caused by such a substance. Correlation of electrocardiographic pattern with myocardial pathology was reported in one child who died of classical vitamin D poisoning; the T waves had been tall and broad and the myocardium showed periarterial necrosis and fibrosis (Hjelt *et al.*, 1956).

The search for left ventricular lesions of a type that could cause electrocardiographic signs such as these included the myocardial fibrosis of uncertain pathogenesis found as a rare accompaniment of fibrocystic disease of the pancreas (Kintzen, 1950; Feer, 1952; Powell, Newman, and Hooker, 1957; Nezelof and Lancret, 1959), for McGiven (1962) described a child in whose published electrocardiogram the ST-T complexes of lead I, V4, and V5 were closely similar if not identical with those described here. In the only other reported electrocardiogram (Nezelof and Lancret, 1959) the T waves were also abnormal. The characteristic lesion affects principally the left ventricular myocardium and consists of muscle fibre degeneration, and replacement by fibrous tissue. Endocardial fibroelastosis has been twice reported (McGiven, 1962; Nezelof and Lancret, 1959). Nezelof and Lancret (1959) did not mention diet or medication but vitamin D had been given in all other instances. Nephrocalcinosis was present in both patients whose renal histology has been reported; this would, according to Shanks and MacDonald (1959), argue the occurrence at some time of hypercalcaemia. Rickets is virtually unknown in fibrocystic disease of the pancreas but extra vitamin D is customarily added to the diet as it is in coeliac disease. It seems possible that there could exist in fibrocystic disease a compensatory mechanism for the conservation of vitamin D and accordingly that toxic effects of overdosage might readily occur.

In regard to the absence of calcification in this cardiac lesion and other lesions ascribed to vitamin D, it is evident from animal feeding experiments (Gillman and Gilbert, 1956), as from some cardiac lesions in infantile hypercalcaemia (Rashkind *et al.*, 1961), and from the natural history of nephrocalcinosis associated with infantile hypercalcaemia (Shanks and MacDonald, 1959), that calcification is neither a primary nor an invariable feature of these lesions.

Prognosis. Spontaneous healing and complete repair have been reported by Gillman and Gilbert (1956) in relation to a wide range of cardiovascular lesions induced in the rat with vitamin D_2. There is no reason to suppose that human cardiovascular lesions similarly produced would behave differently, provided that the toxic stimulus were removed. No conclusion is possible from the present experimental material, because of the apparent long persistence of vitamin D effect as judged from serum calcium levels.

Summary and Conclusions

In infantile hypercalcaemia (infantile hypersensitivity to vitamin D) the high incidence of transient changes in the ST-T complex of the left praecordial electrocardiogram suggests a high incidence of transient damage to the left ventricular myocardium.

Vitamin D given in high dosage to young rabbits causes lesions of the aortic wall, of the mural endocardium (fibroelastosis), and occasionally of the myocardium.

The possibility exists that congenital endocardial fibroelastosis and the myocardial lesion of fibrocystic disease of the pancreas are related to vitamin D.

The persistence of infantile hypercalcaemia and

the possibility of residual cardiovascular lesions is disquieting. Where infantile rickets and infantile hypercalcaemia coexist in different parts of the same community as they do in south western Scotland, it may prove impracticable to eliminate one without increasing the incidence of the other, and in such circumstances it is impossible to evade making a responsible inquiry into the nature of residual effects and their relative importance.

I thank Professor J. H. Hutchison and Dr. R. A. Shanks for access to the clinical material, Dr. A. M. MacDonald for advice on histology, the Departments of Biochemistry and Radiology at the Royal Hospital for Sick Children, and Messrs. W. Mason and J. Devlin for the illustrations. I am indebted to Professor Hutchison for criticism of the manuscript.

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Committee on Nutrition
Pediatrics. 40(1): 89-90, 1967

1116

ABSENCE OF VITAMIN D IN NONFAT DRY MILK

The Committee on Nutrition of the American Academy of Pediatrics has strongly endorsed the use of milk and infant formula products as primary vehicles for vitamin D supplementation to ensure a total vitamin D intake of 400 I. U. per day by all infants and children.[1,2] Although in the United States there are significant regional differences with respect to dairy practices in fortifying fluid milk, almost three-fourths of all whole milk sold at retail is fortified with vitamin D, and almost all evaporated milk and infant formula products contain 400 I. U. of vitamin D per reconstituted quart. In addition, vitamin D is readily available in vitamin supplements, and, undoubtedly as a result of the wide distribution and ready availability of vitamin D, the incidence of infantile rickets in the United States has remained at a very low level.[3]

This report draws attention to the lack of vitamin D in nonfat dry milk, a product used in significantly increasing amounts for preparing milk for home use for children, and possibly for infants. The use of nonfat dry milk has been accelerated recently by the increase in prices of fluid milk. For example, in 1965, the last year for which figures are available, consumer sales of nonfat dry milk amounted to 2.5 billion pounds of fluid equivalent, or about 5% of the total national consumption of milk. This total reflects an increase in consumption of almost 70% over the past 10 years.

The lack of vitamin D in certain major sources of milk for the population invites the reappearance of rickets. In Canada, the addition of vitamin D to fluid milk was not permitted until recently and is not common practice even now. As a consequence, rickets continues to appear and constitutes a continuing and significant cause of morbidity in infancy and childhood. One hospital in Montreal reported almost 200 cases in the latest annual tabulation.[4]

Since nonfat dry milk is not fortified with vitamin D, because this was not included in the definition of the product (Standard of Identity) established by Congress in 1944, the Committee on Nutrition of the American Academy of Pediatrics recommends that infants and children who receive this source of milk also be given a vitamin supplement containing vitamin D to ensure consumption of 400 I. U. of the vitamin per day.

It is of nutritional interest, though obviously unrelated to the problem of vitamin deficiency rickets, that nonfat dry milk contains virtually no vitamin A, the substance having been removed during the manufacturing process. While there are no data in this country on the potential danger from vitamin A deficiency, there is evidence that when nonfat dry milk becomes a major source of nutrition in countries in which the national diet is low in vitamin A content, xerophthalmia has appeared.

While the general level of nutrition of North American children receiving medical supervision by pediatricians appears sound, the facts discussed above preclude complacency. They illustrate the potential threat to nutrition of infants and children when the physician remains uninformed on major changes in feeding practices or manufacturing processes.

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Committee on Nutrition · American Academy of Pediatrics #117

PROPOSED CHANGES IN FOOD AND DRUG ADMINISTRATION REGULATIONS CONCERNING FORMULA PRODUCTS AND VITAMIN-MINERAL DIETARY SUPPLEMENTS FOR INFANTS

THE Food and Drug Administration is currently revising regulations governing composition and labeling of foods for special dietary uses. These proposed revisions should recognize changing practices in infant feeding, new information on nutritional requirements, and major advances in food technology. Of specific interest to the pediatrician is the fact that infant formula products are covered by these regulations. In the opinion of the Committee on Nutrition, it is of great importance that the standards established be consistent with sound nutritional practice and provide adequate levels of all nutrients, since during the first 6 months of life a majority of American infants receive virtually their total dietary intake from products in this category.

The Committee has given much study to the nutrient needs of infants and to the amounts of protein, vitamins, and minerals required for sound infant nutrition. It recognizes that adequate studies are not available to identify in many instances either optimal or safe minimal intakes of these substances. Nevertheless, it is our goal to insure that infant formulas are nutritious and as safe as modern technology and scientific knowledge can accomplish. This requires that the label information should fully inform the physician and the purchaser of the nutrient content of the formula and identify specifically any recognized nutritional inadequacies. The text which follows attempts to specify safe minimal levels of the nutrients contained in proprietary formulations of milk products and applies equally well to formulations with milk substitutes (soya, meat, etc.). The minimal values are not *recommended allowances* of these nutrients. Products which purport to be the sole source of nutrition for infants and which provide less than these minimal specifications are to be so labeled as to identify the need for dietary supplementation.

In addition to proposing regulations governing infant formulas, the Food and Drug Administration has also published proposed revisions relating to formulation and content of vitamin and mineral supplements for infants. The nutritional considerations mentioned above apply with equal force to these products, and it is important that the two sets of regulations concerning these two types of products be consistent.

Average growth performance of full-term infants fed at the breast of well nourished mothers has not been exceeded by that of full-term infants consuming any artificial formulation. Therefore, average values of nutrients in human milk may be considered a rational basis for estimating minimal needs. Nevertheless, experience has proven that levels of several nutrients, notably vitamin D and iron, and possibly also of ascorbic acid, in human milk are even less than those necessary to support normal growth and state of health. In addition, the requirements of the breast-fed infant for vitamin E, thiamine, and vitamin B_6 may be different from those of infants fed formulas having compositions significantly different from that of human milk.

The use of the reference value 100 kcal* for specification of minimal amounts of vitamins and minerals is consistent with modern practice and implies a relation of nutrient needs to total metabolic activity. This

* Kilocalorie, the large calorie, is the calorie commonly used in diets as well as in nutritional and metabolic studies.

TABLE I

MINIMUM VITAMIN AND MINERAL LEVELS PER 100 KCAL OF FORMULA (VALUES GIVEN IN MILLIGRAMS, EXCEPT WHERE OTHERWISE NOTED)

Vitamins	*Minimum Amount**
Vitamin A (U.S.P. units)	250
Vitamin D (U.S.P. units)	40
Vitamin E (I.U.)	0.3
Ascorbic acid	8
Thiamine	0.025
Riboflavin	0.06
Niacin	0.25
Vitamin B_6	0.035
Folic acid (μg)	4
Pantothenic acid	0.3
Vitamin B_{12} (μg)	0.15
Minerals	
Calcium	50
Phosphorus	25
Magnesium	6
Iron	1
Iodine (μg)	5
Copper	0.06

* A number of values have been rounded out.

permits a comparison of these values among infants whose nutritional demands are changing as a function of growth. The tabular summary (Table I) which accompanies this text presents suggested minimal vitamin and mineral levels per 100 kcal. With the exceptions mentioned above, the amounts are similar to those found in human milk.

PROTEIN CONCENTRATION AND QUALITY

Human milk contains 1.8 gm of protein per 100 kcal, or 7.8% of the calories as protein (1.2 gm/100 ml and 66 kcal per 100 ml),[1] and available evidence indicates that, in a food which is the sole source of protein for an infant, such a level of protein is adequate when it is of quality comparable to that of human milk.

The quality of a protein will vary with source and method of processing. If a quantity of formula which supplies 100 available kilocalories as customarily or usually prepared for consumption contains less than 1.8 gm of protein of quality equivalent to that of casein (Protein Efficiency Ratio),[2] or when the quality of the protein expressed as a fraction of that of casein multiplied by the gram protein per 100 kcal is less than 1.8, or if the protein quality is less than 70% of that of casein, the following statement should appear on the label: "This product should not be used as the sole source of protein of the infant diet."

VITAMIN A

No cases of vitamin A deficiency in breast-fed infants have been reported in the United States. The total vitamin A potency of human milk, though variable, averages 250 U.S.P. units per 100 kcal. Several early clinical studies attempted to determine the vitamin A requirements of infants consuming artificial formulations; evaluation of these results is complicated because the vitamin A preparations used were of uncertain potency and stability. A minimal level of 250 U.S.P. units of vitamin A per 100 kcal is therefore recommended for infant formulas.

VITAMIN D

The Committee has recommended an intake of vitamin D of 400 U.S.P. units per day and, at the same time, recognized that normal linear growth without rickets occurs with intakes below this value. The evidence available suggests that a minimum of 250 U.S.P. units be supplied per day;[3] and, when this value is considered in relation to the amount of formula ordinarily consumed, a level of 40 U.S.P. units per 100 kcal appears to be an appropriate value.

VITAMIN E

Human milk contains approximately 2 I.U. of vitamin E per liter (0.3 I.U. per 100 kcal).[4] Although levels of vitamin E in blood of term infants are low, they rise rapidly when the infant is breast-fed, and biochemical evidence of vitamin E deficiency has not been reported in breast-fed infants. Because an increased intake of poly-

unsaturated fatty acids (PUFA) results in an increased vitamin E requirement, formula products with two, three or more times the PUFA content of human milk (approximately 6% of calories) have a depleting effect on the infant's body stores of vitamin E. Such products should probably be supplemented with vitamin E. However, until the relation between vitamin E requirement and PUFA intake is established quantitatively for the infant, the value of 0.3 I.U. of vitamin E per 100 kcal is recommended as a tentative minimum.

ASCORBIC ACID

The requirement for ascorbic acid during early infancy, like the requirement for vitamin B_6, is dictated to a great extent by level of dietary protein. Under these circumstances, needs are determined in relation to tyrosyluria (hypertyrosinemia) rather than to an antiscorbutic effect. Ascorbic acid has been linked to protein metabolism since 1939 when incomplete metabolism of aromatic amino acids was demonstrated in premature infants.[5] Recently the syndrome of transient hypertyrosinemia of the newborn has been reviewed and both clinical and dietary aspects of the problem discussed.[6] This report refers to an unpublished survey of 15,000 infants in which it was determined that 10% of full-term infants had mild tyrosinemia during the first week of life while 30% of premature infants had severe tyrosinemia.

Tyrosinemia of the newborn is related to maturity at birth and protein content of diet and generally remits with age. Tyrosinemia is reduced by a decrease in protein intake or, in some instances, by administration of appropriate amounts of ascorbic acid.

The evidence discussed above indicates that the relative need for ascorbic acid during early postnatal life may be greater than later on. The ascorbic acid content of human milk varies from 2 to 10 mg per 100 ml (3 to 15 mg per 100 kcal). The recommended minimal level, 8 mg per 100 kcal, is consistent with the known antiscorbutic properties of human milk and provides adequate protection against hypertyrosinemia in infants receiving formulas with conventional protein contents.

THIAMINE, RIBOFLAVIN, AND VITAMIN B_6

Average levels of thiamine and riboflavin in human milk are 0.025 and 0.06 mg per 100 kcal, respectively, and no cases of clinical thiamine deficiency have been reported in breast-fed American infants in the past 25 years. In addition, a review by an FAO/WHO expert group supports the position that the level of thiamine and riboflavin in human milk is adequate and consistent with sound nutrition.[7] It is proposed that artificial formulas contain no less than 0.025 mg of thiamine and 0.06 mg of riboflavin per 100 kcal. In formulas supplying more than 40% of the calories from carbohydrate, the level of thiamine should be increased proportionately.

The Committee has defined requirements for vitamin B_6 during infancy[8] and suggested a level of 20 μg of vitamin B_6 per gram of protein in infant formulas. A minimal value of 0.035 mg per 100 kcal of a formula containing 1.8 gm of protein per kilocalorie seems appropriate.

NIACIN

Human milk contains an average of 0.25 mg niacin per 100 kcal and 0.55 mg niacin equivalents (from 1.8 gm protein) per 100 kcal. This amounts to a total of 0.80 mg niacin per 100 kcal of human milk. The recommended dietary allowance of the Food and Nutrition Board[9] for infants is 0.60 mg niacin per 100 kcal. This includes the calculated amount of niacin available from tryptophan in dietary protein (niacin equivalent). Unless the protein supplies less than 0.55 mg niacin equivalent from tryptophan, a content of 0.25 mg of niacin per kcal is adequate.

FOLIC ACID

Folic acid deficiency during infancy is characterized by low levels of folate in serum or blood, morphologic changes in neutrophiles, and, in the severe stage, me-

galoblastic anemia. It is more common among low birth weight infants.[10,11] Low stores at the time of birth, infection, diarrhea or malabsorption, low dietary intake, rapid weight gain, and vitamin C deficiency contribute to the development and manifestations of a folic acid deficiency state.

Estimations of folic acid content of milk products are technically difficult, and results of measurements in different laboratories are not always consistent. This issue has received comment by the Committee and others.[12] On the basis of best available data, a minimal intake of 4 μg per 100 kcal appears satisfactory and in keeping with the average content of human milk.[13]

PANTOTHENIC ACID

Clinical evidence of pantothenate deficiency has not been reported in infants consuming human milk or cow milk formulas. Since human milk contains about 0.3 mg per 100 kcal, there is reason to recommend this as a minimal level.

VITAMIN B_{12}

A dietary deficiency of vitamin B_{12} is unknown in infants in the absence of a specific morbid state. Therefore the amount available in human milk, up to 1 μg per liter, appears adequate (0.15 μg per 100 kcal).

PHOSPHORUS AND CALCIUM

Von Sydow reported that human milk did not provide sufficient phosphorus, 150 mg per liter, to meet the growth needs of infants weighing less than 2,000 gm.[14] Such infants, while consuming adequate amounts of vitamin D, developed chemical or growth rickets characterized by reduced serum levels of phosphorus and calcium, an increase in serum level of alkaline phosphatase, and roentgenographic changes compatible with rickets. Growth rickets occurs as a result of reduced stores of minerals at birth, dietary deficiency, and increased demands for minerals due to rapid growth.

From observations on stable strontium balance in infants,[15] it has been concluded that the ion limiting skeletal mineralization in the human infant is phosphate. It appears that phosphorus may be limiting for the human infant at a level below 150 mg per liter of milk (25 mg per 100 kcal).

The effect of limiting dietary calcium has not been studied in human infants, and the minimal level of calcium required in the diet independent of phosphorus and vitamin D has not been examined. In the absence of such data, and recognizing the adequacy of human milk to support excellent bone growth, calcium content of the diet at the level in human milk is presumed adequate and amounts to 50 mg per 100 kcal.

MAGNESIUM

The content of magnesium in human milk, 6 mg per 100 kcal, is adequate for normal nutrition, and is proposed as the minimum level for infant formulas.

IRON

The human infant will become deficient in iron if only the amount available in human milk, 0.2 mg per 100 kcal, is supplied during the first 12 months of life. A formula must contain 1 mg of iron per 100 kcal if it is to meet the minimum requirement of the infant.

COPPER

While hypocupremia is recognized, it is secondary to primary disturbances in protein metabolism with loss of copper protein complexes. Attempts to produce copper deficiency during infancy have been unsuccessful.

Graham and co-workers have recently described copper deficiency in a limited number of children fed a milk formula low in copper during recovery from kwashiorkor.[16,17] The syndrome produced in these children resembled that described by Cartwright and co-workers in swine fed copper deficient diets.[18]

A reasonable minimum figure for copper is 0.06 mg per 100 kcal, the amount available in human milk.

IODINE

The content of iodine in human milk will not meet the infant's needs when ma-

ternal intake of this element is inadequate. This occurs in many geographic regions where endemic goiter is found. The minimal needs of the infant can be calculated from measurements of iodine content of human milk in goiterous regions and study of the supplements needed to prevent goiters. Available evidence indicates a safe minimal level in formulas is 5 μg per 100 kcal.

GENERAL CONSIDERATIONS

The minimal levels of nutrients identified in the foregoing section apply only to the needs of term infants in good health and cannot be considered as providing safe levels in formulations designed for total dietary intake by premature, low birth weight, or ill infants. The specific nutritional needs of such subjects must be assessed by the physician. Modifications of these formulas by the addition of protein, vitamin or mineral supplements will, in general, be necessary; or, formulations containing higher levels of specific nutrients may be recommended to meet the special needs of these infants.

Improvements in food technology promise the appearance of new infant formulas containing novel types of protein combined with new sources of carbohydrate and lipids. In addition, modifications of milk or other proteins may permit preparation of formulations with either enhanced nutritional value or even in decreased nutritional value. For example, modification of proteins may also remove certain trace elements for which no previous essentiality had been demonstrated. While the Committee welcomes potential benefits to infant nutrition afforded by new products, it believes that properly designed and conducted clinical trials of these products should be mandatory prior to marketing. In the past new formulations have occasionally been sold to the public without prior evidence of efficacy, and occasionally to the detriment of a considerable population of infants. It must be demonstrated that a new formulation does not increase the nutrient requirements for specific substances.

When used for infant feeding, a formula prepared from cows milk (including evaporated and powdered) in essence becomes a complete or partial substitute for human milk. Under these circumstances it would be desirable that milk products represented as an ingredient of infant formulas comply with the minimum standards set forth above. They are, however, specifically exempted by present and proposed regulations and pediatricians should be alert to the need to supplement formulas made from cows milk with (at least) ascorbic acid and iron.

Approximately 600,000 infants per year are fed formulas prepared from evaporated milk. By 3 months of age about 750,000 infants are receiving fluid milk. As a prophylaxis against rickets, we have endorsed the continued addition of vitamin D to fluid and evaporated milk. The Canadian Food and Drug Directorate has recently permitted the addition of ascorbic acid to evaporated milk as a prophylactic measure against scurvy.

VITAMIN AND MINERAL SUPPLEMENTS

Levels of vitamins and minerals in preparations designed for administration as supplements for infants should provide an intake of each nutrient which is consistent with our knowledge of nutritional requirements and should be sufficient to allow for individual variability in dietary habits and food intake. Levels in excess of the maximum levels set forth in Table II appear to have no nutritional advantage for the normal infant. The maximal levels chosen except those for iron and Vitamin D are in general twice the amount present in 1 liter of human milk. This allows an excess considered safe yet permits an infant consuming a formulation devoid of a particular nutrient to receive through a supplement twice the estimated minimal need. Preparations containing more than these amounts should be considered as therapeutic rather than supplemental. Supplements should not provide levels of nutrients which are so low as to be of little value in helping to meet the infant's requirements. The assignment of minimal levels at values approximately one-

TABLE II

SUGGESTED ALLOWABLE MAXIMAL AND MINIMAL AMOUNTS IN VITAMIN AND MINERAL SUPPLEMENTS

VITAMIN CONTENT IN MANUFACTURERS' RECOMMENDED DAILY DOSAGE
(VALUES GIVEN IN MILLIGRAMS, EXCEPT WHERE OTHERWISE NOTED)

Vitamins	*Minimum*	*Maximum*
Vitamin A (U.S.P. units)	850	3,400
Vitamin D (U.S.P. units)	100	400
Vitamin E (I.U.)	1	10*
Ascorbic acid	25	100
Thiamine	0.15	0.6
Riboflavin	0.2	0.8
Niacin	2.0	8.0
Vitamin B_6	0.15	0.6
Folic acid (μg)	12	50
Pantothenic acid	1	4
Vitamin B_{12} (μg)	0.5	2
Minerals		
Calcium	150	600
Phosphorus	75	300
Magnesium	25	100
Iron	4	16
Iodine (μg)	20	80
Copper	0.25	1

* This level is higher than twice the level in 1 liter of human milk to permit supplementation when diets have increased content of polyunsaturated fatty acids.

quarter of the maximum levels will insure against this.

CONCLUSION

This memorandum presents suggested safe minimal levels for vitamin and mineral content of formulas designed to provide total nutrition for infants. These levels must not be equated with recommended daily allowances; rather, they represent levels below which distinct hazards may be encountered. In addition, maximal and minimal values for vitamin and mineral supplements are suggested. The maximal levels will provide intakes close to ideal. A supplement containing less than the stipulated minimal levels will probably serve no useful purpose.

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THE PROPHYLACTIC REQUIREMENT AND THE TOXICITY OF VITAMIN D

It is now more than 40 years since Sir Edward Mellanby[1] demonstrated that cod liver oil would prevent rickets. Since this discovery, rickets due to vitamin D deficiency, once a serious pediatric problem in temperate climates, has become a comparatively rare clinical entity throughout the civilized world. Nonetheless, cases of deficiency rickets still occur in the United States,[2,3] Canada,[4] Great Britain,[5] and elsewhere.

In temperate climates, sunlight cannot be relied upon to provide year-round protection from rickets in infancy and early childhood, and vitamin D must be provided from other sources. Until recently, the intakes of vitamin D recommended by official organizations in a number of countries could only be achieved by administration of oral vitamin D supplements, since the normal diet (even one including butter, milk, and egg yolk) contained relatively little vitamin D. Today, however, the situation is greatly altered, at least in such countries as the United States, Canada, and Great Britain. Because the addition of supplemental vitamin D to various foods is sanctioned, individuals of all ages now receive variable and sometimes considerable amounts of vitamin D from pasteurized, evaporated, or powdered milk, margarine, certain ready-to-eat cereals, and a variety of other foodstuffs. Indeed, in the United States and Canada there is now a reasonable likelihood that the individual, even the small infant, may receive the total recommended allowance of vitamin D from an average diet; in some instances he will receive considerably more than the recommended intake even when no vitamin supplement is given.

It is well known that vitamin D produces serious toxic effects if consumed for a period in grossly excessive amounts. In addition, however, vitamin D, in amounts not much greater than the recommended allowance, has been incriminated as one of the factors in the pathogenesis of idiopathic hypercalcemia of infancy, and in this respect even small excesses of vitamin D may occasionally be injurious. Whereas in the past it was frequently the practice to administer doses of vitamin D in excess of the recommended allowance with the object of providing an extra measure of nutritional safety, it is now evident that all those concerned with the optimal nutrition of the population must bear in mind the possible untoward effects of vitamin D prescribed for prophylaxis. This, in turn, demands that closer attention be paid to the *total* intake of vitamin D, a task that is becoming increasingly difficult as each new "fortified" food is introduced onto the market.

In the report that follows, the Committee on Nutrition of the American Academy of Pediatrics summarizes the evidence regarding the prophylactic requirement and the toxicity of vitamin D, and presents certain recommendations for use of the vitamin.

THE REQUIREMENT OF VITAMIN D AT VARIOUS AGES

Vitamin D is believed to function in minute concentrations by regulating or potentiating certain complex biochemical reactions at various sites in the body. The amount necessary to achieve optimal physiologic function in a given individual is dependent upon a number of factors, among which are believed to be age, rate of growth, efficiency of intestinal absorption (especially of fat), and coexisting disease, but little is known concerning the influence of these factors.

From the sections that follow, it will be apparent that vitamin D is an essential fac-

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tor in the metabolism of the growing human being, and, indeed, probably at all ages. For the healthy premature and full-term infant, fairly reliable estimates of the minimal requirements have been made. This has been possible in part because the newborn infant develops evidence of vitamin D deficiency relatively rapidly, and in part because, by selecting for study infants born in the autumn, the otherwise undefinable amount of vitamin D contributed by sunlight can justifiably be neglected. The minimal requirements during later childhood, adolescence, adulthood, pregnancy, and lactation have been much more difficult to determine because sources of vitamin D become more accessible and more varied, because rickets rarely develops after infancy, and because other satisfactory criteria of minimal vitamin D deficiency have not yet been established.

In early years there was much disagreement regarding the relative efficacies of the various forms of vitamin D. It is now believed that, microgram for microgram, vitamin D_2 (calciferol) and vitamin D_3 (irradiated 7-dehydrocholesterol) have equal potencies in the human.[6] Ordinarily, vitamin D is almost completely absorbed by the intestine, and the vehicle in which the vitamin is administered (be it oil, aqueous suspension, milk, or other food) is believed not to affect absorption[7] unless the intestinal absorption of fat is impaired. In children with steatorrhea, the finely dispersed, water-miscible preparations are thought to be preferable on the basis of indirect evidence obtained from vitamin A absorption studies.[8,9]

The Normal Full-term Infant

Numerous studies, most of them conducted some 20 or 30 years ago, have concerned the efficacy of various amounts of vitamin D in preventing rickets,[7,10-12] in promoting intestinal absorption of calcium[13,14] and in promoting linear growth.[11,15,16]

On the basis of the above criteria, estimates of minimal vitamin D requirement range from approximately 100 U.S.P. units[7,11,12] to approximately 400 U.S.P. units per day[10,16] (1 U.S.P. un[illegible]). Doses of vitamin D greater than 400 I.U. per day did not result in a greater degree of protection against rickets[1,10,11] and did not further increase retention of calcium.[13,14] Although there is not complete agreement on the minimal requirement of vitamin D to promote maximal linear growth (or even whether vitamin D effects linear growth), it would appear that intakes of 250 I.U. daily are at least as effective as greater intakes.[12,16]

Many of these early studies are now open to question from the standpoint of modern experimental design. However, the observation that intakes of 300 to 500 I.U. of vitamin D per day resulted in rapid healing of severe rickets[7,10] provides strong evidence concerning the probable effectiveness of the doses proposed for prophylaxis.

The formerly high incidence of rickets among Negro infants living in the large metropolitan areas of the northern United States gave rise to the impression that sunshine was less effective in preventing rickets in individuals with deeply-pigmented skins; however, sociologic factors made this difficult to prove. On the other hand, data in the American literature supports the opinion that the amount of ingested vitamin D required to prevent[10,11] or to cure[10] rickets in Negro infants does not differ from the requirements of white infants.

The manner in which other factors, such as chronic infection, metabolic disease, and rapid skeletal growth influence vitamin D requirement is not yet accurately known. In particular, there has been argument regarding the relation between calcium intake and vitamin D requirement. Clearly, it is necessary to provide sufficient dietary calcium to meet the requirements of the growing skeleton. Similarly, sufficient vitamin D must be provided to prevent rickets and to promote satisfactory calcium retention. However, having satisfied the minimum needs in these two respects, there is no reason to postulate any specific relation between D requirement and calcium intake.

This principle is of special importance in considering the vitamin dosage of the breast-fed infant, since, per calorie, the calcium content of human milk is approximately one-third to one-quarter that of a cow's milk formula.[21] However, both from theoretical considerations[18] and practical observation, the calcium requirements of the normal full-term infant are satisfactorily met by human milk, and there is no reason to increase the prophylactic dose of vitamin D to more than 400 I.U. per day for the breast-fed infant.

In many parts of Europe, the custom of giving daily vitamin supplements to babies has never been established. In these regions, it is the practice to administer to infants, usually under direct medical supervision, single large oral or intramuscular doses (Stossen) of vitamin D at intervals. Individual doses of 300,000 I.U. at intervals of 1½ to 3 months have been suggested.[19] The Committee endorses the view of Lightwood and Stapleton[20] that such administration of vitamin D is unphysiologic and has no useful place in countries where daily vitamin D prophylaxis has become established practice.

The Premature Infant

Although the various techniques used to determine the vitamin D requirement of full-term infants have been applied to premature infants, it has been more difficult to establish the requirement. In part at least, this has been because of the relatively greater requirement for calcium. The skeleton of the infant born 6 weeks prematurely contains approximately half as much calcium as that of the full-term infant.[21] Clearly, it is axiomatic that the premature infant should be given sufficient calcium to satisfy the needs of the rapidly growing skeleton. Since the average accretion of calcium during the last 2 months of fetal life has been estimated at approximately 200 mg per day,[18] an intake of calcium that will permit retention of this amount must be provided. The relatively low calcium content of human milk makes such retention unlikely, even when the intake of vitamin D is sufficient to permit maximal intestinal absorption. Disregard of this principle by early investigators[22] may explain the statement that premature infants frequently developed rickets when receiving intakes of vitamin D as great as 5,000 I.U. per day.

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More recent studies in which large groups of formula-fed premature infants were observed clinically, roentgenographically, and biochemically indicate that rickets could usually be prevented and normal calcium and phosphorus homeostasis maintained with vitamin D intakes as low as 100 to 200 I.U. per day.[12,23] Rates of growth in body length are reported to have been as rapid with intakes of 100 to 200 I.U. daily as with intakes of 400 to 1,200 I.U. daily.[12,23] Recent studies[24] re-emphasize the necessity of commencing vitamin D prophylaxis within the first 2 weeks of life.

The Pre-school and School Child

There is no doubt that vitamin D is required beyond infancy, since nutritional rickets occurs occasionally in older children.[5,25] However, the requirement of vitamin D is more difficult to ascertain at this age than in infancy because of the slower rate of skeletal growth, the greater opportunity to ingest foods fortified with vitamin D, and the tendency to receive more exposure to sunlight. Because clear-cut evidence of rickets occurs so rarely beyond infancy, this manifestation cannot be employed as a dependable index by which to determine vitamin D requirement in this age group. On the basis of unpublished calcium retention studies, Stearns estimated that the requirement of growing children is of the order of 400 I.U. per day.[26] There is no question that this intake provides an ample supply of vitamin D for normal children. However, it should be recalled that before the current trend toward widespread vitamin D enrichment of food, a balanced diet and outdoor play practically always provided sufficient vitamin D to prevent detectable evidence of vitamin D deficiency even though, in a great many instances, the

youngsters had not received any vitamin supplements, and the computed intake of vitamin D was considerably less than the figure recommended above.

Obviously, additional data are needed to delineate the minimal requirement in this age group.

The Adolescent

The determination of the minimal vitamin D requirement of the adolescent presents the same problems as those met for children. Some years ago, it was considered that rapid skeletal growth during this period necessitated a considerably greater intake of vitamin D (up to 3,900 I.U. per day).[27] However, when studies were made in healthy adolescents under conditions of optimal calcium and phosphorus intake, maximal calcium retention was achieved with intakes of 400 I.U. per day.[28]

The Adult

Calcium retentions have been measured in a number of healthy adults consuming average unfortified diets with standardized intakes of calcium and varying supplemental intakes of vitamin D. In none of these studies was a consistent difference in calcium retention detected before and during the administration of vitamin D supplements.[29] For this reason it has sometimes been implied that healthy adults do not require vitamin D, and no allowances are recommended by the various national committees (Table I).

From physiologic considerations, this is probably not strictly correct. It is more likely that the amount of vitamin D needed for the maintenance of normal calcium and phosphorus metabolism in adults is so low that the requirement is readily met by the average diet and by casual exposure to sunlight. The hypothesis of a small requirement is strengthened by a number of reports of adults with osteomalacia attributed to vitamin D deficiency. One report mentions 15 nuns who developed radiographic and clinical signs of osteomalacia during World War II while confined to a convent under conditions of poor nutrition.[30] The other reports[31,32] record isolated cases, in each of which food faddism had led to extremely low intakes of vitamin D containing foods. In one case[33] the vitamin D intake was estimated to be less than 20 I.U. per day. Although the bizarre diets were also extremely low in calcium, it is probable that vitamin D deficiency was the important factor, particularly since there is evidence that low calcium intakes per se lead to osteoporosis rather than to osteomalacia.[34]

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RECOMMENDED DIETARY ALLOWANCES FOR VITAMIN D, (I.U./DAY)

Age Group	U.S.A.*	Canada†	Great Britain‡
Infants			
Premature	400	400–800§	.. (800‖)
0–24 mo	400	400	800 (400‖)
Children			
2–18 yr	400	400	400
Adolescents (male & female)			
13–19 yr	400	400¶	400
Adults			
Male	..	..	..
Female			
Nonpregnant	..	..	..
Pregnancy, 1st half	..	..	400
Pregnancy, 2nd half	400	400	800
Lactation	400	400	800

* Total daily vitamin D intake to maintain good nutrition in healthy persons (not only in the average person but in substantially all normal persons) in the United States under current conditions of living. The recommendation is meant to afford a margin of sufficiency above minimal requirements and to provide a buffer against added needs of various stresses other than disease or nutrient repletion. (Food and Nutrition Board, National Academy of Sciences—National Research Council: Recommended Dietary Allowances; 1958; Publication 589, Washington, D.C.)

† Recommended total daily vitamin D intake to maintain good nutrition of healthy persons and to provide a margin of safety. (Canadian Bulletin on Nutrition; A Dietary Standard for Canada; Vol. 3, No. 4, Ottawa, August, 1955.)

‡ Total daily vitamin D intake "believed sufficient to establish and maintain a good nutritional state in representative individuals of the groups concerned. It is recognized that in every group there must be cases where the need is greater than that of the average." (British Medical Association; Nutrition Committee; Report; 1950, London.)

§ Recommendation of Medical Research Council Conference on Hypercalcemia in Infants (1956). Not published. Quoted in Reference 5.

‖ Recommendation of Joint Sub-committee on Welfare Foods (1957).[5]

¶ Adolescents 13–15 years.

The Pregnant and Lactating Woman

The reports of osteomalacia in pregnant and lactating Chinese women[35,36] constitute

evidence that these states impose physiologic stresses upon the normal calcium and phosphorus metabolism of the adult. Although the calcium content of the diets of the women was poor, the osteomalacia responded to vitamin D rather than to extra calcium. These studies have therefore been taken to indicate that amounts of vitamin D greater than those needed by the normal adult are required for optimal nutrition during the last trimester of pregnancy and during lactation. However, data regarding the actual requirements are lacking.

In addition, provision should be made in the diet for the extra calcium required by the growing fetus (approximately 200 mg are deposited per day during the last 2 months of pregnancy) and by lactation (approximately 200 to 300 mg are excreted per day).[33]

Summary: Recommended Allowances at Various Ages

Allowances currently recommended in the United States, Great Britain, and Canada are shown in Table I. The recommendations are seen to be in reasonably close agreement. As indicated, there are minor differences in the terms of reference. However, it should be noted that in all instances the figures are intended to be taken as representing the total recommended daily intake of vitamin D from all ingested sources, assuming no exposure to ultraviolet radiation, assuming the consumption of adequate quantities of calcium and phosphorus (values about which, incidentally, there is still much argument[34]) and incorporating a safety factor beyond the minimum daily requirement of the normal individual.

The Committee on Nutrition believes that available data support the view that for full-term infants fed cow's milk formulas or human milk a total intake of 400 I.U. vitamin D per day from all dietary sources is a suitable allowance to prevent rickets and to promote optimal calcium and phosphorus metabolism. The same dose, perferably provided as a water miscible suspension,[31] is considered to be suitable for premature infants fed commonly employed formulas of cow's milk. The higher recommendation of the British authorities (Table I) is intended to provide an additional safety factor, but there does not appear to be scientific proof that the extra amount is actually more effective. For both full-term and premature infants the range of individual variation has been well investigated. It may be assumed that this dose provides a moderate safety factor, and that few, if any, normal infants will develop rickets while receiving such an intake. Vitamin D prophylaxis should be commenced within the first 2 weeks of life.

There is no question that in certain locales and under proper conditions prophylactic amounts of vitamin D can be acquired from sunlight during the summer months. However, to cover all contingencies, the Committee considers that an attempt should be made to ensure the provision of the 400 I.U. allowance from ingestible sources on a year-round basis.

Available data do not provide accurate estimates of the minimum requirement beyond the period of infancy. For children and adolescents, the Committee recommends a total dietary intake of 400 I.U. of vitamin D per day from all sources as a safe allowance. As indicated below, in the United States such an intake will ordinarily be acquired from fortified foods without administration of a vitamin supplement. In Canada, the same situation frequently obtains in individual cases, but cannot be so generally relied upon.

The small requirement of the healthy adult is satisfactorily met by casual exposure to sunlight and by the consumption of usual unenriched foods, and no vitamin D supplementation is necessary unless specifically indicated by reason of occupation or other unusual circumstance. A total daily intake of 400 I.U. of vitamin D is considered adequate to provide for added demands during the second half of pregnancy and during lactation.

It is the opinion of the Committee that there is no necessity to exceed the recom-

mended allowance during convalescence from acute infections. Similarly, for most chronic conditions, the recommended allowance is probably adequate.

THE DANGERS OF VITAMIN D

Notwithstanding lack of knowledge concerning minimal requirements, the Committee believes a daily intake of 400 I.U. of vitamin D can be expected to prevent detectable vitamin D deficiency in the normal infant or child consuming adequate amounts of calcium. Furthermore, there is no evidence to indicate that larger amounts of vitamin D would be more effective in any way. On the other hand, because nowadays many infants and children undoubtedly receive more than 400 I.U. of vitamin D daily, and because there is incontrovertible evidence that prolonged intake of vitamin D in large doses produces hypercalcemia with its numerous sequelae, it has become imperative to define the safe upper limits of vitamin D intake.

Many years ago Hess and Lewis[38] demonstrated that very large doses of vitamin D would produce hypercalcemia. Since that time hypervitaminosis D has been reported in many individuals, a considerable number of whom were infants and children. There appears to be a fairly wide range of susceptibility to the toxic actions of vitamin D and it has been estimated that approximately 20% of normal adults who receive 100,000 I.U. of vitamin D daily for several weeks or months will develop hypercalcemia.[39] Hypercalcemia is accompanied by anorexia, nausea, weight loss, polyuria, constipation, and reversible azotemia. If hypercalcemia continues generalized vascular calcification, nephrocalcinosis and irreversible renal insufficiency eventually supervene. In terms of unit body weight, dosages in the region of 1,000 to 3,000 I.U./kg/day for several weeks or months are toxic in the adult, and evidence suggests that this unit dose manifests approximately the same degree of toxicity in infants under 2 years of age.[39] For a 1-year-old infant, this amount of vitamin D would represent a total dose of between 10,000 and 30,000 I.U. per day.

The Risk of Vitamin D Toxicity in Infancy

The dosages mentioned above are at least 25 times greater than the prophylactic requirement. On the other hand, Jeans & Stearns[40] suggested in 1938 that daily intakes of vitamin D in the range of 1,800 to 6,300 I.U. inhibited linear growth of normal infants and were therefore undesirable. Because the series of infants was small, and because considerable variability was observed in the individual growth patterns of experimental and control subjects, the data are difficult to interpret. The study has not been confirmed by other workers, and there has been little concern until relatively recently about administering prophylactic doses of vitamin D in the order of 2,000 I.U. daily.

However, in 1952 reports from Great Britain and Switzerland drew attention to a small group of infants with unexplained hypercalcemia.[41-47] Shortly after the initial reports were published, cases of infantile hypercalcemia commenced to be diagnosed in increasing numbers throughout the British Isles. In its rare severe form[42] hypercalcemia was associated with mental retardation, osteosclerosis, severe azotemia and other bizarre features. In its relatively more common mild form,[43] which typically commenced between the second and tenth month of life, there was fairly sudden onset of anorexia, vomiting, polydipsia, weight loss, and failure to thrive, and the serum calcium was elevated above 12 mg/100 ml (often to 15 mg/100 ml or higher). Mild, usually reversible azotemia frequently developed.

Because many of the features resembled those of hypervitaminosis D, it was logical that attention should be focused upon the possibility that vitamin D was implicated in the pathogenesis of this new and important health problem. It was found that the hypercalcemia was controlled when all sources of vitamin D were withheld and a very low

calcium intake instituted, although the additional features of the severe form did not disappear.

In 1953 and 1954 at a time when approximately 100 new cases of hypercalcemia were being diagnosed annually in Great Britain, an extensive survey of infant feeding practices in Great Britain revealed that, as a result of the then liberal enrichment of national dried milk powder and infant cereals with vitamin D, a normal infant consuming an average diet plus the recommended daily supplement of vitamin D (at that time 700 to 800 I.U.) might readily ingest 4,000 I.U. of vitamin D per day.[43]

On the other hand, idiopathic hypercalcemia cannot be explained solely on the basis of excessive vitamin D intake. For one thing, it was noted that not all affected infants were ingesting as much as 4,000 I.U. of vitamin D per day. For instance, in a study of 38 cases from Glasgow, 26 (two-thirds) had received less than 2,000 I.U. per day, and 8 (one-fifth) had received less than 1,000 I.U. per day.[44] Further, it should be recalled that, of the large number of British infants who during one period are presumed to have ingested 3,000 to 4,000 I.U. per day, relatively few suffered any detectable ill effects.

To account for some of the contradictory findings, it has been suggested that patients with idiopathic hypercalcemia may be hypersensitive to vitamin D[45] or, alternatively, that they may have an inborn disturbance in cholesterol metabolism[46] which causes the accumulation of vitamin D or of some abnormal metabolite with vitamin D-like action.[47,48] The fact that none of the affected infants had been entirely breast-fed[48] suggests that the higher calcium content or some other feature of cow's milk may also be a factor.

Although it is obvious that infants who develop hypercalcemia differ in some fundamental way from normal infants, it is hard to escape the still-circumstantial evidence which appears to relate the condition with vitamin D. As a result of recommendations of the British Paediatric Association Committee on Hypercalcaemia[45] and of the Joint Subcommittee on Welfare Foods,[5] measures were taken to reduce the average intake of vitamin D of British infants by halving the potency of National Cod Liver Oil and by considerably reducing the amounts of vitamin D added to dried milk powders and to infant cereals. It has been estimated that the average intake of vitamin D has probably been reduced to between one-third and one-half the former quantity as a result of these measures.

Obviously, careful evaluation of the present incidence of idiopathic hypercalcemia in Britain is essential before conclusions can be drawn regarding the importance of vitamin D in the etiology of the condition. Unfortunately, this information is not yet accurately known. However, according to annotations in the *British Medical Journal*[49] and in *Lancet*[50] in 1960, many pediatricians believe that the incidence of the disease has fallen since the recommendations regarding vitamin D intake were carried out. It is very tempting therefore, to suggest that vitamin D and idiopathic hypercalcemia are causally related. On the other hand, because it is not yet possible to weigh the many complex factors, it seems unwarranted to draw final conclusions regarding the etiologic role of vitamin D until the results become available of a comprehensive study which is currently being undertaken by the British Paediatric Association Committee on Hypercalcaemia.

ESTIMATES OF VITAMIN D INTAKES IN THE UNITED STATES AND CANADA AT VARIOUS AGES

The large number of vitamin preparations and of commonplace commercially-fortified foodstuffs on the North American market provide a variable and virtually unavoidable intake of vitamin D which, though difficult to estimate, may often be considerable. Table II gives information concerning some of the foods currently enriched with vitamin D in the United States and Canada. Because of frequent changes by the manufacturers, this information is

TABLE IIA

VITAMIN D ADDED TO FOODS IN U.S.A.

Food	*Product*	*Manufacturer and Trade Name*	*Approximate Vitamin D Content Claimed (I.U./unit measure)*	
Milks	Dairy Milk	Fortified (approx 85% total sales)	1 U.S. quart	400
	Evaporated	All brands	1 U.S. quart (reconstit.)	400
	Powdered	Klim (whole) & most powdered infant formulas	1 U.S. quart (reconstit.)	400
		Skim milks (powdered)		0
Milk flavorings	Powders	Ovaltine (plain or chocolate)	1 glass (approx. 15 gm)	150
		Hershey—Instant Cocoa Mix	1 glass (approx. 15 gm)	150
		Nestle—"Quick"	1 glass (approx. 20 gm)	100
		Borden's Dutch Chocolate	1 glass (approx. 15 gm)	100
		Baker's Instant Choc. Mix	1 glass (approx. 20 gm)	100
	Syrups	Bosco	1 glass (approx. 20 gm)	150
		Cocoa Marsh	1 glass (approx. 15 gm)	50
Margarine		Parkay, Kraft	1 oz	250
		All-Sweet, Blue Bonnet, Fleishman's, Good-Luck, Imperial, Mazola	1 oz	125
Breakfast cereals		Kellogg—All Bran	1 serving (1 oz)	400
		Cornflakes		200
		Special K		200
		Sugar Pops		200
		Pep		200
		Concentrate		200
		General Mills—Hi-Pro		150
		Quaker—Life		80
		Coco-Wheats		80
Miscellaneous	Bread	Special Sunbeam Bread (a premium product only)	1 slice	100
	Candy	Swiss Bar	1 bar	400
	Beverage	Stuart's Instant Vita Drink	1 glass (1 teaspoon powder)	1,000

only approximate. The recorded figures are computed from the manufacturers' label claims and represent minimum values, since it is customary manufacturing practice to add an "overage" to allow for possible deterioration, etc. Overage may constitute as much as 100% or more of the label claim in the United States. In Canada, regulations prescribe that a given food may contain not less than 400 or more than 800 I.U. of vitamin D in a reasonable daily intake of that food,[21] but despite the regulations, the overage permitted within the limits of good manufacturing practice undoubtedly remains a factor to be considered.

There are certain differences between the United States and Canadian figures. In the United States, vitamin D is added to virtually all evaporated milk in a concentration of 400 I.U. per reconstituted U.S. quart, and a similar amount is added to 85% of all dairy milk marketed in the United States (although the latter figure may decrease as a result of the recent re-introduction of unfortified dairy milk by certain supermarkets). In Canada, all brands of evaporated milk contain approximately 600 to 800 I.U. vitamin D per reconstituted Imperial quart (i.e., 500 to 700 I.U. per reconstituted U.S. quart). However, in contrast to custom in the United States, less than 1% of dairy milk on the Canadian market is enriched

TABLE IIB

VITAMIN D ADDED TO FOODS IN CANADA

Food	*Product*	*Manufacturer and Trade Name*	*Approximate Vitamin D Content Claimed (I.U./unit measure)*	
Milks	Dairy milk	Unfortified (approx. 99% total Canadian sales)		None added
		Irradiated or fortified (1% total sales)		400
	Evaporated	All brands	1 Imperial quart (1.2 U.S. quart)	600-800
	Powdered milk	Klim (whole milk powder)		500
		Milko (skim milk powder)		800
		(skim+chocolate)		800
		Instant-Sweet (skim)		700
Milk flavorings	Powders	Cadbury: "Choco"	1 glass (approx. 18 gm)	150
		Ovaltine: plain & chocolate	(approx. 18 gm)	150
		Nestle: "Quik"	(approx. 15 gm)	150
		Bordens: Instant Dutch	(approx. 20 gm)	200
		Instant Toddy	(approx. 20 gm)	200
		J.G.A.: Instant Chocolate	(approx. 15 gm)	150
	Syrups	Bosco	(approx. 20 gm.)	200
		Cocoa Marsh	(approx. 15 gm)	200
Margarine		All brands	1 oz	250-310
Breakfast cereals		Kellogg: Special K	1 oz	400
		Corn flakes		400
		Sugar Pops		400
		Sugar Frosted Flakes		400
		Pep		400
Biscuits		Sun Wheat	1 biscuit	50
Beverages		Happy Henri: Apple drink	8½ oz	200
		Happy Henri: Breakfast drink	8½ oz	200

with vitamin D. For this reason, a considerable number of Canadian babies will fail to receive sufficient amounts of vitamin D in their food, and in a certain proportion of these, rickets may be expected to develop unless vitamin D is given as a supplement or by exposure to sunlight.

By constructing hypothetic menus, and using the information contained in Table II, an estimate has been made in Table III of the amounts of vitamin D that might be ingested under present-day living conditions in the United States and Canada. Two diets have been constructed for each of three age groups, one selected to represent an arbitrarily average situation, the other to represent a situation in which the intake is unquestionably, but not unrealistically high.

In computing the total intakes of vitamin D the amount administered as a vitamin supplement must be included. For several concentrated drop preparations, the daily amount recommended by the manufacturer contains from 800 to 1,000 I.U., but most products now contain 400 I.U. per recommended dose. Most of the candy-type multiple vitamin preparations available for older children in both the United States and Canada contain 400 I.U. per lozenge. Most multiple vitamin capsules intended primarily for adults contain 1,000 I.U. vitamin D or more per capsule.

A glance at Table III will indicate that the well-cared-for infant, receiving an

evaporated milk formula and vitamin supplement (as is customary in North America) will automatically ingest two to four times the recommended daily allowance of vitamin D without taking the question of overage into account. However, it seems unlikely, under present circumstances, that a significant proportion of the infant population in either the United States or Canada would obtain as much vitamin D as many British babies were estimated to have received before the present restrictive measures were implemented. On the other hand, the well-cared-for toddler in North America, still receiving a vitamin supplement, is likely to receive at least three times the recommended allowance and the daily intake might reasonably exceed 2,000 or even 3,000 I.U. For the older child, even higher intakes can be envisaged, especially if he regularly receives one of the candy-like vitamin preparations or has access to a vitaminized candy bar or beverage.

Admittedly these hypothetic examples have no validity as estimates of the national vitamin D intake. They were selected merely to emphasize the diversity of foods that are artificially enriched, and the unexpected ease with which vitamin D can now be acquired in both the United States and Canada.

COMMENT

From the foregoing discussions, it is evident that vitamin D is essential for the maintenance of normal calcium and phosphorus metabolism in humans. On the basis of present evidence, it is considered that a total intake of 400 I.U. of vitamin D per day provides an adequate margin of safety

TABLE III

HYPOTHETIC TOTAL DAILY INTAKES OF VITAMIN D

Age of Child	Intake	Amount of Vitamin D Contributed by Individual Food*									Total Intake I.U./day	
		Vitamin Supplement	Milk, Liquid	Milk, Evaporated	Milk, Powder	Milk, Flavoring	Margarine	Breakfast Cereal	Special Breakfast Drink	Special Candy Bar or Biscuit	Average	High
A. Children in United States												
6 mo	Average	400	..	400	..	..	..	..	..	..	800	..
	High	1,000	.	400	..	..	..	..	..	..	..	1,400
3 yr	Average	(1,000)	500	..	..	..	100	200	..	..	800 (1,800)	..
	High	1,000	400	..	..	450	550	400	..	(400)	..	2,800 (3,000)
8 yr	Average	(400)	400	..	..	..	200	200	..	..	800 (1,200)	..
	High	1,000	400	..	..	600	500	400	(1,000)	(600)	..	2,900 (4,700)
B. Children in Canada												
6 mo	Average	400	..	800	..	..	..	..	..	..	1,200	..
	High	1,000	..	1,000	..	..	..	..	..	..	..	2,000
3 yr	Average	(400)	95	..	..	..	275	..	..	(100)	300 (900)	..
	High	1,000	..	..	800	450	400	400	200	(150)	..	3,950 (8,400)
8 yr	Average	(400)	95	.	..	..	275	400	..	..	700 (1,100)	..
	High	1,000	..	..	800	600	500	800	400	(150)	..	4,100 (4,250)

* When values appear in parentheses, total intake of vitamin D has been calculated twice; once including and once excluding these values

to protect substantially all normal growing individuals from detectable evidence of vitamin D deficiency.

At the same time, it is known that excessive amounts of vitamin D are toxic. The low limit of toxicity is not clearly defined, but British experience with the problem of idiopathic hypercalcemia suggests that this toxic level may, on rare occasions, be as low as 3,000 to 4,000 I.U. per day for susceptible individuals in the infant age group, and sometimes considerably lower.

Until relatively recently, the average diet contained very little vitamin D and could not necessarily be relied upon to prevent vitamin D deficiency during growth, particularly during infancy. The use of vitamin D supplements was therefore promoted, and there was widespread acceptance of this public health measure throughout North America. However, when it became evident that rickets had not been entirely eliminated by this measure, the decision was made to enrich commercial milks with vitamin D, and the outstanding success of this program gave impetus for the addition of this and other vitamins to a variety of foods. At the present time, a wide range of common foodstuffs contain added vitamin D, and it is obvious that the over-all vitamin D intake of all ages of the population has risen appreciably in the United States and Canada. Calculations indicate that the amount of vitamin D now acquired from the diet alone may readily exceed the recommended daily allowance, sometimes by a considerable amount.

Although the exact cause of idiopathic hypercalcemia of infancy remains to be established, there is now a large amount of evidence which incriminates vitamin D as an important etiologic factor. Despite the fact that infantile hypercalcemia has, until now, been much less common on this continent than in Great Britain, more than 30 cases have already been reported in North America since 1954 and existence of a number of unpublished cases is known to members of the Committee.

Thus, in the face of existing evidence, the Committee on Nutrition considers that practitioners and public health authorities should strive to ensure that the total vitamin D intake of all infants reaches the recommended allowance of 400 I.U. per day, but at the same time, they should make concerted efforts to restrict the upper limit of intake for all sources to an amount as close to this figure as is practicable. This demands that cognizance be taken of the amount of vitamin D contributed by the formula and other components of the diet before prescribing a vitamin D supplement, and that attention be paid to the amount of vitamin D provided by the supplement chosen.

It demands, also, that efforts be intensified to dispel from the minds of parents the concept of vitamins as tonics, with the hope of reducing the all-too-prevalent practice of vitamin D "over-insurance" in the home.[12] Although there is no specific evidence that daily intakes of vitamin D in the order of 2,000 to 3,000 I.U. produce deleterious effects on individuals beyond infancy, it should be realized that the present trend in nutrition is imposing a new situation upon the children and adults of North America, the long-term effects of which are entirely unknown.

The addition of vitamin D to practically all commercial milk supplies in the United States has been amply justified by the dramatic reduction which it brought about in the incidence of rickets. By inference, similar benefits would presumably be observed in Canada by broadening the present milk-fortification program to include dairy milk. On the other hand, the milk consumption habits of North American children ensure a uniformity and dependability of intake which does not apply to other foodstuffs. Hence, the enrichment of a large variety of additional foods with vitamin D cannot be similarly justified.

SUMMARY AND RECOMMENDATIONS

Despite inadequacies in information concerning the minimum prophylactic requirement of vitamin D for all age groups be-

yond infancy, there is no doubt that a total intake of 400 I.U. per day is adequate to prevent vitamin D deficiency in substantially all normal children from birth through adolescence.

Evidence derived from the study of idiopathic hypercalcemia suggests that certain infants excessively sensitive to the toxic action of vitamin D may, on rare occasions, be adversely affected by daily intakes of 3,000 to 4,000 I.U. and sometimes considerably less. Because of the prevalent practice of food fortification in the United States and Canada, there is now a definite possibility that the individual, even the young infant, may ingest considerably more than the recommended vitamin D allowance, and intakes of 2,000 to 3,500 I.U. per day are possible, particularly beyond infancy. Although there has been no specific evidence that intakes of this order produce deleterious effects beyond infancy, it is pointed out that the long-term consequences of this new nutritional situation on older children or adults are entirely unknown.

In view of these considerations the Committee on Nutrition recommends that efforts be taken to ensure a total vitamin D intake of 400 I.U. per day by all infants and children. At the same time, an attempt should be made to restrict the intake from all sources to an amount not greatly in excess of this figure.

The value of carefully planned enrichment of commercial milk supplies with vitamin D has been clearly demonstrated. However, the present practice of enriching foods other than milk and infant formula products is not justified, and discontinuation of this practice is to be recommended. Under the present circumstances, cognizance should be taken of the amount of vitamin D acquired by the individual from food sources before prescribing a vitamin D supplement.

In keeping with present concepts regarding vitamin D requirement, commercial vitamin D supplements should be adjusted to contain not more than 400 I.U. per dose.

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VITAMINS

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In an editorial (May, C. D.: Pediatrics 23:883, 1959) this report has been well summarized as follows: "A normal infant requires no more than 30 mg vitamin C and 400 units vitamin D in supplementation of his mother's milk or his artificial formula. (Some prepared formulas include enough of these vitamins.) Normal children should receive 400 units vitamin D, either as a supplement or in vitamin D-fortified milk. Supplementary vitamin A and the B complex are not recommended for ordinary feeding practice, but only in exceptional circumstances of disease and special problems. A supplement is a supplement and not the whole source of supply."

A.A.P. Committee on Nutrition: *Appraisal of the Use of Vitamins B_1 and B_{12} as Supplements Promoted for the Stimulation of Growth and Appetite in Children.* Pediatrics, 21: 860-864, 1958.

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It is concluded that intakes of vitamin D need not be greater than 400 I.U. daily for infants, including premature infants, children and adults. The possible hazards of high doses are discussed.

A.A.P. Committee on Nutrition: *Vitamin K Compounds and the Water-Soluble Analogues. Use in therapy and prophylaxis in pediatrics.* Pediatrics, 28: 501-507, 1961.

Administration of vitamin K_1 (phytonadione), 0.5 to 1.0 mg parenterally of 1.0 to 2.0 mg orally, as a single dose soon after birth is recommended as prophylaxis against hypoprothrombinemia of newborn infants.

A.A.P. Committee on Nutrition: *Vitamin E in Human Nutrition.* Pediatrics, 31: 324-328, 1963.

When dietary intake of fat is markedly reduced (as is sometimes practiced in feeding premature infants) and in the case of prolonged steatorrhea from any cause, it seems desirable to provide dietary supplements of vitamin E. For other individuals, diets ordinarily consumed are believed to provide adequate amounts of vitamin E.

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Circulation 28:653 (1963)

Rapid Production of Atherosclerosis in the Rat

Paris Constantinides, Vancouver, Canada

It is well known that the rat is unusually resistant to the development of true atherosclerosis (with intimal proliferation and foam cells) in response to chronic lipemia alone. Attempts to increase atherogenesis in this species by combining chronic lipemia with systemic ar-

terial injury have so far yielded rather disappointing results, largely because the vasotoxic agents employed in such experiments usually cause extensive medial necrosis and lipoidosis as well as myocardial necrosis and, therefore, a very high mortality. We have recently found that one can produce within 6 weeks true intimal foam cell lesions in rat arteries (particularly coronaries)—with negligible myocardial damage and mortality—if one induces some lipemia during the first 2 weeks with the help of a cholesterol-thiouracil-cholate diet, insults the arteries for only 3 days with viostorol during the third week, and continues the same lipemia-producing diet for another 3 weeks before killing. It appears that the main reason for the success of this particular procedure was the fact that by first adapting the rats to relatively harmless steroids such as cholesterol and bile acids, we increased their general resistance to a much more toxic steroid such as viostorol—thus causing minimal arterial injury and permitting maximal intimal hyperplasia and lipophagia.

Poultry Sci, 26: 30, 1947

The Effect of Different Levels of Manganese and Different Amounts of Vitamin D in the Diet of Hens and of Pullets

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MANGANESE was first recognized as an essential element in poultry nutrition by Wilgus and co-workers (1936, 1937). Almost simultaneously Lyons and Insko (1937) reported that the mineral was necessary for hatchability and for the prevention of nutritional chondrodystrophy. The literature on this subject is very ably reviewed by Wilgus, *et al.* (1939). This review pointed out that a manganese deficiency in poultry resulted in abnormal bone development (perosis), nutritional chondrodystrophy, ataxia, inferior growth, failure to maintain weight, lowered egg production, decreased hatchability, reduced egg-breaking strength and decreased egg-shell ash. The review also enumerated that there was a lower concentration of manganese in the egg, the embryo, the bones and certain organs of the body when a manganese deficient diet was fed. It appeared that the quantity of this mineral required by hens was from 40 to 50 parts per million.

Golding, Schiable and Davidson (1940) studied the manganese requirements of laying hens and used two breeds: White Leghorns and Barred Rocks, They reported a low incidence of chondrodystrophy in the embryos from White Leghorn pullets fed a low-manganese diet. Egg production was improved in both breeds studied by adding 50 parts per million of manganese to a basal diet containing only 9 parts per million of this mineral. Their results showed that there was a breed or at least a strain difference in the manganese requirements of laying birds for hatchability and possibly for egg production. Caskey, Norris and Heuser (1944) observed a chronic congenital ataxia in newly hatched chicks from hens fed manganese deficient diet.

The literature on the vitamin D requirements of hens is reviewed by Evans, Carver and Wilhelm (1944). These workers also studied the effect of different vitamin D levels with Single Comb White Leghorn pullets kept in a laying house and concluded that pullets fed 44 U.S.P. units of vitamin D per 100 grams of diet gave as good production and produced eggs with as good quality as pullets receiving diets containing 103 or 160 U.S.P. units of vitamin D. In a similar study with Single Comb White Leghorn pullets kept in laying cages for six months Evans and co-workers concluded that 60 A.O.A.C. chick units of vitamin D per 100 grams of diet was sufficient to maintain production and quality of the eggshell for this period of time.

It may be noted from the literature cited that pullets have been used in inves-

tigations dealing with the effect of manganese deficiency on laying birds and in studying the manganese requirements for egg production and hatchability.

There were three objectives in the investigation reported herein: (1) to study the effects of low, adequate and high levels of manganese with an adequate amount of vitamin D; (2) to study the effect of low, medium and adequate levels of vitamin D with a high level of manganese; and (3) to determine whether the requirements for manganese and vitamin D of hens in the second year of production are different from those of pullets in the first year of production.

EXPERIMENTAL

All birds used in the study to be reported here were New Hampshires of like breeding which had been selected for four generations for high hatchability and low embryonic mortality. The incidence of chondrodystrophic embryos from the hens and from the dams of the pullets was very low.

The pullets* and hens* were divided into six groups, each group containing twenty-one birds. The pullets and hens had access to direct sunlight and range during the summer and fall prior to the start of the experiment. The egg production and hatchability records of the dams of the pullets and of the hens were considered in making the distribution among the various groups. Two cockerels were placed in each pen and were rotated among the pens twice weekly. Mortality records were kept on the hens and the pullets. Birds dying were autopsied within twelve hours after death occurred.

* For the purpose of this paper pullets that were six to seven months of age at the start of the experiment will be referred to as "pullets" and hens that had finished their first laying year will be referred to as "hens."

The pullets and hens were housed in a laying house without access to direct sunlight and were kept under as nearly identical conditions as it was possible to maintain. Each pen had one ordinary glass window through which a small amount of ultraviolet light might be transmitted. Litter was not used on the floor of the pens. The duration of the experiment was 235 days.

Eggs were hatched each month throughout the experimental period. The eggs were candled and the approximate age of the embryos which failed to hatch was determined. All eggs, which failed to hatch, were broken at the end of the incubation period and the embryonic abnormalities were classified. Embryos classified as chondrodystrophic in this paper exhibited characteristics similar to those described by Lyons and Insko (1937) for nutritional chondrodystrophy.

Representative samples of eggs were collected during each month of the last 120 days of the experiment. These eggs were studied and grouped according to shell-quality grades using the method of Lyons (1939). Eggs, which when candled, that appeared to be intermediate between grades 2 and 4 (Figure 1) were recorded as grade 3. Grade 5 was likewise intermediate between grades 4 and 6 (Figure 1). Figure 1 is reproduced through the courtesy of the Arkansas Agricultural Experiment Station.

Both hens and pullets were fed a basal diet composed of 75.5 percent ground yellow corn, 3 percent dried whey, 5 percent dehydrated alfalfa leaf meal, 8.5 percent fish meal, 1 percent salt, 3 percent poultry bone meal and 4 per cent chick size oyster shell. This diet contained 15.89 percent protein, 3.82 percent fat, 2.34 percent crude fiber, 60.10 percent nitrogen-free extract, 11.89 percent water, 2.79 percent calcium, 0.82 percent phosphorus and 41

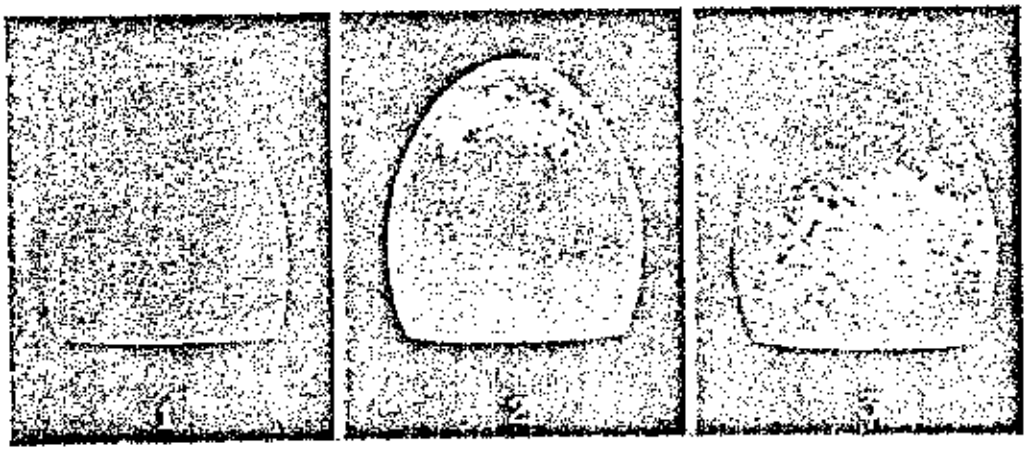

FIG. 1. The candling appearance of eggs of different shell-quality grades. (1) Shell-quality grade 2 (normal); (2) shell-quality grade 4; (3) shell-quality grade 6. These eggs were photographed in a photographic dark room, dimly lighted with a low-powered red glass bulb. The large end of the eggs was placed in the aperture of an upright lighted candler. (Photographed by A. R. Patton.) (Reproduced through the courtesy of The Arkansas Agricultural Experiment Station).

parts per million of manganese. The tap water supplied the birds to drink contained less than 0.02 parts per million of manganese.

In studying the effect of a low, adequate and high level of manganese with adequate vitamin D the first group of pullets and the first group of hens received the basal diet unsupplemented. The second, third and fourth group of pullets and hens received the basal diet supplemented with 30, 60 and 200 parts per million of manganese ($MnSO_4 \cdot 2H_2O$) respectively. All of the first four groups received 76 A.O.A.C. chick units of vitamin D per 100 grams of diet. Two additional groups of hens were fed the basal diet supplemented with 19 and 38 A.O.A.C. chick units of vitamin D per 100 grams of diet and with 200 parts per million of manganese.

It may be noted in the tables which follow that the data from the group of hens and pullets which received 200 parts per million of manganese and 76 A.O.A.C. chick units of vitamin D per 100 grams of diet is used in studying the effect of varying amounts of manganese and is also used in studying the effect of varying amounts of vitamin D.

RESULTS AND DISCUSSION

Egg Production

Hens of like breeding appear to require more manganese for egg production than do pullets (Table 1). The group of hens fed the basal ration, which contained 41 parts per million of manganese produced 37.0 eggs per bird during the experiment. Hens fed supplements of manganese produced from 56.6 to 64.4 eggs per bird. There was not an appreciable difference in the egg per bird produced by pullets fed the basal diet and those fed 30, 60 and 200 parts per million of manganese. From these data it appears that pullets in their first year of production do not require over 41 parts per million of manganese which was supplied by the basal diet. This is in agreement with previous work reviewed by Wilgus, *et al.* (1939). Hens in the second year of production appear to require between 41 and 71 parts per million of manganese for egg production.

Pullets in the first year of production

TABLE 1.—*Average number of eggs per bird, average number pounds diet consumed per bird and manganese content of eggs from hens and pullets fed varying amounts of manganese**

P.p.m. of manganese** added to basal diet***	Average number of eggs per bird		Average number pounds of diet consumed per bird		P.p.m. of manganese in dried egg yolk	
	Hens	Pullets	Hens	Pullets	Hens	Pullets
0	37.0	89.4	44.1	52.7	0.7	0.4
30	64.4	92.7	47.5	52.9	0.6	0.8
60	56.6	88.4	48.4	51.3	0.9	0.8
200	62.0	86.1	50.8	54.0	1.0	0.9

* 76 A.O.A.C. chick units of vitamin D per 100 grams of diet fed to all groups shown in this table.
** Manganese was added in the form of $MnSO_4 \cdot 2H_2O$.
*** The basal diet contained 41 parts per million of manganese.

appear to have a lower requirement for vitamin D than do hens in the second year of production (Table 2). Pullets fed 38 A.O.A.C. chick units of vitamin D per 100 grams of diet produced a smaller number of eggs per bird than those fed 76 A.O.A.C chick units of vitamin D per 100 grams of diet. From these data, it appears that pullets require from 38 to 76 A.O.A.C. chick units of vitamin D per 100 grams of diet for egg production. This is in agreement with the work of Evans and coworkers (1944). Hens fed 38 A.O.A.C. chick units per 100 grams of diet produced a decidedly smaller number of eggs per bird than did those fed 76 A.O.A.C. chick units of vitamin D per 100 grams of diet. From these data, it appears that hens require approximately 76 units of vitamin D per 100 grams of diet for egg production.

From table 2, it may be noted that hens fed 38 A.O.A.C. chick units of vitamin D per 100 grams of diet with 241 parts per million of manganese produced a larger number of eggs per bird than did the hens fed 41 parts per million of manganese with 76 A.O.A.C. chick units of vitamin D (Table 1). This suggests a supplementary relationship between manganese and vitamin D when a sub-optimum amount of the vitamin is fed.

Egg Analysis

Eggs were collected about one month prior to the close of the experiment and the manganese content was determined by the Chemistry Division of the Texas Agricultural Experiment Station (Table 1). There was in most cases an apparent relationship between parts per million of manganese in the dried egg yolk and the amount of manganese which the hens and pullets had been fed. This is in agreement with work previously reported and reviewed by Wilgus, *et al.* (1939). The

TABLE 2.—*Average number of eggs per bird, average number pounds diet consumed per bird and manganese content of eggs from hens and pullets fed varying amounts of vitamin D**

A.O.A.C. chick units of vitamin D per 100 gms. of diet	Average number of eggs per bird		Average number pounds of diet consumed per bird		P.p.m. of manganese in dried egg yolk	
	Hens	Pullets	Hens	Pullets	Hens	Pullets
19	25.1	73.4	47.1	50.1	0.9	1.0
38	44.0	81.5	45.9	53.7	1.3	1.6
76	62.0	86.1	50.8	54.0	1.0	0.9

* 241 parts per million of manganese was fed with each level of vitamin D.

TABLE 3.—*Distribution of shell-quality grades of eggs from hens and pullets fed varying amounts of manganese*

P.p.m. manganese* added to basal diet**	Total number eggs		2		3		4		5		6	
	Hens	Pullets	Hens %	Pullets %	Hens %	Pullets %	Hens %	Pullets %	Hens %	Pullets %	Hens %	Pullets %
0	293	524	13.3	24.0	19.5	32.5	17.0	22.5	21.2	13.8	29.0	7.2
30	570	675	36.5	36.7	32.1	33.6	20.9	18.1	6.8	6.8	3.7	4.8
60	423	568	26.0	36.1	32.9	37.5	15.8	20.6	14.4	3.9	10.9	1.9
200	442	459	23.8	26.8	34.6	42.5	26.2	22.2	8.8	5.0	6.6	3.5

* Manganese was added in the form of $MnSO_4 \cdot 2H_2O$.
** The basal diet contained 41 parts per million of manganese.

amount of manganese in the egg yolk (Table 2) did not appear to be correlated with the vitamin D added to the diet.

Mortality

The mortality within groups of hens and pullets could not be attributed to the manganese or vitamin D in the diet.

Shell-quality

Hens and pullets apparently required between 41 and 71 parts per million of manganese for the production of eggs with the best shell-quality (Table 3). This is in agreement with the work of Lyons (1939). There is an increase in the percentage of eggs in the shell-quality grades 2 and 3 and a decrease in the percentage of eggs in the shell-quality grades 5 and 6 when 30 parts per million of manganese was added to the diet of both hens and pullets (Table 3). The difference in the percentage of eggs in the shell grades 2, 3, 4, 5 and 6 between hens fed the basal diet unsupplemented and those fed the basal diet supplemented with 30 parts per million of manganese was greater than that of comparable groups of pullets. This again indicates that hens may require more manganese for the production of eggs of the best shell-quality. Additions of manganese to the basal diet above 30 parts per million did not tend to improve shell-quality of eggs from hens or pullets (Table 3).

Levels of vitamin D studied did not tend to affect the shell-quality of eggs from hens or pullets (Table 4). This does not agree with the work of Evans, *et al.* (1944) where it was reported that 60 A.O.A.C. chick units per 100 grams of diet was required for maintenance of egg production and eggshell-quality. It is believed that suboptimum levels of vitamin D failed to have an effect on eggshell-quality because of the interrelationship of manganese and vitamin D observed in this study.

TABLE 4. *Distribution of shell-quality grades of eggs from hens and pullets fed varying amounts of vitamin D**

A.O.A.C. chick units of vitamin D per 100 grams of diet	Total number eggs		2		3		4		5		6	
	Hens	Pullets	Hens %	Pullets %	Hens %	Pullets %	Hens %	Pullets %	Hens %	Pullets %	Hens %	Pullets %
19	151	430	40.4	31.9	29.8	30.7	17.2	23.5	8.0	9.0	4.6	4.9
38	419	405	34.1	34.1	34.4	32.1	20.1	24.7	8.1	5.2	3.3	3.9
76	442	459	23.8	26.8	34.6	42.5	26.2	22.2	8.8	5.0	6.6	3.5

* 241 parts of manganese per million was fed with each level of vitamin D.

Fertility and Hatchability

The fertility and hatchability of eggs from New Hampshire hens and pullets was increased by adding 30 parts per million of manganese to a basal diet which contained 41 parts per million of manganese (Table 5). This increase in fertility and hatchability was more pronounced in the hens than it was in the pullets. The fertility of eggs from hens fed the basal diet was 84.8 percent and of eggs from the pullets fed this diet was 87.7 percent. When the basal diet was supplemented with 30, 60 or 200 parts per million of manganese the fertility of eggs from hens varied from 90.5 to 93.6 percent and the fertility of comparable groups of pullets varied from 90.4 to 94.2 percent. The fertile eggs hatched from the hens fed the basal diet was 54.2 percent and total eggs hatched was 46.0 percent; from pullets receiving the same diet there was 68.9 percent of fertile eggs hatched and 60.5 percent of total eggs hatched. The fertile eggs hatched from the hens fed the basal diet supplemented with 30, 60 or 200 parts per million of manganese varied from 71.6 to 76.6 percent and the total eggs hatched varied from 64.8 to 71.7 percent; in comparable groups of pullets the fertile eggs hatched varied from 72.5 to 77.5 percent and the total eggs hatched varied from 66.5 to 70.0 per cent. The embryos which died prior to the eighteenth day of incubation from hens fed the basal diet was 16.8 percent and from hens fed the basal diet supplemented with 30, 60 or 200 parts per million of manganese varied from 11.4 to 11.5 percent. The percentage of chicks dead in the shell at the completion of the incubation period was decreased by adding 30 parts per million of manganese to the basal diet of the hens but was not decreased by adding it to the diet of the pullets.

TABLE 5.—*Hatchability data of hens and pullets fed varying amounts of manganese**

P.p.m. manganese** added to basal diet***	Total eggs set		Percent fertility		Percent fertile eggs hatched		Percent total eggs hatched		Percent dead embryos prior to 18th day		Percent dead in shell on 22nd day		Percent chondrodystrophy	
	Hens	Pullets	Hens	Pullets	Hens	Pullets	Hens	Pullets	Hens	Pullets	Hens	Pullets	Hens	Pullets
0	224	473	84.8	87.7	54.2	68.9	46.0	60.5	16.8	15.4	29.0	15.7	0.5	1.0
30	421	552	93.6	91.7	72.3	72.5	67.7	66.5	11.4	12.3	16.3	15.2	1.0	0.8
60	369	481	90.5	94.2	71.6	74.2	64.8	69.9	11.4	11.7	17.0	14.0	0.3	0.9
200	325	530	93.5	90.4	76.6	77.5	71.7	70.0	11.5	11.9	11.9	10.6	0.7	0.4

* 76 A.O.A.C. chick units of vitamin D per 100 grams of diet fed to all groups shown in this table.
** Manganese was added in the form of $MnSO_4 \cdot 2H_2O$.
*** The basal diet contained 41 parts per million of manganese.

There is a greater difference between the hatchability data of the hens fed the basal diet unsupplemented and those where the diet was supplemented with 30 parts per million of manganese than between comparable groups of pullets. This suggests again that hens may require more manganese than pullets for fertility and hatchability (Table 5). It appears that New Hampshire hens and pullets require between 41 and 71 parts per million of manganese for fertility and hatchability.

The incidence of chondrodystrophic embryos from hens varied from 0.3 to 1.0 percent and from pullets varied from 0.4 to 1.0 percent and did not appear to be related to the manganese content of the diet (Table 5). It has been mentioned earlier that the hens and pullets used in

this test had been selected for high hatchability for four generations and that the incidence of chondrodystrophy was very low in these blood lines. It is realized that the level of manganese in the basal diet, 41 parts per million, was high enough to prevent chondrodystrophy according to work previously reported, Wilgus, *et al.* (1939).

Hens appear to require more vitamin D for hatchability than do pullets (Table 6). The basal diet in this case was supplemented with 200 parts per million of manganese. There was an increase in the percentage of fertile eggs from hens with each increase in vitamin D fed (Table 6). The percentage fertility, percentage of fertile eggs hatched and percentage of total eggs hatched from hens increased with each addition of vitamin D to the diet. There was an increase in the percentage fertility, percentage of fertile eggs hatched and percentage of total eggs hatched from pullets where 38 A.O.A.C. chick units of vitamin D was added to the diet but a further increase was not noted with the addition of 76 A.O.A.C. chick units to the diet (Table 6). There was a decrease in the percentage of dead embryos prior to the eighteenth day with both hens and pullets fed 38 A.O.A.C. chick units of vitamin D as compared with those fed 19 A.O.A.C. chick units of vitamin D per 100 grams of diet. There was a decrease in the percentage of chicks dead in the shell at the end of the incubation period from hens fed 38 A.O.A.C. chick units of vitamin D per 100 grams of diet as compared with those fed 19 A.O.A.C. chick units of vitamin D per 100 grams of diet; this decrease was not so apparent in comparable groups of pullets. Further decreases, in the percentage of dead embryos prior to the eighteenth day and in the percentage of chicks dead in the shell were not observed either in the hen groups or the pullet groups when 76 A.O.A.C. chick units of vitmin D were added to the diet. The percentage of chondrodystrophy was low and did not appear to be related to the vitamin D content of the diet although a slight decrease in the percentage of chondrodystrophy was noted with each increase in the amount of vitamin D added to the diet.

TABLE 6.—*Hatchability data of hens and pullets fed varying amounts of vitamin D**

A.O.A.C. chick units of vitamin D per 100 grams of diet	Total eggs set		Percent fertility		Percent fertile eggs hatched		Percent total eggs hatched		Percent dead embryos prior to 18th day		Percent dead in shell on 22nd day		Percent chondrodystrophy	
	Hens	Pullets	Hens	Pullets	Hens	Pullets	Hens	Pullets	Hens	Pullets	Hens	Pullets	Hens	Pullets
19	131	479	80.3	86.4	49.5	71.3	39.7	61.6	28.6	15.5	21.9	13.2	2.9	0.5
38	263	411	90.9	95.1	73.6	80.3	66.9	76.4	12.6	9.2	13.8	10.5	1.3	0.0
76	325	530	93.5	90.4	76.6	77.5	71.7	70.0	11.5	11.9	11.9	10.6	0.7	0.4

* 241 parts of manganese per million was fed with each level of vitamin D.

A supplementary relationship between manganese and vitamin D is indicated from hatchability data of the hens when a sub-optimum amount of vitamin D is fed (Tables 5 and 6). Hens fed the basal diet with 76 A.O.A.C. chick units of vitamin D added had a lower percentage fertility, lower percentage of fertile eggs hatched, lower percentage of total eggs hatched, a higher percentage of embryos that died prior to the eighteenth day and a much higher percentage of chicks dead in the shell (Table 5) than hens fed 38 A.O.A.C. chick units of vitamin D per 100 grams of diet supplemented with 200 parts per mil-

lion of manganese (Table 6). The fact that 38 A.O.A.C. chick units of vitamin D per 100 grams of diet did not appear to meet the vitamin requirements of hens in this study was discussed above.

SUMMARY AND CONCLUSIONS

1. Hens in the second year of production appear to require more manganese and more vitamin D for egg production, for eggshell-quality and for fertility and hatchability than do pullets in the first year of production.

2. Pullets do not require over 41 parts per million of manganese in the diet for egg production.

3. Hens require approximately 71 parts per million of manganese for egg production.

4. Hens and pullets require between 41 and 71 parts per million of manganese for eggshell-quality and for fertility and hatchability.

5. Pullets require from 38 to 76 A.O.A.C. chick units of vitamin D per 100 grams of diet for egg production and a minimum of 38 A.O.A.C. chick units of vitamin D per 100 grams of diet for fertility and hatchability.

6. Hens require a minimum of 76 A.O.A.C. chick units of vitamin D per 100 grams of diet for egg production.

7. Amounts of vitamin D fed in this study did not affect eggshell-quality when an excess of manganese was fed.

8. There is an apparent relationship between the manganese content of dried egg yolk and the manganese content of the diet.

9. This study indicates that a supplementary relationship exists between manganese and vitamin D when an excess of the mineral is fed with a sub-optimum amount of the vitamin.

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Improvement of the Nutritive Quality of Foods

General Policies

The maintenance of a high level of nutritional health requires the continuous availability of a wholesome, nourishing food supply that can provide all of the essential nutrients in amounts sufficient to meet human needs. If the nutritional quality of foods is insufficient to meet these needs, means are available to enhance the quality by addition of specific nutrients. General policies and guidelines are desirable for such enhancement.

Recommended Dietary Allowances serves as a dietary standard of achievement in the United States and forms the basis for consideration of the improvement of the nutritive quality of foods.

Principles of Nutrient Improvement

Enrichment, restoration, and fortification illustrate the principles of the improvement of the nutritive quality of foods.

Enrichment is the addition of more than one nutrient to a food, such as a standard developed by the government for enriched bread, flour, and certain other cereal products. Restoration is the addition of nutrients to conventional foods in order to restore those nutrients that are present naturally, but have been destroyed or lost in processing. Fortification is the addition of one or more nutrients that were not present or were present in small amounts in foods before processing.

Efforts to improve the nutritional status of populations by introduction of new foods and by attempts to change food habits often have not been effective. Improvement of the nutritive value of widely used foods through enrichment, restoration, or fortification can provide good nutrition to populations at risk, without the necessity of extensive efforts to change food consumption patterns.

In estimating nutritional benefits that may be derived from the incorporation of specific nutrients in certain foods, the composition of the overall diet must be considered. The level of nutrient intake in the diet that is attained by such incorporation should not be normally excessive for those who consume the food in usual patterns of use. The addition of nutrients to foods commonly used should not put imbalanced diets for a major proportion of the population.

Foods can be altered in nutritive content during processing when this seems desirable. For example, foods can be produced containing varying proportions and types of proteins, fats, and carbohydrates. In the pro-

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duction of such foods, consideration of nutritional values such as protein quality must receive attention. The nutritional alterations should be appropriate for the types of food in question. Technological progress that is nutritionally and economically advantageous can provide significant benefits.

Classification of Foods

The rapid changes and innovations introduced by modern technology make the development of a universally applicable system of nomenclature of foods difficult. For the purposes of this article, foods will be classified as conventional, formulated, or fabricated.

Conventional foods are the traditional, primarily single entity foods identified as dietary staples. Formulated foods are mixtures of two or more foodstuffs or ingredients, other than seasonings, processed or blended together.

The two classes, conventional and formulated, adequately embrace all foods. For the appropriate discussion of nutritional improvement, however, it is desirable to consider a third class, fabricated foods. These are prepared principally from ingredients specifically designed to achieve a particular function not possible with common food ingredients. Fabricated foods may or may not be analogues of conventional or formulated foods. An analogue duplicates or approximates the structure or composition or both of a food as well as its role in the diet, e.g., imitation orange juice. Fabricated foods may be single substances or complex mixtures. An example might be foods derived from textured vegetable proteins.

Improvement of Conventional Foods

Good processing practices that conserve the nutritional values of foods should be encouraged. The preservation of nutrients natural to the food should always be an important consideration. Certain operative contingencies, however, may result in loss of substantial amounts of nutrients. Restoration of the lost nutrients is acceptable when the product in question is nutritionally important, that is when the amount of affected nutrients originally present provided at least 5% of the Recommended Dietary Allowances (RDA) in a serving. The added nutrients should be stable and biologically available, and the cost of restoration should be reasonable. Industry should constantly strive for improvement of processing techniques that conserve nutritional quality rather than depend on restorative addition.

In addition to nutrient addition as restoration, nutrients are sometimes added to conventional foods to accomplish specific nutritional objectives for the population. By way of illustration, nutrient additions defined as enrichment, fortification, and restoration have been endorsed by the Council on Foods and Nutrition and the Food and Nutrition Board. Specifically, the following practices in the United States continue to be endorsed:

> The enrichment of flour, bread, degerminated cornmeal, corn grits, whole grain cornmeal, and white rice (with thiamin, riboflavin, niacin, and iron); the restoration or restoration of thiamin, riboflavin, niacin, and iron in processed foods; and the addition of vitamin D to milk, fluid skim milk, and nonfat dry milk; the addition of vitamin A to oleomargarine, fluid skim milk, and nonfat dry milk; and the addition of iodine to table salt. The preventive action of fluoride against dental caries is recognized and the standardized addition of fluoride to water is endorsed in areas in which the water supply has a low fluoride content.

These practices meet sound principles that have been in effect for many years. The following criteria and guidelines for the nutritional improvement of conventional foods constitute these principles:

1. The intake of a nutrient considered for addition to food should be judged to be below a desirable level in the diets of a significant number of people.

2. The food that is to carry the nutrient should be consumed by the segment of the population in need and the added nutrient should make an important contribution to the diet.

3. The addition of the nutrient should not create a dietary imbalance.

4. The nutrient added should be stable under customary conditions of storage and use.

5. The nutrient should be physiologically available from the food.

6. There should be reasonable assurance that an excessive intake to a level of toxicity will not occur.

7. The additional cost should be reasonable for the intended consumer.

Improvement of Formulated Foods

Formulated foods, being mixtures of two or more foodstuffs or ingredients other than seasonings, include a very wide range of products. Examples are the so-called convenience foods formulated to resemble standard recipes but that require little or no preparation in the home, complete frozen dinners, meal replacers such as liquid meals, and prepared breakfast cereals. Snack foods may be formulated from conventional foods and become important when they displace serious quantities of nutrients in the diet.

The principles of nutritional improvement of formulated foods should be similar to those applied to conventional foods.

When the formulated food has no easily identifiable counterpart among conventional foods and contributes 5% or more of the RDA of calories or any essential nutrient in a serving, its composition becomes especially important. Under these circumstances, additions of nutrient should be made according to a concept of a calculated nutrient density.

Nutrient density is an expression of nutrient content in terms of [illegible] value of a serving of food as [illegible] to a standard such as the [illegible] "Application of General Policy" (p. 1115) for a discussion of a [illegible] standard) for nutrients and calories. For example, a serving containing 230 kilocalories might be [illegible] a calorie standard of 2,200 kilocalories/day. In this instance the food represents 10% of the calorie value of the standard and should be expected to contain this percentage of each of the nutrients in the standard. If a given nutrient is naturally present in an amount equal to three [illegible] more of that percentage, no further adjustment is needed.

A product designed primarily as a meal replacer should provide the consumer with 25% to 50% of the allotment of nutrients, except calories, established for all nutrients included in the RDA. Prepared breakfast cereals

[illegible] considered to be formulated foods, because their primary use is as [illegible] fast entrée, it is suggested that they may be designed to provide approximately of the RDA percentage of the [illegible] common to cereals. Other [illegible] that are not properly listed [illegible] RDA table but are known or [illegible] suspected, with proof scientific [illegible], to be dietary essentials [illegible] included in proportions similar [illegible] those consumed in a diet [illegible] to be of good quality. The contribution of calories by the meal replacement should be determined by our description of its intended use (e.g., whether to provide the full proportion of the calorie allowance or to be used in weight control).

Improvement of Fabricated Foods

Fabricated foods are complex mixtures of ingredients specially designed for a particular use and may or may not closely resemble existing foods. By this definition, two criteria for [illegible] additions are necessary, [illegible] fabricated products (analogs) that have the appearance, [illegible] of use of conventional or formulated foods and the [illegible] for fabricated foods not resembling existing [illegible].

[illegible] should contain on [illegible] the variety and [illegible] of the essential nutrients [illegible] in important amounts in [illegible] class to which the food belongs. Nutrients [illegible] present in quantities not [illegible] the calculated average [illegible] given [illegible] "class" of foods. [illegible] to encompass all types of commercial or formulated foods recognized in the ordinary diet, [illegible], fruits, vegetables, and [illegible].

The quality of the protein and the amount and composition of the fat in fabricated foods must receive attention and should be nutritionally appropriate to the food and its intended use.

A fabricated new food should provide one to one and one-half times the representative RDA for improved nutrients in proportion to the calorie intake it is intended to replace. Selection of nutrients and the amounts used should be based upon the producer's suggested pattern of use of the food by consumers, the stability of the nutrients in the foods, and good manufacturing practice. Should the pattern of use of the fabricated new food change with time, the nutrient formulation may need to be revised appropriately where feasible.

Snack and other nonmeal food items become nutritionally important when they provide at least 5% of the RDA for calories. The nutritional improvement of such items should be in keeping with the general principles outlined in this statement.

Exceptions to these guidelines should be made for foods intended for therapeutic or modified diets in which the nutrient content is deliberately modified qualitatively or quantitatively.

Application of General Policy

The implementation of these general policies will require the refinement of the RDA in order to make them more useful as guidelines for the nutritional improvement of foods. This refinement would include the synthesis of age and sex categories to a minimum. Recommended Dietary Allowances, designed to [illegible] guidelines, should include all nutrients recognized as being required for [illegible]. Some judgment will be needed to determine which nutrients should be considered in the development of the adjusted nutrient density for all foods. The use of certain nutrients should be specifically restricted. [illegible] the margin between the guideline allowance and a toxic level is relatively narrow.

One such derivation of the NAS-NRC *Recommended Dietary Allowances* has been proposed by the Food and Drug Administration for use with nutritional labeling. The derived values for protein, vitamins, and minerals are called US Recommended Daily Allowances. With the adoption of a caloric base (the Council recommends 2,000 calories), the US-RDA could serve as the basis for calculation of nutrient density as defined in the statement.

The Council on Foods and Nutrition urges continued development of new and improved foods that will assure for the total population an overall diet of superior nutritional quality (including greater variety and acceptability) and economic advantage.

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TOXIC EFFECTS OF OVERDOSAGE OF VITAMIN D_2 IN CHILDREN

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A LITTLE MORE than ten years ago, W. Gerlach wrote: "The time has come when the internist must, in cooperation with the pediatrician, determine the effects of vitamin D intoxication in the human race." Since Hess and J. H. Lewis reported for the first time two typical cases of disease due to excessive intake of viosterol, more precise knowledge has been acquired about this matter. Similar facts have been reported in different countries. But since then the concentrated preparations have still been given repeatedly for a long period, especially in France. Physicians have come to prescribe considerable amounts of vitmain for children affected with tuberculous processes such as primary infection, pleural effusion or tuberculosis of the skin.

I had the opportunity to witness the dreadful effect of that therapy, especially the death of a doctor's child who had been literally stuffed with vitamin D by his father.

For all these reasons, I thought that it would be useful, in an important meeting of pediatricians, to speak once more about this well known disease, a true experimental disease indeed. My present report is based on 21 personal cases, some of which were serious, 2 ending in death.

CLINICAL MANIFESTATIONS

Peculiar manifestations are observed. Anorexia occurs first. Its appearance is especially sudden. During a meal the child will refuse all food, and the father of one of our patients could point to the date on the calendar when this happened. The child refuses all nourishment for days and weeks. Loss of appetite may subside for a while, but will suddenly reappear. The diagnosis of mental disease has often been considered because of such sudden and unexpected anorexia. Anorexia is the main symptom. It was present in all of our cases.

Nausea sometimes appears at the same time. Vomiting occurs some days later, and with remarkable suddenness. Though it is rare in the beginning, it may occur repeatedly from the first. Its repetition is also a special feature of the illness. Some of our patients vomited ten, fifteen or more times a day.

Thirst is nearly as frequent. Many of our children got up several times during the night to drink and urinate. Sometimes this thirst was great. A 2 year old child had polydipsia and resulting polyuria of 3 liters daily. Frequent periods of intense constipation were noted sometimes after short periods of diarrhea.

Such clinical features are typical. But often many other symptoms may be observed, mainly pain. First of all, there is headache. It is frontal or occipital and is generally severe. Some children complained of pain in different parts of the body, such as the limbs, the trunk, the abdomen, the loins or the back of the neck, with a feeling of stiffness. Abdominal pain caused mistakes with the most serious consequences. Some children had muscular cramps. One child limped because of pain in his feet; a roentgenogram showed marked osteolysis.

If the intoxication is discovered soon enough, the symptoms recede quickly. Otherwise, they become rapidly worse, and the appearance of these children becomes striking. The child is pale; his wan look and tallow complexion strike one at first sight. The pallor is not always connected with a reduction in hemoglobin or anemia. The child loses weight quickly because of the loss of subcutaneous adipose tissue and because of dehydration. One of our children, 7 years old, had lost 11 pounds (5 Kg.) in two months. The skin is remarkably dry, as are also sometimes the tongue and other mucous membranes.

Meanwhile, the child loses his animation and becomes irritable and depressed. Finally, lying on his side, he becomes indifferent to his surroundings and falls into a stupor. In 2 cases, heavy and deep breathing was noted.

In spite of such alarming symptoms, no meningeal signs (no stiffness) or visceral trouble can be found.

The temperature is sometimes elevated; it may not be very high, between 27 and 38 C. (98.6 and 100.4 F.); sometimes, it is higher, above 39 C. (102.2 F.). Wrong diagnoses may be considered on account of the fever.

CLINICAL FINDINGS

Examination of such children brings out precise clinical and biologic data. As a rule, the blood pressure is high. Although it is not always so in the early stages, it may be very high; 130 systolic, and 80 or 70 diastolic are usual findings.

The blood urea level is nearly always high; 0.5, 0.55, 0.6 or 0.65 Gm. is usual. A level of 0.45 or 0.48 Gm. may be difficult to interpret at first examination. A later fall to normal will confirm the former elevation. Ambard's coefficient is abnormal. Results of the phenolsulfonphtalein test are also abnormal.

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The urine may be normal. In half of the cases, slight albuminuria may be found, as well as high calcium and phosphorus levels. Results of the water concentration and dilution test deviate from normal.

In the blood the amount of calcium is usually high, and this may appear before the urea level rises; very high calcium levels may be found (129, 137 or 155 mg.; in 1 case it rose to 205 mg.). In this connection, I shall point out three facts: Elevation of the calcium level is not constant and may be normal or even low; elevation does not imply a serious prognosis; repeated examinations show marked variations.

The phosphorus level also is often high. It was over 60 mg., in 3 cases. It is often variable, and may be normal. The amount of serum phophatase is normal. No changes are found in the serum protein or serum globulin.

There is often a low red cell count. In 4 cases, it was below 3,000,000 and in another case, 2,250,000, with 50 per cent hemoglobin. A high leukocyte count is often found. Westergreen's method shows an accelerated sedimentation rate.

Finally, I point out these facts: Gastric hydrochloric acid is diminished or absent after an injection of histamine. I mentioned before having observed osteolysis in the roentgenogram of a boy who limped. In that case, calcareous deposits were seen with the ocular biomicroscope. They formed a fringe on the limbus cornealis, with granular white infiltrations under the epithelium of the cornea, and small deposits on the conjunctiva.

COURSE AND PROGNOSIS

The course and prognosis are not always favorable. Generally speaking, when treatment with vitamin is stopped all symptoms disappear quickly. Vomiting will cease in eight or fifteen days, often suddenly. Thirst diminishes little by little for about ten days. During the same time constipation is relieved, and the child begins gaining weight regularly. Pallor is slower to disappear; apathy also, for it is due to severe intoxication.

In such favorable cases, the blood urea level falls to normal in two weeks or a month; the reduction is regular and begins as soon as the treatment is stopped. The calcium level decreases more slowly, in two or three months. The rise of the blood phosphorus level is not parallel to that of the calcium, and decreases more quickly in three weeks or a month. The blood pressure may remain high as long as six months.

On the other hand, there are more serious cases in which the intoxication is more severe; recession is slower, more irregular, with possible relapses. Nervous symptoms may then occur: hypotonia, loss of reflexes, loss of equilibrium and inability to sleep. I have observed, in several instances, small muscular contractions, and in 3 cases, convulsions. Such severe cases have a long course. Thirst, anorexia and fever may persist as long as six months or more. Lastly, serious accidents may occur. Fortunately, they are rare. I have heard that 10 fatal cases have been reported since 1930.

In my personal experience, I had 2 fatal cases: that of an infant with a blood urea level of 3.57 Gm. and that of a child who died from subarachnoid hemorrhage with continuous convulsions. A third child is still alive, but has hemiplegia with a serious mental deficiency. Thus, the two main dangers are renal and cerebral impairment.

What are the signs by which one can make a prognosis?

The total dosage of the drug is not the main factor. However, it is true that the children who died (at the age of 20 and 16 months) had received respectively 11,200,000 and 18,200,000 units. The mild cases occurred when only 3,000,000 to 6,000,000 units had been given.

In the 2 cases in which serious nervous trouble occurred, the blood pressure was extremely high, whereas the blood calcium and urea were low. In my second fatal case, there was also a very high blood pressure. So it seems that a high blood pressure and high urea level in the blood are alarming findings. Neither phosphorus or calcium levels nor anemia have any importance as regards the prognosis.

Such are the accidents of severe intoxication. I believe that, on the other hand, there are many cases of slight intoxication, attested only by anorexia, fatigue, depression and pallor.

All the symptoms are caused by vitamin D_2 and not by its impurities. The same may be said of the troubles reported earlier with preparations which, before 1935, were perhaps impure.

PATHOLOGIC PROGRESS

Postmortem examination of a 20 month old infant was performed, and in another case biopsy of a kidney was made. The following lesions were found: calcaneous precipitates in the renal tubules and changes in the tubular cells, glomeruli and intersititial tissue of the kidney. There was the beginning of atherosclerosis in a renal artery. Calcaneous deposits in the tubules or even in the vessels, the heart muscle, the dura, the lungs, the laryngeal and tracheal cartilages, the gastric glands, the synovia and the subcutaneous tissue were found.

CAUSATION

Do these anatomic findings explain the clinical signs? It is not certain. Vomiting may result from a disturbed mineral balance. Thirst and dehydration may not have a renal origin. The fever may result from a

metabolic disturbance. The most important fact is perhaps dehydration, and it has been proved, in experimental intoxication, that dehydration is connected with disturbance of the mineral balance of blood and tissues.

A second point is now being investigated: A mild intoxication increases the fixation of calcium in the bones. The richer in phosphatase a soft tissue is, the more easily will calcium precipitate in it. That is why sediments are found in the kidneys, the aorta and the intestine. On the contrary, if the intoxication is severe, osteolysis will appear. It is still unknown how and why the massive doses of vitamin D_2, stored in the liver, will be destroyed or set free little by little. Spontaneous aggravations appearing some months after the end of the intoxication necessitate great care in the prognosis.

TREATMENT

One must first prevent intoxication; great care must be taken with the dosage of the vitamin. A child with full growth certainly needs no more than 1,000 units a day. A vial of 15 mg. contains 600,000 units, that is to say enough vitamin for several years.

The diet is important; indeed, experimentation has shown that the blood calcium level increases only if during the treatment the diet is rich in calcium phosphate.

Irradiation of the skin produces considerable amounts of vitamin D. That is why sunshine or ultraviolet rays may cause aggravation.

But the main prophylactic measure is to make sure that vitamin D_2 in massive doses is prescribed only in selected cases under medical supervision. This is all the more important as laymen usually think that the drug is harmless and can be used freely, as it can be bought without a prescription.

When the symptoms of intoxication appear, what must be done? Of course stop the administration of the vitamin, protect the child from sunlight, stop the exposure to ultraviolet rays and prescribe a low calcium and low milk diet.

Charpy tried to use the well known antagonism between vitamins A and D. The fact that vitamin D has an acidotic action led him to inject sodium bicarbonate, though perhaps it would cause calcium to precipitate. Finally, because of the antagonism between calcium and sodium, Fruchard tried to inject isotonic solution of sodium chloride.

The most important thing, however, is to recognize the first signs of this intoxication in order to check it at the right time. That is why I have given a full description of the disease.

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DONATH, W. F. and DE LANGEN, C. D. **Vitamin D sclerosis of the arteries and the danger of feeding extra vitamin D to older people, with a view on the development of different forms of arteriosclerosis.** *Koninkl. Nederl. Akad. Wetenschappen Proc., Ser. C*, 1957, 60, 15–21.

Rabbits on stock diet were given by stomach tube 25 mg. cholesterol in 2 ml. oil daily for 150 days, and the experimental group received also 5 drops of standardised Vigantol solution and a little more than 1 mg. irradiated ergosterol for the first 10 or 25 days. The earlier findings of Pfleiderer (Abst., 1957, Vol. 2) were confirmed, that the combination of Vigantol and cholesterol stimulated the development of arteriosclerosis, especially in older rabbits. Results with an interval between administration of the 2 substances require more study. The rabbits described in the preceding Abst., which had 100,000 I.U. pure vitamin D_2 or vitamin D_3 weekly, showed high serum cholesterol. It is considered that vitamin D should be prescribed with great caution for adults, since an injurious effect might appear years later.

D. Duncan.

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2301

The Compared Antirachitic Effects of Lactose and Vitamin D, Given Alone or Simultaneously

by Yvonne Dupuis and Paul Fournier

Vitamin D and lactose are the types of compounds whose presence in the diets of young rats is indispensable to the usage of calcium. In their absence, various problems arising from overwhelming calcemia, as well as disturbances of the endochondral ossification process, appear and develop rapidly.

Lactose has a powerful curative effect on such symptoms (3), as does vitamin D whose effect on rickets is classically well known.

The fact that there exists a particular relationship between the amount of vitamin D administered and the effect obtained is so widely recognized that it serves as a basis for determination of the biologically proper dosages of this vitamin (4). In the rat, the existence of a certain proportionality between ingested quantities of lactose and the degree of prevention against rickets has also been observed (5).

The aim of this work was to precisely identify the respective effects of various doses of lactose and vitamin D in order first, to be able to establish a quantitative relationship between these two compounds, and then, having this scale of values, to study to what degree the antirachitic effect of one of these compounds can add to that of the other.

Experimental Conditions

Wistar rats of laboratory breeding were divided into 13 lots of 6 each.

Each lot was fed a particular diet corresponding to one of the four following variations:

1. Diet without any factor of calcium utilization (lot 1): centesimal composition; wheat starch: 69.5; devitaminized casein: 18; peanut oil: 8; vitamin mixture without vitamin D (6): 1; saline mixture (7): 3; TiO_2: 0.5.

2. Vitamin D diets, of the same composition as the preceding one, but containing, in addition, calciferol. Four diets belong to this group which differ from each other by the quantity of calciferol contained so that the animal received each day: 1/16 (lot 2), 1/8 (lot 3), 1/4 (lot 4) or 1/2 IU (lot 5).

3. Lactose diets differing from the first type of diet only by the replacement of an equal quantity of starch by lactose introduced in the respective proportions of 2,4,8 and 16% (lots 6,7,8 and 9).

4. Mixed diets containing at the same time both vitamin D and lactose:

	Vitamin in IU per day	Lactose in % of diet
Lot 10	1/16	2
Lot 11	1/16	4
Lot 12	1/8	2
Lot 13	1/8	4

After three weeks of the experiment, the rats were sacrificed. The serum calcium was measured by flame spectrophotometry. The tibias were examined after being split longitudinally. The consistency was noted. The appearance of the cartilage and the subjacent metaphyseal zone enabled us to estimate the degree of rachitis to which we assigned an arbitrary value between 0 and 6 with the highest value corresponding to the most pronounced anomalies.

Results

The animals whose diet contained no factor for the utilization of calcium had very low amounts of calcium (60 mg per liter) in the blood (lot 1, without the factor). Their bones showed all the symptoms of severe rachitis. The other lots showed indications of two different types:

1. Establishment of a relationship between the respective antirachitic effects of vitamin D and lactose. The presence in the diet of vitamin D or lactose exerted a certain preventive action against rachitis, and this effect is greater, the larger the quantity of vitamin D or lactose administered to the animal.

Diets + Lots		Calcium in blood in mg/l	Characteristics of Tibias			Degree of Rachitis
Characteristics	#		Consistency	Cartilage	Ossification Zone	
No factor	1	60	soft	very thick & very irregular	areas are rare & twisted	6
Lactose 2%	6	68				
Vit. D 1/16 ...	2	68	fairly soft	thick, forming one or two large sacs	areas very irregularly placed	4
Lactose 4%	7	70				
Vit. D 1/16 + Lactose 2%	10	76	firmer	very large + irregular in spots	poorly organized across from cartilage irregularities	3
Vitamin D 1/16 + lactose 4% ...	11	89				
Vit. D. 1/8 ...	3	87	fairly hard	often a little too big at the edge	generally well distributed except where cartilage is enlarged	from 2 to 1
Lactose 8%	8	85				
Vit. D.1/8 + lactose 2% ...	12	85				
Vit. D. 1/8 + lactose 4% ...	13	98				
Vit. D 1/4	4	94	hard	essentially normal thickness + regularity	fine and parallel zones	from 1 to 0
Lactose 16% ...	9	96				
Vit. D 1/2	5	101	very hard	ends are edged + nacreous	well ordered	0

In looking at the data in the table, it can be seen, for example, that the animals of lots 3 and 8 reacted in essentially the same way, whether they received 1/8 IU or about 1 g of lactose per day. Lots 4 and 9 are very similar themselves, indicative of the similarity between 1/4 IU of calciferol and 2 g of lactose.

2. The antirachitic properties of vitamin D and lactose are additive. The rats of three of the four lots whose diets contain simultaneously small quantities of both vitamin D and lactose are much better protected from the afflictions of rachitis than those whose diet contains this same small quantity of one or the other of these substances.

This was particularly clear for lot 11 in which the rats received 1/16 IU per day and whose diet included 4% of lactose. Calcium in the blood was up to 89 mg; the signs of rachitis are very subtle. The sole presence of either 1/16 IU of vitamin D (lot 2) or of 4% of lactose (lot 7) had only slight effects against the appearance of rachitis. In the case of lot 11, the additive effect of the vitamin and the lactose is so pronounced that the animals had essentially the same reaction as those who received twice the amount of the vitamin (lot 3, vitamin 1/8) or twice the amount of lactose (lot 8, lactose 8%). The same thing was seen with lot 13, that the joint effect of the vitamin (1/8 IU. per day) and of lactose (4% of diet) almost completely protected the animal, which was not the case when separate doses of the same amounts were administered alone (lots 3 and 7). In only one case (lot 12), the presence of the very small proportion of 2% of lactose, along with the administration of 1/8 IU of the vitamin, did not improve the already very significant effect produced by the sole presence of the vitamin (lot 8).

Discussion

It is known that by definition, the international unit of vitamin D is the daily amount necessary to protect or cure the young rat of rachitis. The preventive doses which are completely effective are, based on this present experiment, between 1/4 and 1/2 IU, in other words, significantly less than one unit. But it should be noted that our experiment included a balanced diet with normal levels of calcium and phosphorus, conditions which diminish greatly the animals' vitamin needs.

In the present experiment, the effect of lactose, relative to previous results, is fairly weak. An amount of lactose of 16% does not entirely prevent rickets. In a graph of the curves of the effects of vitamin D and lactose as a function of the dose, it can easily be shown that about 23% of lactose is necessary in order that the animal, under the present conditions, is entirely protected. But, various experiments have shown us that sometimes 12% of lactose is sufficient to obtain this protective effect (5) and that in practice, a proportion of 20% puts the great majority of the animlas under protection from rachitis. The present relatively passive effect of the lactose upon the animal seems to be related to the well-known fact that animals of the same stock react differently from one time to another to the same dose of vitamin D (8).

These few considerations do not prevent this present work from being able to establish a relationship of effects between glucide and the vitamin. This is a strange relationship if one considers that 1/4 IU or 0.006 ug of calciferol and 2 g of lactose have the same antirachitic effect. It can be seen as well that it is necessary to have on the order of one billion (10^9) times more lactose than vitamin to obtain the same effect.

Such a disparity would cause one to suppose that it is an infection

which is the at the origin of the antirachitic effect of lactose. And the present experimental conditions do not allow us to reject this assertion. But other circumstances show the specific role of lactose in the utilization of calcium and in ossification: from the time of adulthood on, vitamin D assures no more than a state of equilibrium in the calcium exchanges, the morphology of the skeleton being subject only to the smallest changes. It is different with lactose whose constnat presence in large proportions in the diet of the animal is accompanied by a significant retention of calcium, which leads progressively, with age, to a generalized thickening of the skeleton (9). This is certainly not vitamin D which is itself ineffective under such conditions, but surely the lactose, which in this case, is responsible for such osteogenic activity.

Conclusion

In the young rat, vitamin D and lactose possess antirachitic properties so similar that it can be estimated what quantity of one is equivalent to a certain quantity of the other. For small dosages, the effect of one of these factors adds to that of the other.

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Physiologie.

Effets antirachitiques comparés du lactose et de la vitamine D, seuls ou associés.

par Yvonne Dupuis et Paul Fournier.

La vitamine D, le lactose, sont le type du composé dont la présence dans le régime du jeune Rat est indispensable à l'utilisation du calcium. En leur absence apparaissent et se développent rapidement les divers troubles consécutifs à l'effondrement de la calcémie, ainsi que des perturbations de l'ossification endochondrale (1, 2).

Contre de tels symptômes, le lactose est doué d'un puissant effet curatif (3), tout comme la vitamine D dont l'action à l'égard du rachitisme est classique.

Le fait qu'il existe un certain rapport entre la dose de vitamine D administrée et l'effet obtenu est si connu qu'il sert de base aux dosages biologiques de la vitamine (4). L'existence chez le Rat d'une certaine proportionnalité entre les quantités de lactose ingéré et le degré de prévention du rachitisme a été aussi observée (5).

Le présent travail a pour but de préciser les activités respectives de diverses doses de lactose et de vitamine D afin d'abord d'établir une correspondance quantitative entre ces deux composés, puis, en possession de cette échelle de valeurs, de rechercher dans quelle mesure l'action antirachitique de l'un de ces composés peut s'ajouter à celle de l'autre.

Conditions expérimentales. — Des rats Wister de l'élevage du Laboratoire sont répartis en 13 lots de 6. A chaque lot correspond un régime particulier, appartenant à l'un des 4 types suivants :

1. Régime sans facteur d'utilisation calcique (lot 1) : composition centésimale : amidon de blé : 69,5 ; caséine dévitaminée : 18 ; huile d'arachide : 8 ; mélange vitaminique sans vitamine D (6) : 1 ; mélange salin (7) : 3 ; O_2Ti : 0,5.

2. Régimes « vitamine D » de même composition que le précédent. Ils contiennent, en plus, du calciférol. Quatre régimes appartiennent à ce type, qui diffèrent entre eux par la quantité de calciférol incorporé de telle sorte que l'animal en reçoit par jour : 1/16 (lot 2), 1/8 (lot 3), 1/4 (lot 4) ou 1/2 U.I. (lot 5).

3. Régimes « lactose » ne diffèrent du régime 1 que par le remplacement d'une quantité égale d'amidon par du lactose introduit dans les proportions respectives de 2, 4, 8 et 16 % (lots 6, 7, 8 et 9).

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4. Régimes mixtes renferment à la fois de la vitamine D et du lactose :

	Vitamine en U.I. par jour	Lactose pour 100 du régime
lot 10	1/16	2
lot 11	1/16	4
lot 12	1/8	2
lot 13	1/8	4

Après 3 semaines d'expérience, les rats sont sacrifiés. Le calcium sérique est mesuré au spectrophotomètre de flamme. Les tibias sont examinés après avoir été fendus longitudinalement. On note la consistance, l'aspect du cartilage et de la zone métaphysaire sous-jacente permettant de juger le degré de rachitisme, auquel on attribue une valeur arbitraire comprise entre 0 et 6 d'autant plus élevée que les anomalies sont plus prononcées.

Résultats. — Les animaux dont le régime ne comporte pas de facteur d'utilisation du calcium ont une calcémie très abaissée à 60 mg par

Régimes et lots		Calcémie en mg/l	Caractères des tibias			Degré du rachitisme
Caractères	N°		Consistance	Cartilage	Zone d'ossification	
Sans facteur	1	60	molle	très épais et très irrégulier	travées directrices rares et tordues	6
Lactose 2 %	6	68	assez molle	épais formant une ou deux saillies jointes	travées très irrégulièrement orientées	4
Vit. D 1/16	2	68				
Lactose 4 %	7	70				
Vit. D 1/16 + lactose 2 %	10	79	plus ferme	trop large et irrégulier par endroit	mal organisée face aux irrégularités du cartilage	3
Vit. D 1/16 + lactose 4 %	11	89	assez dur	souvent un peu trop large sur le tout	généralement bien disposée sauf au niveau des élargissements du cartilage	de 2 à 1
Vit. D 1/8	3	87				
Lactose 8 %	8	83				
Vit. D 1/8 + lactose 2 %	12	85				
Vit. D 1/8 + lactose 4 %	13	98	dur	épaisseur et régularité sensiblement normales	travées droites et parallèles	de 1 à 0
Vit. D 1/4	4	94				
Lactose 16 %	9	96				
Vit. D 1/2	5	101	très dur	épais, large, régulier	bien ordonnée	0

litre (lot 1, sans facteur). Leurs os présentent tous les signes d'un rachitisme grave. Les autres lots fournissent des indications de deux ordres différents :

1. Établissement d'une correspondance entre les activités antirachitiques respectives de la vitamine D et du lactose. — La présence dans le régime de vitamine D ou de lactose exerce un certain effet préventif à l'égard du rachitisme, et cet effet est d'autant plus

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marqué que la quantité de vitamine D ou de lactose administrée à l'animal est plus grande.

A consulter les renseignements groupés dans le tableau, on peut relever par exemple que les animaux des lots 3 et 8 réagissent sensiblement de la même façon, qu'ils reçoivent soit 1/8 d'U.I. ou environ 1 γ de lactose par jour. Les lots 4 et 9, très comparables eux aussi, indiquerait de même l'activité semblable de 1/4 d'U.I. de calciférol et de 2 g de lactose.

2. Les propriétés antirachitiques de la vitamine D et du lactose sont comparées. — Les rats de trois des quatre lots dont les régimes comportent simultanément de petites quantités de vitamine D et de lactose vont beaucoup mieux [illegible] du rachitisme que lorsque le régime contient cette petite quantité de l'un ou l'autre de ces facteurs.

Ceci est particulièrement net pour le lot 11 dont les rats reçoivent 1/16 U.I. par jour et dont le régime renferme 4 % de lactose. La calcémie est à 88 mg ; les signes de rachitisme sont discrets. La présence isolée, soit de 1/16 d'U.I. de vitamine D (lot 2), soit de 4 % de lactose (lot 7) n'avait eu que de discrets effets à l'encontre de l'apparition du rachitisme. Dans le cas du lot 11 l'effet additif de la vitamine et du lactose est si prononcé que les animaux ont sensiblement la même réaction que ceux qui reçoivent deux fois plus de vitamine (lot 3, vitamine 1/8) ou 2 fois plus de lactose (lot 8, lactose 8 %). On voit de même avec le lot 13, que l'effet conjugué de l'administration de la vitamine (1/8 U.I. jour) et du lactose (régime à 4 %) protège presque complètement l'animal, ce que ne font pas séparément ces quantités de l'un ou de l'autre de ces facteurs (lots 3 et 7). Dans un seul cas (lot 12), la présence de la très petite proportion de 2 % de lactose en plus d'une administration de 1/8 d'U.I. de vitamine n'améliore pas l'effet déjà très important produit par la seule présence de cette dose de vitamine (lot 8).

Discussion. — On sait que, par définition, l'unité internationale de vitamine D est la quantité journalière qui protège ou guérit le jeune rat du rachitisme. Les doses préventives complètement efficaces sont, dans l'expérience présente, comprises entre 1/4 et 1/2 U.I., c'est-à-dire notablement inférieures à l'unité. Mais il convient de remarquer que nous expérimentons en régime équilibré, de teneur normale en calcium et en phosphore, conditions qui diminuent beaucoup le besoin vitaminique de l'animal.

Dans la présente expérience, l'activité du lactose est, relativement à des résultats antérieurs, assez faible. Une teneur en lactose de 16 % ne préserve pas entièrement du rachitisme. Par le tracé des courbes d'activité de la vitamine D et du lactose en fonction de la dose, on montrerait aisément qu'il faudrait environ 23 % de lactose pour que l'animal soit, dans les conditions présentes, entièrement protégé. Or, diverses expériences avec soi montré que, parfois, 12 % de lactose suffisaient à obtenir cet effet protecteur (5) et que pratiquement une proportion de 25 % n'était l'immense majorité des animaux à l'abri du rachitisme. La présente passivité relative du lactose vis-à-vis de l'animal semble être à rapprocher du fait, bien connu, d'après lequel

les animaux d'un élevage homogène réagissent différemment, d'une saison à une autre, à la même dose de vitamine D (8).

Ces quelques considérations n'empêchent pas que le présent travail permet d'établir une correspondance d'activité entre le glucide et la vitamine. Correspondance étrange si l'on considère que le 1/4 d'U.I. soit 0,006 μg de calciférol et 2 g de lactose ont la même activité antirachitique. On voit aussitôt qu'il faut de l'ordre de 1 milliard (10^9) fois plus de lactose que de vitamine pour obtenir le même effet.

Une telle disparité pourrait laisser supposer que c'est une contamination qui est à l'origine de l'activité antirachitique du lactose. Et les présentes conditions expérimentales ne permettent pas de rejeter cette assertion. Mais d'autres circonstances montrent le rôle propre du lactose dans l'utilisation du calcium et sur l'ossification : à partir de l'âge adulte, la vitamine D n'assure plus qu'un état d'équilibre dans les échanges calciques, la morphologie du squelette ne subissant que d'infimes variations. Il en va autrement du lactose dont la présence constante en forte proportion dans le régime de l'animal s'accompagne constamment d'une importante rétention calcique, ce qui conduit progressivement avec l'âge à l'épaississement généralisé du squelette (9). Ce n'est certes pas la vitamine D, elle-même inopérante dans de telles conditions, mais bien le lactose qui est dans ce cas responsable de telles activités ostéogènes.

Conclusion. — Chez le jeune Rat, la vitamine D et le lactose possèdent des propriétés antirachitiques si semblables que l'on peut dire à quelle quantité de l'un équivaut une certaine quantité de l'autre. Pour les petites doses, l'activité de l'un de ces facteurs s'ajoute à celle de l'autre.

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Biochimie.

Comparaison de l'action biologique de différents œstrogènes administrés par voie sous-cutanée, orale ou transcutanée.

par M. F. Jayle, S. Vandel et B. Desfosses.

Nous avons comparé l'action de l'œstrone (O_I), de l'œstradiol (O_{II}), de l'éthynyl-œstradiol (Et. -O_{II}), des quatre isomères de l'œstriol : œstriol (16α, 17β-O_{III}), 17-épi-œstriol (16α, 17α-O_{III}), 16-épi-œstriol (16β, 17β-O_{III}), 16, 17-épi-œstriol (16β, 17α-O_{III}), de la 16α-hydroxy-œstrone (16α-O_I) et de la 16β-hydroxy-œstrone (16β-O_I) sur la kératinisation vaginale de rattes Wistar castrées (réaction d'Allen et Doisy).

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editorials

Cancer and Immune Mechanisms

The idea that natural immunologic mechanisms play a role in protecting against malignant neoplasms gains credence from reports of higher incidences of cancers in persons having immunologic deficiencies, whether congenital, acquired as part of another disease, accidental, or iatrogenic.

The outstanding example of accidental exposure to immunosuppression by irradiation derives from studies by the Atomic Bomb Casualty Commission of survivors of the atomic bombings of Hiroshima and Nagasaki. For example, Sampson et al[1] reported a significantly higher incidence of thyroid carcinomas among those persons exposed to 50 rads or more of direct atomic radiation. Other incidental-accidental generations of carcinoma of the thyroid have resulted from radiation therapy for adolescent acne vulgaris[2] and from irradiation of children having enlargement of the thymus[3,4] or other conditions of the head, neck, or thorax.[4]

From these earlier experiences, it is not too surprising to learn that long-term immunosuppressive therapy in recipients of renal transplants increases the risk of development of malignant neoplasms.[5] And, more recently Arseneau and co-workers,[6] during a one-year period, witnessed three Hodgkin disease patients in whom a second malignant tumor developed and was followed by a rapidly fatal clinical course. All these patients had received intensive radiotherapy and had been treated with combination chemotherapy soon after radiotherapy.

Knowing that Hodgkin disease patients have evidence of immunologic deficiency, but that there is divided opinion about the prevalence of second unrelated malignant neoplasms in this disease, Arseneau et al nevertheless undertook a review of the records of the 425 Hodgkin disease patients treated at the National Institutes of Health from July 1, 1953 to July 1, 1971. They found a significantly higher ratio of observed to expected second malignant neoplasms in the total population. The ratio was especially higher for patients who had received intensive radiotherapy plus intensive chemotherapy. They suggest that the role of the cytotoxic drugs may simply have compounded the radiation effect or may have interfered with cellular repair mechanisms.

In conclusion, these and similar published reports on the subject encourage continuation of research in the area of immunotherapy for malignant neoplasms.

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From Vitamin to Hormone

While extravagant, unwarranted claims for ascorbic acid and vitamin E resound shrilly through the media, vitamin D has largely escaped public notice. Yet, recent developments in vitamin D research are truly portentous. They are currently engaging the attention of physicians concerned with calcium metabolism and the treatment of its disorders.

Renewal of interest in vitamin D began recently when DeLuca[1] had demonstrated that the major part of the vitamin circulating in the blood is not calciferol (D3), but its more potent metabolite, 25-hydroxycholecalciferol (25-HCC), to which it is converted in the liver by side-chain hydroxylation at carbon 25. For a while, it appeared that 25-HCC might be the functional form of vitamin D. But it soon became clear that 25-HCC was only an intermediary—the first step in its formation. Kodicek and his University of Cambridge team[2-4] demonstrated the presence of a much more potent metabolite in cell nuclei of the intestinal mucosa and other tissues—only a minimal amount is found in the blood—and subsequently, traced to the kidney its synthesis by hydroxylation of 24-HCC to 1,25-dihydroxycholecalciferol. In the manner of a true hormone, 1,25-dihydroxycholecalciferol is secreted in small amounts and then dispatched to the intestinal mucosa, bone, and skeletal muscle to be stored for regulating calcium absorption and metabolism.

To study the potency and action of the newly discovered renal hormone, Brickman and associates[5] administered it in 0.6- to 270-mg daily doses for six to ten days to four normal subjects, five patients with uremia, two with pseudohypoparathyroidism, and three with hypophosphatemic D resistant rickets. The values for urinary calcium and ^{47}Ca absorption rose both in normal subjects and patients, while serum calcium and phosphorus levels increased only in the latter group. The hormone proved much more potent than other

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vitamin D metabolites, but similarly ineffective in restoring responsiveness to parathyroid extract in pseudohypoparathyroidism and in correcting the renal phosphorus wasting in renal rickets.

It is not unreasonable to assume that in addition to its therapeutic promise, 1,25-dihydroxycholecalciferol has a potential for clarifying the pathogenesis of some as yet poorly understood disorders of calcium metabolism, such as vitamin D resistance of familial rickets and chronic uremia, in which the kidney may be implicated in the failure to convert vitamin D to its functional form. S.V.

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Electrical Safety in the Operating Room

The life-saving results achieved by a team of skillful surgeons and anesthetists, reinforced by a well-trained team of nurses and operating room personnel, may be nullified by a simple and avoidable accident of a mechanical or electrical nature in the operating room. Properly equipped carts, well-executed transfer of the patient from the cart to the operating table and back again, and strategic placement of furniture and equipment in the surgical rooms will partially ensure the patient's safety. The most difficult and invisible dangers to the patient lurk in the electrical system of the operating room, in its electrical and electronic equipment.

For years now, we have controlled the dangers of static electricity, which used to cause explosions and fires in operating rooms; we avoid static electricity-producing materials, clothing, and equipment; nurses and physicians wear nonconductive shoes, and hospital floors are made of nonconductive material. This aspect of electrical safety hardly needs to be discussed.

Another danger arises with the use of high-powered electrical equipment, especially for electrosurgery or electrocautery. Becker et al[1] describe nine patients who suffered burns at the sites of electrocardioscope electrodes in one hospital within a period of only ten months. The causes of these burns were multiple: broken ground wires, defective rectifiers, improper equipment, improper use of active electrodes, capacitative coupling of cables, and radio frequency current division. All of this happened in a well-supervised, university-affiliated hospital. A detailed study of various electrical defects led to corrections in the electrical system and to revision of procedures, and the safety of the patients and of the operators was restored.

Becker et al suggest that all physicians in hospitals using radio frequency currents and electric surgical equipment should pay attention to the following [illegible] safety: ferrite-core inductors or chokes should be inserted at the patient-end of each electrocardiographic wire; the alarm system of the electrosurgery unit should be checked before each operation; the electrocardiographic electrodes on the patient should be placed as far away from the site of electrosurgical incision as possible; electrocardioscope cables should never be coiled or clipped with conductive material; the electrosurgery unit alarm circuit should be checked for integrity and safety of its silicone or plastic parts; a preventive educational program should be presented to all operating room personnel working with electrosurgery; the use of needle-electrocardiographic electrodes should be avoided when electrosurgery is anticipated; periodic rechecking of the equipment and procedures would insure that electrical burns or accidents will not occur.

Atkin and Orkin[2] report an incident concerning near-fatal electric shock to a patient, which occurred during a routine surgical procedure with the patient under spinal anesthesia. The patient had been properly monitored, including the electrocardiographic tracing. When the monitor was affected by interference and the anesthetist was unable to evaluate the tracing, the electrical connection was transferred from one wall socket to another. When the change in connection was made, the anesthesiologist felt a shock in the hand checking the patient's pulse, the patient made a sudden jerking movement and then became cyanotic, apneic, and pulseless. The power connection of the electrocardiographic monitor was immediately disconnected and after energetic measures of resuscitation were instituted by the alert anesthetist, the patient recovered fully, the operation was completed, and no permanent damage was done. The happy ending of this unpleasant incident could be credited to the quick and skillful action of the anesthetist and of the operating room team.

The follow-up investigation of the electrical system of the operating room produced evidence that an improperly wired electrocardiographic monitor had been in use for some time. The careful check of the wall sockets throughout the surgical department showed that there was no standardized polarity wiring in the majority of the sockets. Twenty percent of all receptacles in the operating rooms had reversed polarity; they had been put in 15 years previously at the time the hospital was constructed. All the defects were immediately corrected.

Isolated power systems are highly recommended for the patient-care areas in all hospitals. The isolation transformers and the line-isolation monitors are also recommended as a protection against macroshock hazards. Proper grounding of all the equipment and of all the electrical systems is also a must. Frequent checks of all electrical aspects of the operating rooms by skilled engineer-surgeon teams would be a good measure of protection. Z.P.

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DEGENERATIVE CHANGES IN THE AORTA OF SHEEP INJECTED WITH ONE MILLION UNITS OF VITAMIN D_3

By

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INTRODUCTION

In 1962 we examined histologically several organs from each animal in a small group of adult Blackface ewes which had received a single intramuscular injection of one million units vitamin D_3 in 2 ml. ethyl oleate approximately eleven months before slaughter. These sheep had been used in an experimental study of blood levels of vitamin D_3 after its injection (Quarterman, Dalgarno and Adam, 1963). The histological studies were made to investigate possible pathological effects of the treatment, and tissues from twelve normal Blackface ewes that were believed to have had no more than ordinary amounts of dietary vitamin D were used as controls.

All the animals in both groups had small calculi in the renal pelvis. Non-calcified arteriosclerotic intimal lesions were found in the aorta of two of the treated sheep. The remaining five had a mild, diffuse, medial fibrosis of the aortic wall.

We supposed that the arteriosclerotic lesions were due to vitamin D toxicity. Whilst it appeared unlikely that such lesions would have shortened the useful life of the sheep or have other economic disadvantages, we felt obliged to make a similar study on a larger group of animals in order to check this observation.

MATERIALS AND METHODS

Forty Blackface ewe hoggs, with no history of disease, were bought from a reputable source when one year old. They were born in Strathdon, Aberdeenshire, kept on hill grazing during the summer, and on lowland grazing supplemented with turnips and hay in winter. They had not been injected with vitamin D or with prophylactic vaccines.

The animals were weighed, ear-marked, divided into groups and injected with vitamin D_3 on 25.5.62 as shown in Table 1. After injection the sheep were kept on grass for a short time until sheared, when they were transferred to marginal hill grazing until the end of September. Early in October they were returned to grass, receiving silage from 28.12.62 to 8.1.63, hay and ½ lb. concentrates from 8.1.63 to 5.2.63.

Immediately after slaughter the heart, thoracic aorta and pieces of lung were placed in neutral buffered 4 per cent. formaldehyde solution. After about two hours the heart was freed from blood clots and adventitious tissue, examined and weighed. Tissues for further fixation and processing for histological examination were then selected from each chamber of the heart, from similar areas of the aortic arch and from the lung. Three specimens were taken from each aorta.

Paraffin wax sections from each specimen obtained at two and four months after the injections were stained by the von Kossa method for calcium salts. Sections from all specimens were stained by van Gieson's picro-fuchsin method for collagen and by the Weigert-Sheridan method for elastic tissue. In addition, selected tissues were stained by the Periodic-acid-Schiff and haemalum method, by Heidenhain's iron haematoxylin method for the study of smooth muscle cells and by the silver impregnation of reticular fibres. Frozen sections were stained by Sudan IV.

RESULTS

The treated and control groups did not show a significant difference in average carcase weights. Neither macroscopic nor microscopic lesions were found in the heart or lung of any animal, except for parasitic lung lesions. The heart weights from the three groups were not significantly different. The von Kossa preparations were all negative for calcium salts. Although no changes were seen with the unaided eye microscopic lesions were present in many of the sections of aorta and were classified as localised or diffuse. The former were found in animals from all three groups and were apparently unrelated to the treatment, whereas the latter occurred in all the animals injected with 1,000,000 units vitamin D_3 and we believe were attributable to this cause.

TABLE 1

Group	No. of ewes	Intramuscular injection	Slaughter: time after injection			
			2 months	4 months	6 months	8 months
1	16	1,000,000 units vitamin D_3 dissolved in 2 ml. ethyl oleate (Robert Young & Co. Ltd.)	4	4	4	4
2	8	500,000 units vitamin D_3 in 1 ml. ethyl oleate	—	4	—	4
3	16	2 ml. ethyl oleate	4	4	4	4

Spontaneous Arteriosclerotic Lesions occurring in Treated and Untreated Animals

The non-specific changes were localised arteriosclerotic lesions. These occurred as intimal scars and plaque-like elevations of the intima.

Intimal scars. Of the sixteen animals injected with 1,000,000 units vitamin D_3, seven had one or more small sub-endothelial scars in the aorta. In two of the seven affected animals multiple lesions were found. Three out of eight receiving half the above dose showed similar areas of fibrosis.

In five of the sixteen controls there were lesions similar to those in the treated groups. The typical appearance of these arterio-

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sclerotic areas is shown in Fig. 1. Calcified, atheromatous or active inflammatory lesions were not found.

Intimal plaques in the aorta. In four of the treated ewes and three of the controls there were intimal plaques in the aorta (Fig. 2). These plaques consisted of smooth muscle cells, elastic fibres and collagen. They were covered with endothelium and did not contain demonstrable fat. They were superficial to the inner elastic layers of the vessel wall and appeared to be localised thickenings of the subendothelial tissues.

Diffuse Lesions in the Aorta occurring only in Animals Injected with Vitamin D_3

Staining for elastic tissue, collagen and reticulum showed a variable but generally slight increase in these tissues in the aortic media in the region of the larger smooth muscle bundles at six and eight months after the injection of 1,000,000 units vitamin D_3. In the same specimens, but not in the controls, there was a diffuse, patchy degeneration and atrophy of smooth muscle. This diffuse muscular lesion was present at two months after the injection, when vacuolar degenerative changes were present; these became progressively worse up to six months and at eight months the main appearance was of mild, diffuse atrophy of smooth muscle (Figs. 3 to 8). In some of the affected areas there was a considerable increase in fine elastic fibrils (Fig. 9). The wall of the aorta was slightly oedematous in these specimens, but fatty change was not present.

There were also lesions in the vasa vasorum of the aorta. These were infrequent and consisted of swelling or proliferation of vascular endothelium sufficient to reduce effectively the vessel lumen (Fig. 10). This change appeared to be confined to the group injected with 1,000,000 units vitamin D_3; only half of the sheep in this group were found to be affected and in them we were unable to find more than one or two occluded vessels per animal. There was no direct evidence of medial ischaemia even in areas of smooth muscle which showed relatively marked degenerative changes. There was no significant perivascular fibrosis in comparison with the control specimens.

DISCUSSION

It is possible that the "spontaneous" aortic scars and plaques were caused by ethyl oleate, the vitamin injection vehicle. Our data do not resolve this question, but since intimal plaques in the aorta have been noted incidentally in other species it seems probable that they are non-specific.

Spontaneous, non-atheromatous, arteriosclerotic lesions in the aorta are fairly common in captive wild mammals (Fox, 1933, 1939; Ratcliffe and Cronin, 1958; Lloyd, 1959; Ratcliffe, Yerasimides and Elliott, 1960; Finlayson, Symons and T-W-Fiennes, 1962). Similar lesions have been recorded in several domesticated species (Fox, 1933). Gottlieb and Lalich (1954) examined more than 2,000 fresh

aortas from pigs slaughtered in American abattoirs. Arteriosclerotic lesions were found in all age-groups, reaching an incidence of 35 per cent. in pigs over 3 years old. Principally the lesions were fibrous thickenings of the intima. In one middle-aged pig, Jennings, Florey, Stehbens and French (1961) found plaque-like intimal lesions, superficial to the internal elastic lamina of the aorta. These plaques were relatively acellular, but contained intra- and extracellular fat and areas of calcification. Non-fatty, fibrous intimal plaques in the thoracic aorta have been recorded in the cat (Lindsay and Chaikoff, 1955) and in the dog (Lindsay, Chaikoff and Gilmore, 1952), but the rat appears to be relatively free from spontaneous vascular disease (Wilens and Sproul, 1938) except in repeatedly bred animals, where spontaneous arteriosclerotic lesions commonly occur in the main arteries of both sexes (Wexler, 1964). Wexler found that the lesions were more common and more severe in the female; 40 per cent. of a large group showed grossly detectable lesions and 80 to 90 per cent. had microscopic lesions. In the course of other investigations, Hulland (1960) observed a high incidence of arterial changes in the visceral arteries of both normal and diseased sheep. Sudanophilic areas were found in the aortic intima in some cases, but the incidence of arteriosclerotic changes in this organ was not recorded. Lesions of the media were not common in any vessel.

Generally, these spontaneous intimal lesions in younger mammals resemble those which we found in sheep in this experiment, being mainly fibrous thickenings of the intimal tissues, containing elastic fibres and occasionally some smooth muscle cells, but rarely lipid. In older animals and particularly in the aging pig, there is a tendency to atherosclerosis (French, Jennings, Poole, Robinson and Florey, 1963). The aetiology of the condition is obscure.

We believe the diffuse changes in the arterial wall to be genuine pathological effects of the treatment. The actual cause of these microscopic degenerative lesions in smooth muscle is not clear. Presumably the vasa vasorum play an essential part in supplying the outer parts of the arterial wall with nutrients and it seems probable that atrophy of smooth muscle could be a consequence of an interrupted local blood supply. It is unlikely, however, that the few abnormal vasa vasorum which we were able to find could cause a serious circulatory deficiency. On the other hand, these could be the residue of a more widespread vascular lesion such as the arteritis described by Gillman and Gilbert (1956) and Hass, Trucheart, Taylor and Stumpe (1958) in vitamin D intoxication. If so, it is surprising that the coronary arteries and myocardium escaped injury.

Alternatively, the changes in aortic smooth muscle could possibly be the sequel to a direct injury sustained soon after the vitamin injection. Gross calcification probably did not occur, since such deposits tend to be relatively persistent in the aorta and cause distinct focal scarring of the media (Gillman, Grant and Hathorn, 1960; Quarterman, Dalgarno, Adam, Fell and Boyne, 1964). It should be

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noted, however, that the aorta can accumulate considerable amounts of calcium before mineralisation can be detected by routine histochemical procedures (Gillman and Gilbert, 1957).

Finally, the medial degenerative change could possibly have some relationship to the lesions of skeletal muscle observed by Gillman and Gilbert (1957) in rats suffering from vitamin D poisoning. In this case the skeletal muscle underwent atrophy and extensive degeneration with dissolution, due possibly to a direct toxic action of the vitamin. On the other hand, similar changes found in rabbits given very high levels of Vitamin D were thought to be due to arteritis (Hass *et al.*, 1958).

CONCLUSIONS

A single intramuscular injection of one million units vitamin D_2 in normal sheep resulted in degeneration of the smooth muscle of the aortic media. No histological lesions due to this treatment were detected in the heart or lung. The pathogenesis of the lesion is not understood; damage to the vasa vasorum of the aorta or direct toxic action of vitamin D are suggested as possible causes. Both the injected and control animals showed a high incidence of spontaneous intimal arteriosclerotic lesions.

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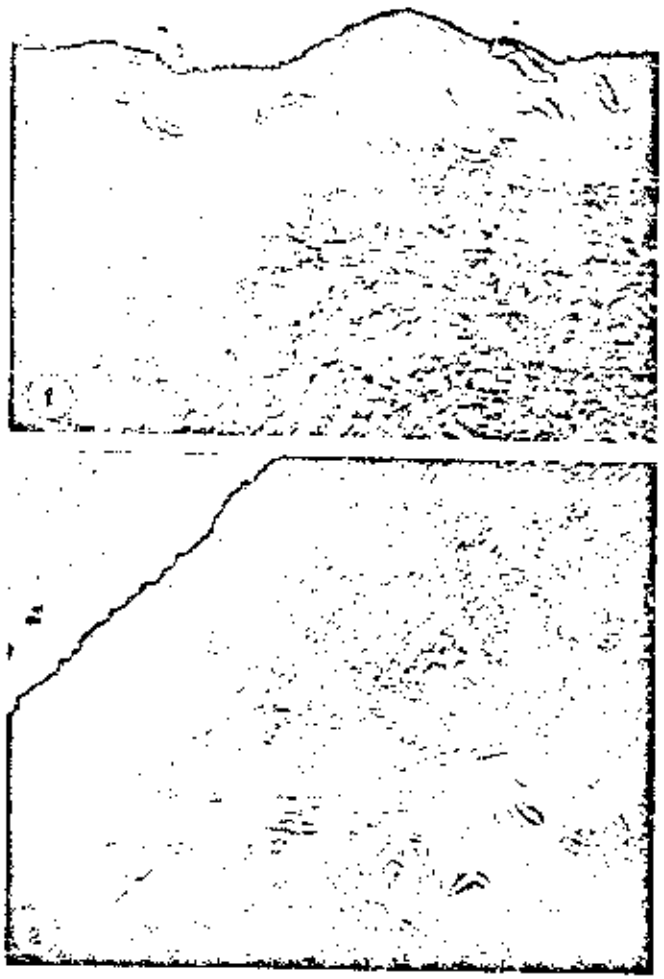

Fig. 1. Spontaneous sub-endothelial scar in the aorta of a control sheep. Van Gieson × 150.

Fig. 2. Fibrous plaque in the aorta of a control sheep. Van Gieson × 150.

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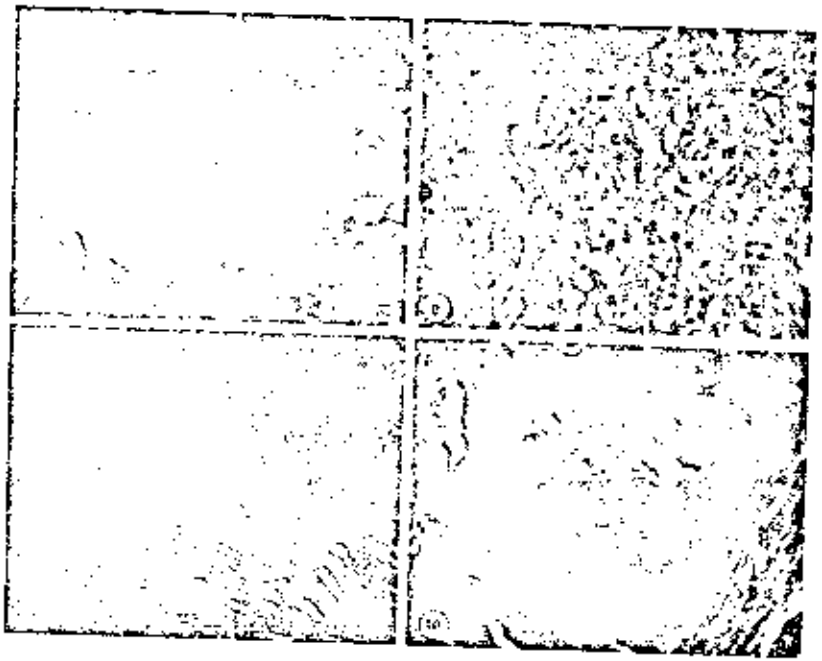

Fig. 7. Degenerating smooth muscle in the aorta 6 months after vitamin D_3 injection.
Van Gieson × 600.

Fig. 8. As above.
Van Gieson × 150.

Fig. 9. Proliferation of elastic tissue in an area of degenerating muscle 8 months after vitamin D_3 injection.
Weigert-Sheridan × 150.

Fig. 10. Occluded vessel in the wall of the aorta 4 months after vitamin D_3 injection.
Van Gieson × 600.

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JOINT FAO WHO EXPERT COMMITTEE ON NUTRITION

Eighth Report

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Geneva, 9–18 November 1970

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JOINT FAO/WHO EXPERT COMMITTEE ON NUTRITION

Eighth Report

GENERAL INTRODUCTION

The Joint FAO/WHO Expert Committee on Nutrition met in Geneva from 9 to 18 November 1970. The meeting was opened, on behalf of the Directors-General of FAO and WHO, by Dr P. Dorolle, Deputy Director-General of WHO. In his introductory remarks, Dr Dorolle mentioned that the meeting should concentrate its attention on two main subjects of international importance: (1) food fortification programmes within the context of overall national food and nutrition policies; (2) protein-calorie malnutrition, one of the most serious public health problems confronting the developing countries today.

Dr Dorolle added that FAO and WHO would welcome suggestions from the Committee on other nutritional topics that it considered of particular importance for future programmes.

PART I. FOOD FORTIFICATION

1. INTRODUCTION

1.1 General considerations

Depending upon the causative factors of malnutrition and the resources available, different approaches to the improvement of nutrition may be adopted. It is always important to consider the costs and effectiveness, both short-term and long-term, of a particular approach or group of approaches. The first requisite is a supply of nutritionally adequate food. Owing to inequalities in distribution, the required supply is always greater than a simple sum of individual requirements.

Food fortification is only one of the measures affecting the quality of food supply that are worthy of consideration in the design of programmes intended to improve the health of the population. Thus, at the level of the food producer, the long-term possibilities of improved agricultural practices should be considered. Such measures as selective plant breeding may produce crops with higher nutritive value as well as higher yield, while improved animal feeding practices coupled with a programme of genetic improvement may provide an increased supply of foods of animal origin.

Between their leaving the producer and their reaching the consumer, foods may be treated in many ways, several of which may affect their nutritional quality. Adequate storage facilities not only prevent total loss of food but may reduce nutritional deterioration of some foods. Similarly, care in the selection of processing methods can ensure retention of nutrients, obvious examples being the parboiling of rice and the undermilling of wheat, both of which increase the retention of vitamins of the B group. Another example is high-temperature short-time sterilization resulting in decreased loss of nutrients. Other processing methods may lead to the destruction of antimetabolites or toxic materials or to the release of such bound nutrients as niacin, as well as improving the digestibility of the food. A further approach to the improvement of quality can be described as " biological ennoblement " — the improvement that takes place following sprouting and fermentation procedures.

At the level of the consumer, the way in which food is prepared can alter nutrient intake and bad practices can be corrected through education. Cooking losses can be minimized and parboiling or fermentation processes can be encouraged. Suitable mixtures of available foods to improve the quality of dietary protein can be prepared in the home. Education on how to choose foods for maximum nutrient value should be part of any nutrition programme.

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The educational approaches mentioned above must be regarded as long-term measures. However, it is also possible to improve the quality of the food supply immediately, without necessarily changing food habits, by adding nutrients directly to food. The guidelines given below for consideration in implementing a programme of this type are based on the wide experience already gained in the use of this procedure.

1.2 Definition

Of the names in current use in various parts of the world, the Committee considered the term *fortification* to be the most appropriate to describe the process whereby nutrients are added to foods to maintain or improve the quality of the diet of a group, a community, or a population.

The nutrients may be added as extracts or concentrates of materials of biological origin, or as products of chemical or biochemical synthesis. Although it is not possible to draw a sharp line of demarcation between fortified products and some kitchen or factory formulations of food mixtures, the term "fortification" is intended to apply principally to the use of relatively small quantities of additives.

The Committee did not discuss the fortification of salt with iodine nor the fluoridation of water, since these subjects have already been extensively considered in WHO publications.[1]

1.3 Objectives

Fortification is a public health measure aimed at improving and maintaining the health of individuals in the population through the provision of adequate levels of nutrient intake. Although fortification programmes will often cover the entire population, they may also be designed to meet the needs of people in particular geographic areas, of particular socio-economic groups, or of particular age and sex groups. Some fortification programmes may have still more limited objectives, such as those concerned with the specification of quality standards for the foods used in special feeding programmes (e.g., school lunch programmes).

For the purposes of the present report, it is assumed that a fortification programme will usually be intended to meet a demonstrable need in the population, apparent from either dietary, biochemical, or clinical evidence, and that the severity of the nutritional deficiency and its prevalence will be factors influencing the priority to be assigned to such a programme. It is recognized, however, that while the basic purpose of fortification, i.e., the improvement or maintenance of health, remains unaltered, the approach

[1] *Endemic goitre*, by various authors, Geneva, World Health Organization, 1960 (*Monograph Series*, No. 44); *Fluorides and human health*, by various authors, Geneva, World Health Organization, 1970 (*Monograph Series*, No. 59).

to fortification may change as the technological capabilities and economic resources of a country increase. The different approaches have been reviewed by various authors;[1] here it need only be stated that, in countries that have highly developed food industries and where food fortification is customary, less emphasis may be placed on the use of this process to meet demonstrated needs than on its use as a method of maintaining the quality of foods consumed by the population. For example, nutrients may be added to foods to replace those lost in processing; they may be added to "imitation foods" to provide nutritive value equivalent to the foods that they imitate; or they may be added to "ready to eat" meals or similar preparations. Foods may also be classified into groups and fortified to provide the nutrients expected in members of that group. Finally, nutrients may have to be added to food mixtures compounded for special dietary needs, e.g., infant foods, weaning foods, or emergency rations.

Some of these considerations were recently discussed by the Committee on Food Standards of the International Union of Nutritional Sciences.[2]

The Joint FAO/WHO Committee on Nutrition noted that at present there is no unanimity among nutritionists about the role to be assigned to food fortification in solving the nutrition problems of a country. Whereas there would appear to be close agreement on the potential value of fortification in a community where nutritional diseases are prevalent, there is a divergence of views on the action to be taken in situations where nutrient intakes are low but there is little or no evidence of actual nutritional disease, or where there is concern that new trends in food processing and food usage may lead to nutritional problems, again without specific evidence of ill health for the time being. In these situations, it may be very difficult to choose between various approaches, which might include not only conventional nutrition education and programmes for improving agricultural production but also national policies relating to the whole food processing industry and the extent to which the processing of foods is deemed desirable. Clearly, all these aspects of the problem must be considered before a decision can be reached on the value of a fortification programme. Attention is drawn to the following specific points that have led to differences in opinion about the relative merits of fortification and it is suggested that these should be considered before implementing a programme.

(1) Knowledge of human nutrient requirements remains incomplete, particularly as it is very difficult to obtain precise information about actual

[1] Causeret, J. (1946) *Bull. Soc. Sci. Hyg. Aliment. rat.*, 34, 122; Causeret, J. (1957) *Enrichissement des aliments*. In: *Nutrition et alimentation tropicales*, Rome, Food and Agriculture Organization of the United Nations (Réunions de la FAO sur la Nutrition, Rapport No. 20), p. 709; Harris, R. S. (1968) *J. agric. Food Chem.*, 16, 149; Bigwood, E. J. & Gérard, A. (1968) *Objectifs et principes fondamentaux d'un droit comparé de l'alimentation*. Vol. 2: *Eléments de motivation et éléments de qualification*, Basle, Karger.

[2] Unpublished report.

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nutrient intakes and to interpret the data correctly (see section 3.1). Serious doubts may remain about the actual benefits to be gained when there is no definite evidence of a nutritional disease problem in the population. Introduction of fortification programmes may divert limited resources and efforts from more important approaches.

(2) In most cases, fortification is carried out with a small number of nutrients and it may be forgotten that other nutrients lost during processing or reduced in supply with the introduction of new types of foods have not been replaced. For example, in the usual fortification of white bread only a few nutrients are added while many are partially removed.

(3) Since, with a multiplicity of fortified foods, it would be very difficult to maintain the nutrient ratios found in a usual mixed diet, the possibility remains that widespread fortification could lead to nutrient imbalances perhaps unrecognized at present.

(4) Experience in some countries has suggested that the introduction of fortified foods, unless undertaken for specific reasons and carefully controlled, may lead to a much broader range of such foods than had been originally envisaged and, in some instances, to unwarranted claims for their health advantages. This might, in turn, lead to confusion in the minds of the public and thwart the efforts of other nutrition programmes. As pointed out elsewhere (see section 4.2, p. 27), such a situation may give rise to special problems, particularly in the case of potentially harmful nutrients.

(5) There is always a danger that the apparent simplicity and early effectiveness of fortification may lead to a slackening of efforts directed towards the maintenance of the basic quality of food products and the development of nutrition education programmes. Thus, a balanced approach to a sound food and nutrition policy may be difficult to maintain.

In view of the above considerations, the Committee stressed that the final decision on food fortification must be made at the national level, as a part of the national food and nutrition policy. It urged, however, that in formulating a national policy on food fortification appropriate to a particular situation due attention should be paid to the arguments set forth in this report.

2. FOOD FORTIFICATION IN PRACTICE

2.1 A review of past programmes

Some 50 years ago, the fortification of salt with iodine compounds was recommended for the prevention of goitre. Since then, many countries have adopted programmes of this kind.[1]

The next major development in this field came with the identification of vitamins A and D and their availability as fish liver oils or concentrates and later as pure vitamins. The serious consequences of the replacement of dietary milk fat with certain substitutes were dramatically demonstrated in Denmark between 1909 and 1920, when the substitution of unfortified margarine for butter produced eye lesions in children.[2] As a result, the fortification of margarine, first with concentrates and later with purified or synthetic forms of vitamin A or provitamin A, was introduced in many countries.

The third nutrient that was added to foods to prevent a clearly defined nutritional deficiency disease was vitamin D. In the 1920s it became feasible to prevent rickets by providing vitamin D in the diet through the irradiation of sterol-containing foods. Later the addition of purified vitamin D to milk and such foods as cereals and margarine was widely adopted, but this practice has recently been reconsidered (see section 4.2, p. 27).

Between 1920 and 1940 rich sources of the B-vitamins, such as yeast, wheat germ, and liver extract, were used to fortify various food products. The fortification of bread, pasta, and other cereal products with purified and synthetic B-vitamins — particularly thiamine, niacin, and riboflavin — together with iron and calcium was first introduced in Newfoundland in the 1940s. The USA followed a few years later when it passed federal regulations permitting the fortification of wheat and maize products.

Rice fortification also received considerable attention, particularly in the 1940s. Processes were being developed for impregnating foods with nutrients through a parboiling process, for adding nutrients to the surface of rice kernels in a protective coating, and for making vitamin-mineral concentrates in the form of simulated rice kernels. Rice-enrichment programmes were started in Japan, the Philippines, Taiwan, and the Territory

[1] *Endemic goitre*, by various authors, Geneva, World Health Organization, 1960 (Monograph Series, No. 44); *Seminario sobre yodización de la sal para prevención del bocio endemico, Salta, Argentina, 21-26 de Junio de 1965 — Informe final*, Washington, Pan American Health Organization, 1965; *Report of a Seminar on Goitre Control, New Delhi, 30 October – 4 November 1967*, New Delhi, WHO Regional Office for South-East Asia, 1967 (unpublished document SEA/Nut/22).

[2] Vitamins: a survey of recent knowledge (1932) *Spec. Rep. Ser. med. Res. Coun. (Lond.)*, 167, 231.

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of Papua and New Guinea. In the last of these, comprehensive programmes are still being implemented, while in Japan and the Philippines enrichment is mostly being continued on a small scale on a voluntary basis. In some countries of eastern Asia, the implementation of rice fortification programmes has been hampered by such factors as dispersion of milling facilities and unsatisfactory economic arrangements from the point of view of the millers, the government, or the consumer.

During the Second World War, when an undermilled flour was introduced in the United Kingdom to increase the nutrient content of bread, it was also enriched by the addition of calcium in anticipation of a possible deficiency. In the same period, Canada introduced the addition of vitamin C to canned apple juice in anticipation of a decreased supply of citrus fruits. Vitamin C has now been added to a wide range of fruit drinks and juices as well as to canned milk and other products.

The discovery of vitamin B_{12} and its ready availability in pure form removed one of the chief barriers to the development of complete vegetarian formulations.

Fortification of bread with protein concentrates from soybean, milk, or other sources is practised in various countries. Only in Japan has the addition of lysine to cereal products been practised on a wide scale. In India it is still in the experimental stage.

During the years since the first introduction of fortification programmes, considerable experience has been gained in the technology of fortification.[1] Some idea of the variety of procedures that have been used can be obtained by reference to Table 1 (p. 14).

2.2 Evaluation of some past programmes

In considering the history of fortification programmes, the following should be borne in mind. These programmes have been evolved gradually over the past 40 years as suitable sources of nutrients became available. At the time that many of the programmes were instituted, techniques for the evaluation of nutritional status were relatively crude and background data for evaluating the specific effects of the programmes were fragmentary. Most of these programmes were implemented in developed countries where the prevalence of many of the deficiency diseases was not very high. Furthermore, their implementation often coincided with a variety of social, economic, agricultural, educational, and public health developments—

[1] Harris, R. S. *Mineral fortification of foods and food products.* In: *Proceedings of the Third International Congress of Food Science and Technology, Washington, August 9–14, 1970* (in press); Aylward, F. & Morton, I. D. *Vitamin fortification of foods.* In: *Proceedings of the Third International Congress of Food Science and Technology, Washington, August 9–14, 1970* (in press).

TABLE 1. SOME TYPES OF FOOD USED IN FORTIFICATION PROGRAMMES *

Nutrient	Type of food	Comments
Ascorbic acid	Canned, frozen, and dried fruit drinks, canned and dried milk products, dry cereal products	Must be protected from air if in neutral solution
Thiamine, riboflavin, niacin	Dry cereals, flour, bread, pasta, milk products	With materials like rice, may be impregnated into or coated on the kernel; riboflavin may colour the food; nicotinamide is usually preferred to nicotinic acid
Vitamin A (or β-carotene)	Dry cereal products, flour, bread, pasta, milk products, margarine, vegetable oils	Must be protected from air and must be added in water-miscible form to non-fat products — may be added in gelatin beadlets together with stabilizer coated on product or mixed in simulated kernel (e.g., rice); carotene may colour product; heat losses in cooking oils may be great
Vitamin D	Milk products, margarines, dry cereal products, vegetable oils, fruit drinks	See comments for vitamin A; multiple sources may be undesirable (see section 4.2, p. 27)
Calcium	Cereal products, bread	The quantity to be added usually limits the scope of vehicles
Iron	Cereal products, bread, canned and dried milks	Availability varies with form used (see section 4.1, p. 25); may cause colour or flavour changes
Iodine	Salt	Iodine usually used; iodate more stable in crude salt
Proteins	Cereal products, bread, cassava flour	Protein concentrates of various types used; the amount to be added usually limits the scope of vehicles
Amino acids	Cereals, bread, meat substitutes	Other vehicles have been proposed but not yet used; lysine or methionine now authorized in some areas

* In addition a wide range of nutrients have been added to infant foods and prepared formulas.

for example, in Newfoundland,[1] where one of the early trials of fortification was carried out. Under such conditions, it is not surprising that the specific effects of the fortification programmes can rarely be identified. Nevertheless,

[1] Adamson, J. D. et al. (1945) Medical survey of nutrition in Newfoundland. *Canad. med. Ass. J.*, 52, 227; Metcoff, J. et al. (1945) Nutritional survey in Norris Point, Newfoundland. *J. lab. clin. Med.*, 30, 475; Aykroyd, W. R. et al. (1949) Medical resurvey of nutrition in Newfoundland 1948. *Canad. med. Ass. J.*, 60, 329.

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improvements in nutritional status did occur. The prevalence of the classical deficiency diseases fell to very low levels, and several were practically eliminated. Many public health and nutritional authorities concluded that these fortification programmes provided a safeguard against shortcomings in the national diet and should therefore be continued.

In this brief review it is convenient to consider the evidence available with regard to each specific nutrient, although it is realized that fortification will be effective only if the vehicle delivers the nutrient to the target group in adequate amounts. It must also be remembered that single deficiency diseases are rare.

Thiamine

The Philippine experiments in the 1940s[1] provided some indication of the effectiveness of thiamine fortification in reducing the prevalence of beriberi and improving tissue thiamine levels. This work has not been followed up by a programme of rice fortification in the Philippines. Such programmes have been implemented, however, in Japan, Korea, and Taiwan.

Riboflavin

It is difficult to assess the efficiency of riboflavin fortification programmes because clinical signs of the deficiency lack specificity. However, in a study carried out in the Chinese Army in Taiwan in the 1950s, 12 companies were examined before and after the implementation of a fortification programme in 6 of the companies. At the beginning of the study many of the men had a severe form of the "orogenital syndrome" and were presumed to be severely deficient in riboflavin. Improvements were noted and were probably attributable to the increased riboflavin intake, although the effect of other changes that were made in the diet is not completely clear.

Niacin

Epidemiological studies of the effects of niacin fortification in pellagrous areas do not appear to be available. The virtual disappearance of pellagra in the USA in the 1930s and 1940s clearly began before the implementation of widespread fortification programmes. Although the role of the fortification is not discounted, such other factors as economic improvements, nutrition education, and improved medical care undoubtedly played a major role.

[1] *Rice enrichment in the Philippines*, Rome, Food and Agriculture Organization of the United Nations, 1954 (FAO Nutritional Study No. 12).

Vitamin C

Although ascorbic acid has been added to many products in some countries and there is no reason to doubt the effectiveness of such products in the control of scurvy, data do not appear to be available from areas where special health benefits would have been expected.

Vitamin D

See section 4.2, p. 27.

Vitamin A

The addition of vitamin A and/or carotene to margarines has long been practised in many countries. In the Newfoundland surveys,[1] clinical evidence of vitamin A deficiency was reported. A programme for the fortification of white bread with B vitamins, iron, and calcium, and of margarine with vitamin A, was begun. A repeat survey in 1948[2] indicated a reduced incidence of symptoms suggestive of nutritional deficiency. Mean serum vitamin A levels had risen from 20 µg/100 ml in 1944 to 45 µg/100 ml in 1948. However, there were rather large economic improvements during these years. These were associated with increased supplies of various foods — milk, fruits, vegetables, etc. — nutrition education programmes, free distribution of skim milk to schools, and free cod liver oil to expectant mothers and infants. These changes undoubtedly obscure the direct effect of the vitamin A fortification programme.

Calcium

Since neither clinical nor biochemical methods for the evaluation of dietary calcium deficiency are available, there is no evidence on the effect of calcium fortification upon the health of populations.

Iron

See section 4.1, p. 25.

Protein and amino acids

See section 4.3, p. 29.

2.3 Fortified food formulations for infants

Up to fifty years ago, it was common paediatric practice in many industrialized countries to feed infants on diets composed of cow's milk

[1] For references, see p. 14.

[2] Aykroyd, W. R. et al. (1949) Medical resurvey of nutrition in Newfoundland 1948. *Canad. med. Ass. J.*, 60, 329.

diluted with corn syrup or a similar source of carbohydrate to give approximately the same composition as breast milk. Later, various milk preparations became available in powder form.

From 1920 onward, with the discovery and identification of the vitamins and increased knowledge of the role of vitamins, minerals, and proteins in nutrition, improved methods of processing and more adequate formulations were developed, under the guidance of paediatricians, in many industrialized countries. Studies in major hospital paediatric clinics, orphanages, and other centres, involving co-operation between the paediatrician and the nutritionist-biochemist, demonstrated that the newer knowledge of nutrition could be successfully applied in practice, both in the hospital and later in the home.

The addition of nutrients to infant foods was among the first successful examples of food fortification. In more recent years, both infant foods and weaning foods based wholly or partly on foods from plant sources have been formulated and have been fortified with nutrients. These formulations have been tested and used extensively with success.

The earlier work on formulated infant and weaning foods has provided a background for the large number of formulations developed in many different countries, e.g., Incaparina and Superamine. These preparations are fortified foods designed for specific purposes.

3. DESIGN OF A FORTIFICATION PROGRAMME

In designing a fortification programme it is first necessary to decide (*a*) what nutrients to add and (*b*) what food to use as the vehicle. To answer these questions, nutrient needs and food usage in the population must be determined as precisely as possible. It is then necessary to consider several subsidiary points concerning the technological, legislative, control, and economic aspects before assessing the potentialities of the programme and reaching a final decision on its design. Provision should also be made for the evaluation and monitoring of programmes after implementation.

3.1 Definition of nutrient needs and target groups

If estimates indicate gross inadequacy in the availability of a particular nutrient, this may be sufficient indication of the need for action. However, it must be recognized that national food balance sheet data provide no information about the distribution of nutrients within the population. Since the portion of the population with low intakes constitutes the target group, the intake distribution must be known. Data may be available from food consumption surveys, but their usefulness will depend both on the reliability

of the information and on the basic unit of investigation. Unfortunately for the present purpose, many such studies examine food usage at the family level, thus leaving unanswered the question of distribution within the family and making precise definition of the risk group impossible. In spite of these limitations, such data do permit a rough identification of target groups.

There are three overlapping situations in which improvements in the nutrients of the diet must be considered:

(*a*) when there is clinical evidence of deficiency diseases;

(*b*) when estimates indicate low availability of nutrients;

(*c*) when deficiencies are thought likely to develop because of changes taking place in food habits.

The immediate objective of a fortification programme is the maintenance or improvement of the nutrient intake of a population or a segment of it (the target group) to meet an established or anticipated need. To make sure that the programme is directed at those most in need, it is important to define the target group in terms of age, sex, socio-economic status, geographical location, and other significant variables.

Where a fortification programme has been decided on because of the known prevalence of a deficiency disease, the presence of the disease itself will serve to describe the primary target group. It is then only necessary to establish that the disease will respond to the nutrient in question (i.e., that it is attributable to a dietary deficiency) and to determine whether other segments of the population have low intakes of the nutrient (i.e., to define the extent of the target group).

Sometimes, however, the dietary intakes may be low but not low enough for there to be clear evidence of a significant prevalence of identifiable disease. In such circumstances, the dietary information must be critically examined and should be reinforced whenever possible by biochemical and other studies. As has already been noted (section 1.3) there is a divergence of opinion about the action that might be taken in such situations. Before taking a decision on food fortification, therefore, its relationship to other programmes designed to improve nutrient intakes should be thoroughly assessed.

If fortification is accepted as a possible solution, the costs must be examined carefully. Should the programme prove to be relatively inexpensive, there may be no justification for spending additional money in order to define the target group precisely; it may be more economical to aim at a rather wide group of the population.

It is well to remember that the cost of adding a second or third nutrient may be relatively low, once the initial programme is established. In reviewing the dietary information, therefore, a search might be directed toward

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other nutrients possibly in short supply. Before deciding on the addition of a second nutrient, it is important to be sure that persons with low intakes of the second nutrient fall within the primary target group or that it is economic to expand the target group.

3.2 Selection of a vehicle and level of fortification

Once the target group is known, it is possible to consider possible vehicles for fortification. It is obvious that the food selected must be one consumed by members of the target group. For maximum efficiency it is desirable to select a food used preferentially by the target group ; in addition, the intake should be similar for all members of the target group and should be expected to remain stable. While these conditions cannot always be met, their significance can be assessed by examination of data on the intakes of various foods believed to reach the population group in question. Only those foods that pass through central, regional, or communal processing points, and hence might be fortified, need to be considered for this purpose. While a staple food, consumed in relatively large amounts, is often chosen as a vehicle, it is always wise to consider other perhaps unusual foods that reach the target group and might be suitable. It is again stressed that knowledge of the distribution of individual intakes is essential, since the level of nutrients to be added is a function both of the estimated individual need for the nutrient — which may be affected by environmental conditions — and of the lowest intakes of the chosen food by individuals in the target group.

It should be noted that in practice it will be impossible to ensure that those with the lowest intakes of the vehicle food receive adequate quantities of the nutrient. The decision on how complete a coverage should be attempted will probably be based on the considerations of cost-effectiveness. The use of multiple vehicles for the nutrient(s) may increase the effectiveness of coverage but necessitates careful consideration of the level of nutrient to be added to each food in order to minimize waste. Such estimates can be made, but only with detailed information about the range of intakes of vehicle foods by the individuals in the target group and the costs of multiple fortification.

In the case of potentially toxic nutrients (see section 4.2, p. 27) it is also important to have information about the upper range of intake of the vehicle food in all segments of the population. This information is needed to ascertain the safe upper limit of fortification.

Special mention should be made of sugar, soft drinks, and alcoholic beverages as potential vehicles. One or the other of these may meet the specifications described above. Special problems are raised when foods whose excessive consumption is discouraged by nutritionists are selected as potential vehicles for fortification. Fortification of these products with

properly selected nutrients could increase their nutritive value and thus reduce to some extent the disadvantages of their consumption in large quantities. It must be borne in mind, however, that there is a danger that such fortification might frustrate the endeavours that are being made, or might be made, to check excessive consumption of these products and might even be used as publicity in their favour.

The respective advantages and disadvantages of fortification of these foods will need to be weighed in each case. The Committee considered that, as a general rule, it is preferable not to include such products among the possible vehicles for fortification programmes. However, it recognized that, in the case of populations that are in the habit of consuming large quantities of sugar and whose habits in that respect seem unlikely to change in the near future, the fortification of sugar cannot be ruled out *a priori*.

Before reaching a final decision on the selection of the vehicle, the technological aspects set forth below should also be considered.

3.3 Technological considerations

The most important technological factor to be considered in designing a fortification programme is, of course, the stage or stages in the processing or handling of the vehicle at which the nutrient(s) can be added. For this purpose, it is desirable that a single centre be designated within the region or country, in order to facilitate control of the programme and minimize the costs (see section 3.6, p. 23). In the case of flour fortification, this centre might be a milling plant, but if no suitable centre is available, the nutrient may be added in a local mill or at a local bakery, making use of pre-mixes that can be handled with moderate cost and dispensed with sufficient accuracy. Tests should be run periodically to ensure that the rate of addition is uniform and mixing is thorough. The range of vehicles used in past fortification programmes is briefly described in section 2.1, p. 12.

It must also be established that the nutrient is biologically available in the form in which it is added ; that the availability is not adversely affected by the vehicle ; and that the nutrient does not convey unwanted characteristics to the food (colour, taste, flavour, cooking properties) or unduly increase the cost of the product. As discussed in section 4.1, p. 25, iron presents serious problems in several of these respects. Protein fortification involves the addition of relatively large quantities of material and may influence the cooking properties of the fortified foods, the acceptability of which may depend upon the cooking practices of the region. Since some nutrients may be unstable and either be destroyed (or affected by specific antimetabolites or other agents in the food) or gradually confer undesirable colours, flavours, or odours to the food through a variety of reactions, it [illegible]ential to examine the stability and food quality under the conditions [illegible] temperature, humidity, and light exposure that might be expected

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in adverse storage situations. Provided that the nutrient remains biologically available, reluctance to accept slight changes in colour, flavour, or cooking properties may be overcome by an education programme. However, this will add to the cost. The effect of home cooking practices upon the retention of the nutrient should be checked. If such practices have a serious adverse effect (e.g., the washing of rice when the nutrients have been added in a coating on the kernel) they may need to be corrected by an education programme.

3.4 Assessment and monitoring of fortification programmes

To determine the effectiveness of a fortification programme in meeting a specific nutritional need, appropriate monitoring procedures should be employed. These will presumably include either clinical or biochemical assessment of the nutritional status of large groups and/or dietary surveys to evaluate the food and nutrient intake of target groups. Such assessment will need to be made periodically, since changes in the food supply or economic or other factors may alter food usage by the target group. Consequently, the kinds and amounts of nutrients used in the fortification programme may become inappropriate. Furthermore, the effectiveness of the vehicles used may change. The history of iron fortification provides an example of a programme that proved less successful than had been anticipated from information available at the time it was conceived and implemented.

Attention is also drawn to the need for periodic review of fortification policy, legislation, and other related matters. In some countries, outdated policies and legislation provide barriers to more effective programmes and there is no simple mechanism to provide for review and modification as the situation changes.

3.5 Legislation and control measures

3.5.1 *Legislation*

Countries have made laws and regulations governing the fortification of foods. Examination of these laws and regulations shows that they are extremely varied. Bigwood & Gérard,[1] reviewing the laws and regulations on fortification of foods in 12 countries, speak of a "truly chaotic situation".

This situation has resulted in part from the difficulties experienced by some countries that already had complex food legislation in modifying their legislation when new procedures were proposed. Other factors have

[1] Bigwood, E. J. & Gérard, A. (1968) *Objectifs et principes fondamentaux d'un droit comparé de l'alimentation*. Vol. 2: *Éléments de motivation et éléments de qualification*, Basle, Karger.

been the diversity of views on the advisability of fortifying foods — views that did not invariably have a scientific basis — and the pressures of various kinds exerted in some countries in favour of or against fortification. It should also be remembered that terminology in regard to fortification differs from country to country.

It will be seen from the foregoing that it is difficult on the basis of existing legislation to prepare a "model" for countries that as yet have no legislation of their own on the subject. It may be pointed out, however, that several approaches are possible. Regulations may be designed:

(*a*) to make a particular programme compulsory;

(*b*) to *permit* fortification;

(*c*) to provide incentives to firms that produce fortified products; or

(*d*) to ensure appropriate standards in food contracts for hospitals, schools, and other institutions.

The advantages and disadvantages of these different approaches should be considered at the national level. It would appear, however, that in the developing countries approach (*a*) will frequently be preferred. In some cases a programme encouraging a small firm to manufacture on its own initiative a special product with a formula that has been carefully investigated might also have good results.

3.5.2 *Control*

The best devised regulations can be ineffective if they are not accompanied by proper means of control. To permit the enforcement and control of a food fortification programme, procedures and facilities for assaying the levels of nutrients in the food are essential. Properly trained technical staff must also be available. There should be periodic scrutiny of the levels of nutrients added at the fortification point (and in the pre-mix if used) to ensure proper functioning of the equipment, and at the level of the consumer distribution centre to ensure that there has not been loss during storage.

Obviously, the greater the number of fortified foods and the nutrients put into them, the more problems control will involve. It will be noted also that more resources will be required if fortification is carried out by small firms than if it is done centrally.

3.5.3 *Labelling and publicity*

In some industrialized countries, the regulations contain specific provisions on these two points. In others, the manufacturers are left almost entirely free. In the absence of specific regulations, there is always a danger that unwarranted, inaccurate, and misleading statements concerning a

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product . . . made in labelling and advertising, possibly interfering with the development of a sound nutrition programme.

The Committee considered that, in principle, the consumer has a right to be informed concerning the characteristics of the products he is buying.

3.5.4 *Possibilities of co-ordinating national fortification legislations*

There is a danger that the variety of national laws on fortification may make international trade in certain commodities difficult. Consequently, it would seem desirable to try to harmonize the legislation of different countries.

Complete harmonization, however, does not seem feasible, since the fortification measures to be prescribed or encouraged, the amounts of nutrients to be put into the selected vehicles, and the nature of the vehicles themselves have to be decided upon in relation to the special needs and eating habits of the population or population group whose state of nutrition it is desired to improve.

There seems to be a possibility that harmonization of legislation can at least be achieved on a regional basis, several countries having decided to promote identical measures to deal with comparable difficulties. FAO and WHO might assist in this effort.

3.6 Economic considerations

It has been stressed in the introduction to this report that the probable costs of nutritional programmes should be weighed against their predicted benefits and that various approaches to the same problem might be compared on this basis before a final decision is reached. It should be made clear that this is of major concern only when the proposed expenditure is large: some fortification programmes can be accomplished at a relatively low cost, while others are quite expensive and warrant close examination.

In considering these economic aspects of fortification programmes, the Committee examined the document recently prepared by the FAO/WHO/UNICEF Protein Advisory Group on amino acid fortification.[1] It is recommended that this document be consulted for a discussion of some of the specific factors to be considered in the evaluation of programmes of amino acid and protein fortification. The following are some general comments applicable to programmes involving most nutrients.

Evaluations of the costs and benefits of a fortification programme should always be conducted in the context of the special situation. Ideally each country should have well-defined short-term and long-term nutritional goals and a co-ordinated plan for achieving these goals. Such a plan

[1] FAO/WHO/UNICEF Protein Advisory Group (1970) *PAG Statement on amino acid fortification of foods*, New York, United Nations.

permits consideration of the cost of a fortification programme in terms of a re-allocation of limited resources and the effect of the programme upon the rate of progress toward long-term goals. For example, if the long-term goal is the improvement of the quality of the basic food supply, the fortification programme should not be permitted to interfere with the achievement of this goal.

Certain of the costs of a fortification programme, such as the capital costs of fortification equipment and the cost of purchase and transport of the nutrients, are direct and easily recognized. These costs may be passed on to the consumer in the form of an increase in the cost of the food. Programmes that cause even a moderate increase in the retail price of a staple food can be justified only if it can be shown that the expected benefits outweigh the additional food expenditure of the family; if the change in price is substantial, or if fortified and unfortified products appear on the market with a price differential, the programme may defeat itself.

Whether these costs are passed on to the consumer or met by the government (subsidized programme), there are other less direct costs that should also be recognized. To control the programme, laboratory facilities must be created and technologists trained and employed. As previously mentioned, in certain circumstances there may also be a need for educational programmes to ensure that the fortified product is used and that the nutrients are preserved.

As with all other programmes, there is a cost attached to preliminary field testing and continuous monitoring of the effectiveness of the programme. It will be recognized that many of these costs are related to the number of installations where fortification is carried out. For this reason, fortification at a central processing plant represents the ideal situation, whereas fortification at the communal mill represents the limit of decentralization that can be considered practical. Some authors have suggested a programme of "home fortification" through the supply of fortified wafers to be added to the cooking pot. In most instances, however, this would offer little or no advantage over the provision of direct supplements to each member of the target group, through periodic oral dosing or injection of the nutrient.

It should also be remembered that, since costs must be related to the number of persons in the target group who are reached by the programme and benefit from it, for maximum efficiency the food chosen as the vehicle should be one consumed specifically by the target group.

An economic consideration that should not be neglected in reviewing possible fortification programmes is the potential drain upon foreign currency reserves if vitamin and mineral preparations have to be purchased from sources outside of the country. In some instances, this problem may be avoided by developing special sources of the needed nutrients for

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addition to foods (e.g., red palm oil as a source of vitamin A, locally produced protein concentrates, etc.).

4. NUTRIENTS REQUIRING SPECIAL CONSIDERATION

Other WHO publications have dealt with the particular approaches to and problems of the fortification of salt with iodine and the addition of fluoride to water supplies.[1] These are not considered in the present report. The following three types of fortifications, currently of interest to the international agencies, warrant special consideration.

4.1 Iron

The Committee has recommended that one of the factors to be considered in designing a fortification programme is the availability of the nutrient in the proposed vehicle (see section 3.3, p. 20). This factor presents considerable difficulty in the case of iron.

While iron fortification programmes have been in effect in North America and the United Kingdom for many years, there appear to have been few attempts to evaluate their effectiveness until recently. In recent years, several groups have questioned the availability of iron in flour and bread.[2] It is now apparent that the form of iron added, the nature of the vehicle, and the nature of other dietary constituents may all affect the availability of iron added to foods. Reports in the literature suggest that all these factors operate and may contribute to confusion of interpretation.

It now appears that some but not all preparations of "reduced iron" or "powdered iron", as well as sodium iron pyrophosphate and perhaps other iron salts that have been used in fortification programmes, are relatively unavailable to the human being. Most of the evidence suggests that ferrous sulfate, used in some fortification programmes, is among the most available forms. Data on the availability of other forms of iron that might be used for fortification are too limited for them to be placed in a definite rank order. Furthermore, the availability may be affected by the nature of the vehicle, the processing to which the vehicle is subjected, and the diet with which it is consumed.

[1] For references, see p. 9.

[2] Elwood, P. C. (1968) *Iron in flour*, London, H. M. Stationery Office (*Reports on public health and medical subjects*, No. 117); Committee on iron nutritional deficiencies, Food and Nutrition Board (1970) *Workshop on measures to increase iron in foods and diets*, Washington, National Academy of Sciences — National Research Council.

Selection of the iron salt to be used may be based on cost (Table 2) and on technological factors. Ferrous sulfate and some other forms that seem

TABLE 2. RELATIVE COST OF SOME FORMS OF IRON SALTS *

Iron source	Cost per lb of salt ($)	Cost per lb of elemental iron ($)
Ferrum reductum	0.40	0.41
Ferrous sulfate, anhydrous	0.28	0.47
Ferric orthophosphate	0.54	1.88
Sodium ferric pyrophosphate	0.39	2.69
Ferrous fumarate	0.95	2.39
Ferric ammonium citrate	0.67	3.94
Ferrous gluconate	0.91	7.85

* Committee on iron nutritional deficiencies, Food and Nutrition Board (1970) *Workshop on measures to increase iron in foods and diets*, Washington, National Academy of Sciences — National Research Council.

to be biologically available may produce disadvantageous colour or flavour problems when added at moderate levels to some foods, whereas with certain sparingly soluble (and presumably unavailable) forms these problems are not encountered. Interaction of foods with iron compounds might be prevented by such techniques as incorporation of the compounds in gelatin beadlets, as is done with certain of the vitamins, but this would further increase the cost.

In view of the uncertainty about the physiological availability of the various iron preparations, serious doubt must be cast upon the efficiency of at least some of the present iron fortification programmes and it is difficult to offer specific recommendations for future programmes. The Joint FAO/WHO Expert Group on Requirements of Ascorbic Acid, Vitamin D, Vitamin B_{12}, Folate and Iron[1] has recently made new recommendations for daily intakes of iron. In view of the prevalence of iron deficiency anaemia[2] and the size of the recommended intakes in relation to the usual iron intakes, the Committee agreed with the Joint FAO/WHO Expert Group that there is a clear need to increase the dietary iron intakes in many, if not most, populations. This might be most easily accomplished through fortification programmes, the alternatives being either the provision of direct iron supplements for vulnerable groups or a major change in dietary habits. The Committee endorsed the recommendations of the Joint FAO/WHO Group that research into effective methods of iron fortification be given high priority. In particular the Committee recom-

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1970, No. 452, p. 54; *FAO Nutrition Meetings Report Series*, 1970, No. 47, p. 54.

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1968, No. 405.

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mended continuation and expansion, if possible, of current human studies and suggested that it might be advantageous to encourage comparative studies of absorption of iron in various species in the hope that information applicable to man might be developed. The Committee stressed that such studies, whether in man or in animals, should be concerned with the availability of iron when added in the form proposed for fortification to the proposed vehicle and fed in conjunction with usual diets. As a final confirmation of studies of this type, the Committee recommended a careful field evaluation of the ability of the fortified foods to maintain normal values for all blood constituents.

Concern has also been expressed about possible toxic effects of high levels of iron fortification and it has been suggested, though not established, that haemosiderosis is associated with the characteristically high iron intakes of the Bantu. The Committee was unaware, however, of any evidence of haemochromatosis or haemosiderosis associated with dietary iron intake in other populations except in occasional patients with absorptive abnormalities. Consequently, there does not seem to be any need for serious concern about the safety of iron fortification, but it is suggested that the possibility be considered when collecting data in field studies.

4.2 Vitamin D

The discovery of vitamin D, followed shortly afterwards by the availability of irradiated or fortified milk and other foods, resulted in a marked decline in the prevalence of rickets in many countries. Although the relative importance of the fortification programmes, better paediatric care, use of fish liver oils, etc., cannot be clearly defined, there is abundant evidence that milk and infant foods fortified with vitamin D are effective antirachitic agents. Nevertheless, the number of cases of rickets due to vitamin D deficiency reported from developed countries is sufficiently high to indicate that the preventive programmes are not totally effective. In many of the developing countries rickets is frequent in hospitalized infants. In a recent WHO survey in North Africa,[1] 45–60% of the infants examined were found to show signs of rickets.

The toxic effect of excessive intake of vitamin D has long been known but the possibility that the amounts used in fortification programmes might be hazardous was discounted until an increased incidence of hypercalcaemia in infants was reported in the United Kingdom.[2] This phenomenon was associated with an increase in the vitamin D content of the fortified national dried milk from 280 IU/oz (10 IU/g) to 500 IU/oz

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 377, p. 31; *FAO Nutrition Meetings Report Series*, 1967, No. 42, p. 31.

[2] *Brit. med. J.*, 1956, 2, 149.

(18 IU/g). In 1957, the Ministry of Health in the United Kingdom recommended that the vitamin D content of the national dried milk be reduced to 90–100 IU/oz (3.2–3.5 IU/g); that of infant cereal from 1000 IU/oz (35 IU/g) to 300 IU/oz (10 IU/g); and that of cod liver oil preparations from 800 IU to 400 IU per teaspoon. The prevalence of hypercalcaemia has apparently fallen to low levels since these changes were made. Another way of controlling the problem of toxicity due to high intake has been to prohibit the addition of vitamin D to a wide variety of foods. In Canada, vitamin D may be added only to milk (400–530 IU per imperial quart; 350–465 IU/litre) and to margarine. Following the reports of vitamin D toxicity in the United Kingdom some authors suggested that congenital supravalvular aortic stenosis might be related to excessive vitamin D intake. The Committee on Nutrition of the American Academy of Pediatrics[1] recommended that efforts be made to ensure an intake of approximately 400 IU daily for infants, but that fortification of foods other than milk and infant formulas be discontinued. In the United Kingdom, infants who happened to be receiving vitamin D from all three sources mentioned above (1½ pints of milk, 1 oz of cereal, and one teaspoon of cod liver oil) would at present have a total intake of the order of 1000–1200 IU/day.

It should be noted that during the time when foods highly fortified with vitamin D were available in the United Kingdom, the number of reported cases of hypercalcaemia was comparatively small compared to the large number of infants who must have consumed rather excessive amounts of vitamin D. Thus, it can be assumed that there are relatively large differences in susceptibility. These differences may have a genetic basis, but unidentified environmental factors may also play a role.

The continued occurrence of rickets in an appreciable number of infants in Canada, the United Kingdom, and the USA, where certain products fortified with vitamin D, especially fortified milks, are available on demand, indicates that such preventive programmes are not entirely satisfactory. The Committee assumed that such optional programmes would be even less satisfactory where standards of paediatric care and its utilization by the community are relatively poor. The prevalence of rickets in many countries is of great concern and should receive immediate attention. Because of the great differences in the severity of the problem and in the availability of suitable vehicles for fortification, the appropriate decisions must presumably be made upon a regional or national basis, considering both the need for increased vitamin D intakes and the dangers of excessive levels.

[1] American Academy of Pediatrics. Committee on Nutrition (1953) The prophylactic requirement and the toxicity of vitamin D. *Pediatrics*, 31, 512.

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4.3 Protein and amino acids

In recent years, the possibility of fortifying foods with proteins or amino acids has attracted much attention among national and international bodies. The subject of amino acid fortification was recently discussed by the FAO/WHO/UNICEF Protein Advisory Group.[1] There are special problems to be considered in evaluating the need for and the benefits that may be derived from amino acid and protein fortification programmes.

It has been suggested earlier (section 1.3) that, as a first principle, fortification should be considered when a need for a nutrient can be demonstrated. Most of the vitamins and minerals can be considered in isolation from the rest of the diet. However, in the case of protein-caloric malnutrition the protein supply must be judged in relationship to other aspects of the diet, notably, the calorie intake (see Part II of this report). When the protein intake is found to be low in a particular population, it is likely that the calorie intake is equally inadequate, if not more so. In such circumstances, it is uncertain whether there is any benefit to be gained from providing only additional protein or amino acids. As has already been stressed in the introduction, the relative merits of such fortification programmes must be judged against the other approaches that might be undertaken. In the example cited, the question might be whether it would be better to increase the food supply in general or merely to provide additional protein. However, the general principle applies that the protein or amino acid intake must be a limiting factor in the diet of a significant proportion of the population if beneficial results are to be expected from a fortification programme.

Should a protein fortification programme be under consideration, attention should be given to the possible complementary effect of the added protein on the proteins already being consumed. If it is possible to provide a protein source of the limiting amino acids in the diets consumed in a particular region, the total protein supply as well as the quality of the protein in the diet will be improved. In this regard, the Committee considered the search for locally available protein sources that can be used in fortification programmes to complement indigenous dietary proteins to be a desirable approach that may have economic as well as health benefits.

The unique advantage of amino acid fortification as compared with protein fortification lies in the fact that such fortification is unlikely to affect the properties of the food and hence the acceptability of the fortified product, which is a potential problem in the case of protein fortification. However, prediction of the benefit that may result from such a

[1] FAO/WHO/UNICEF Protein Advisory Group (1970) *PAG Statement on amino acid fortification of foods*, New York, United Nations.

programme is even more difficult than in the case of protein fortification. The amino acid requirements for various population groups are not well established. Although the protein score can prove useful in assessing the relative value of national supplies, it is not fully satisfactory for screening an amino acid deficiency in the diets actually consumed by a particular group.

It is well known that amino acid fortification of cereals increases the quality of the available protein supply when cereals are the only food consumed, but experience with mixed diets is very limited. The practical significance of the findings is questionable, since they relate to dietary conditions that differ from those that may exist in population groups.

The response that may be expected from the fortification of a diet with a single amino acid, such as lysine, will presumably be determined by the degree to which this amino acid is limiting and the supply of the second or even the third most limiting amino acid. If the supply of the second most limiting amino acid is not appreciably higher than that of the first, the benefit may be expected to be marginal. Since relatively few essential amino acids are now available at reasonable cost, a careful analysis of the specific conditions is required. There is a clear need for more experimentation and practical field experience before firm recommendations can be made.

At the present time, the costs of amino acid fortification — in particular, lysine fortification — are relatively high compared to the usual vitamin and mineral fortification programmes. Current estimates of the increase in the cost of bread are in the range of 5–7% for lysine fortification. These costs may fall markedly in the future with increased amino acid production. As has been discussed previously (section 3.6) and in the statement of the FAO/WHO/UNICEF Protein Advisory Group, the effects of such costs upon food consumption patterns, foreign exchange, cost of living index, etc., may be serious and may nullify the expected benefits.

Attention is again drawn to the Committee's basic recommendation that the benefits of a fortification programme be weighed against its costs and those of alternative approaches to the same objective.

5. IMPLEMENTATION OF PROGRAMMES IN DEVELOPING COUNTRIES

5.1 Introduction

In its approach to food fortification, the Committee constantly had in mind the need to differentiate between three groups of countries: (*a*) those with well-developed food industries, (*b*) those where food preservation and processing are carried out largely in the household or as cottage or village

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industries, and (c) those in a transitional stage. This section of the Committee's report is devoted to some of the special problems of developing countries, particularly to the questions that should receive special consideration in fortification programmes.

The Committee recognized that there are considerable differences even between neighbouring regions in the developing world — for example, Central and South America; South-East Asia and other parts of Asia; and the Middle East and Africa south of the Sahara. Moreover, there are wide differences from country to country within each region and from one part of a country to another. For example, many countries are in a transitional stage and include, on the one hand, large areas based on subsistence agriculture, and, on the other hand, urban and other centres of industrialization based on petroleum or other national resources. In many of these countries, food industries are growing rapidly in parallel with other industries.

It has always to be borne in mind, therefore, that the developing countries vary greatly in size, total population, population density and distribution, natural resources and financial reserves, climatic conditions, agricultural systems and potentialities, and general cultural patterns.

Because of these differences it is emphasized that improvement of food supplies and nutritional status will depend in large measure on decisions made in the light of circumstances and possibilities within individual countries. Furthermore, within any one country it will in many cases be necessary to take into account differences from one part of the country to another. Because of poor systems of communication, differences in agricultural patterns and in food consumption patterns may be much more pronounced than in an industrialized country of the same size.

5.2 Basic information for a national food fortification policy

Food fortification programmes are one of several approaches to the improvement of diet and nutritional status. As such they are part of a food and nutrition policy at national level. Where a food fortification programme is under consideration, special attention must be given to the following factors that normally constitute the basis for a food and nutrition policy:

(a) estimated food and nutrient requirements, taking into account ecological conditions and environment;

(b) food production, distribution, and consumption, and food habits at various income levels;

(c) agriculture patterns and possibilities of change;

(d) national and local food preservation and processing industries;

(e) food imports and exports;

(f) assessment of nutritional status in various areas of the country and in different socio-economic groups.

National food and nutrition committees, established on an interministerial and interdisciplinary basis and serviced by competent specialists, are appropriate bodies for advising the governments on food fortification policies and programmes.

The estimates of requirements are all-important. It is necessary to obtain as precise a picture as possible for various groups of the population and to assess the situation in terms of requirements based on physiological standards, taking into account environmental factors.

The survey of needs must clearly indicate those of special groups, such as pregnant and nursing mothers and children, and the special problems associated with protein-calorie malnutrition in infants and young children; it should cover not only proteins and calories but also vitamins and minerals. Deficiencies in the nutrients available may vary, depending on the locality; the identification of geographic areas with special problems is important in enabling remedial action to be taken.

5.3 Food imports

Many developing countries import considerable quantities of food through commercial channels; they may also receive food supplies through the World Food Programme, UNICEF, or bilateral aid schemes, or from voluntary bodies. Decisions are required as to whether or not such foods should be fortified. These decisions can be based in part on the general criteria discussed earlier (section 1.3) but some special points should be noted.

The Committee considered it desirable that donor agencies or groups should discuss fully with recipient countries the question of fortification of donated foods and should hold consultations with FAO and WHO. Although in general fortification should be based on local needs, there may be good social and psychological reasons for ensuring that food donations should reach the quality (including nutritional quality) of foods actually used in the exporting country. In many cases (e.g., dried milk, white flour) fortification may be important.

5.4 Scientific and technical personnel and facilities

Fortification programmes require scientific and technical personnel (a) to carry out the practical operations within the food industries; (b) for laboratories concerned with control at factory and governmental level; (c) for research and developmental work; and (d) to provide nutritional guidance at all stages from agricultural production to consumption.

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Attention has already been drawn to the importance of nutrition education and training programmes, both for medical and paramedical personnel and for those engaged in agriculture. The FAO Conference in 1967 stressed the importance of renewed efforts to promote nutrition teaching in colleges and schools dealing with agriculture.

The Committee noted that there is an acute shortage of personnel trained in food science and technology in the majority of developing countries, but that efforts are being made in many countries to establish food science departments in universities or associated institutions and courses in food processing in technical and other colleges. It is clearly of importance that provision should be made for the teaching of nutrition in these departments of food science and technology. The Committee recommended that the international agencies should give increased support to such developments.

PART II.
PROTEIN-CALORIE MALNUTRITION

1. INTRODUCTION

Seven Joint FAO/WHO Expert Committees on Nutrition have met since 1949. The first Committee recommended that studies be made of the clinical characteristics of PCM and its relation to food habits, especially during pregnancy, lactation, infancy, and early childhood.[1] As a result, a joint FAO/WHO team made a quick survey in Africa.[2]

The report of the second Committee[3] stressed the need for the dissemination of knowledge on kwashiorkor, which is a form of PCM, described practical measures for the prevention and treatment of PCM, including the use of skimmed milk, and recommended that information be obtained on the prevalence of the condition in Latin America, South-East Asia, and the Western Pacific Region. As a result, two joint FAO/WHO surveys were carried out in Central America and Brazil[4,5] and a WHO-sponsored study was conducted in southern India.[6]

The meeting of the third Committee[7] was devoted to malnutrition in mothers and children, special attention being paid to protein malnutrition and its effects on child health.

The fourth Committee discussed protein-rich foods other than milk for feeding infants and children, and recommended that FAO and WHO encourage and assist in the development of such foods.[8] The criteria for the selection of these foods were laid down by the fifth Committee in 1957,[9]

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1950, No. 16.

[2] Brock, J. F. & Autret, M. (1952) *Kwashiorkor in Africa*, Geneva, World Health Organization (*Monograph Series*, No. 8) and Rome, Food and Agriculture Organization of the United Nations (*FAO Nutritional Studies*, No. 8).

[3] *Wld Hlth Org. techn. Rep. Ser.*, 1951, No. 44 (*FAO Nutrition Meetings Report Series*, No. 5).

[4] Autret, M. & Behar, M. (1954) *FAO Nutritional Studies*, No. 13; *Bull. Wld Hlth Org.*, 11, 891-966.

[5] Waterlow, J. & Vergara, A. (1956) *FAO Nutritional Studies*, No. 14; *Bull. Wld Hlth Org.*, 15, 165-201.

[6] Rao, K. S. et al. (1959) *Bull. Wld Hlth Org.*, 20, 603.

[7] *Wld Hlth Org. techn. Rep. Ser.*, 1953, No. 72 (*FAO Nutrition Meetings Report Series*, No. 7).

[8] *Wld Hlth Org. techn. Rep. Ser.*, 1955, No. 97 (*FAO Nutrition Meetings Report Series*, No. 9).

[9] *Wld Hlth Org. techn. Rep. Ser.*, 1958, No. 149 (*FAO Nutrition Meetings Report Series*, No. 19).

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when the activities of the FAO/WHO/UNICEF Protein Advisory Group were reviewed for the first time since its establishment in 1955.

The sixth Committee[1] suggested that not only kwashiorkor but all the forms of protein-calorie deficiency disease be investigated, and advocated a greater emphasis in all nutrition and public health programmes on the major role of infections in the development of nutritional deficiencies. This Committee also drew attention to the need (*a*) to improve feeding practices during and immediately after weaning, (*b*) to organize regional programmes for the evaluation of the treatment of various forms of protein-calorie deficiency, and (*c*) to develop locally available foods of satisfactory protein content for feeding young children. Finally, the Committee proposed a new classification of nutritional diseases that placed emphasis on the responsible nutrient; some of these proposals were adopted in the eighth revision of the International Classification of Diseases.[2]

The seventh Committee[3] discussed protein-rich foods for infant feeding, nutrition in pregnancy and lactation, and the effect of nutrition on mental development.

PCM and its implications have also been discussed at a number of seminars and international meetings sponsored by FAO, WHO, UNICEF, and other bodies.

In recent years much new information has accumulated on the etiology, prevention, and treatment of PCM and on its sequelae, with their socio-economic implications. Differences of opinion in certain areas have an important bearing on the preventive measures to be adopted and need to be resolved as far as possible. In convening the present Committee, FAO and WHO therefore considered that the time was ripe for a comprehensive review of PCM.

The present report gives special attention to the following points:

(1) The urgent need to study the incidence and prevalence of PCM. A prerequisite for such studies is agreement on definitions and classification.

(2) The possibility of a world-wide trend towards an earlier age incidence of PCM and the causes of this trend.

(3) The assessment of the relative importance of deficiencies of protein, calories, and other nutrients in the etiology of PCM.

(4) The role of infection in the etiology of PCM.

(5) The increasing evidence that malnutrition may permanently impair mental and physical development.

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1962, No. 245 (*FAO Nutrition Meetings Report Series*, No. 32).

[2] *Manual of the international statistical classification of diseases, injuries, and causes of death, 1955 revision*, Vol. 2, Geneva, World Health Organization, 1967.

[3] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 377 (*FAO Nutrition Meetings Report Series*, No. 42).

(6) The influence of malnutrition in childhood and its sequelae on the socio-economic development of a country.

(7) The re-assessment of practical approaches to the prevention of PCM.

(8) The relationship between PCM and maternal and fetal nutrition.

2. NOMENCLATURE AND CLASSIFICATION

2.1 Nomenclature

The term "protein-calorie deficiency diseases", adopted by the sixth Committee,[1] is inappropriate, since the word "diseases" suggests too narrow a concept.

In malnutrition in the pre-school child, not only protein and calorie deficiencies but also other nutrient deficiencies and infections are involved. Thus the term "protein-calorie malnutrition" does not fully reflect the etiology of the entire spectrum of infant and child malnutrition. However, since this term is widely used the Committee considered it convenient to retain it for the time being.

2.2 The current concept of PCM

PCM includes many different clinical syndromes, all of which are accompanied by retardation of growth and development. The severe cases show clinical and metabolic changes, which vary according to the severity and duration of the nutritional deficiency, whereas the more frequent, milder forms only manifest retardation of growth and development, with few clinical symptoms.

The manifestations of PCM vary widely according to the nature of the causative factors, the time for which they operate, and the age of the patient. Two severe clinical forms are recognized: nutritional marasmus and kwashiorkor. Between these a wide range of variation and graduation exists. Moreover, a child with nutritional marasmus may develop kwashiorkor, and a child with kwashiorkor may present a picture of nutritional marasmus after losing oedema. In general, kwashiorkor is a more acute condition than marasmus. However, neither of these conditions presents such an important problem as does the widespread occurrence of mild and moderate PCM in a community.

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1962, No. 245 (*FAO Nutrition Meetings Report Series*, No. 32).

2.3 Classification of PCM

The definition and classification of PCM present an extremely difficult problem for several reasons:

(*a*) in common with other nutritional states, there is no sharp dividing line between the normal and the pathological;

(*b*) PCM is often associated with deficiencies of vitamins and minerals;

(*c*) infection may cause a marginal state of nutrition that permits survival and perhaps moderate growth to change into frank malnutrition; and

(*d*) the earliest sign of PCM in infants and children is failure of growth, but it follows from (*b*) and (*c*) that this is a non-specific effect.

The choice of a system of classification will depend upon whether it is to be used for prevalence studies in the field, for clinical diagnosis and investigation, for recording the causes of death, or for the development of preventive measures. A complete description of PCM should include the age of the patient and the severity, type, and duration of the condition.

2.3.1 *Classification in field studies*

The terms kwashiorkor and nutritional marasmus are of little relevance in field studies, as the number of frank cases of either condition is always small compared with the total number of children who are malnourished by any acceptable criteria.[1]

The original classification by Gomez[2] into three degrees of malnutrition based on weight deficit takes account of the age of the child and the severity, but not the type or duration, of the condition. The cardinal criterion, on which all authors are agreed, for distinguishing between kwashiorkor and marasmus is the presence or absence of oedema. It has been suggested by some workers that cases with oedema be classified as third degree malnutrition, regardless of the weight loss or extent of the growth failure. This suggestion implies that a child who is 50% of standard weight without oedema and a child who is 80% of standard weight with oedema are in the same category as regards the severity of their condition.

[1] Beaton, J. M. (1970) *Recent trends in public health nutrition. Protein-calorie malnutrition.* In: *Nutrition. Proceedings of the Eighth International Congress, Prague, September 1969*, Amsterdam, Excerpta Medica.

[2] In 1942 Gomez devised a classification of malnutrition in children aged 1–4 years using weight for age as the criterion. The purpose of the classification was to group cases of similar prognosis and to guide physicians in selecting the place of treatment (home, out-patient clinic, or hospital). It was not originally intended to use the classification as a diagnostic tool in community surveys, nor to extend its application to other age groups. However, Gomez' classification is now used also in community surveys for determining degrees of malnutrition. See: Gomez, F. (1956) *J. trop. Pediat.*, **2**, 77.

In recent years it has been increasingly realized that the measurement of deficit in height or body length gives valuable information about the chronicity of malnutrition, complementary to that given by the measurement of weight deficit. A reduction or stunting of height is evidence of past malnutrition; a low body weight in relation to height is evidence of present malnutrition. It is therefore important to take account, whenever possible, of height as well as of weight. However, the combination of weight deficit, height deficit, and the presence or absence of oedema in a single index for evaluation is unsatisfactory, because children with the same evaluation may differ in their clinical picture, history, and prognosis.

In view of these difficulties it does not seem feasible to devise a workable system of classification that fulfils all the criteria or that takes account of the age of the patient and the severity, type, and duration of the condition.

The Committee therefore considered that, in community surveys, attention should be concentrated on the systematic collection and grouping of data (see section 3.2, p. 42) and that it is not necessary to try to fit these data into preconceived categories.

2.3.2 *Classification for clinical purposes*

In clinical investigations the situation is different. It is often convenient to group the conditions of patients as kwashiorkor, nutritional marasmus, or the intermediate state of marasmic kwashiorkor for the purpose of analysing etiological factors, comparing biochemical and metabolic changes, etc. By way of example, a simplified scheme for defining these terms is set out in Table 3.

TABLE 3. SIMPLIFIED CLASSIFICATION OF PROTEIN-CALORIE MALNUTRITION[1]

	Body weight as % of standard [a]	Oedema	Deficit in weight for height [b]
Underweight child	60–80	0	Minimal
Nutritional dwarfing	< 60	0	Minimal
Marasmus	< 60	0	++
Kwashiorkor	60–80	+	++
Marasmic kwashiorkor	< 60	+	++

[a] Standard taken as 50th percentile of the Harvard values. See: Nelson, W. E. (1964) *Textbook of pediatrics, 8th edition*, Philadelphia, W. B. Saunders, pp. 48–53.

[b] Weight for height = $\frac{\text{weight of patient}}{\text{weight of normal subject of same height}} \times 100$

[1] Unpublished report to the Wellcome Trust, 1968.

2.3 ... *M in the International Classification of Diseases*

At its meeting in 1961, the Committee reviewed the classification of diseases of nutritional origin in the International Classification of Diseases [1] and suggested the following classification of PCM : [2]

1. Protein-calorie deficiencies
   - A. Kwashiorkor (including marasmic kwashiorkor)
   - B. Marasmus (e.g., athrepsia, cachexia, extreme wasting)
   - C. Unspecified (including starvation in adults, famine oedema).

However, the 1965 revision of the International Classification of Diseases [3] includes :

267 Protein malnutrition
Kwashiorkor
Kwashiorkor — marasmus type
Multiple deficiency syndrome
Protein deficiency

268 Nutritional marasmus
Athrepsia
Cachexia
Extreme wasting
Marasmus
} NOS [4] or stated to be due to malnutrition

269 Other nutritional deficiency
269.9 *Other and unspecified*
Famine oedema
Malnutrition NOS
Nutritional oedema
Underweight

The Committee felt that the present revision does not fully reflect the opinions of most specialists in this field.

In March 1970, the classification of nutritional diseases was considered at a meeting of the Pan American Health Organization (PAHO) held to discuss the Inter-American Investigation of Mortality in Childhood (unpublished report). It was recommended that PCM be consider ...der the following sub-headings :

260 Kwashiorkor
261 Nutritional marasmus
262 Severe PCM, unqualified
263 Other and unspecified PCM (particularly of moderate type)
264 Late effects of PCM (which include dwarfism, physical retardation, arrested development)

The Committee endorsed the classification proposed by PAHO with slight alterations, as given in Annex 1, and suggested that it be taken into consideration in preparing the 1975 revision of the International Classification of Diseases.

[1] *Manual of the international statistical classification of diseases, injuries, and causes of death, 1955 revision*, Vol. 1, Geneva, World Health Organization, 1957.

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1962, No. 245, p. 27 (*FAO Nutrition Meetings Report Series*, No. 32, p. 27).

[3] *Manual of the international statistical classification of diseases, injuries, and causes of death, 1965 revision*, Vol. 1, Geneva, World Health Organization, 1967, p. 128.

[4] Not otherwise specified, i.e., cause not specified.

3. PREVALENCE OF PCM

3.1 Present knowledge

Community surveys, representative of the population under study, are the only satisfactory method of estimating the prevalence of PCM. The existing data are incomplete and scanty ; the available literature, covering 80 surveys in over 39 countries from 1966 to 1969, has recently been reviewed.[1]

3.1.1 *Prevalence of severe PCM*

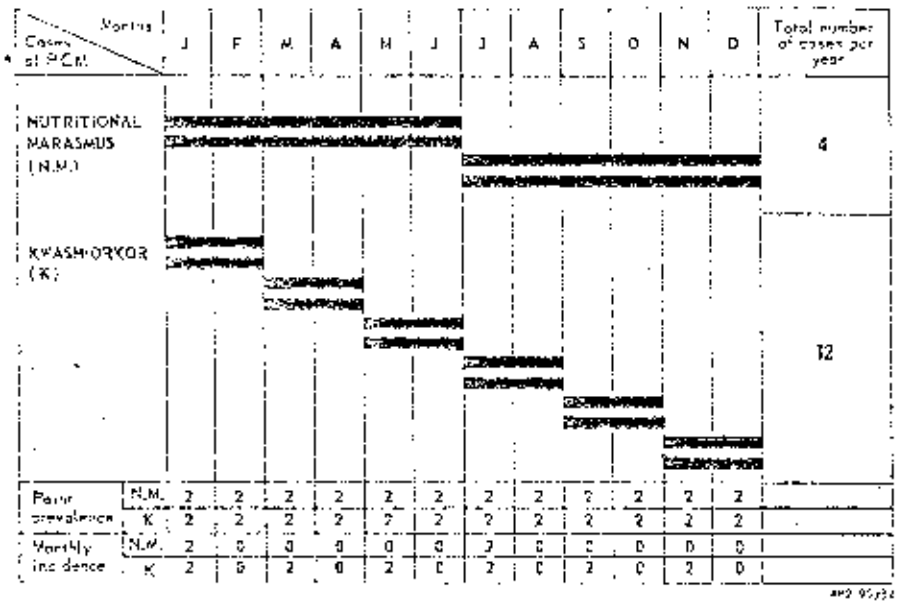
Different authors have used different criteria for defining the clinical manifestations of kwashiorkor and nutritional marasmus and different methods for sampling populations. The limited data available seem to indicate that, in some of the surveys, the point prevalence of nutritional marasmus was higher than that of kwashiorkor, but this question requires careful examination.

Kwashiorkor is an acute and severe condition and the child who does not receive medical attention usually dies within a few days or weeks. A child who is treated and survives recovers rapidly and no specific signs of kwashiorkor can be found a few weeks later. In nutritional marasmus, however, the child may survive for several months without showing any significant change, even if no medical attention is given. The turnover rate of cases in a community may therefore be significantly higher in kwashiorkor than in nutritional marasmus, and thus even where the two conditions have the same point prevalence more children are affected with kwashiorkor

[1] Beaton, J. M. (1970) *Recent trends in public health nutrition. Protein-calorie malnutrition.* In : *Nutrition. Proceedings of the Eighth International Congress, Prague, September 1969*, Amsterdam, Excerpta Medica.

than with nutritional marasmus in any one year (Fig. 1). The difference in turnover rates will depend, among other factors, on seasonal variations and on the fatality rates of the two conditions.

FIG. 1. THEORETICAL EXAMPLE OF THE DIFFERENCE BETWEEN POINT PREVALENCE, MONTHLY INCIDENCE, AND TOTAL NUMBER OF CASES PER YEAR *



* Reproduced from: Bengoa, J. M. (1969) *Recent trends in public health nutrition. Protein-calorie malnutrition.* In: *Proceedings of the Eighth International Congress on Nutrition, Prague, Czechoslovakia, August 28–September 5, 1969, Amsterdam* (Excerpta Medica International Congress Series No. 213), p. 15.

The point prevalence of severe PCM (either kwashiorkor or nutritional marasmus) in the limited surveys conducted between 1966 and 1969 ranges between 0% and 7.6% in children below 5 years of age.[1] The prevalence of severe PCM has also been estimated from the proportion of children who are below 60% of the standard weight for a given age (corresponding to third-degree malnutrition in the Gomez classification). These surveys showed the prevalence of severe malnutrition to be 0.5–4.6%.

3.1.2 *Prevalence of moderate PCM*

Both from the public health and socio-economic points of view, the prevalence of moderate cases of PCM is even more important than is that of severe cases.

[1] See footnote 1, page 40.

The terms that have been used to define moderate PCM, such as "pre-kwashiorkor", "general under-nutrition", and "poor nutritional status", are vague and difficult to interpret and should no longer be used. Using the Gomez classification, the prevalence of moderate PCM (second-degree malnutrition) varied from country to country between 4.4% and 43.1% in children up to 5 years of age.[1]

It is clear from the above considerations that existing knowledge is inadequate and that, from the available data, it is difficult to obtain even a rough estimate of the total number of malnourished children in the world.

The following general requirements must be met so that field studies can provide an accurate picture of the prevalence of PCM:

(*a*) Studies should be concerned with the total picture of nutrition in the community, including important forms of malnutrition that commonly coexist with PCM in many regions, e.g., anaemia or vitamin A deficiency.

(*b*) Studies must be planned on a sound epidemiological and statistical basis if they are to be representative of the country or region concerned. If possible, sampling methods and the age groups to be studied should be standardized. Allowance must be made for both seasonal and geographic variations.

(*c*) Uniform criteria should be adopted for the recording, grouping, and reporting of clinical data (see section 3.2 below).

The data that can be obtained from point prevalence studies are limited and should be supplemented wherever possible by information obtained from longitudinal studies.

3.2 Guidelines for community studies of prevalence

The Committee considered that, in the collection of data in field studies, a pre-defined classification serves no useful purpose and recommended that attention be given instead to obtaining basic data in a standardized manner. There are four stages in this process:

3.2.1 *Recording of basic data*

The basic data are age, weight, height, presence or absence of oedema, and, when available, birth weight.

This information should be obtained from all subjects in the group to be studied, without selection or bias. Other clinical signs of PCM,

[1] Bengoa, J. M. (1970) *Recent trends in public health nutrition. Protein-calorie malnutrition.* In: *Nutrition. Proceedings of the Eighth International Congress, Prague, September 1969*, Amsterdam, Excerpta Medica.

e.g., hepatomegaly and changes in skin, mucosae, and hair, should be recorded.

As has been emphasized above, it is essential not to overlook the presence of associated deficiency states, e.g., anaemia or avitaminosis A, which are common in some regions.

3.2.2 *Grouping and presentation of data*

In order to make international comparisons possible, the data must be related to some common standard. For this purpose, the Harvard standards and percentile distribution of height and weight [1] have been widely used. Standards of this kind should be regarded as a yardstick and not necessarily as a target or norm. Individual countries or regions may find it convenient to adopt local standards for additional comparisons.

The Committee recommended that FAO and WHO examine the feasibility of developing and adopting international standards for heights and weights and that, as an interim measure, the Harvard standards be used in field studies on PCM.

3.2.3 *Analysis of data*

The data must be analysed in order to show the severity, duration, and type of PCM and any associations between different sets of observations. Such analyses can be greatly facilitated by the use of computers. The Committee recommended that, as a necessary first step, WHO promote a uniform system for the collection of basic data for community studies on the prevalence of PCM.

3.2.4 *Additional information*

Additional anthropometric, haematological, and biochemical information can be obtained on the whole sample or on a sub-sample, according to local facilities, conditions, and problems. Since anaemia is an urgent public health problem in many countries, measurements of haemoglobin or haematocrit should always be made, at least on a sub-sample.

The Committee emphasized that the basic data are in themselves of great value and that their collection must represent a primary objective. Simplicity and accuracy must not be sacrificed for an elaboration of detail that may increase costs out of proportion to any extra useful information gained.

The anthropometric and biochemical methods that are most useful

[1] Jelliffe, D. B. (1966) *The assessment of the nutritional status of the community*, Geneva, World Health Organization (*Monograph Series*, No. 53).

for the appraisal of nutritional status have been considered in detail in previous publications.[1, 2, 3, 4]

3.3 Indirect indicators of the prevalence of PCM

3.3.1 *Age-specific death rates*

As emphasized in the report of the WHO Expert Committee on Medical Assessment of Nutritional Status,[1] age-specific mortality rates in children between 1 and 4 years of age are related particularly to malnutrition and can be used to indicate the nutritional status of this age-group. However, the relative importance of malnutrition and infectious diseases in causing this mortality varies in different circumstances. Moreover, the reliability of vital statistics varies greatly from one country to another. Under-reporting, particularly in the younger age-group, is likely to occur more frequently in the developing countries.

Infant mortality is greatly influenced by malnutrition in the first year of life, particularly in those countries where weaning is early and the weaning foods are inadequate.

Neonatal deaths, occurring in the first 28 days of life, are due mainly to congenital defects, obstetric complications, prematurity, and neonatal tetanus. Although these causes are not influenced to any great extent by the child's diet, the neonatal mortality rate is higher among children of low birth weight for length of gestation, which suggests that it may be affected by the nutritional status of the mother.

Post-neonatal infant mortality, i.e., the mortality between 28 days and 1 year of life, is higher in the developing countries than in the advanced, industrialized areas. This difference, like that in the entire second year, is due mainly to a combination of factors: malnutrition causes a lowered resistance to infection, while frequent episodes of infectious diseases precipitate clinical malnutrition. The same factors continue to operate, but with decreasing importance, during the remaining pre-school years; the magnitude of their consequences obviously depends on the medical services provided.

The Committee discussed the preliminary results of the Inter-American Investigation of Mortality in Childhood, which is a continent-wide collaborative study started in 1968 by PAHO. The overall objective of this study is to determine accurate and comparable mortality rates for children

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1963, No. 258, p. 11.

[2] Jelliffe, D. B. (1966) *The assessment of the nutritional status of the community*, Geneva, (*World Health Organization: Monograph Series*, No. 53).

[3] Committee on procedures for appraisal of protein-calorie malnutrition of the International Union of Nutritional Sciences (1970) *Amer. J. clin. Nutr.*, **23**, 807.

[4] Simmons, W. K. & Bohdal, M. (1970) *Bull. Wld Hlth Org.*, **42**, 897.

1944

under 5 years of age in 15 widely separated areas of the Americas. Information will be collected on about 35 000 deaths, using hospitals, clinics, private physicians, pathology records, and family interviews as sources to determine both the multiple causes of deaths occurring over a two-year period and also the relevant ecological, socio-economic, and demographic conditions.

The preliminary results indicate that nutritional deficiency is directly or indirectly responsible for a high proportion of deaths under 5 years of age, excluding neonatal deaths. The death-rates in the age-group 1–4 years are principally due to the synergistic effects of malnutrition and infection, regardless of what is the terminal event; close relationship was found between nutritional deficiency and the death rate from all causes in this age-group. Among all deaths associated with nutritional deficiency, this condition was classified as a contributory cause of death in approximately 70% of cases.

The Committee felt that the final results of the Inter-American Investigation of Mortality in Childhood will help to clarify many questions related to the interaction of malnutrition and infection.

In the developing countries, deaths in the second year of life commonly account for one-half of all deaths between 1 and 4 years of age. After the second year death rates decline progressively, at a rate depending upon local conditions.

The Committee recommended that countries present their mortality data separately for each of the first 5 years of life. Much valuable information about PCM in the pre-school child can be obtained from the analysis of data presented in this manner.

3.3.2 *Disease-specific death rates*

In principle, death rates by cause should be extremely useful in assessing nutritional status and the effects of programmes for the improvement of nutrition. In practice, however, the classification of deaths associated with malnutrition is so unreliable as to be almost worthless. Moreover, few nutritional disorders are diagnosed and reported as such. In a two-year study in Guatemala, all but one of 40 deaths from kwashiorkor in children aged 1–4 years were attributed in the official records to worms, parasites, or dysentery.[1] The one case diagnosed as due to malnutrition was the only one to die in hospital, and the only one to be medically certified.

The most striking example of the value of disease-specific death rates as an indicator of the nutritional status of children is the variation in the mortality rate from measles, which is several hundred times higher in some developing countries than in North America and Europe. This variation

[1] Béhar, M., Ascoli, W. & Scrimshaw, N. S. (1958) *Bull. Wld Hlth Org.*, **19**, 1093.

is not due to differences in the incidence of measles, since nearly everyone acquired the disease in childhood prior to the recent advent of measles vaccine, nor to differences in virulence, since the higher-income groups in the developing countries do not share the high fatality rates. Neither is it due to differences in medical care alone, since few children in the industrialized countries need any treatment for measles in order to ensure their survival. Almost certainly it is due primarily to differences in resistance to disease, resulting from differences in nutritional status. An earlier age of onset of measles among less privileged children is often due to their contracting the disease as weanlings, when they are most likely to be malnourished and consequently more susceptible.

In longitudinal or prospective population studies, investigation of the causes of all child deaths can yield valuable information on disease-specific mortality rates if the sample is large enough and the duration of study sufficiently long. Even when the period of observation is too short for mortality rates to be obtained, the ratio of fatalities to cases can give valuable information on the susceptibility of a population to disease. Such investigations can be made in studies of epidemics or in prospective field studies in which accurate mortality data are obtained.

If the proposed change (see section 2.3.3, p. 39 and Annex 1, p. 70) in the International Classification of Diseases to include severe unqualified PCM and moderate unspecified PCM is accepted, more useful data on these different types of PCM may become available in the vital statistics of countries, particularly if the associated causes of deaths are included. In general, it can be assumed that it will be some time before most deaths from malnutrition in developing countries are recorded under a specific diagnosis. This is because many deaths are not medically certified and lay knowledge is insufficient to identify malnutrition as a primary, or even a secondary, cause of death.

3.3.3 *Occurrence of disease*

There is extensive evidence that PCM in children, as judged by low weight for age, is associated with a greater frequency and severity of some infectious diseases, especially in populations living under conditions of poor environmental sanitation and personal hygiene.[1] Since PCM is often related to certain infectious diseases, properly collected and interpreted data on the frequency and duration of infectious diseases, by broad categories, can provide an indirect indication of the prevalence of PCM.

The most common condition among malnourished pre-school children is weanling diarrhoea.[1] This is caused by reduced resistance to a variety of organisms due to malnutrition, which is common during the weaning period.

[1] Gordon, J. E., Chitkara, I. D. & Wyon, J. B. (1963) *Amer. J. med. Sci.*, **254**, 345.

1844

The amount of respiratory illness and the severity of the common communicable diseases of childhood and their complications are also influenced by nutritional status. Recent studies have shown a decrease in the total days of infectious diseases, as well as in the morbidity from diarrhoeal diseases, after the introduction of supplementary feeding.[1]

Moreover, in situations where PCM occurs a seasonal increase in the frequency of diarrhoeal disease, or an epidemic of an acute communicable disease, is usually followed by an increase in severe PCM. Information on the morbidity due to infectious diseases may therefore contribute to the planning of public health measures for the prevention of PCM.

Since published data on the incidence of infectious disease in population groups are unreliable and incomplete, this kind of information must be collected by special surveys. It appears that 2 weeks is about the longest interval over which mothers can remember accurately episodes of common diseases, such as respiratory infections and diarrhoea, in their pre-school children. The collection of reliable morbidity data over a long period of time, therefore, requires a personal interview at least every 2 weeks. Mothers can be interviewed either during home visits or at a maternal and child health centre when they are participating in a programme requiring them to visit the centre regularly. In nutritional surveys for point prevalence or 2-week period prevalence, data on morbidity from diarrhoeal and other infectious diseases in children under 5 years of age provide valuable supplementary information relevant to the extent of PCM in this age-group.

4. RELATIONSHIP BETWEEN SOCIO-ECONOMIC DEVELOPMENT AND PCM

4.1 Socio-economic development and the prevalence of PCM

The factors that influence the nutritional state of a population and those that determine its socio-economic development are complex and interrelated. Food consumption is regulated by its availability to families, its cost in relation to income, its acceptability, and the degree of development of the chain of distribution. Other factors that influence the nutritional state include the degree of literacy, the development of environmental sanitation, and the extent to which a change has occurred from a subsistence to a cash economy and from rural to urban living.

Income and purchasing power affect food consumption. As incomes rise, the proportion spent on food tends to decrease, although there may

[1] Gordon, J. E., Ascoli, W., Mata, L. J., Guzman, M. A. & Scrimshaw, N. S. (1968) *Arch. environm. Hlth*, 16, 424; Scrimshaw, N. S., Béhar, M., Guzman, M. A. & Gordon, J. E. (1969) *Arch. environm. Hlth*, 18, 51.

be an increase in the total expenditure on food. Food consumption habits tend to be formed early in life and to persist.

Migration from rural to urban areas, for whatever reason, has socio-economic implications that may affect the nutritional state of families and young children. The Committee emphasized the urgent need for studies on the incidence and prevalence of PCM and on its epidemiology during the change from a subsistence to a cash economy and from rural to urban living.

A "socio-economic development index" has been developed by the United Nations Research Institute for Social Development. When this index is compared with the mortality rate in the age-group 1–4 years, an inverse correlation is observed (Table 4).

TABLE 4. INFANT MORTALITY RATE, MORTALITY RATE FOR THE AGE-GROUP 1–4 YEARS, AND "SOCIO-ECONOMIC DEVELOPMENT INDEX"

Country	Infant mortality rate per 1000 live births	Mortality rate in children 1–4 years old per 1000 children of the same age	Socio-economic development index*
United States of America (1964)	24.8	1.0	111
England (1964)	20	0.8	104
Sweden (1963)	14.2	0.6	103
Australia (1964)	19.1	1.0	93
Argentina (1963)	60	3.7	73
Venezuela (1962)	30	6.0	62
Chile (1962)	114	7.2	61
Costa Rica (1964)	75	7.8	50
Panama (1964)	42.7	8.0	48
Colombia (1964)	84	13.7	46
Mexico (1964)	64	12.7	44
El Salvador (1962)	70	16.0	32
Ecuador (1962)	39	20.2	31
Guatemala (1962)	91	26.9	21

* Calculated by United Nations Research Institute for Social Development, Geneva, July 1970.

Malnutrition in this age-group appears to be closely related to the socio-economic level of the community. However, the reported infant mortality rates do not show such a clear tendency to fall with a rise in the socio-economic development index. Chile has one of the lowest mortality rates for the age-group 1–4 years and yet has a very high infant mortality rate. This apparent contradiction results from the fact that, in Chile, weaning tends to occur very early. In countries with a high level of socio-

econ. development, early weaning does not produce malnutrition; in the less highly developed countries, however, where people cannot afford to buy milk, where milk substitutes are not readily available, and where sanitary conditions are poor, PCM prevails.

4.2 Regional differences in the pattern of PCM

Socio-economic factors influence not only the prevalence of PCM, but also its pattern, e.g., the age of onset and its clinical characteristics. Other factors, such as climatic and seasonal conditions and food habits, result in marked geographic differences in the pattern of PCM.

In recent years, many authors have emphasized that, because of the rapid growth of cities and the migration from rural to urban areas, it is likely that, in general, malnutrition will develop at an increasingly early age and will tend more and more towards the marasmic type. It is important to establish whether this tendency exists and, if so, its extent. The pattern of PCM, and in particular its age of onset, is relevant to the organization of preventive measures, since different measures will be required in the first months of life and in pre-school children. There is an urgent need, therefore, to obtain more information on the pattern and age incidence of PCM and to monitor the changes that may be occurring in different parts of the world.

Conditions are likely to vary widely in different regions and generalizations may be misleading. For example, although migration to towns is a widespread phenomenon, the reasons for it and the nature of the people migrating are different in different countries. Because of wide variations in socio-economic conditions, although the principles for prevention remain the same, the detailed measures may differ from place to place and must be based on a sound appreciation of the local situation.

Information on patterns of PCM must be obtained as a matter of urgency, ideally through community surveys of incidence and prevalence. However, such surveys require careful epidemiological planning, the employment of special staff, and much time for their organization and financing. In the meantime, certain information that can be obtained more quickly may be useful. For example, the age of onset of malnutrition can be obtained from two sources that should be readily available in most places:

(*a*) The records of infant welfare or other centres. These usually represent a highly selected population.

(*b*) The records of hospital inpatient and outpatient departments. These too represent a highly selected population. Hospital statistics on the relative frequencies of kwashiorkor and marasmus are difficult to interpret because they depend on variable criteria for admission and

diagnosis. Some data reported from selected hospitals in different countries are presented in Table 5.

TABLE 5. REGIONAL DIFFERENCES IN THE PATTERN OF PCM (HOSPITAL CASES)

Country	Percentage of cases classified as:			Percentage of cases less than 1 year of age
	kwashiorkor	marasmus	unspecified	
Chile (Santiago)	3	97	—	83
Iran	33	66	—	45
Jamaica	32	34	34	52
Jordan	21	15	61	
Senegal (Dakar)	45	55	—	
South Africa (Johannesburg)	96	4	—	48
Sudan	92	8	—	50
Thailand	44	46	10	18

Large differences are seen in the patterns of PCM in Chile (Santiago) at one extreme and in South Africa (Johannesburg) at the other, with other countries showing intermediate patterns. One cannot be certain whether the differences are real or whether, in some hospitals, marasmus is regarded as a condition not requiring admission.

Despite their shortcomings, these data give presumptive evidence of regional differences; they would be more valuable if diagnostic criteria were more uniform.

In view of the urgency of the problem, the Committee recommended that WHO explore ways of obtaining, within the shortest possible time, a wider range of information about the ages of onset and the clinical types of PCM in different countries and regions of the world. The information to be collected in such studies should be of the simplest kind and need not include more than the basic data recommended for prevalence studies in section 3.2, p. 42.

5. INTERRELATIONSHIP OF DIETARY FACTORS IN THE ETIOLOGY OF PCM

Malnutrition in childhood is not only due to protein and calorie deficiency. Although these may be the major deficiencies in the developing countries, other nutrients, such as vitamin A, iron, folic acid, and riboflavin, are often lacking to varying degrees depending upon local conditions.

The clinical picture varies widely, reflecting the complex interrelationship of the causative factors, and is influenced mainly by the age of weaning. There has been a tendency to over-emphasize the importance of either protein or calorie deficiency alone, whereas in fact the two almost always occur together. The questions of whether the clinical pictures of kwashiorkor and nutritional marasmus reflect real differences in etiology and whether the nature of the predominant deficiency can be deduced from the clinical, biochemical, and other changes have an obvious bearing on diagnosis and on the planning of preventive measures.

Much research has been devoted to this problem in recent years and there is a large amount of literature on the significance of the various clinical and biochemical changes. Although there is a wide area of agreement on the significance of these changes, opinions differ on the importance to be attached to many of them. It is generally accepted that some clinical and biochemical changes probably reflect a state of protein depletion, notably oedema, hypo-albuminaemia, a reduction of some serum enzymes, and distortion of the serum amino acid pattern. However, a state of protein depletion does not result from dietary protein deficiency alone; calorie deficiency may act as a contributory factor by promoting the utilization of protein for energy production, and infections can cause protein loss (see section 6.3, p. 55). The condition of a child frequently reflects a recent episode superimposed on a more chronic state of malnutrition.

Thus the clinical and biochemical pictures in PCM are not reliable indicators of the relative importance of protein and calorie deficiency as causative factors.

Dietary studies fail to provide conclusive evidence. In the early descriptions of "sugar babies" in the West Indies, both the dietary histories and the presence of ample amounts of subcutaneous fat suggested that the calorie intake had been relatively high. On the other hand, a detailed study in India of the dietary habits of patients with the clinical pictures of nutritional marasmus and kwashiorkor did not show any quantitative or qualitative difference in the previous diets of the two groups.

However, isolated instances are on record in which it has been possible to show a relationship between the clinical picture and the recent dietary history. For example, cases have been described of typical kwashiorkor developing in children whose diets were adequate in calories but deficient in protein.[1]

It must therefore be concluded that the information about dietary background that can be derived from clinical and biochemical findings and retrospective dietary histories is usually of limited value and is unlikely to provide a sound basis for the planning of preventive measures.

[1] Monckeberg, F. (1968) *Adaptation to caloric and protein restriction in infants.* In: McCance, R. A. & Widdowson, E. M., ed., *Calorie deficiencies and protein deficiencies*, London, Churchill, p. 91.

The next question to arise is the value of information that can be derived at the level of the community, from studies on the family and the pre-school child in their natural environment.

In general, the clinical picture of PCM tends to be that of kwashiorkor in regions where breast-feeding is continued into the second year of life, whereas marasmus is the typical result when weaning occurs early, as in Chile.[1] However, the type of diet available is not always a good indicator of the type of PCM that develops. In the Amazon region of Brazil, where cassava is the staple food, the prevailing form of PCM was found to be marasmus, whereas in areas of Brazil where maize is the staple food the prevailing picture was kwashiorkor, in spite of the higher protein content of the diet.[2]

Although the staple foods differ in different regions, the type of PCM is influenced mainly by the age of the child, the feeding practices before and during weaning, the age at which weaning is completed, the age at which various supplementary foods are introduced, and the form in which these are given. Some weaning diets are bulky and have a high water content with a low concentration of calories and proteins, so that the child cannot eat enough to satisfy his needs. Finally, it should be emphasized that the frequency and severity of infections at the time of weaning may be important in determining the type of PCM.

In view of the complexity of these interrelationships, the only way to ascertain which are the most important causative factors in PCM, and hence to establish priorities for preventive measures, is by studying conditions in a particular community.

While recognizing the difficulties and limitations involved, the Committee stressed the need to obtain more information about the diets of pre-school children, which might serve as a basis for the planning of preventive programmes.

6. OTHER FACTORS OF SPECIAL IMPORTANCE IN THE CAUSATION OF PCM

6.1 The significance of fetal nutrition

Birth weight in relation to length of gestation is generally accepted as one of the main indices of fetal development. The relationship between birth weight, even at full term, and maternal nutrition during pregnancy

[1] Monckeberg, F. (1970) *Factors conditioning malnutrition in Latin America, with special reference to Chile.* In: György, P. & Kline, O. L., ed., *Malnutrition is a problem of ecology*, Basel, Karger, p. 23.

[2] Waterlow, J. & Vergara, A. (1956) *Bull. Wld Hlth Org.*, 15, 165; *FAO Nutritional Studies*, No. 14.

require further investigation, with regard to the following problems in particular:

(1) Fetal development and the outcome of pregnancy may be influenced by many factors other than maternal nutrition, e.g., genetic factors, infections, parity, pregnancy interval, multiple births, and the state of the placenta.

(2) It is difficult to isolate the effects of maternal malnutrition from those of the many social and economic factors that accompany poverty. Therefore it is hard to draw valid conclusions from the generally observed close correlation between low birth weight and poor socio-economic status, for the purposes either of international comparisons or of comparisons between different population groups within a country.

(3) It is generally believed that women who have suffered from chronic, moderate under-nutrition in childhood produce children of low birth weight more commonly than do women who were formerly well nourished and are subjected to acute food restriction. This belief is based on: (*a*) observations of pregnancy under famine conditions; (*b*) the observed positive correlation between birth weight and maternal height; this leads to the assumption that the median height of a socio-economic group is a valid index of its nutritional status in infancy and childhood; and (*c*) the fact that prematurity is more strongly associated with the social class of a child's maternal grandfather than with that of its father.

(4) There is controversial evidence on the effectiveness of dietary supplements during pregnancy, among severely undernourished women in developing areas, in increasing birth weight.[1, 2, 3]

Recent research has led to a greater understanding of the significance of fetal nutrition and its influence on mental and physical development in infancy and childhood.

The most convincing evidence comes from longitudinal studies of monozygotic twins of different birth weights, which showed marked differences both *in utero* and in their physical and mental development throughout the observation period of over 15 years.[4]

Studies on fetal growth have shown that, up to 26 weeks, the mean growth curve is similar in the developing countries to that in industrialized countries, such as the USA, but that, in the developing countries, fetal growth slows down after 26 weeks, resulting in a greater proportion of small-for-dates babies.

[1] Acosta-Sison, H. & Villanueva, J. (1954) *J. Philipp. med. Ass.*, 30, 151.
[2] Neven, M. & Holemans, K. (1959) *Ann. Soc. belge Méd. trop.*, 39, 299.
[3] Venkatachalam, P. S. (1962) *Bull. Wld Hlth Org.*, 26, 193.
[4] Willerman, L. & Churchill, J. A. (1967) *Child Develop.*, 38, 623.

Small-for-dates babies not only have high rates of perinatal mortality and morbidity but also suffer a high incidence of sublethal damage that has long-lasting effects.

In the absence of conclusive evidence on which to base specific preventive measures, the following general principles, which are likely to improve fetal nutrition, the outcome of pregnancy, and lactation, should be observed:

(1) The life stages of adolescence, motherhood, fetal life, infancy, and childhood should be viewed as a continuous cycle, thus indicating the need to initiate dietary improvement as early as possible in the life of the future mother.

(2) Antenatal, natal, and postnatal care should include general advice on food, dietary habits, personal hygiene, and the additional nutritional requirements during pregnancy and lactation.[1]

(3) Nutritional advice should be integrated into broader maternal and child health advisory activities on such topics as (*a*) family planning to space pregnancies and to defer the first pregnancy until adolescent growth and development have been completed; (*b*) the management of common infections; and (*c*) the avoidance of hard physical work during the latter part of pregnancy.

(4) Small-for-dates babies should be considered at high risk and appropriate measures taken for their care.

The Committee recommended that studies on the etiology of fetal malnutrition and on effective preventive measures for reducing the incidence of low birth weight be encouraged and vigorously pursued.

6.2 Premature weaning

In developing countries, particularly in rural areas, babies are usually suckled until the age of 2 years or more or until the mother becomes pregnant again. This practice is favoured in some societies by polygamy or by rigid customs that enforce a long period of sexual abstinence after childbirth. These social controls are rapidly disappearing, however, and early weaning is becoming increasingly common, but the complex and interrelated factors that predispose to early weaning are not clearly understood and require further study.

In the developed countries, the present fashion is either not to breast-feed at all or to do so for only a month or two. This fashion has spread rapidly to the towns and cities of the developing countries, where the period of breast-feeding is becoming shorter. Mothers in towns and cities

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1965, No. 302.

generally have to work away from home in circumstances that make it difficult for them to have their children with them, and they have no alternative, therefore, but to resort to artificial feeding. Moreover, most of them cannot afford the milk and milk substitutes, nor the time for proper home care of the infant, that are required for successful artificial feeding; thus PCM may be expected to show a tendency to occur at an earlier age. There is also a general shortening of the duration of breast-feeding in response to advertisements, in the mistaken belief that this is good for infants, and from a desire to imitate the better-educated section of the community. Short spacing of pregnancy is also a contributory cause of early weaning. Even where a mother would normally continue breast-feeding for a long time she may stop as soon as she misses her period; thus weaning takes place earlier as the intervals between pregnancies become shorter.

Artificial feeding may be unsatisfactory for several reasons. The formulation of the food may be wrong, because of mistaken motives of economy or lack of true understanding of what is involved. Most of those responsible for preparing the feeds for the infants of working mothers use unhygienic methods, and artificially fed infants are thus more predisposed than are breast-fed infants to attacks of gastroenteritis, hence to early PCM.

Breast milk by itself is adequate as food for the young infant. However, all the available evidence indicates that breast-feeding alone is not adequate for infants aged 4 months or more, who weigh less and make poorer progress when supplements are not given. No good supplementary foods are at present available in most developing countries, and efforts to develop foods suitably concentrated in calories and proteins need to be intensified. The Committee emphasized that, in spite of some evidence that there are foods not based on milk that can be used as a milk substitute for very young infants, the general consensus is that milk is still the best basis for food for infants aged 6 months or less.

The Committee deprecated the universal trend towards the early weaning of infants and recommended that every effort be made to encourage mothers, particularly in the developing countries, to breast-feed their infants at least throughout the first year of life. The advantages of breast-feeding should be taught to health and community workers at all levels, and they in turn should educate mothers. There is a need for health education, incentives, and legislation to slow down or stop this trend, which is having such disastrous consequences especially in the developing countries.

6.3 Interrelationships between nutrition and infection

In developing countries, a major factor in PCM is the effect of the frequent infections experienced by children during the first years of life.

Recent studies in areas as widely separated as Guatemala,[1] Ethiopia,[2] and India have shown that diarrhoeal diseases, respiratory infections, the common communicable diseases of childhood, and other infectious diseases are frequent in most children in rural low-income groups, and that intestinal parasitism is also widespread.

The way in which infections adversely affect nutritional status are well known, but difficult to quantify. They include decreased appetite, reduced food intake, and diminished absorption when diarrhoea is present. The reduced food intake frequently results in calorie deficiency, hence in the less efficient utilization of the dietary protein, since some of this is used to meet energy requirements.

The metabolic loss of nitrogen in the urine is increased with even the mildest infections, e.g., bronchitis, otitis media, pharyngitis, and small staphylococcal abscesses, and has also been shown to occur with immunization against smallpox, measles, and yellow fever.[3] The nitrogen that is lost must be replaced once the infection is over. Infection also increases needs for calories, vitamin A, vitamin C, and probably for most other essential nutrients. If the diet does not provide adequate quantities of these nutrients the child may recover slowly or not at all and be left more depleted than before.

There is extensive evidence that resistance to infections is markedly reduced in severe PCM and that even a mild degree of PCM in the pre-school child increases susceptibility to diarrhoeal and respiratory diseases and to other serious infections of childhood. There is evidence that the following factors contribute to this lowering of resistance in severe PCM: reduced antibody formation; reduced leucocyte activity; altered epithelial integrity; changes in the nature and distribution of the gastrointestinal flora; and, possibly, endocrine changes. However, the relative importance of these mechanisms in mild or moderate PCM needs clarification.

There is some evidence that supplementary feeding of pre-school children with mild or moderate PCM will reduce the incidence and severity of diarrhoeal and respiratory diseases.[4] In one study over a 2-year period, no deaths from measles were observed in children receiving supplementary food, although there was a high case-fatality rate from this disease among the children in the surrounding villages.

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[1] Scrimshaw, N. S., Salomon, J. B., Bruch, H. A. & Gordon, J. E. (1966) *Amer. J. trop. Med. Hyg.*, 15, 625.

[2] Eksmyr, R. (1969) *A trial to change infant feeding practices in an Ethiopian village (a longitudinal study of 11 cases)*, Addis-Ababa, Ethiopian Nutrition Institute.

[3] Scrimshaw, N. S., Taylor, C. E. & Gordon, J. E. (1968) *Interactions of nutrition and infection*, Geneva, World Health Organization (*Monograph Series*, No. 57).

[4] Gordon, J. A., Ascoli, W., Mata, L. J., Gusman, M. A. & Scrimshaw, N. S. (1968) *Arch. environ. Hlth*, 16, 424.

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PCM and infection act synergistically and must both be taken into consideration, therefore, in programmes of prevention. Thus programmes of environmental sanitation, personal hygiene, and immunization can decrease the frequency and severity of PCM, while measures to improve the nutritional status of pre-school children are likely to reduce the frequency and severity of infectious diseases. Programmes to improve nutrition and to reduce infectious disease are thus complementary and should proceed simultaneously in populations with PCM.

7. TREATMENT AND REHABILITATION

7.1 Treatment

The treatment of mild or moderate cases of PCM includes an adequate diet and the control of infection. These can be achieved routinely on an ambulatory or semi-ambulatory basis and should, in all cases, be accompanied by nutritional and health education of the mother and of other persons taking care of the child.

Severe cases may require hospitalization, particularly when, as frequently occurs, they are complicated by dehydration and when infection demands immediate attention.

A suitable diet is the mainstay of treatment. Complications may arise, however, especially in the early stages of treatment, that require special therapeutic measures.

The basic principle of treatment is to raise the child's nutritional level as quickly as possible by providing sufficient calories and high-quality protein. The child may initially have to be tube-fed and the diet must therefore be in a form that allows this. It is generally agreed that a daily intake of 100–200 kcal per kg body weight, depending on the clinical form of PCM and the age of the child, and 3–4 g of good quality protein per kg body weight are sufficient to cover the child's needs. With such levels of protein intake there is evidence that the weight gain depends on the caloric intake.[1] There is no advantage in giving much larger amounts of protein, as is sometimes recommended.

The protein used in treatment will depend on the local resources that are available. Whole milk is generally considered to be one of the best sources of protein, the most easily administered, and the best assimilated. Skimmed milk, being less expensive, is generally used but has the disadvantages of a lower calorie value and a high lactose content, which may cause diarrhoea if lactase deficiency exists. The latter disadvantage can be partly corrected by the addition of casein, which reduces the proportion

[1] Waterlow, J. C. (1961) *J. trop. Pediatr.*, 7, 16.

of lactose as compared to proteins. The lack of vitamin A in skimmed milk can easily be overcome by adding it to the milk or by administering it as a supplement.

It is impossible to overestimate the importance of correct and vigilant care and strict hygiene as essential prerequisites to successful treatment. Sufficient auxiliary staff are therefore needed to take care of the children. Most of this staff do not need extensive training or much technical knowledge, since their functions consist almost entirely of feeding the children properly and keeping them clean. After a short period of training, even young girls can easily perform these duties under the supervision of trained nurses.

In many parts of the world the mother is usually hospitalized with her sick child. This provides an excellent opportunity for educating the mother in the consequences of inadequate diets, the importance of proper feeding and hygiene, and how these can be achieved within the cultural and socio-economic conditions of the family. Where mothers are not hospitalized with their children, rehabilitation centres for the treatment of uncomplicated cases of PCM might offer similar opportunities.

The period of hospitalization for severe cases of PCM, both kwashiorkor and marasmus, should be kept to a minimum. After 2–4 weeks a large majority of such children are out of danger and well on their way to recovery. A paediatric ward is not the best environment for children who are at the stage of "consolidation of cure", when the only treatment being provided is an adequate diet, since they are at risk of cross infection, which delays recovery. When children cannot be properly cared for after discharge, then rather than being kept in hospital they should be transferred to a nutritional rehabilitation centre; here they can stay until they have completely recovered, which may take from 2 to 4 months. Such rehabilitation costs much less than hospital treatment. The use of nutritional rehabilitation centres also reduces the length of stay in hospital, thereby freeing beds in the paediatric wards, which are usually in short supply. (For further details of treatment, see Annex 2, p. 71.)

7.2 Nutritional rehabilitation services

In recent years, many countries have established nutritional rehabilitation services in order to educate mothers through their active participation in the nutritional rehabilitation of their children. These services cost very little to run compared with hospital treatment.[1]

Malnourished children can be treated by even less expensive means, such as supplementary feeding or long-range nutritional education programmes, but the treatment usually takes much longer and during this

[1] Bengoa, J. M. (1967) *J. trop. Pediatr.*, 13, 169.

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time the children may be exposed to the additional risks of infectious diseases. To be effective, both supplementary feeding and nutritional educational programmes require very close home supervision.

The Committee felt that, in areas with a high prevalence of PCM, nutritional rehabilitation services may be a useful means of reducing mortality and morbidity rates through education of the mother and adequate dietary treatment of the malnourished child. They should be viewed as an integral part of the basic health services, particularly of the maternal and child health services, and as only one link in the chain of activities aimed at the prevention of PCM (see section 9).

The nutritional rehabilitation services now in operation have already achieved results in some areas. The Committee recommended that further cost-benefit studies be undertaken to compare the results that can be achieved through different types of activities aimed at the nutritional rehabilitation of malnourished children and the education of their mothers.

8. LONG-TERM CONSEQUENCES OF PCM

Experiments on animals of various species have shown that moderate or severe deprivation of food imposed shortly after birth has permanent and obvious effects on subsequent growth, whereas malnutrition that arises after weaning suppresses growth only during the period of malnutrition, without affecting the ultimate size of the animal. Whether the effect on growth is transient or permanent appears to depend on the severity and duration of deprivation and the age of the animal, the effect being greatest when malnutrition occurs early in life.

For man, information on the later effects of early malnutrition is both fragmentary and scanty. This is so largely because malnutrition in man does not occur in isolation and many of the non-nutritional factors that accompany it or contribute to it may themselves influence growth and development. It is difficult, therefore, to assess the specific effect of an episode of inadequate nutrient intake on ultimate body size and function. The interpretation of the few published data is further impeded by a lack of knowledge about the genetic potential of the children under study, the adequacy of their dietary intake, and the health care given to them in their home environment.

Studies published with these limitations show that children with chronic malnutrition and an acute episode of superimposed deficiency have a height and weight corresponding to those of children in their own social class, as represented by their siblings. It takes them several years to reach the growth norms for the upper social class of their ethnic group. A controlled study showed that compensatory growth was very rapid at the beginning of the recovery period, but that when the expected weight for height was

reached food intake fell abruptly by 30% and growth rates dropped to the level of normal children of the same weight and height but of younger age.[1] Considering both the calories needed for new tissue formation, i.e., 8 kcal per g,[2] and the home conditions under which children at high risk of malnutrition live, it is extremely unlikely that these children will consume adequate diets and therefore stunting of growth may continue for a long time.

The stunting of growth due to malnutrition early in life should be clearly documented, in view of data showing that, in industrialized societies, the risk of perinatal death is strongly associated with maternal height. For example, among the offspring of primigravidae less than 29 years of age in Aberdeen, Scotland, the perinatal death rate due to birth trauma was 2.7 per 1000 infants born to mothers whose height was about 64 inches, and 8.6 per 1000 for mothers whose height was 60 inches or less.[3]

Evidence has accumulated in the past dozen years that leads to the general conclusion that malnutrition in the first year of life, if severe enough markedly to retard physical growth and to necessitate admission to hospital, may cause mental impairment.[4] If the deprivation is severe and prolonged and occurs during the first months of life, the retardation of brain development and function so produced may be so severe that it cannot be completely cured by nutritional rehabilitation. The lag in mental development is not restricted to the motor components of behaviour. Problem-solving ability, language, personal-social development, general intelligence, intersensory integration, and perceptual visual competence are significantly below the level obtained by siblings or matched controls. When extra mental stimulation is provided during recovery the performance obtained is better, but usually not up to the normal level expected for the particular age.[5]

The lower intellectual performance and inferior learning capacity found in survivors of severe malnutrition in early life must be seen in the context of the growing body of evidence on the vulnerability of the central nervous system to nutritional insults at critical periods of development. It has been shown in experimental animals that changes in the number of brain cells, in cellularity patterns, and in myelin lipids occur only when the animal is

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[1] Ashworth, A. (1969) *Brit. J. Nutr.*, 23, 835.

[2] McCracken, K. J. (1968) *Proc. Nutr. Soc.*, 27, 40A.

[3] Thomson, A. M. (1968) *The later results in man of malnutrition in early life.* In: McCance, R. A. & Widdowson, E. M., ed., *Calorie deficiencies and protein deficiencies*, London, J. & A. Churchill, p. 289.

[4] Cravioto, J. & Robles, B. (1962) *The influence of protein-calorie malnutrition on psychological test behaviour.* In: *Mild and moderate forms of protein-calorie malnutrition, First Symposium of the Swedish Nutrition Foundation*, Uppsala, Almquist & Wiksells, p. 135; Scrimshaw, N. S. & Gordon, J. E., ed. (1968) *Malnutrition, learning and behaviour*, Cambridge, Mass., The M.I.T. Press; Cravioto, J., DeLicardie, E. R. & Birch, H. G. (1966) *Pediatrics*, 38, 319.

[5] Yakin, U. S. & McLaren, D. S. (1970) *J. trop. Defic. Rev.*, 14, 25.

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malnourished during the period of rapid growth, when the rate of cellular multiplication is close to its maximum. Permanent effects have been obtained under these conditions, even with moderate levels of food restriction.[1]

Although many non-nutritional factors that either accompany or precipitate malnutrition can themselves probably influence mental development, it is important to remember that the age of hospitalization for severe malnutrition early in life coincides with critical periods in behavioural development, which depends on an adequate environment and opportunities for acquiring experience. The effects of malnutrition on mental performance may, therefore, also reflect interference with the gaining of experience and the differentiation of behaviour.

At present, there are not enough data to identify the particular contributions of nutritional and non-nutritional factors to defective mental function following malnutrition. Both types of factor probably have effects that are independent but that interreact. Longitudinal studies, now in progress, should enable the differential effects of these factors to be assessed. The data so far available indicate that survivors of severe malnutrition in the first year of life frequently show low levels of intelligence and a low level of competence in learning basic skills.

9. PREVENTION

Because of the global scale of PCM as a public health problem and its adverse effects on the survival, health, performance, and progress of population groups, preventive action on a world-wide basis is urgently needed. The prevention of PCM should usually be planned at the national level. Emphasis in this report is therefore on national efforts, but these can be facilitated by international action.

In many parts of the world, the main obstacles to the effective control and prevention of PCM have been the failure to apply preventive measures on a community basis, as a result of deficiencies in programme planning and organization: the poor development and utilization of material resources; the shortage of personnel and the inadequacy of their training; and a growing demand for food, health, and nutritional services due to a rapidly expanding population.

PCM can ultimately be controlled only by general economic and social development and a co-ordinated approach in the fields of agriculture, education, the social services, and public health. Since the control of

[1] Dobbing, J. (1968) *Vulnerable periods in developing brain.* In: Davidsen, A. N. & Dobbing, J., ed., *Applied neurochemistry*, Oxford, Blackwell Scientific Publications; Winick, M., Fish, I. & Rosso, P. (1968) *J. Nutr.*, 95, 623.

PCM is a long-term process, it should be incorporated within the overall objectives of national development plans and national food and nutrition policies, not only as an ultimate goal but also as an immediate and intermediate goal.

There is a reasonable chance that the average quantity of food needed per person will be available over the next few decades through the achievements of the "green revolution", which has led to greater yields of some new varieties of cereal provided they are properly cultivated. However, the concentration and quality of protein in existing cereals is not fully adequate for the needs of the young child. Where roots and tubers are staple foods this is an even more serious problem. Quantitatively, legumes could provide the most important vegetable protein supplements to staple diets in most developing countries.

There is a serious danger that government support for, and the greater profitability of, cereal production will lead to a lowering in the production of grain legumes; this would have serious repercussions on the quantity and quality of protein in diets. It is important, therefore, to increase the production of grain legumes as well as of cereals. It is highly desirable too that the quality and quantity of protein in cereals be improved by genetic means. For example, the incorporation of the Opaque-2 gene into maize greatly improves the quality of its protein, and varieties of rice with higher protein content can be produced.

There is no simple solution to the problem of PCM. Indeed, it is so complex that many approaches have to be explored to meet the various requirements of particular countries, of different areas, both rural and urban, within countries, and of different population groups, particularly those that are vulnerable.

Many types of action are necessary: the increased production of foods that will provide adequate protein, calories, and other essential nutrients, by means of agriculture, animal husbandry, and fisheries; the reduction of losses during harvesting and storage and the better distribution and utilization of supplies; the development of nutritious food mixtures for weaning and of industries for the preservation and processing of food; education and training, including the education of consumers; a broad programme of environmental sanitation and disease prevention, especially in childhood; and studies to provide better information on dietary intake and the factors that influence it. All these activities must fit into national plans for economic development, aimed at increasing the purchasing power of the population. Where practicable, assistance to vulnerable groups through institutional feeding or other programmes should be included in such plans.

9.1 Preventive programmes: objectives and strategies

The following guidelines may help in making the best possible use of available preventive measures under prevailing conditions:

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(*a*) To be effective, a preventive programme should be based on a knowledge of the epidemiology of PCM in the community. Some aspects of the programme may also need to be related to the level of social and economic development, the local patterns of administration and service, food production and supply, dietary habits, cooking practices, environmental and housing conditions, the purchasing power of the population, etc.

(*b*) The aim of a preventive programme is to eliminate the morbidity and mortality due to PCM. A control programme may be considered to be successful if it reduces PCM to a low level and eliminates mortality from this cause, even if less severe forms continue to occur.

(*c*) In formulating a strategy for a preventive programme, one must clearly define immediate and ultimate goals, specific objectives, target groups, priority measures, and criteria for the periodic evaluation of programme effectiveness and efficiency.

For the purpose of achieving the above objectives, priority should be given to the following target groups:

(*a*) Population groups who are undergoing periods of physiological stress, namely, pregnant and lactating women, and infants and children in the growth period;

(*b*) Mothers and children who are considered to be at high risk through living in low-income, high birth rate areas;

(*c*) Families in transition from a rural to an urban environment and low-income families in peri-urban and rural areas, where most high-risk infants and children live. The Committee emphasized the need for preventive measures to start in the home, where the mother must know how to prepare locally available foods in such a way as to satisfy the nutritional needs of her children. Means of educating mothers in this respect must be developed through every possible approach.[1]

In planning preventive measures, the most important causes of PCM should be given priority. This report emphasizes the health aspects of prevention, since previous reports[2] have already stressed the importance of economic and educational measures and food production (including the production of processed foods).

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[1] Autret, M. (1964) *Nutrition of the pre-school child: a consideration of new approaches*, Rome, Food and Agriculture Organization of the United Nations, (unpublished document); FAO/WHO/UNICEF Protein Advisory Group (1971) *Report of an ad hoc group on feeding the pre-school child* (unpublished document, available from the FAO/WHO/UNICEF Protein Advisory Group, United Nations, New York).

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 277 (*FAO Nutrition Meetings Report Series*, No. 42); *International action to avert the impending protein crisis. Report to the economic and social council of the advisory committee on the application of science and technology to development*, New York, United Nations, 1968; Johnston, B. F. & Greaves, J. P. (1969) *Manual on food and nutrition policy*, Rome, Food and Agriculture Organization of the United Nations (*FAO Nutritional Studies*, 1969, No. 22).

9.2 Specific public health measures

Specific preventive measures have the following main objectives:

(*a*) the improvement of nutrition and the maintenance of good nutritional status;

(*b*) the control of infectious and diarrhoeal diseases;

(*c*) minimizing the effects of infectious diseases;

(*d*) the early detection and management of mild cases of malnutrition;

(*e*) the improved treatment, and the early and complete rehabilitation, of moderate and severe cases of malnutrition.

Preventive measures should aim at interrupting the natural course of PCM at the three basic levels of prevention: health promotion and specific protection (primary prevention); early detection and treatment (secondary prevention); and the prevention of disability and the restoration of functions (tertiary prevention). In this context the basic health services should be given special consideration, while integrated preventive measures, to attack simultaneously as many vulnerable points as possible in the chain of causation, should be initiated early in life.

Since the problem is nearly always too large to be dealt with by local resources, the programme needs to be implemented in stages. The *first stage* is directed mainly towards the treatment and nutritional rehabilitation of young children, while efforts towards specific protection and health promotion are progressively increased. The *second stage* should aim at achieving a better balance between treatment and rehabilitation, on the one hand, and specific protection and health promotion, on the other. The purpose of the *third stage* is specific protection and the general improvement of the nutritional status and food habits of the community, rather than the treatment of PCM and rehabilitation.

The various activities that could form part of a comprehensive preventive programme at the community level, with the above objectives, have recently been described by Bengoa,[1] based on experience from various countries. These activities are briefly described below as they provide a useful guide in relating preventive measures to objectives, local conditions, and available resources (see also Table 6).

(*a*) *Improvement of nutrition or maintenance of good nutritional status*

(1) Measures directed towards pregnant and lactating women: education, with more emphasis on maternal nutrition in antenatal, natal, and postnatal care; the management of lactation; the distribution of local foods as supplements to family diets; and family planning.

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[1] Bengoa, J. M. (1970) *WHO Chronicle*, 24, 560.

TABLE 6. OBJECTIVES AND ACTIVITIES IN THE CONTROL OF PCM IN YOUNG CHILDREN

Levels of intervention	Objectives	Activities
I. GENERAL	To establish a food and nutrition policy, to develop agriculture, to expand education, to improve environmental sanitation, and to raise standards of living	Establishment of an interministerial food and nutrition board Food production programme, including development of agricultural industries Fundamental education Socio-economic measures directed towards the less privileged groups, and the development of opportunities for employment Policies on food prices and minimum wages
II. SPECIFIC Health promotion	(a) Improvement of nutrition or maintenance of nutritional status	1. Measures directed towards pregnant and lactating women (education, distribution of supplements, etc.) 2. Promotion of breast-feeding 3. Development of low-cost weaning foods 4. Measures to improve family diets, including food distribution programmes 5. Education on nutrition, in co-operation with health and agricultural extension services, schools, and social and home economics services
Health protection	(b) Control of infectious and diarrhoeal diseases	6. Expansion of immunization programmes 7. Promotion of hygienic food preparation and personal hygiene 8. Promotion and provision of more suitable domestic utensils for the preparation of infant foods and for feeding infants
Treatment and rehabilitation	(c) Minimizing the effects of infectious diseases	9. Development of programmes for the early rehydration of young children with diarrhoea 10. Development of supplementary feeding programmes during epidemics 11. De-worming of heavily infested children
	(d) Early detection and management of mild cases of malnutrition	12. Periodic surveillance of the population at risk
	(e) Improved treatment, and early and complete rehabilitation, of moderate and severe cases of malnutrition	13. Ambulatory treatment; nutritional rehabilitation services 14. Hospital treatment 15. Follow-up care, through supplementary feeding and nutrition education

(2) The promotion of breast-feeding, initiated shortly after birth and continued as the sole source of food and water for at least the first 4 months of life. It should be continued as a supplement to other baby foods during the weaning period, up to the age of 9, 12, 18, or 24 months or more, depending on the local customs and on the availability of good quality protein; efforts to slow the trend towards the early cessation of breast-feeding, particularly among lower socio-economic groups, should be intensified.[1]

Every effort should therefore be made to discourage early weaning, particularly among low socio-economic groups. In countries that are developing industrially, and particularly in urban populations, the trend towards early weaning is already so evident that immediate measures are necessary to combat it and counter its ill effects.

(3) The development of low-cost weaning foods. This subject was discussed at the seventh meeting of the Joint FAO/WHO Expert Committee on Nutrition.[2] In practice, some of these mixtures have also been used as supplements to breast milk for children under 6 months of age.

The Committee considered that, where the need is established, pre-cooked and easily assimilated food mixtures might be used as supplements for infants under 6 months of age, provided that they are properly tested.

The Committee recommended that FAO and WHO continue investigations to develop suitable processed protein food mixtures that can be used as a supplement to or a substitute for breast milk when lactation fails, and in countries where cow's milk is not available. The development of such mixtures should not, however, be allowed to interfere with efforts to encourage and promote prolonged breast-feeding. Substitutes for breast milk should not be unduly promoted by advertising.

(4) The improvement of weaning foods, and measures to improve family diets in general, including food distribution programmes.

(5) Education on nutrition, including the production, preservation, storage, buying, and preparation of food; such education should be directed to mothers and/or their substitutes (grandmothers, elder sisters, domestic helpers, baby-sitters, etc.).

(b) Control of infectious and diarrhoeal diseases

(6) The expansion of immunization programmes, and the intensification of combined nutrition and mass immunization campaigns for infants under one year of age, are high priority measures. In some developing countries,

[1] World Health Organization Regional Office for the Eastern Mediterranean (1969) *Report on the seminar on the nutritional problems in the weaning period, Addis Ababa, Ethiopia, 5-15 March 1969* (unpublished document No. EM/NUTR/43).

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 377, p. 59 (*FAO Nutrition Meetings Report Series*, No. 42, p. 59).

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compulsory immunization may be considered a prerequisite for the control of PCM in young children.

(7) The promotion of hygienic food preparation and good personal and domestic hygiene, and general sanitary measures.

(8) The promotion and provision of more suitable domestic utensils for the preparation of infant foods and for feeding infants.[1]

(c) Minimizing the effects of infectious diseases

(9) The development of programmes for the early rehydration of young children with diarrhoea; the development of oral rehydration schemes and of ambulatory rehydration units may be very useful in some areas.

(10) The development of supplementary feeding programmes during epidemics, especially of measles, enteric fever, bacillary dysentery, and pertussis.

(11) The de-worming of heavily infested children and the effective treatment of lambliasis, amoebiasis, and other infestations. Selective chemoprophylaxis in countries with a high prevalence of tuberculosis or malaria or both.

(d) Early detection and management of mild cases of malnutrition

(12) Periodic surveillance of the population at risk, for the early detection and management of mild cases, especially in the weaning and post-weaning periods. Simple screening indicators (including growth curves), trained personnel, and mobile units to reach high-risk children in peri-urban, rural, and low-income areas are urgently needed.

(e) Improved treatment, and early and complete rehabilitation, of moderate and severe cases of malnutrition

(13) The improved ambulatory treatment and nutritional rehabilitation of moderate cases of PCM, to reduce the need for hospitalization and the occurrence of severe PCM.

(14) The improved hospital treatment of severe PCM should be given high priority in some areas. In many developing countries hospitals are constantly overcrowded with young children suffering from PCM, although only a proportion of such children are able to gain admission to hospital. In some instances mortality rates are 50% or more, and cases of PCM may be detained in hospital for 8–10 months. This situation imposes a tremendous financial burden on countries with limited resources.

(15) Follow-up care through supplementary feeding and intensive nutritional education, to prevent relapses or the occurrence of PCM in

[1] Welbourne, H. F. & DeBeer, G. (1964) *J. trop. Med. Hyg.*, 67, 155.

siblings, should receive high priority after ambulatory or hospital treatment.

9.3 Agricultural and socio-economic measures

The following general principles apply to the planning of agricultural and socio-economic measures for the prevention of PCM:

(1) Food production at the national level must take into account the special needs of vulnerable groups for food containing high-quality protein, e.g., milk, eggs, legumes, and oilseeds. National plans must therefore be devised that improve the nutrient value of diets according to ecological and regional conditions.

(2) Food distribution is uneven; it varies between socio-economic groups and between children according to the family income and the parents' education. The distribution of free or subsidized food supplements is necessary in order to alleviate PCM. Such supplements must supply the amount of calories and nutrients that are lacking in the traditional home diet.

(3) In a subsistence economy, the family must be shown ways of improving the production, storage, and preparation of foods at home.

(4) In planning food production and applied nutrition programmes, priority must be given to those local foods or food varieties that are needed for the preparation of food mixtures suitable for the pre-school child.

(5) Special financial support or tax incentives may be required to encourage the production of processed weaning foods.

(6) The purchase of foods by governmental or non-governmental organizations may be needed when a new food industry begins, to help in reducing the cost of production and distribution, thereby keeping prices at a reasonable level.

(7) Purchasing power depends on employment and is influenced by economic development. However, some agricultural development programmes, e.g., of re-afforestation, irrigation, and drainage, assisted by the World Food Program, give priority to the employment of parents who are poor or have large families.

(8) Parents should be provided with education through all possible channels and services.

9.4 Priority services

The following community services should be developed preferentially as means of providing preventive care:

(1) Services whose organizational patterns make it possible for mothers, young children, and families to be reached for education on nutrition and child spacing, and for preventive care.

(2) Integrated preventive, curative, and rehabilitation services providing adequate follow-up care.

(3) Services that are provided to those most in need, through the selection of high-risk groups.

The following services meet most of the above requirements but urgently need strengthening and expanding:

(1) Maternal and child health services based on health centres and simple facilities.

(2) Nutritional rehabilitation services or mothercraft centres.

(3) Mobile units to reach low-income, high-risk groups in rural and peri-urban areas for immunization, periodic surveillance, nutritional education, follow-up, and referral.

(4) Outpatient services equipped with rehydration facilities and providing education on public health and nutrition.

(5) Applied nutrition; agricultural and home economics extension programmes; and social work.

9.5 Personnel

Shortage of manpower (health personnel, nutritionists, dieticians, home economists, etc.) at all levels, and inadequate training in nutrition, are obstacles to the planning, organization, and implementation of programmes for the prevention of PCM. These obstacles can be partly overcome by improved pre-service and in-service training and by the increasing use of auxiliary personnel.

9.6 Programme evaluation

The aim of programmes for the prevention of PCM is to secure the greatest efficiency per unit cost, i.e., the maximal result from the minimal cost. However, there are certain technical difficulties in the evaluation of cost effectiveness in this field. For example, one major variable that is impossible to quantify is the ability and motivation of those providing services. In comparing inpatient and outpatient treatment, it must be remembered that some ambulatory services, such as nutritional rehabilitation, have an educative value, so that the costs of prevention and cure cannot be separately assessed. Furthermore, some hospitals and many pilot projects attached to universities have elements of teaching and research,

the cost of which is difficult to isolate. Many preventive services, e.g., clinics for young children, are concerned with other diseases in addition to malnutrition, and the cost of the purely nutritional component cannot always be isolated. Variations between countries in the costs of transport and personnel restrict the comparability of results.

Indicators that can be used in the evaluation of public health programmes for the prevention of PCM are: (*a*) improved diets and their effect on the duration of hospitalization; (*b*) the use of inexpensive auxiliaries or volunteers in feeding hospitalized children; (*c*) the frequency of re-admission of children to hospital as a result of relapses; (*d*) the relative effectiveness of intensive education given to mothers admitted to hospital with their sick children, as compared to such education given after discharge by home visitors or through nutritional rehabilitation services; (*e*) changes in mortality rates and in the prevalence of PCM in the community; and (*f*) anthropometric studies on school-children before and after the institution of public health and feeding programmes.

A recent review was made by Cook [1] of the available literature on the cost-benefit analysis of treatment in hospital and outside (i.e., in nutritional rehabilitation centres or in clinics for young children) from several parts of Africa, Asia, and Latin America.

Cost must obviously be an important consideration in extending preventive programmes on a nation-wide scale. Further research on programme evaluation is therefore urgently needed, to compare not only hospital and other services providing treatment but, even more important, alternatives in the field of prevention.

Annex 1

PROPOSED CLASSIFICATION OF PROTEIN-CALORIE MALNUTRITION [2]

260 Kwashiorkor

Multiple deficiency syndrome
Nutritional oedema
Severe protein deficiency
Severe protein malnutrition

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[1] Cook, R. (1971) *J. trop. Pediat.*, 17, 15.

[2] Adapted from the classification proposed by the Pan American Health Organization in 1970 (unpublished report) for the next revision of the WHO *Manual of the international statistical classification of diseases, injuries, and causes of death*, expected in 1975.

261 Nutritional marasmus
Athrepsia
Nutritional cachexia
Nutritional atrophy
Severe under-nutrition
Severe caloric malnutrition

262 Severe protein-calorie malnutrition, unqualified
Marasmus-kwashiorkor
Hunger oedema
Inanition
Severe malnutrition
Malnutrition of third degree

263 Moderate and unspecified protein-calorie malnutrition

263.0

Malnutrition of moderate degree
Malnutrition of second degree
Under-nutrition
Moderate dystrophy

263.8

Other protein-caloric malnutrition

263.9

Malnutrition, unspecified
Calorie malnutrition
Dystrophy

264 Lasting effects of protein-caloric malnutrition
Nutritional dwarfism

Annex 2

TREATMENT OF PROTEIN-CALORIE MALNUTRITION

Dietary treatment

A liquid diet, based on milk and supplemented with sugar and oil, is usually given at the beginning of treatment. It is often necessary to divide the diet into a number of small meals given by spoon; this is time-consuming and may require the almost constant presence of nursing staff, whose devotion to the task has a direct bearing on the results that can be expected. If anorexia is severe, tube feeding is required: after a few days, the child has generally regained sufficient appetite and strength to take nourishment

by himself, and tube feeding can be discontinued. In severe cases it is often advantageous to increase the quantities of protein and calories progressively, the rate of increase depending upon the clinical picture. The regimen should be supplemented with the ingredients of the traditional local diet as soon as the child has sufficiently recovered.

Vitamins should be given whenever there are signs of deficiency; in particular, signs of vitamin A deficiency should be watched for in regions where it is prevalent. An oral supplement of iron is also required after the first few days of treatment, to correct any iron deficiency that may exist or that may develop in the presence of intense erythropoiesis. Folic acid is indicated in the presence of macrocytosis. In most cases a supplement of potassium is necessary during the first days of treatment, owing to the rapid protein synthesis that is taking place. The administration of other minerals may also be necessary in severe cases of kwashiorkor, in regions where associated mineral deficiencies have been reported.

Treatment of complications

Hypothermia and shock

In severe cases of PCM, hypothermia and shock are frequently observed on admission. The child must be kept warm, and treated as promptly as possible for shock by the continuous intravenous infusion of an electrolyte-dextrose solution; in the most severe cases, plasma may also be required.

The administration of whole blood is indicated only when the child has severe anaemia; such cases are rare.

Intravenous dextrose-saline solution is also indicated if the child loses his oedema rapidly under treatment. As a working rule, administration should begin as soon as the child has lost more than 10% of his body weight in 24 hours.

Hypoglycaemia

This may develop without the typical signs of restlessness, shivering, and sweating. Glucose by the intragastric route or, better, by continuous intravenous infusion is indicated, but in some cases these measures are of no avail.

Pulmonary and gastrointestinal infections

Pulmonary infections can develop rapidly and can quickly prove fatal; therefore several authors administer antibiotics routinely during the first few days of hospitalization. Although good results have been reported, the advantages are difficult to assess accurately. As children with PCM are highly susceptible to infection, routine treatment with antibiotics

is probably advantageous when close surveillance is not feasible or when diagnostic facilities are inadequate.

The presence of a hidden infection, such as tuberculosis, should be suspected whenever a child fails to respond to treatment.

Diarrhoea is usually non-specific but occasionally is due to a specific infective enteritis or to intestinal sugar intolerance, particularly to lactose. It usually responds to dietary therapy alone.

In the great majority of cases diarrhoea is not a justification for postponing dietary treatment. Only if it is profuse, or if there is intense vomiting, may it be necessary to postpone dietary treatment by one or two days; the child should be given some liquid during this period, e.g., Darrow's solution in 2.5% glucose, by the intravenous or intragastric route.

Malaria, like bacterial infections, calls for immediate drug therapy. On the other hand, with intestinal parasitic infections it is preferable to wait until the child has recovered sufficiently before administering anthelmintics. Finally, the need must be emphasized for strict hygiene during hospitalization.

Results of treatment

With the above treatment and the levels of calorie and protein intakes indicated on p. 57 the child, after losing his oedema (in 7–10 days) may gain 10–15 g/kg/day; this rate of growth is 5 times that of a normal child of the same height, and 10 times that of a normal child of the same age. Nitrogen balance studies and measurements of total body potassium indicate that the tissue gained is of normal composition. It is of great importance to secure these rapid weight gains; a child can only be considered as nutritionally cured when he is symptom-free and has achieved the expected weight for his height, although he may still be below the normal height for his age.

PART III.

FUTURE RESEARCH AND ACTION

The Committee discussed other aspects of nutrition that, in its opinion, deserve immediate attention by FAO and WHO and that are relevant not only to food fortification and PCM but also to several other nutritional activities and programmes being developed by the two organizations.

1. THE INTERPRETATION OF NUTRITIONAL STANDARDS

Difficulties arise in interpreting dietary intakes and biochemical measurements in relation to nutritional status. Changes in dietary intake, biochemical measurements, and nutritional status according to the clinical picture do not necessarily occur simultaneously. More sensitive tests of deficiency would allow a better estimation of human requirements, while a more precise definition of human requirements might lead to a better interpretation of nutrient intakes.

The Committee considered that the problem of the interpretation of nutrient intakes and biochemical measurements should be kept constantly under review. Certain aspects of the problem are considered below.

1.1 Nutritional requirements: interpretation of nutrient intakes and planning of food supplies

The Joint FAO/WHO Expert Groups on the requirements for calcium,[1] protein,[2] vitamin A, thiamine, riboflavine, and niacin,[3] and ascorbic acid, vitamin D, vitamin B_{12}, folate, and iron[4] have adopted the concept of "recommended intake", which is defined as "the amount considered sufficient for the maintenance of health in nearly all people".[5] Whenever

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1962, No. 230 (*FAO Nutrition Meetings Report Series*, No. 30).

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1965, No. 301 (*FAO Nutrition Meetings Report Series*, No. 37).

[3] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 362, p. 73 (*FAO Nutrition Meetings Report Series*, No. 41, p. 73).

[4] *Wld Hlth Org. techn. Rep. Ser.*, 1970, No. 452 (*FAO Nutrition Meetings Report Series*, No. 47).

[5] The "recommended intakes" are not intended to be adequate under extreme environmental conditions, nor to cover any additional requirements that may result from abnormal conditions, such as infections, malabsorption syndromes, metabolic abnormalities, or the effects of food additives or other chemicals.

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data permitted, the Groups predicted the average requirement of a population group and then estimated the variability of this requirement within subgroups of similar age, sex, and physiological state. They then recommended an intake that they felt would meet the requirements of all but a small proportion of individuals.

Thus an individual regularly ingesting the recommended intake of a nutrient must be considered at low risk of receiving less than his true requirement. As his intake falls, the risk of inadequate intake (i.e., of dietary deficiency) increases. Thus, it may be stated that:

(1) dietary intakes can be interpreted only in terms of the risk of deficiency to the individual that might be associated with a particular level of habitual intake;

(2) the long-term objective of a nutrition programme is the maintenance of all individuals at as low a risk of deficiency as is consistent with national resources.

At the individual level dietary intakes can be assessed in relation to the "recommended intakes" as outlined above. However, other considerations must be taken into account in judging the adequacy of the average intake of a population. In a population, both the requirement for and the usual intake of a nutrient vary among individuals. In the case of calories, there is evidence that, if there are no limitations with regard to food supply or socio-economic or other factors, the intake is proportional to the requirements. Thus the average calorie intake of a population can be compared directly with the predicted average requirement. On the other hand, in the case of almost all nutrients there is no reason to believe that the intake parallels the requirements. In general, the distribution of intakes and of requirements for specific nutrients within a population are quite unrelated. Therefore, in assessing the risk of deficiency in a population (i.e., the proportion of persons who are predicted to ingest less than their true requirements) it is necessary to consider:

(1) the variation of nutrient requirements among individuals; and

(2) the variation of habitual nutrient intake among individuals.

The Committee noted that FAO had attempted to investigate the variation of habitual intake of nutrients and hoped that these efforts would continue. However, it drew attention to the need for the further investigation of methods of dietary study that will provide reliable estimates of the usual intakes of individuals; the variation of daily intakes depends upon the nutrient and probably also upon age or socio-economic group. It recommended that both FAO and WHO increase their efforts to obtain information about individual variation as regards nutrient requirements and habitual nutrient intakes, so that both factors can be considered simultaneously in interpreting the adequacy of reported nutrient supplies

A joint FAO/WHO Expert Group recommended that "in order to ensure that nearly all individuals in the population are adequately nourished, it would be advisable for the average intakes of population groups to be in general higher than the weighted average recommended intake".[1] The question of how much higher requires consideration of both the above independent variables (calorie intakes excepted) as well as of predicted wastage.

The predictions of the desirable calorie content of gross food supplies and of their quality in terms of nutrients must be based on different theoretical considerations.

1.2 The problem of "normal" in tests of nutritional status

In most biochemical and haematological measurements it is usual, for practical reasons, to specify ranges and "cut-off" points that distinguish "normal" individuals or groups from those who are "at risk" or "deficient". This is an arbitrary procedure, since most parameters vary continuously.

It is usually assumed that the same criteria can be applied to all populations under all conditions; an exception is the variation of "normal" haemoglobin levels with altitude.

The major difficulty arises from the concept that certain levels of measurement indicate a risk, and from the diagnoses made as a result, for example, a diagnosis of "subclinical rickets" based on a value of serum alkaline phosphatase above a certain level. In fact, it has been shown that a large proportion of children who do not have rickets but who are receiving regular supplements of vitamin D have serum alkaline phosphatase levels above the so-called limit of normal.[2] Conversely, children with PCM may have rickets without a raised level of serum alkaline phosphatase.

There are three possible conditions in which biochemical measurements indicate a deficiency or risk without any evidence of clinical disease or functional impairment. These are: a state of adaptation, without any impairment of health or function; a state of compensation, with diminished reserve; and a state of slow deterioration, leading to future breakdown. The classical way of distinguishing between these possibilities is by observing the natural evolution of the condition. In this way it has been established that certain levels of blood sugar may be regarded as pre-diabetic, and that certain levels of blood pressure are likely to end in hypertensive disease. In assessing the significance of biochemical and haematological indices of

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[1] *World Hlth Org. techn. Rep. Ser.*, 1970, No. 452, p. 68 (*FAO Nutrition Meetings Report Series*, No. 47, p. 68).

[2] Stephen, J. M. L. & Stephenson, P. (1971) *Arch. Dis. Childh.*, 46, 185.

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nutritional status, therefore, longitudinal studies are of the greatest importance.

A second approach is by statistical evaluation, but this cannot by itself distinguish between what is normal and abnormal in the biological sense. Nevertheless, as a first step it is desirable that data be expressed in such a way that the distribution patterns for different populations and groups can be compared.

The Committee recommended that FAO and WHO give high priority to supporting research on this general problem.

2. NUTRITIONAL VALUE AND PHYSIOLOGICAL EFFECTS OF FATS

During the last decade there have been great advances in knowledge of the nutritional value and physiological effects of different fats. Some of these advances may soon lead to a revision of current views about the quality of fats and to a more precise approach to the problem of lipid requirements, thereby influencing recommendations on the kinds of fat suitable for use in human diets and on the conditions governing their use.

Current research on dietary fats includes research on the nutritional value and physiological effects of (*a*) erucic acid and other long-chain fatty acids, (*b*) fats containing a high proportion of linolenic acid, and (*c*) heated and oxidized fats.

For example, investigations have shown that rape oil, which is rich in erucic acid, is poorly utilized in various species of animals and may in some cases cause an accumulation of lipids in cardiac tissues, followed by myocardial lesions. It is not known whether similar effects are produced in man. However, on the basis of findings in animals, some countries are aiming to produce new varieties of rape seed, the oil from which is expected not to have these harmful effects.

The Committee recommended that the existing knowledge on this subject be reviewed, that the possible practical implications for agriculture and for health be considered, and that it be determined what aspects require more thorough research, including epidemiological studies, in animals and man.

3. IMPACT OF FAMILY PLANNING PROGRAMMES ON FETAL AND MATERNAL NUTRITION

Recognizing (*a*) the advantages of incorporating nutrition and family planning into the basic health services, and (*b*) the increased opportunity for education in nutrition and family life, and for investigations into fetal

and maternal nutrition, offered by such incorporation,[1, 2] the Committee recommended:

(1) that guidelines be prepared to orient health personnel, nutritionists, and home economists to the above-mentioned advantages;

(2) that existing and anticipated family planning services be utilized for collecting data on lactation, maternal metabolism, reproductive efficiency, and the possible side effects of contraception;

(3) that consideration be given to maternal and infant nutrition in programmes already initiated by FAO to study the impact of child spacing on the nutrition of families and on their general economic and social development.

4. TRAINING AND EDUCATION IN NUTRITION

4.1 Training of professional and para-professional personnel in food and nutrition

The science of nutrition is concerned primarily with the part played by nutrients in body growth, development, and maintenance. Foods are the prime source of these nutrients. In many colleges and universities, nutrition is taught by biochemists, as a part of biochemistry. Because physiology, endocrinology, and other biomedical sciences, as well as a special knowledge of foods, are also basic to nutritional education, a teacher of broad training and experience, such as a nutritional biochemist, is needed for this task.

In universities where nutrition is taught well, the subject is presented in courses on basic, clinical, applied, and comparative nutrition. Various aspects of human nutrition should be included in the training of nutritional biochemists, nutritional physiologists, clinical nutritionists, public health nutritionists, and dietitians, because nutrition is their primary professional concern. The broader aspects of human nutrition and food science should receive greater emphasis in the curricula of physicians, dentists, food scientists, agriculturists, home economists, and nurses, because this subject is basic to their professional practice.

Throughout the world the nutritional education of professional and para-professional personnel is seldom good, often fair, and frequently poor. This is because (*a*) those who teach this subject are inadequately prepared or too narrow in their approach, or their knowledge is obsolete, and (*b*)

[1] *WHO Hlth Org. techn. Rep. Ser.*, 1969, No. 428.

[2] *Maternal nutrition and family planning in the Americas. Report of a PAHO Technical Group meeting, Washington, D.C., 20-24 October 1969*, Washington, Pan American Health Organization, 1970 (Scientific Publication No. 204).

students have insufficient time allocated to nutrition to obtain the knowledge and understanding required.

The amount and quality of education in food and nutrition given to professional personnel is fundamental to the solution of the food and nutritional problems of the world's population. This problem is not new; FAO and WHO, as well as other organizations, have been concerned with it for many years, and yet little progress has been made towards its solution. Today, nutrition is inadequately taught in medical, dental, and veterinary schools throughout the world and to students of food science and technology, agriculture (especially agronomy and plant breeding), dietetics, and home economics.

The Committee recommended that FAO and WHO study again the problem of nutritional education, identify those attempts to solve it that are succeeding, and decide what new approaches should be made. The problem is serious throughout the world; inadequate training is probably an important reason why nutritional efforts have failed in the past.

4.2 Evaluation of nutritional education of the community

Education in nutrition is a major strategic method for the prevention of malnutrition and must receive emphasis in community nutrition programmes. Yet there is surprisingly little evidence that existing techniques of nutritional education are effective or represent a good utilization of limited resources, and it appears that some conventional programmes of nutritional education have not succeeded in influencing food habits in the desired manner.

The Fourth Joint FAO/WHO Expert Committee on Nutrition [1] recommended that governments be helped in developing methods and materials suitable for the teaching of nutrition in schools and community and health centres, in training personnel, and in convening conferences on the subject. Nutritional education was discussed also by the fifth [2] and sixth [3] Committees.

The present Committee recommended the investigation of whether nutritional education in its present form is as effective as is generally assumed, and of whether additional techniques could be employed to improve its effectiveness and the evaluation of its results.

The effectiveness of educational approaches using the mass media and peer groups of volunteers should be compared with that of such traditional

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1955, No. 97 (*FAO Nutritional Meetings Report Series*, No. 9).

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1958, No. 149 (*FAO Nutritional Meetings Report Series*, No. 19).

[3] *Wld Hlth Org. techn. Rep. Ser.*, 1962, No. 245 (*FAO Nutritional Meetings Report Series*, No. 32).

approaches as group conferences, demonstrations, and individual counselling. The results of using various types of graphic presentation of nutritional information also need critical evaluation.

In evaluating the nutritional education of the community and in developing guidelines for the future, the adequacy of the teaching methods used, of the content of the programme, and of the methods used for evaluation must be considered. In judging the response to a nutritional education programme, due consideration must be given to the economic and other constraints upon the response of individuals to advice on food habits.

There is also a need to review the nature and effectiveness of educational material on nutrition presented to children in primary and secondary schools.

Future expert groups on various nutritional problems should be provided with a realistic appraisal of the effectiveness of nutritional education as a preventive measure, of the time scale involved, and of the resources required.

The need to evaluate the results and methodology of nutritional education programmes does not detract from the value of such programmes as a major approach to community nutritional problems; on the contrary, they deserve more vigorous support.

SUPPLEMENT

FAO NUTRITION PROGRAMME

1967-1970

COMPLETED PROJECTS

Long-term country projects

1967

Algeria	Organization of nutrition education and school feeding programmes	1967
Central African Republic	Organization of applied nutrition programmes	1961-1967
Ceylon	Organization of applied nutrition programmes	1967
Dahomey	Organization of applied nutrition programmes	1967
Gabon	Organization of nutrition services and applied nutrition programmes	1967
Honduras	Organization of applied nutrition programmes	1962-1967
Liberia	Home economics development	1967
Madagascar	Home economics and nutrition	1967
Mauritania	Organization of applied nutrition programmes	1963-1968-1969-1970
Mexico	Food canning and packing	1967
Philippines	Organization of applied nutrition programmes	1967
China (Taiwan)	Institute of food science and technology	1967
United Arab Republic	Developing training in home economics at higher institutes of training	1967

1968

Central African Republic	Development of food technology	1967-1968
Central African Republic	Organization of applied nutrition programmes	1967-1968
Chad	Organization of applied nutrition programmes	1966-1968
Chile	Institute of food science and technology	1967-1968 (continuing with AGS)
Colombia	Home economics development	1968
Colombia	Organization of applied nutrition programmes	1961-1970
Cuba	Organization of nutrition education programmes	1968
El Salvador	Organization of applied nutrition programmes	1964-1968
Ghana	Food research and development unit	1963-1968 (continuing with AGS)
Guyana	Organization of nutrition education programmes	1967-1968
India	Food science and technology	1967-1968
India	Development of an applied nutrition programme	1967-1970
Libya	Development of home economics	1965-1968
Malawi	Applied nutrition and training	1967-1968
Morocco	Organization of nutrition services, food technology development	1968

Morocco	Organization of applied nutrition and school feeding programmes	1963-1968
Niger	Development of food industries	1968
Niger	Organization of applied nutrition programmes	1966-1968
Sudan	Food technology	1966-1968
Swaziland	Organization of applied nutrition programmes	1967-1968
Thailand	Development of home economics and applied nutrition programmes	1967-1968
Thailand	Community development	1965-1968
Venezuela	Organization of nutrition education programmes	1967-1968

1969

Ivory Coast	Organization of nutrition education and school feeding programmes	1966-1969
Korea	Organization of nutrition education programmes	1967-1969
Nigeria	Development of nutrition education programmes and family gardens	1966-1969
Panama	Organization of applied nutrition programmes	1964-1969
Poland	Research and extension services for food production, processing and industrialization	1965-1969
Sierra Leone	Developmers of home economics	1964-1969
Tunisia	Development of home economics	1961-1969
Tunisia	Organization of applied nutrition programmes	1964-1969

1970

Caribbean	Nutrition education and training (CFNI)	1967-1970
Ecuador	Development of home economics	1968-1970
Haiti	Nutrition education and feeding programmes	1969-1970
India	Organization of applied nutrition programmes	1959-1970
Lesotho/Botswana/Swaziland	Development of home economics (group country project)	1969-1970
Lesotho	Organization of applied nutrition programmes	1962-1970
Mexico	Development of home economics	1969-1970
Paraguay	Organization of applied nutrition programmes	1960-1970
Saudi Arabia	Food quality control project, 1st phase	1967-1970
Senegal	Organization of school feeding programmes	1965-1970
Somalia	Development of home economics	1969-1970
Spain	Organization of nutrition education programmes	1962-1970
Thailand	Nutrition education and training	1966-1970
Togo	Development of home economics	1964-1970
Upper Volta	Organization of nutrition education and school feeding programmes	1964-1970
Yemen	School feeding and nutrition education	1965-1970
Yugoslavia	Integrated services for mothers and children	1965-1970

Short-term country projects and consultants' assignments

1967

India	Applied nutrition programme (Consultant)
Iraq	Refrigeration plants (Consultant)
Ivory Coast	Food Chemistry (Consultant)

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Ivory Coast	Home economics programme (Consultant)
Panama	Home economics (Consultant)

1968

Burma/Cambodia/Indonesia/Pakistan/Philippines/Taiwan	Nutrition education in schools (Consultant)
Cameroon	Nutrition training in agricultural institutions (Consultant)
Colombia	Feeding of workers (Consultant)
Ivory Coast	Nutrition training in agricultural institutions (Consultant)
Morocco	Nutrition training in agricultural institutions (Consultant)
Sierra Leone	Food promotion (Consultant)
Sudan	Evaluation of nutrition programme (Consultant)

1969

Qatar	Study of current food control set up (Consultant)
Korea	Mass feeding (Consultant)
Pakistan	Assistance with food policy planning Consultant)
Philippines	Food catering training (Consultant)
Thailand	Nutrition education in schools (Consultant)
Zambia	Study of workers' feeding (Consultant)

1970

Indonesia	Survey of food and nutrition situation (Consultant)
Mauritania	Home economics (Consultant)
Nepal	Food microbiology (Consultant)
Turkey	Urban nutrition (Consultant)
Sudan	Applied nutrition (1969-70) (Consultant)

Training centres, seminars and committees

1967

Sixth Session of the FAO/UNICEF Joint Policy Committee (JCP), Rome, 6-8 February

FAO/WHO/UNICEF Secretariat Meeting, Rome, 9-10 February

FAO Regional Seminar of Educators in Agriculture and Home Economics, Kampala, Uganda, 22-27 May

Ad Hoc Inter-Agency Working Group on Home Economics, Rome, 3-5 July

Regional Seminar on Rural Home Economics in French-speaking Countries in Africa, Dakar, Senegal, 3-13 July

International Advisory Committee of the FAO International Food Technology Training Centre, Mysore City, India, 11 July

Eleventh Session of the Joint FAO/WHO Expert Committee on Food Additives, Geneva, Switzerland, 21-28 August

Second Board Meeting of the ECA Working Party on Home Economics for Europe, Rome, 28 August-1 September

National Seminar for the Development of Home Science Education and Extension Programmes, Accra, Ghana, 28 August-1 September

National Training Seminar for the Development of Home Economics Education and Extension Programmes, Limuru, Nairobi, Kenya, 1-14 October

Fourteenth Session of the FAO/WHO/UNICEF Protein Advisory Group (PAG), New York, 12-14 October

1968

Regional Centre on Food Economics and Applied Nutrition, Lima, Peru, March-December

Second Session of the Ad Hoc Inter-Agency Working Group on Home Economics, Geneva, Switzerland, 24-26 January

Twelfth Session of the Joint FAO/WHO Expert Committee on Food Additives, Geneva, Switzerland, 1-8 July

FAO/Danish Seminar on Long-term Planning of Home Economics Education for Selected English-speaking Countries in Africa, Aarhus, Denmark, 23 June-13 July

Fifteenth Session of the FAO/WHO/UNICEF Protein Advisory Group (PAG), Rome, 9-13 September

FAO/WHO/UNICEF Inter-Secretariat Meeting, Rome, 17-21 September

Meeting of Representatives of the International Union of Nutrition Societies (IUNS), International Biological Programme (IBP), World Health Organization (WHO), and the Food and Agriculture Organization of the United Nations (FAO), Rome, 19-20 September

FAO Ad Hoc Consultation on Programme Development in Home Economics in Africa, Accra, Ghana, 28 October-2 November

1969

Regional Centre on Food Economics and Applied Nutrition, Bogotá, Colombia, February-June Meeting of FAO Regional Nutrition Officers, Rome, 10-18 April

FAO/WHO Joint Expert Group on Vitamin and Mineral Requirements, Geneva, Switzerland, 21 April-2 May

Ad Hoc Working Group on Amino Acid Fortification of the Joint FAO/WHO/UNICEF Protein Advisory Group (PAG), Rome, 7-8 May

PAHO/FAO Technical Meeting on Food and Nutrition Policy in Latin America, Washington, D.C., USA, 19-23 May

Thirteenth Session of the FAO/WHO Expert Committee on Food Additives, Rome, 27 May-4 June

Sixteenth Session of the FAO/WHO/UNICEF Protein Advisory Group (PAG), Geneva, Switzerland, 8-11 September

Second Session of the ECA Working Party on Home Economics for Europe, Rome, 22-26 September

Regional Training Centre on Home Economics Education for English-speaking Countries in West Africa, Accra, Ghana, 15 September-2 October

Meeting of the Ad Hoc Working Group of the Protein Advisory Group (PAG) on Feeding the Pre-school Child, Geneva, Switzerland, 15-18 December

1970

FAO/WHO/UNICEF High-Level Regional Food and Nutrition Seminar, Near East, Beirut, Lebanon, 26-29 January

FAO/WHO/UNICEF PAG Steering Committee, Rome, 11-12 February

Ad Hoc Consultation on Food Promotion, Rome, 24-26 March

Third Meeting of the Board of the Working Party on Home Economics, Rome, 1-3 April

FAO/WHO/UNICEF PAG Ad Hoc Working Group on Single-Cell Protein, Marseille, France, 8-10 April

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Seventeenth Session of the FAO/WHO/UNICEF Protein Advisory Group of Experts, New York, 25-29 May

Regional Training Centre on Food Economics and Applied Nutrition, Bogotá, Colombia, February-June

Fourteenth Session of the Joint FAO/WHO Expert Committee on Food Additives, Geneva, Switzerland, 24 June-2 July

FAO/WHO/UNICEF Regional Nutrition Training Centre, Beirut, Lebanon, July-August

FAO/WHO/UNICEF Maghrebin Seminar on the Food and Nutrition Problems of the Pre-School Child, Algiers, 9-13 October

FAO/WHO/UNICEF PAG Ad Hoc Working Group on Feeding the Pre-School Child, London, 19-23 October

Meeting of members of Board of ECAWP on Home Economics, Portugal, 29-31 October

FAO/WHO/UNICEF PAG Ad Hoc Working Group on Marketing, Rome, 2-5 November

Eighth Session of the Joint FAO/WHO Expert Committee on Nutrition, Geneva, Switzerland, 9-18 November

PAG Ad Hoc Working Group on Fish Protein Concentrates (FPC), London, 9-13 November

FAO/WHO/UNICEF PAG Steering Committee, Geneva, Switzerland, 19 November

Asia and the Far East Seminar on Strengthening Family Life (Planning for Better Family Living), Los Baños, Philippines, 7-17 December

PAG Ad Hoc Working Group on Feeding the Pre-School Child, Geneva, 14-18 December

PROJECTS IN PROGRESS OR PLANNED FOR 1971

Algeria	Food promotion programme
Brazil	Organization of applied nutrition programmes
Brazil	Nutrition education and training
Brazil	Protein-rich foods (Consultant)
Caribbean	Applied nutrition and nutrition services
Central African Republic	Development of home economics
Chile	Organization of applied nutrition programmes
Colombia	Food biochemistry
Colombia	Integrated services for mothers and children
Congo	Development of home economics
Costa Rica	Organization of applied nutrition programmes
Dahomey	Organization of applied nutrition programmes – family gardens
Ecuador	Development of home economics
Ethiopia	Development of home economics
Gambia	Organization of nutrition education programmes
Ghana	Food legislation
Guatemala	Training for rural development
Haiti	Development of home economics
India	Catering training programmes
India	Organization of nutrition education programmes

Indonesia	Organization of applied nutrition programmes
Iran	Food policy and planning
Iran	Formulated protein foods for infants and young children
Iraq	Development of home economics
Iraq	Establishment of nutrition services
Ivory Coast	Development of home economics
Kenya	Home economics project, Egerton College
Korea	Organization of applied nutrition programmes
Madagascar	Protein-rich foods (Consultant)
Madagascar	Formulated protein foods for infants and young children
Madagascar	Human nutrition and food economics
Malaysia	Food analysis and control
Malawi	Development of home economics
Mali	Development of home economics
Mauritania	Nutrition education and school feeding programmes
Mauritania	Household consumption surveys
Morocco	Nutrition training in agriculture
Morocco	Formulated protein foods for infants and young children
Nepal	Food and Nutrition policy (Consultant)
Nicaragua	Organization of applied nutrition programme
Niger	Foods habits (Consultant)
Nigeria	Applied nutrition home gardens
Pakistan	Food and nutrition programme
Papua & New Guinea	Development of home economics
Peru	Development of home economics
Peru	Food consumption and household budget survey at national level
Peru	Organization of applied nutrition programmes
Philippines	Food and nutrition policy and planning
Saudi Arabia	Food quality control project — 2nd phase
Senegal	Food legislation
Sierra Leone	Organization of applied nutrition programmes
Sierra Leone	Formulated protein foods for infants and young children
South Pacific	Nutrition training and education
Sudan	Organization of school feeding programmes with WFPA
Sudan	Organization of applied nutrition programmes
Syria	Development of home economics
Syria	Establishment of national nutrition services
Tanzania	Training centre on home economics (Musoma)
Tanzania	Formulated protein foods for infants and young children
Turkey	Formulated protein foods for infants and young children
Upper Volta	Development of home economics
Yemen	Food and nutrition programme
Zambia	National food and nutrition programme
Zambia	Strengthening food control services
Zambia	Workers' feeding programme

Meetings Planned for 1971

February 8	FAO/WHO/UNICEF PAG Inter-agency meeting, Rome, Italy
February 9-13	FAO/WHO/UNICEF Protein Advisory Group of Experts (18th Session), Rome, Italy

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February 16-19	FAO/UNICEF Seminar on the production, utilization and marketing of soybeans.
February 22-23	Session of the Board of the Working Party on Home Economics of the ECA, Paris, France
March 22 - April 2	FAO/WHO Ad Hoc Committee of Experts on Energy and Protein: Requirements and Recommended Intakes, Rome, Italy
April 28 - May 7	FAO/UNDP Asia and Far East Seminar on Strengthening Family Life (Planning for Better Family Living), Los Baños, Philippines
May 10-14	FAO/ILO/WHO Ad Hoc Expert Consultation on Workers' Feeding, Rome, Italy
May 4 weeks	Seminar on Nutrition in Food Policy and Planning, Moscow, USSR
June 7-10	FAO/WHO/UNICEF PAG Ad Hoc Working Group on Single-Cell Proteins (inter-agency); Moscow, USSR
June 16-24	Joint FAO/WHO Committee of Experts on Food Additives (15th Session), Rome, Italy
September 23-29	FAO/WHO Travelling Seminar on Protein Food Mixtures: Development Production and Distribution, Ethiopia/Algeria
October 4-13	Seminar on Food Promotion, Istanbul, Turkey
December 13-18	FAO/WHO/UNICEF Protein Advisory Group of Experts (19th Session), Geneva, Switzerland

PUBLICATIONS

Nutritional Studies

No. 20	*Learning Better Nutrition*, by J. Ritchie (E, F, S)
No. 21	*Food and Nutrition Procedures in Times of Disaster*, by G. B. Masefield (E, F, S)
No. 22	*Manual on Food and Nutrition Policy*, by B. F. Johnston and J. P. Greaves (E, F, S)
No. 23	*Wheat in Human Nutrition*, by W.R. Aykroyd and Joyce Doughty (E, F, S)
No. 24	*Amino-Acid Content of Foods and Biological Data on Proteins* (trilingual)

Nutrition Meetings Report Series

No. 41	*Requirements of Vitamin A, Thiamine, Riboflavin and Niacin — Report of a Joint FAO/WHO Expert Committee* Rome, 6-17 September 1965
No. 42	*Joint FAO/WHO Expert Committee on Nutrition — Seventh Report* Rome, 12-20 December 1966
No. 43	*Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Emulsifiers and Stabilizers and Certain Other Substances — Tenth Report of the Joint FAO/WHO Expert Committee on Food Additives* Geneva, 11-18 October 1966

No. 44	*Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Flavouring Substances and Non-nutritive Sweetening Agents — Eleventh Report of the Joint FAO/WHO Expert Committee on Food Additives* Geneva, 21-28 August 1967
No. 44A	*Toxicological Evaluation of Some Flavouring Substances and Non-Nutritive Sweetening Agents* (Monograph)
No. 44B	*Specifications and Criteria for Identity and Purity of Some Flavouring Substances and Non-Nutritive Sweetening Agents* (Monograph)
No. 45	*Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Antibiotics — Twelfth Report of the Joint FAO/WHO Expert Committee on Food Additives* Geneva, 1-8 July 1968
No. 45A	*Specifications for Identity and Purity of Some Antibiotics* (Monograph)
No. 46	*Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Food Colours, Emulsifiers, Stabilizers, Anticaking Agents and Certain Other Substances — Thirteenth Report of the Joint FAO/WHO Expert Committee on Food Additives* Rome, 27 May — 4 June 1969
No. 46A	*Toxicological Evaluation of Some Food Colours, Emulsifiers, Stabilizers, Anticaking Agents and Certain Other Substances* (Monograph)
No. 46B	*Specifications for the Identity and Purity of Some Food Colours, Emulsifiers, Stabilizers, Anticaking Agents and Certain Other Substances* (Monograph)
No. 47	*Requirements of Ascorbic Acid, Vitamin D, Vitamin B_{12}, Folate and Iron — Report of a Joint FAO/WHO Expert Committee*
No. 48	*Evaluation of Food Additives — Fourteenth Report of the Joint FAO/WHO Expert Committee on Food Additives* — Geneva, 24 June-2 July 1970

Food Additive Control Series

No. 8	*Food additive control in the USSR*, by A. I. Sterbeng et al.

Miscellaneous

Planning, Building and Equipping Home Economics Centers, by E. Haglund and L. E. Nagnusson (E, F, S)

Child Care — A Handbook for Village Workers and Leaders, by M. E. Keister (E, F, S)

Index (1945-1966) *Nutrition* (trilingual)

Human Nutrition in Tropical Africa, by M. Latham (French edition)

Food Composition Table for Use in Africa (E and F)

Visual Aids in Nutrition Education, by A.C. Holmes (E, F, S)

Food Composition Tables — Annotated Bibliography (E)

Lives in Peril — Protein and the Child, by T. Loftas (E, F, S)

List of Foods Used in Africa (E and F)

Sewing for Children (E and F)

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Reports of meetings, seminars and conferences

Report on the Seminar in Home Economics Extension in the Near East Region, Teheran, Iran, 4-13 November 1965

Report of an FAO/WHO Seminar on Planning and Evaluation of Applied Nutrition Programmes in Latin America, Popayan, Colombia, 10-17 November 1966

Report of a Joint FAO/WHO Seminar on the Planning and Evaluation of Applied Nutrition Programmes in Asia and the Far East, New Delhi, India, 30 November-30 December 1966

Rapport d'un séminaire régional pour la formation des cadres en économie familiale rurale pour les pays africains d'expression française au sud du Sahara, Dakar, Sénégal, 3-13 juillet 1967

Report on a National Seminar for the Development of Home Science Education and Extension Programmes, Accra, Ghana, 28 August-1 September 1967

Report on the FAO/Danish Seminar on Long-Term Planning of Home Economics for Selected English-speaking Countries in Africa, Aarhus, Denmark, 23 June-13 July 1968

Report on the FAO ad hoc Consultation on Programme Development in Home Economics in Africa, Accra, Ghana, 22 October-2 November 1968

Report of the Second Session of the Working Party on Home Economics — European Commission on Agriculture — Rome, 22-26 September 1969

Report of the Regional Food and Nutrition Seminar for the Near East, Beirut, Lebanon, 26-29 January 1970

Reports by experts

Rapport intérimaire au Gouvernement de Haute-Volta sur "L'Economie Familiale en Haute-Volta"	AT/67/3	M. Dessibourg
Report to the Government of India on "High Level Nutrition Training within the Applied Nutrition Program"	TA 2233	M.E. McDivitt
Rapport au Gouvernement de la Côte-d'Ivoire sur "Un Programme de Nutrition Appliquée"	AT 2212	B.C. Stainier
Rapport au Gouvernement de la Côte-d'Ivoire sur "L'implantation du Laboratoire de Nutrition de l'Institut National de Santé Publique de Côte-d'Ivoire"	AT 2267	J. Abraham
Report to the Government of Swaziland on an "Applied Nutrition Program"	TA 2269	A.J. O'Hara May
Rapport au Gouvernement du Gabon sur un "Programme de Nutrition Appliquée"	AT 2345	P.R. Yang
Report to the Government of Greece on "The Fruit and Vegetable Processing Industry"	ZIT No. 6	C.C. Strachan
Rapport au Gouvernement du Dahomey sur "Les Revenus et les Niveaux de Consommation Alimentaire de la Population"	AT 2377	M. Cresta
Rapport au Gouvernement de l'Algérie sur un "Programme de Nutrition Appliquée"	AT 2383	R. Lancen

SPC/FFHC Project for the Extension of Educational Facilities in the South Pacific Region	FFHC 24	M. Crowley
Informe sobre una Facultad de Economia del Hogar para Colombia y Paises Vecinos	CEP 39	C. Peterson
Informe sobre Enseñanza de la Economia Doméstica en el Centro Regional de Educación Fundamental para América Latina (CREFAL)	AT 2374	H. Segarra-Ortiz
Rapport sur une Session de Pédagogie de la Coupe-Couture	CMCF 25	M. Mathieu
Informe al Gobierno de Colombia sobre un "Programa Integrado de Nutrición Aplicada"	TA 2431	N. Paga
Informe al Gobierno del Perú sobre un "Programa de Nutrición Aplicada en la Región Andina"	TA 2390	C. Faesky
Report to the Government of India on "Nutrition Education and Training in the Applied Nutrition Program With Special Reference to South India"	CEP 42	D.E. Atkinson
Rapport au Gouvernement de la Côte-d'Ivoire sur un "Programme d'alimentation scolaire et de nutrition appliquée"	AT 2406	S. Liebrecht
Rapport au Gouvernement du Sénégal sur "Tentatives de Promotion d'Aliments Nouveaux"	AT 2396	Y. Claret
Rapport au Gouvernement du Gabon sur le "Développement de l'éducation alimentaire et de l'économie familiale"	AT 2469	J. Mondot
Report to the Government of the Philippines on a "Food and Nutrition Education Programme"	TA 2475	H.Y. Yang
Report to the Government of Thailand on a "Programme for Nutrition Training in Thailand"	TA 2507	M. Roush
Rapport au Gouvernement du Mali sur une "Mission d'économie familiale"	AT 2501	M.T. de la Rivière
Report to the Government of the Republic of Liberia on FAO/UNICEF assisted Home Economics Project, Department of Agriculture	CEP 44	A.M.M. Hiel
Report to the Government of Swaziland on an "Applied Nutrition Programme"	TA 2508	J.M. Tripp
Informe al Gobierno de Cuba sobre "Un programa de Nutrición Aplicada"	CEP 54	M. Espinoza
Report to the Government of Ceylon on "The Applied Nutrition and Agricultural Extension Project"	CEP 49	R.G. MacIntosh
Rapport au Gouvernement du Niger sur le "Développement de l'éducation alimentaire"	AT 2511	P.R. Yang
Report to the Government of Malawi on "The Family and Child Welfare Training Applied Nutrition Training Programme"	TA 2564	M.S. Kotnis

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Rapport au Gouvernement du Burundi sur "Le Développement de l'économie familiale en milieu rural"	AT 2505	M. Larour
Rapport au Gouvernement de la République Malgache sur le "Développement d'un programme d'économie familiale"	AT 2509	M. Rakotomalala-Laurens
Report to the Government of Thailand on the "Community Development Programmes for Women and Youth"	TA 2528	J. Acton-Smith
Rapport au Gouvernement du Tchad sur "Une mission d'économie familiale et de nutrition appliquée"	AT 2550	F. Visserias
Report to the Government of the United Arab Republic on "Food and Nutrition Education within Rural Home Economics Department in the Faculty of Agriculture, Cairo University"	CEP 48	A. Molander
Report to the Government of Malawi on the "Development of Programmes for Women in Community Development and Agriculture Extension"	CEP 50	L. Engberg
Report to the Government of Guyana on an "Applied Nutrition Programme"	TA 2592	N. Puga M. Keyser
Rapport au Gouvernement du Maroc sur un "Programme de nutrition appliquée"	AT 2582	R. Tallier
Rapport au Gouvernement de l'Algérie sur "Les foyers d'animation de la jeunesse"	CEP 55	A. Maire
Rapport au Gouvernement du Maroc sur "Le Développement de l'économie familiale"	CEP 51	J. Parisot
Report to the Government of Iran on "Food Policy and Planning Based on Household Food Consumption and Nutrition Surveys"	TA 2598	P.N. Sen Gupta
Informe sobre "Enseñanza de la Economia del Hogar en el CREFAL y los Servicios de Extensión de la Economia del Hogar en México"	AT 2613	P.H. Dino
Rapport au Gouvernement du Togo sur "L'économie familiale dans le cadre de la protection sociale et du développement communautaire"	AT 2635	M.L. Dammron
Preliminary Report to the Government of the Sudan on "Food Consumption Surveys"	TA 2636	S. Arora
Rapport au Gouvernement de Mauritanie sur un "Programme d'alimentation scolaire et d'éducation en nutrition"	AT 2618	L.J. Roumeas
Rapport au Gouvernement du Tchad sur un "Programme de nutrition appliquée"	AT 2640	G. Le Guèles
Report to the Government of India on "The Applied Nutrition Programme"	TA 2642	K. Ekenes

Rapport au Gouvernement de la République Centrafricaine sur un "Programme de nutrition appliquée"	AT 2646	J.P. Heisler
Report to the Government of Tanzania on a "Home Economics Training Centre for Women and Girls, Musoma, Tanzania"	FFHC 41	G. Berstrom
Report to the Government of Tanzania on "Training at the Rural Home Economics Training Centre for Women and Girls, Musoma, Tanzania"	FFHC 37	K. Baeckmark
Report to the Government of Nigeria on "Home Gardens for Improved Human Nutrition (Nutrition Section)"	FFHC 52	A.T.E. Wijnhoven A.M. Ter Heersche
Report to the Government of Tanzania on "Training in Textiles and Clothing of Rural Development Instructors — Home Economics Training Centre for Women and Girls, Musoma"	FFHC 53	G. Winka
Report to the Government of India on "Aspects of the Applied Nutrition Programme 1964-1969"	CEP 57	B.M. Purvis
Report on "Development of Home Economics at the Arab States Training Centre for Education and Community Development (ASFEC)"	TA 2696	E.A. Elwy
Report to the Government of Tanzania on "Training in Food and Nutrition of Rural Development Instructors"	FFHC 54	G. Berg
Report to the Government of Ethiopia on "Community and Social Services Development and Training Project"	CEP 56	M. Morton
Report to the Government of Sierra Leone: "The Role of the FAO/UNICEF assisted Family Nutrition and Home Development Programme in Community and Social Development"	CEP 58	J.F. Asare
Rapport au Gouvernement de la Côte-d'Ivoire sur un programme d'alimentation scolaire et d'éducation nutritionnelle	AT 2813	R. Lancen Helenon
Rapport au Gouvernement de la Tunisie sur "Une expérience de nutrition et planification alimentaire (1965-1969)"	AT 2808	G. Le Bideau
Report to the Government of Thailand on "Community Development Programs for Women and Youth"	TA 2301	J. Acton Smith
Report to the Government of the United Arab Republic on "Development of the Curriculum at the Higher Institute of Home Economics, Boulac, Cairo"	TA 2343	G.C. Eastwood
Rapport au Gouvernement du Sénégal sur "Un programme d'éducation nutritionnelle et agricole dans les écoles primaires sénégalaises"	AT 2898	S. Prosper
Report to the South Pacific Commission, Noro, Fiji, on "Home Economics in the Community"	TA 2903	E. Eden

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Report to the Government of the Republic of Korea on the Applied Nutrition Project	TA 2841	Y.H. Yang
Informe al Gobierno del Perú sobre la Nutrición Aplicada	AT 2860	B. Stainier
Report to the Government of Somalia on the Development of a National Programme of Home Economics	TA 2892	L.L. Almira

Reports by Consultants

Rapport au Gouvernement du Cambodge sur "Les centres de conservation de denrées alimentaires"	AT 2299	E.J. Lavan
Rapport au Gouvernement de la Yougoslavie: "La récolte et la conservation par le froid des fruits et légumes en Macédoine"	AT 2280	M. Audéma
Rapport au Gouvernement de la République Centrafricaine sur "Les industries alimentaires en République Centrafricaine"	TA/6714	G. d'Avout
Report to the Government of the Republic of Korea on "The Development of Nutrition Projects"	TA 2236	Y.H. Yang
Report on a Mission to Libya	CEP 40	E.A. Elwy
Report to the Government of the U.A.R. on Home Economics Extension	CEP 35	H. Strow
Rapport au Gouvernement de l'Algérie sur le "Développement de l'économie familiale"	CEP 37	J. Parisot
Rapport au Gouvernement du Maroc sur le "Développement de l'économie familiale"	CEP 38	J. Parisot
Report to the Government of Ceylon on "Organization of Food Research"	TA 2325	E.C. Bate-Smith
Report to the East Africa Common Services Organization (EACSO) on "Edible Protein Resources in East Africa"	TA 2365	P. Voiro
Report to the Government of Zambia on "Nutrition Improvement"	TA 2368	D.M. Lubbock/ J.A. Clague
Informe al Gobierno del Paraguay sobre un "Programa de Alimentación y Educación en Nutrición (PAEN)"	CEP 43	J. Crosnier
Report on an Assignment by FAO of the United Nations to serve as Deputy-Director of the Caribbean Food and Nutrition Institute	TA 2855	J.I. McKigney
Rapport au Gouvernement du Cameroun sur une "Mission d'économie familiale"	CEP 45	S. Lambert
Rapport au Gouvernement de la République Centrafricaine sur une "Mission d'économie familiale"	CEP 47	S. Lambert
Rapport au Gouvernement de la Côte-d'Ivoire sur une "Mission d'économie familiale"	CEP 53	S. Lambert

Rapport au Gouvernement du Gabon sur "Les possibilités de développement des industries alimentaires au Gabon"	NCRS 1	C. Vacherot
La Enseñanza Superior de la Economía Doméstica en Costa Rica, El Salvador, Guatemala y Panamá	NCRS 2	G.E. Vail
"Long-Term Planning in Home Economics for Europe" A Summary Report of Information Collected from an FAO Enquiry Sheet to European Member Governments	NCRS 3	A. Prantila
Rapport d'une mission en République de Côte-d'Ivoire: "L'enseignement supérieur agronomique et la formation en alimentation-nutrition"	NCRS 4	G.J. Bretones
Rapport d'une mission au Royaume du Maroc: "L'enseignement supérieur agronomique et la formation en alimentation-nutrition"	NCRS 5	G.J. Bretones
Informe al Gobierno de Colombia: "Estudio del Servicio Nacional de Aprendizaje y Otros Servicios de Alimentación de Instituciones"	NCRS 6	L.H. Kotschevar
Rapport au Gouvernement du Cameroun sur "L'enseignement universitaire agronomique et la formation en alimentation-nutrition"	NCRS 7	G.J. Bretones
Home Economics in the Context of Economic and Social Development	NCRS 8	H.K. Stiebeling
Report to the Government of Zambia on "A Study of Workers' Feeding"	NCRS 9	K. Waltenberg
Final Report Poland — Research and Extension Services for Food Production, Processing and Utilization	FAO/SF: 79/POL. 2	B. Coulson
Report to the Government of Nepal on "Food Research and Food Production"	TA 2864	J. Hany
Report to the Government of the Philippines on "Training of Personnel for the School Feeding Programme and the Feeding of Workers"	NCRS 10	L.H. Kotschevar
Report to the Government of the Republic of Korea on "Feeding Problems in Schools, Factories and Other Institutions and the Need for Training Programmes for Food Service Workers"	NCRS 11	L.H. Kotschevar
Report to the Government of Qatar on "A Study of Current Food Control in Qatar, with Proposals for Expanding the Programme"	NCRS 12	D.S. Chadha
Report to the Government of Thailand on "School Feeding Programmes and Catering Training"	NCRS 13	L.H. Kotschevar
Report to the Government of Indonesia on "Food and Nutrition Policy in Relation to the Food Needs"	NCRS 14	A.G. van Veen
Report to the Governments of Guyana, Jamaica and Trinidad and Tobago on "Industrial Production of Protein Foods for Infants and Young Children in the Caribbean — A Feasibility Study"	NCRS 15	F. Avera *et. al.*

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College of Agriculture and Forestry, Liberia. A pre-investment study on home economics development planning	Techn. Rep. No. 2	J. Janelid
Report to the Government of Nigeria: "A preliminary study on home economics planning for rural development"	NCRS 16	I. Janelid
Informe al Gobierno de Cuba sobre Alimentación de Colectividades	NCRS 17	K. Waitenberg

List of Articles and Other Documents Published by Nutrition Division Staff Members in Scientific Journals, Proceedings of Conferences, etc., during 1967/1970

Title	*Published in*	*1967*
Les farines de poisson dans l'alimentation humaine	Le Maroc médical, 1967	M. Autret
La maladie aux cent noms	Alfa-Laval International, 1967	M. Autret
La nutrition appliquée	Compte rendu de la 4e Conférence de Rehovot (15-25.8.67) sur "Les problèmes de la santé dans les pays en voie de développement". Published also in the Israel Journal of Medical Sciences, Vol. 4, No. 3, May-June 1968	M. Autret
Note on the Programme of Work of the Nutrition Division	Nutrition Newsletter Vol. 5, No. 4, Oct.-Dec. 1967	M. Autret
An Indicative Plan for the World and the Protein Problem	Alfa-Laval International, 1967	B.M. Nicol
Problems of Reaching the Pre-School Child	Proceedings of the Third Far East Symposium on Nutrition, 1967	B.M. Nicol
The Nutrition Division World Food Programme	Nutrition Newsletter, Vol. 5, No. 1, Jan.-Mar. 1967	J.F. Góngora
The FAO/UNICEF Joint Programme	Nutrition Newsletter, Vol. 5, No. 2, April-June 1967	P. Lunven
Hiérarchie dans la valeur nutritionnelle des aliments calculée sur une base iso-calorique	Médecine tropicale, Vol. 27, No. 1, Jan.-Fév. 1967	C. Jardin/ J. Adrian/ J. Périssé
Les modifications des habitudes alimentaires - Leur importance pour la conception des programmes d'éducation en nutrition	Proceedings of the Symposium Internazionale d'Informazione Dietologica, Rome, 27/28 April, 1967	H.P. Teulon
Problems and Perspectives in Introducing Human Nutrition into the Curricula of Faculties of Agriculture	First issue of Review of Agricultural Education and Training (FAO)	T.B. Morgan
Industrial Food Services Problems Abroad	Journal of American Dietetic Association, Vol. 50, No. 4, April 1967	M. Ross

Title	*Published in*	*1967*
Aspects socio-culturels des comportements alimentaires	Le Maroc médical, No. 508, décembre 1967	I. de Garine
A World Organization Linking Nutrition Experts and Food Procedures	Alfa-Laval International, 1967	J.S. Russell
Development of Canned Food Industries in the World	Proceedings of the 5th International Congress of Canned Food (Vienna, Oct. 1967)	J. Guzman/ M. Autret
Oilseeds and Protein Concentrates	Alfa-Laval International, 1967	G.D. Kapsiotis
Struggling to Meet the Protein Needs	Nutrition Newsletter, Vol. 5, No. 3, July-Sept. 1967	G.D. Kapsiotis
Traditional Foods: Their Present Production and Use	Indian Journal of Nutrition and Dietetics, Vol. 4, p. 331, 1967	S.S. De
The Effect of Cafestol and Kahweol Esters on the Behaviour of Coffee Oil	Journal of American Oil Chemists Society, 1967	L. Hartman (UNDP/SF Expert, Campinas, Brazil)
Family Life and Food Habits	Alfa-Laval International, 1967	J. Steckle
Some Implications of the Activity Component in the Calorie Requirements of Adult Africans	Occasional Paper No. 1 of African Food and Nutrition Commission	P.L.H. Davey
Asia's Nutrition Problems: Progress Towards Their Solution	Sintesis (Switzerland), 1967	F.M.S. Hansell
Food and Nutrition Problems and Activities in Asia and the Far East	Journal of Korean FAO Association, March 1967	F.M.S. Hansell
Down to Earth Nutrition in Thailand	Housecraft (UK), 1967	J. Acton Smith
		1968
Protein Value of Different Types of Diets in the World: Their Appropriate Supplementation	Nutrition Newsletter, Vol. 6, No. 4, Oct.-Dec. 1968	M. Autret/ M. Cresta/ J. Périssé/ F. Sizaret
International Tables on Amino Acids Contents and Protein Quality of Foods	Nutrition Newsletter, Vol. 6, No. 2, April-June. 1968	M. Cresta
The Nutritional Approach in Food Policy and Planning	Nutrition Newsletter, Vol. 6, No. 1, January-March 1968	J. Périssé
Nutrition — A New Operational Technique	Nutrition Newsletter, Vol. 6, No. 1, January-March 1968	P. François
The Caribbean Food and Nutrition Institute	Nutrition Newsletter, Vol. 6, No. 2, April-June 1968	J.I. McKigney
Changes in Dietary Habits: Their Importance in Nutrition Education Programmes	Nutrition Newsletter, Vol. 6, No. 1, January-March 1968	H.P. Teulon

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Title	*Published in*	
		1968
The Role of Promotion in the Marketing of low-cost Protein-rich Foods	Nutrition Newsletter, Vol. 6, No. 4, Oct.-Dec. 1968	N.A. Wilkie
The Need for Human Nutrition Studies in the Curricula of Universities and Colleges of Agriculture	Nutrition Newsletter, Vol. 6, No. 3, July-September 1968	T.B. Morgan
Some Principles of Teaching and Learning	Nutrition Newsletter, Vol. 6, No. 3, July-September 1968	J. McNaughton
The Role of International Organizations in the Development of Food Technology in Latin America	Food Technology (USA), Vol. 22, No. 2, (Feb. 1968)	J. Guzman
Sources potentielles de protéines comestibles	Revue "Industries Agricoles et alimentaires", Oct. 1968	G.D. Kapsiotis
The Food Additives Programme	Nutrition Newsletter, Vol. 6, No. 4, Oct.-Dec. 1968	H.P. Mollenhauer
Industrial Processing of Millet and Sorghum in Niger	Nutrition Newsletter, Vol. 6, No. 2, April-June 1968	J. Mocquard
An Introduction to the World Food Situation and the Indicative World Plan	Report of a Seminar on Better Living in Community, Country and Continent, Associate Country Women of the World	K.K.P.N. Rao
Home Economics in the Service of International Cooperation	Nutrition Newsletter, Vol. 6, No. 3, July-September 1968	J. Steckle
International Debate at CENECA	Nutrition Newsletter, Vol. 6, No. 2, April-June 1968	H. Stevens
The South Pacific Commission Community Education Training Center	Nutrition Newsletter, Vol. 6, No. 3, July-September 1968	E. Eden
Human Nutrition and Involvement of FAO in World Food Programme	Journal of Canadian Institute of Food Technology 1 (2), A 37, 1968	M. McRoberts
For Your Fellow Man	Journal of the American Dietetic Association, 1968	M. McRoberts
Some Influences of Economic Factors on Nutrition in Africa	Bulletin of the Africa Food and Nutrition Comm., 1968	P.L. H. Davey
		1969
La production mondiale de protéines et les besoins alimentaires mondiaux présents et futurs. (Paper delivered at the 13th International Symposium on New Sources of Protein in Human Nutrition, Amsterdam, 26-29 November 1968)	VOEDING, Year 30, No. 4 (15 April 1969)	M. Autret

Title	*Published in*	
		1969
Food Technology Contribution to Solve the Nutritional Problems Created by the Population Explosion. (Paper delivered at the 12th International Congress of Pediatrics, Mexico, 1-7 December 1969)	Proceedings of the Congress (1969 — pp. 389-395)	M. Autret/ J. Guzman
World Protein Supplies. (Paper delivered at the Symposium on Proteins as Human Food, Nottingham, UK, 30 March-3 April 1969)	"Proteins as Human Food" — Proceedings of the 16th Easter School in Agricultural Science, Univ. of Nottingham (edited by R.A. Lawrie, 1969, pp. 3-19	M. Autret
The Programme of Work of the Nutrition Division	Nutrition Newsletter, Vol. 7, No. 4, Oct.-Dec. 1969	M. Autret
Nutritive Value of Cereals and Other Main Staple Foods	Nutrition Newsletter, Vol. 7, No. 2, (April-June 1969)	K.K.P.N. Rao
Nutrimetric Study on Dietary Disparities	Nutrition Newsletter, Vol. 7, No. 3, July-Sept. 1969	P. François
Projection of an Income Frequency Distribution in Relation to Dietary Patterns	Nutrition Newsletter, Vol. 7, No. 4, Oct.-Dec. 1969	P. François
Study of the Correlations between Ecological and Chemical Measurements of Food Protein Quality	Nutrition Newsletter, Vol. 7, No. 2, April-June 1969. (Expanded version published in "Annales de l'alimentation et de la nutrition", Vol. 24, No. 5, 1970)	M. Cresta/ J. Périssé/ M. Autret
The Effect of Income on the Structure of the Diet. (Paper delivered at the VIIIth International Nutrition Congress, Prague, 26 Aug.-3 Sept. 1969)	Nutrition Newsletter, Vol. 7, No. 3, July-Sept. 1969, to be published in the proceedings of the Congress	J. Périssé/ P. François/ F. Sizaret
Nutrition in Food Policy and Planning (Paper delivered at the Conference on "Protein Foods for the Caribbean", held at Georgetown, Guyana, 29 July-1st Aug. 1968)	Proceedings of the Conference, 1969	J.I. McKigney
Food Economics in Nutrition Policy and Planning	Nutrition Newsletter, Vol. 7, No. 4, Oct.-Dec. 1969	J.I. McKigney
Nutrition and Productivity	CAJANUS, Vol. 2, No. 3, May-June 1969	J.I. McKigney
Food Imports: Milk	CAJANUS, Vol. 2, No. 4, July-August 1969	J.I. McKigney

Title	*Published in*	*1969*
Cultural Adaptations in the Development and Introduction of Protein-rich Foods (Paper delivered at Conference on "Protein Foods for the Caribbean", held at Georgetown, Guyana, 29 July-1st Aug. 1968)	Proceedings of the Conference, 1969	S.K. Reddy
Planning for Better Family Living	Nutrition Newsletter, Vol. 7, No. 2, April-June 1969	L. Marin/ M. Moore
Writing Styles of Food Faddists and Nutritionists (Report of M. S. research)	Canadian Home Economics Journal, Vol. 19, No. 2, April 1969	W.J. Fewster
Readability and Selected Style Variables in Nutrition Books Written for the Public by Food Faddists and Recognized Nutritionists. (Abstract of M. S. research)	Journal of the Canadian Dietetic Association, Vol. 30, No. 3, September 1969	W.J. Fewster
Nutrition Education in Developing Countries	Nutrition Newsletter, Vol. 7, No. 3, July-Sept. 1969	P.L.H. Davey/ J. McNaughton
The Present Position of Applied Nutrition Programmes	Nutrition Newsletter, Vol. 7, No. 4, Oct.-Dec. 1969	P.L.H. Davey
Food, Nutrition and Urbanization	Nutrition Newsletter, Vol. 7, No. 1, Jan.-March 1969	I. de Garine
Population Growth and Food Supplies	"Explosion Hunger — 1975", Vol. 2, No. 1, Oct.-Dec. 1969, India	T.B. Morgan
Applied Nutrition Programme, Brazil	Nutrition Newsletter, Vol. 7, No. 4, Oct.-Dec. 1969	S. Stefanini
Applied Nutrition Programme, India		B. Purvis
Applied Nutrition Programme, Senegal		S. Prosper
Applied Nutrition Programme, Peru		B. Stainier
History and Status of Specific Protein-rich Foods	Monograph "Protein-rich Cereal Foods for World Needs" edited by American Assoc. of Cereal Chemists, 1969	G.D. Kapsiotis
Les aflatoxines : (I) Les agents responsables	Oléagineux - - 24e année, N° 1, janvier 1969	P. Lunven/ Adrian
Les aflatoxines : (II) Les manifestations de la toxicité	Oléagineux — 24e année, N° 2, février 1969	P. Lunven/ Adrian
Lupiste : (I) Lupiste des oiseaux - une source exceptionnelle de tryptophane	Annales de la nutrition et de l'alimentation, Vol. 26, n° 6, 1969	P. Lunven/ Adrian

Title	*Published in*	*1969*
Prévention de l'infestation des aliments par les mycotoxines	Proceedings of the 8th Int. Congress of Nutrition, Prague	P. Lunven
		1970
Besoins et disponibilités futurs de protéines. (Paper delivered by Dr Cresta at the Colloque sur l'amélioration de ressources en protéines d'origine végétale hors de méthodes nucléaires, Vienna, 8-12 June 1970)	The proceedings of the Symposium	M. Autret
Perspectives of Human Nutrition. (Paper delivered at the closing ceremony of the VIIIth International Nutrition Congress, Prague, 26 Aug.-3 Sept. 1969)	The proceedings of the Congress	M. Autret
Indicative World Plan for Agricultural Development — Nutritional Digest	Nutrition Newsletter, Vol. 8, No. 2, April-June 1970	M. Autret/ J. Périssé
Philosophy and Main Lines of Orientation of the Programme of Work of the Nutrition Division	Nutrition Newsletter, Vol. 8, No. 2, April-June 1970	M. Autret
Nutrition et agriculture. (Paper delivered at the Third African Conference on Nutrition and Child Feeding, Tunis, 25-29 May 1970)	The proceedings of the Conference	J. Périssé/ M. Autret
Alias Kwashiorkor	"World Review of Animal Production" by the World Association for Animal Production, FAO special issue, No. 26, 1970	B.M. Nicol
Calorie Requirements and Physical Activity	8th International Congress of Nutrition, Abstracts of Papers, Prague	K.K.P.N. Rao
Agriculture, Food and Nutrition in Developing Countries. (Paper delivered at the Symposium of the Royal Society of Medicine on "Problems of World Medicine", London, 7 May 1970)	Proc. Roy. Soc. Med. Vol. 63, p. 1177, 1970	B.M. Nicol
Causes of Famine in the Past and in the Future. (Paper delivered at the Symposium on "Nutrition and Relief Operations in Times of Disaster", organized by the Swedish Nutrition Foundation and the Swedish International Development Authority, Saltsjöbaden, Sweden, 24-27 Aug. 1970)	Xth Symposium of the Swedish Nutrition Foundation, pp. 10-16, 1971	B.M. Nicol

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Title	Published in	1970
Food and Nutrition Situation in Western Samoa, Eastern Samoa and Tonga	SPC Nutrition Manual, 1970	F.M.S. Hansell
Nutrition in Food Policy and Planning	International Encyclopaedia of Food and Nutrition, Vol. 5 (Pergamon Press) and in the proceedings of the VIIIth International Congress of Nutrition	K.K.P.N. Rao
Cassava in the Human Diet	Nutrition Newsletter, Vol. 8, No. 3, July-Sept. 1970	K.K.P.N. Rao
The Protein Problem in the Asia and Far East Region	Journal of Nutrition and Dietetics of India	D.L. Bucoho
Education and Training in Food Control	Annual Review of Selected Developments, pp. 13-19 (Agricultural Education and Training, FAO, 1970)	R.K. Malik
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GENERAL POLICIES

in regard to

IMPROVEMENT OF NUTRITIVE QUALITY OF FOODS

A Policy Statement of the Food and Nutrition Board of the National Academy of Sciences-National Research Council. This statement supersedes "General Policies in Regard to Improvement of Nutritive Quality of Foods" published in 1968 (1).

1973

FOOD AND NUTRITION BOARD
NATIONAL ACADEMY OF SCIENCES
NATIONAL RESEARCH COUNCIL
2101 Constitution Avenue
Washington, D. C. 20418

NOTICE

The project that is the subject of this report was approved by the Governing Board of the National Research Council, acting in behalf of the National Academy of Sciences. Such approval reflects the Board's judgment that the project is of national importance and appropriate with respect to both the purposes and resources of the National Research Council.

The members of the committee selected to undertake this project and prepare this report were chosen for recognized scholarly competence and with due consideration for the balance of disciplines appropriate to the project. Responsibility for the detailed aspects of this report rests with that committee.

Each report issuing from a study committee of the National Research Council is reviewed by an independent group of qualified individuals according to procedures established and monitored by the Report Review Committee of the National Academy of Sciences. Distribution of the report is approved, by the President of the Academy, upon satisfactory completion of the review process.

The recommended dietary allowances (RDA), (2) first developed in 1943 by the Food and Nutrition Board, are levels of nutrient intake estimated to be adequate for the maintenance of good nutritional status in practically all healthy people in the United States. The recommended allowances have been revised as new data on nutritional needs accrued. Ideally, adequate amounts of the nutrients for which RDA are specified and of the other essential nutrients will be provided by the consumption of a diet appropriately selected from the wide variety of foods commonly available to the general public.

The availability and low cost of nutrients produced industrially and advances in food technology have enhanced the potential for the improvement of existing foods and for the formulation of new foods. Enrichment, fortification, and restoration of nutritional value of certain foods have been endorsed by the Council on Foods and Nutrition of the American Medical Association and the Food and Nutrition Board. (1) Specifically, the following practices in the United States continue to be endorsed:

> The enrichment of flour, bread, degerminated corn meal, corn grits, whole grain corn meal, white rice, and certain other cereal grain products with thiamin, riboflavin, niacin, and iron; the addition of vitamin D to milk, fluid skim milk, and nonfat dry milk; the addition of vitamin A to margarine, fluid skim milk, and nonfat dry milk; and the addition of iodine to table salt. The protective action of

fluoride against dental caries is recognized and the standardized addition of fluoride to water in areas in which the water supply has a low fluoride content is endorsed.

As population groups are identified as being potentially at risk of deficiency of various nutrients, standards for nutrient additions should be appropriately modified.

In assessing nutritional benefits to be derived from the modification or the formulation of foods, the composition of the overall diet must be considered. When levels of nutrient intake are increased by concentrates or foods in any form, the amounts provided should bear a realistic relationship to needs. Enhanced levels attained in the total diet should not be harmfully excessive for those who may employ the food(s) in varied patterns of use. The need for sound nutritional understanding by the consumer should be stressed as there is no one measure that can correct all possible dietary faults.

RESTORATION OF NUTRIENTS IN CONVENTIONAL FOODS

The appropriate selection of a wide variety of conventional foods is important in assuring adequate intake of all essential nutrients. It is, of course, desirable that foods having undergone some form of post-harvest processing contain the variety and amounts of essential nutrients that were present at significant levels in the raw, preprocessed food. A significant level of a nutrient is considered to be 5 percent or more of the RDA of that nutrient in an average serving. Restoration of nutrients

lost in the processing of foods has been recognized for many years as an appropriate method for improving their nutritional value. Under circumstances where restoration is undertaken through the addition of nutrients as specific chemical entities, it is important that all essential nutrients present at significant levels in the original preprocessed food be restored to the extent that is technically feasible. The nutrient level achieved through restoration should be 1.0-1.5 times preprocessed levels. The principle of extensive restoration is of special importance when a label claim for the content of an added nutrient is made.

ENRICHMENT AND FORTIFICATION OF FOODS

The Food and Nutrition Board endorses the addition of nutrients to foods in order to achieve enrichment or fortification when all of the following conditions are met:

1. The intake of the nutrient(s) is below the desirable level in the diets of a significant number of people;

2. The food(s) used to supply the nutrient(s) is likely to be consumed in quantities that will make a significant contribution to the diet of the population in need;

3. The addition of the nutrient(s) is not likely to create a dietary imbalance;

4. The nutrient(s) added is stable under customary conditions of storage and use;

5. The nutrient(s) is physiologically available from the food;

6. The enhanced levels attained in the total diet will not be harmfully excessive for those who may employ the foods in varying patterns of use; and

7. The additional cost is reasonable for the intended consumer.

NEW AND FORMULATED FOODS

The composition of common foods can be improved by alterations in formulation or in processing. For example, foods can be produced containing varying proportions and types of protein, fat, and carbohydrate. When nutritionally or economically advantageous to a significant segment of the population, such technological progress in producing good food is endorsed.

Scientific and technological advances have made it possible to produce a variety of new foods that combine acceptability and good nutritional value. These products frequently use as a base blended cereal grains, oil seeds, legumes, roots, or tubers as sources of protein and energy.

New foods can imitate common foods in appearance, texture, flavor, and odor. Examples are foods resembling dairy products, fruit juices, and meats. Other such foods do not physically resemble conventional foods. Examples are non-meal items such as certain snack foods. Another type of product is produced primarily to serve as a meal replacement. Because these products are replacing foods that make significant nutrient contributions, their nutritional value should at least equal the foods replaced.

The composition of a new or formulated food becomes especially important when an average serving of the product it imitates or replaces contributes 5 percent or more of the recommended daily allowance of any essential nutrient or energy. The formulated food should contain on a caloric basis at least the variety and the amounts of important nutrients (as defined above) provided by the food it replaces. Exceptions should, of course, be made for foods intended for therapeutic or modified diets in which the nutrient content is deliberately modified quantitatively or qualitatively.

A product designed primarily as a meal replacer should provide in the portion designated as a "meal" 25 to 50 percent of the RDA or, for those essential nutrients for which RDA have not been established, 25 to 50 percent of the estimated requirement. Caloric value of such products will depend upon recommended use.

It is recommended that efforts be continued to develop new foods that will provide superior nutritional quality, greater variety and acceptability, and that will be economically advantageous.

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RECOMMENDED DIETARY ALLOWANCES
Publication #1694, Food & Nutrition Board N.R.C.
National Academy of Sciences, Seventh Edition 1968

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VITAMIN D

Vitamin D is essential at all ages for maintenance of calcium homeostasis and skeletal integrity. Vitamin D can be acquired by ingestion of vitamins D_2 (ergocalciferol) or D_3 (cholecalciferol) and by exposure to certain ultraviolet wavelengths of light, which convert 7-dehydro-cholesterol in the skin to vitamin D_3. Vitamin D deficiency can occur only when the amount supplied by both sources is inadequate. One international unit of vitamin D is 0.025 μg of pure vitamin D_3.

Requirements and Recommended Allowances at Various Ages

The requirement for vitamin D can be met entirely by skin irradiation, so that the need for ingested vitamin D is influenced by the amount of exposure to ultraviolet light. There are few reliable data concerning minimum vitamin D requirements, except for infants. However, long experience has shown that 400 IU/day are sufficient to meet the requirements of practically all healthy individuals, assuming no exposure to ultraviolet light. There is also good evidence that such intakes are nontoxic. The allowances recommended during infancy are well documented [1]; however, those recommended for children and adolescents and during pregnancy and lactation are not well documented and may exceed actual requirements.

In normal full-term infants, intakes of as little as 100 IU/day have prevented rickets.[2-4] Actively rachitic infants have been cured with 300 IU/day.[2] With inadequate vitamin D intake, prematurely born infants develop rachitic lesions more readily than full-term infants, because of their more rapid growth rate. However, the administration of as little as 100 to 200 IU daily has been adequate to prevent biochemical evidence of rickets and to sustain normal skeletal growth in premature infants ingesting adequate amounts of calcium and phosphorus.[4,5] Assuming a satisfactory calcium intake, the ingestion of 400 IU/day provides for excellent skeletal growth, promotes satisfactory

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calcium absorption and metabolism, and prevents rickets in practically all normal full-term and premature infants.[1,6,7]

Vitamin D is required throughout the growth period, as evidenced by the rare occurrence of vitamin D-deficiency rickets in adolescents.[8] However, the requirement has been more difficult to determine beyond infancy, because of the greater access to sunlight and various food sources of vitamin D, and because skeletal changes occur less rapidly. In the past, the recommended intake for children and adolescents has been 400 iu/day,[1,9,10] and this is reaffirmed here. Ingestion of this amount of vitamin D protects practically all normal children from deficiency, and there is no evidence that this intake is harmful in any way.[1]

Diet surveys indicate that the majority of adolescents[11,12] do not take vitamin supplements. Because of inconsistency in the addition of vitamin D to commercial foods in North America (in Canada, until very recently, fortification of fluid milk was not permitted), many such individuals ingested much less than 400 iu of vitamin D per day, the total oral intake from all sources amounting to less than 100 iu/day in a number of instances.[13] Despite this, the general health and growth of these individuals were satisfactory. Furthermore, clinical rickets owing to vitamin D deficiency is virtually unknown beyond infancy in North America. These observations should be borne in mind when evaluating the nutritional adequacy of diets of American children and adolescents.

The requirement of vitamin D in adult life is not known. The occurrence of deficiency states (though very rare) indicates that a small need exists,[1] but it is considered that the amounts required are so small that under normal circumstances they are met by the vitamin D content of the usual mixed diet and by exposure to sunlight. For persons working at night, and for nuns and others whose clothing or customs shield them from sunlight, the regular consumption of vitamin D-fortified milk is recommended.

The requirement for vitamin D during pregnancy and lactation also is not known. On the basis of the small amount of evidence,[7] a daily intake of 400 iu is recommended during pregnancy and lactation.

Toxicity

It has long been recognized that excessive amounts of vitamin D (of the order of 1,000 to 3,000 iu/kg/day) are potentially dangerous to children[14] and adults[15] and may lead to hypercalcemia and attendant

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complications. It had first been thought that the margin of safety between the vitamin requirement and its toxic dosage was very great. However, relatively recently, circumstantial evidence derived from epidemiologic and metabolic studies of infantile hypercalcemia has tended to implicate vitamin D in the pathogenesis of both the mild and severe forms of this disease.[1,16-19] Vitamin D intakes implicated in hypercalcemia have usually been in the range of 2,000 to 3,000 IU/day, but sometimes the intake has been considerably less than this, and hypersensitivity to vitamin D has been postulated as an additional factor in affected individuals.[1,20] Although the relationship with vitamin D has not been completely proved, and recent estimates[20] place the incidence of infantile hypercalcemia at an extremely low figure, there is a justifiable basis for recommending that vitamin D intakes should not appreciably exceed a total of 400 IU/day during infancy.[1] There is no evidence that intakes of the order of 2,000 to 3,000 IU/day produce hypercalcemia beyond infancy. However, because the long-range effects of small excesses of vitamin D have not been extensively studied in vitamin D-sensitive individuals, the above recommendation for infants is also justified for older children and adults.

Considerations

The decline in the incidence of rickets in the United States in recent decades can largely be credited to the addition of vitamin D to fluid and evaporated milk. Few natural foods contain more than minute amounts of vitamin D. However, in the United States, a number of other foods, such as margarine, milk-flavorings, and certain breakfast cereals, have vitamin D added.[1] In evaluating the total vitamin D intake of the individual in terms of the RDA, the vitamin D content of the milk, of the vitamin supplement, and of all fortified foods must be taken into account. In North America, individuals of all ages consuming an ordinary diet may ingest an appreciable amount of vitamin D without taking a vitamin supplement, and in some individuals the recommended allowance may be considerably exceeded.[1,13] Calculation of individual intakes is often difficult. Vitamin D-addition practices vary from country to country. In the United States, the types of foodstuffs being fortified and the vitamin D concentrations of individual food items are continually being altered by the manufacturers. It is estimated that 85 percent of the fluid milk marketed in the United States has vitamin D added to a concentration of 450 IU/1,000 ml, and almost all brands of evaporated milk and commercial infant formulas

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contain this concentration when reconstituted.[1] Thus, within a few weeks of birth, most formula-fed full-term babies will acquire the RDA of vitamin D from the formula, and additional vitamin D is not necessary. Human milk and unfortified cow's milk contain minimal amounts of vitamin D, and, at the present time, fortification of nonfat dry milk is not permitted in the United States. Rickets may occur in breast-fed infants and in those fed unfortified formulas[2]; hence, for these infants a daily vitamin supplement providing 400 IU of vitamin D is recommended. During childhood and adolescence, consumption of vitamin D-fortified milk will usually provide a large proportion of the individual's recommended allowance of vitamin D. Milk consumption tends to be less during adult life, but the vitamin D requirement is also much lower. During pregnancy and lactation, however, many women consume at least one quart of milk per day. If vitamin D-fortified milk is used, the RDA of this nutrient is ingested.

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FOOD AND NUTRITION BOARD, NATIONAL ACADEMY OF SCIENCES–NATIONAL RESEARCH COUNCIL
RECOMMENDED DAILY DIETARY ALLOWANCES,[a] REVISED 1968

Designed for the maintenance of good nutrition of practically all healthy people in the U.S.A.

	Age[b] (years)	Weight		Height				Fat-Soluble Vitamins			Water-Soluble Vitamins							Minerals				
	From Up to	(kg)	(lbs)	cm	(in.)	kcal	Protein (gm)	Vitamin A Activity (IU)	Vitamin D (IU)	Vitamin E Activity (IU)	Ascorbic Acid (mg)	Folacin[c] (mg)	Niacin (mg equiv)[d]	Riboflavin (mg)	Thiamin (mg)	Vitamin B_6 (mg)	Vitamin B_{12} (μg)	Calcium (g)	Phosphorus (g)	Iodine (μg)	Iron (mg)	Magnesium (mg)
Infants	0 – 1/6	4	9	55	22	kg × 120	kg × 2.2[e]	1,500	400	5	35	0.05	5	0.4	0.2	0.2	1.0	0.4	0.2	25	6	40
	1/6 – 1/2	7	15	65	25	kg × 110	kg × 2.0[e]	1,500	400	5	35	0.05	7	0.5	0.4	0.3	1.5	0.5	0.4	40	10	60
	1/2 – 1	9	20	72	28	kg × 100	kg × 1.8[e]	1,500	400	5	35	0.1	8	0.6	0.5	0.4	2.0	0.6	0.5	45	15	70
Children	1 – 2	12	28	81	32	1,100	25	2,000	400	10	40	0.1	8	0.6	0.6	0.5	2.0	0.7	0.7	55	15	100
	2 – 3	13	34	91	36	1,250	25	2,000	400	10	40	0.2	8	0.7	0.6	0.6	2.5	0.8	0.8	60	15	150
	3 – 4	16	35	100	39	1,400	30	2,500	400	10	40	0.2	9	0.8	0.7	0.7	3	0.8	0.8	70	10	200
	4 – 6	19	42	110	43	1,600	30	2,500	400	10	40	0.2	11	0.9	0.8	0.9	4	0.8	0.8	80	10	200
	6 – 8	23	51	121	48	2,000	35	3,500	400	15	40	0.2	13	1.1	1.0	1.0	4	0.9	0.9	100	10	250
	8 – 10	28	62	131	52	2,200	40	3,500	400	15	40	0.3	15	1.2	1.1	1.2	5	1.0	1.0	110	10	250
Males	10 – 12	35	77	140	55	2,500	45	4,500	400	20	40	0.4	17	1.3	1.3	1.4	5	1.2	1.2	125	10	300
	12 – 14	43	95	151	59	2,700	50	5,000	400	20	45	0.4	18	1.4	1.4	1.6	5	1.4	1.4	135	18	350
	14 – 18	59	130	170	67	3,000	60	5,000	400	25	55	0.4	20	1.5	1.5	1.8	5	1.4	1.4	150	18	400
	18 – 22	67	147	175	69	2,800	60	5,000	400	30	60	0.4	18	1.6	1.4	2.0	5	0.8	0.8	140	10	400
	22 – 35	70	154	175	69	2,900	65	5,000	—	30	60	0.4	18	1.7	1.4	2.0	5	0.8	0.8	140	10	350
	35 – 55	70	154	173	68	2,600	65	5,000	—	30	60	0.4	17	1.7	1.3	2.0	5	0.8	0.8	125	10	350
	55 – 75+	70	154	171	67	2,400	65	5,000	—	30	60	0.4	14	1.7	1.2	2.0	6	0.8	0.8	110	10	350
Females	10 – 12	35	77	142	56	2,250	50	4,500	400	20	40	0.4	15	1.3	1.1	1.4	5	1.2	1.2	110	18	300
	12 – 14	44	97	154	61	2,300	50	5,000	400	20	45	0.4	15	1.4	1.2	1.6	5	1.3	1.3	115	18	350
	14 – 16	52	114	157	62	2,400	55	5,000	400	25	50	0.4	16	1.4	1.2	1.8	5	1.3	1.3	120	18	350
	16 – 18	54	119	160	63	2,300	55	5,000	400	25	50	0.4	15	1.5	1.2	2.0	5	1.3	1.3	115	18	350
	18 – 22	58	128	163	64	2,000	55	5,000	400	25	55	0.4	13	1.5	1.0	2.0	5	0.8	0.8	100	18	350
	22 – 35	58	128	163	64	2,000	55	5,000	—	25	55	0.4	13	1.5	1.0	2.0	5	0.8	0.8	100	18	300
	35 – 55	58	128	160	63	1,850	55	5,000	—	25	55	0.4	13	1.5	1.0	2.0	5	0.8	0.8	90	18	300
	55 – 75+	58	128	157	62	1,700	55	5,000	—	25	55	0.4	13	1.5	1.0	2.0	6	0.8	0.8	80	10	300
Pregnancy						+200	65	6,000	400	30	60	0.8	15	1.8	+0.1	2.5	8	+0.4	+0.4	125	18	450
Lactation						+1,000	75	8,000	400	30	60	0.5	20	2.0	+0.5	2.5	6	+0.5	+0.5	150	18	450

[a] The allowance levels are intended to cover individual variations among most normal persons as they live in the United States under usual environmental stresses. The recommended allowances can be attained with a variety of common foods, providing other nutrients for which human requirements have been less well defined. See text for more-detailed discussion of allowances and of nutrients not tabulated.

[b] Entries on lines for age range 22–35 years represent the reference man and woman at age 22. All other entries represent allowances for the midpoint of the specified age range.

[c] The folacin allowances refer to dietary sources as determined by *Lactobacillus casei* assay. Pure forms of folacin may be effective in doses less than ¼ of the R.D.A.

[d] Niacin equivalents include dietary sources of the vitamin itself plus 1 mg equivalent for each 60 mg of dietary tryptophan.

[e] Assumes protein equivalent to human milk. For proteins not 100 percent utilized factors should be increased proportionately.

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Vitamins—(Continued)

Table 1. Properties—(Continued)

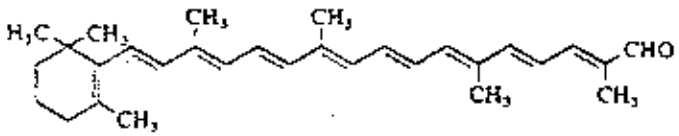
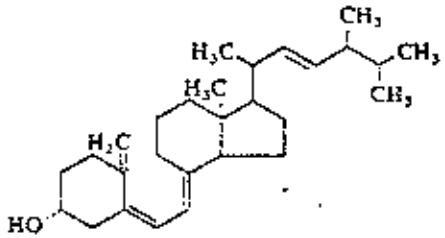
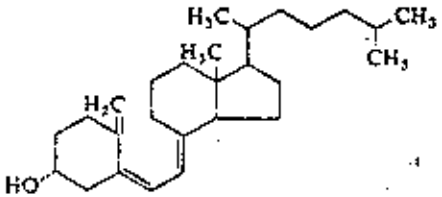
Compound	Formula	Properties	Solubility (g/100 ml)	Stability
Provitamin A—(Continued)				
β-apo-8'-Carotenal	 $C_{30}H_{42}O$ mol. wt. 416.65	Dark violet crystals; m.p. 136–140°C (all-*trans*); λ_{max} = 461 and 488 mμ (cyclohexane); $E^{1\%}_{1cm}$ = 2,640 at 461; 1 mg = 1,220 IU (rat).	s. most org. solv.; sl.s. alcohol; ins. glycerol, water.	Unstable to oxygen; can be used, however, as food color.
VITAMIN D_2				
Activated ergosterol Calciferol Ergocalciferol Oleovitamin D_2 Viosterol 9,10-Secoergosta-5,7,10(19),22-tetraen-3-ol Irradiated ergosta-5,7,22-trien-3β-ol	 $C_{28}H_{44}O$ mol. wt. 396.66	White, prism crystals; m.p. 115–118°C; λ_{max} = 264 mμ (hexane); $E^{1\%}_{1cm}$ = 459; $[\alpha]_D^{25}$ = +103 to 106° (alcohol); 1 mg = 40,000 IU or USP units.	s. most org. solv.; sl. s. oils; ins. water.	Crystals relatively unstable to air, accelerated by unsaturated fat and trace mineral contact; can be stabilized
VITAMIN D_3				
Activated 7-dehydrocholesterol Cholecalciferol Oleovitamin D_3 22,23-Dihydro-24-demethylcalciferol Activated 5,7-cholesta-dien-3β-ol	 $C_{27}H_{44}O$ mol. wt. 384.65	White, fine needle crystals; m.p. 84–88°C; λ_{max} = 264 mμ (hexane); $E^{1\%}_{1cm}$ = 450, 490; $[\alpha]_D^{25}$ = +105 to 112° (alcohol); 1 mg = 40,000 IU or USP units.	like D_2.	Similar to D_2.

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TABLE 2. BIOLOGICAL CHARACTERISTICS

Compound	Function	Deficiency symptoms	Hyper-use symptoms	Coenzyme and enzyme involved	Remarks
Vitamin A	Primary role in vision as 11-cis retinal; synthesis of mucopolysaccharides; maintenance of mucous membranes and skin; bone development and growth; maintenance of cerebrospinal fluid pressure; production of corticosterone	Retarded growth, xerophthalmia, nyctalopia, hemeralopia, ataxia, tissue keratinization, corneification, desquamation, emaciation, lachrymation, impaired reproduction or hatchability (eggs), increased susceptibility to infection, optic nerve degeneration, odontoblast atrophy	Weight loss, bone abnormalities, inflammations, exfoliated epithelium, liver enlargement, pain, loss of hair, facial pigmentation	None identified; may be involved with NADP	Other carotenes, α, β, γ and β-zeacarotene, cryptoxanthin, echinenone, certain apocarotenals, cis isomers of retinol, and dehydroretinol have fractional Vitamin A activity, differing for various animal species. Retinoic acid may be metabolically active form for certain functions.
Vitamin D	Absorption and transport of calcium and phosphorus; synthesis of calcium protein carrier; interrelationship with parathyroid hormone; maintains alkaline phosphatase levels at bone site	Rickets, enlarged joints, softened bones, stilted gait, arched back, thin-shelled eggs, disturbed reproduction and hatchability, osteomalacia, faulty calcification of teeth, tetany, convulsions, raised plasma phosphatase, parturient paresis (dairy cattle)	Abnormal calcium deposition in bones and tissues, brittle or deformed bones, vomiting, abdominal discomfort, renal damage, weight loss	None identified	Other calciferols (D_4, D_5, D_6, D_7) exist, differing in the structure of the side chain and having some biological activity. D_3 is the most effective form for the avian species. For man D_2 and D_3 are fully active.
Vitamin E	Biological antioxidant, interrelated with Se; metabolism of nucleic and sulfur amino acids; ubiquitone and ascorbic acid synthesis; cytochrome c reductase function; detoxicant and oxidation-reduction action	Muscular dystrophy, encephalomalacia, hepatic necrosis, erythrocyte hemolysis, hock disorders, steatitis, reduced reproduction and hatchability, exudative diathesis, liver dystrophy, anemia, degeneration of testicular germinal epithelium, creatinuria	None identified; relatively non-toxic	None identified	Other tocols (β, γ, δ-tocopherol) and trienols (α, β, γ, δ-tocotrienol) exist differing in ring substituents and in the side chain and having fractional vitamin activity; presence of unsaturated fat in the diet increases dietary Vitamin E requirements
Vitamin K	Hepatic synthesis of prothrombin; synthesis of thromboplastin; needed in RNA formation and electron transport	Hemorrhage, impaired coagulation (low prothrombin levels), increased blood clotting time	Vomiting, albuminuria, porphyrinuria, polycythemia, splenomegaly, kidney and liver damage	None identified	K_1 is plant form of the vitamin; K_2, the microbiologically synthesized form; K_1 and K_2 are metabolically active forms; they also occur with longer or shorter side chain (isoprene) units; coumarin compounds and excess sulfa drugs are dietary stress agents for vitamin K.

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Vitamins—(Continued)

TABLE 3. ANIMAL REQUIREMENTS (per kilo ration) (NAS-NRC)

Animal	Purpose	A (IU)	D (IU)	E (IU)	K (mg)	AA (mg)	Bi (μg)	Ch (mg)	Cy (μg)	FA (mg)	Ni (mg)	Pa (mg)	Py (mg)	Ri (mg)	Th (mg)
Cat (1962)	Growing	25,000	1,000	34-136	-	*	--	3,000	–	*	40	5	2	4	4
Dog (1962, revised)	Growing	5,000	250	50[a]		--	--	1,200	22	0.18	10	2.2	1	2.2	0.7
Fox[b] (1963)	Growing	2,410	*	*	—	...	*	*	*	0.2	10	8	2	2.6	1.0
Guinea pig (1962)	Growing	16,000	*	60	10	200	—	1,500	--		50	20	4	16	6
Hamster (1962)	Growing	13,000	*	25	*	*	*	*	-		*	40	6	6	6
Mink[c] (1968)	Growing	3,500	*	25	--	—	*	*	30	0.5	20	6	1.1	1.5	1.2
Monkey	Growing	*	*	*	--	25	250		25	1.3	38	*	1.2	0.8	0.8
Mouse (1962)	Growing	500	150	20	*	*	*	570-1,140	5	*	30	8.5	1	4	2.9
	Pregnancy and lactation	500	*	*	*	*	*	*	4.5	*	*	10.2	*	7	5
Rat (1962)	Growing	2,000	*	60	1	*	*	750	5	*	15	8	1.2	2.5	1.3
	Gestation	12,000	*	30	*	*	*	< 1,000	5	*	*	8	0.6	4	4
	Lactation	12,000	*	20	*	*	*	< 1,000	5	*	*	10	0.6	4	4
Poultry															
Chicken (1966)	Growing 0-8 weeks	2,000	200	*	0.5	--	90	1,300	9	1.2	27	10	3	3.6	1.8
	Growing 8-16 weeks	2,000	200	*	*	—	*	*	*	*	11	10	*	1.8	*
	Laying	4,000	500	*	*	—	*	*	*	0.25	*	2.2	*	2.2	*
	Breeding	4,000	500	*	*	—	150	*	3	0.35	*	10	4.5	3.8	0.8
Duck (1966)	Growing	*	220	*	*	—	*	*	*	*	55	11	2.6	4	*
Pheasant (1966)	Growing	*	1,200	*	*	—	*	*	*	*	60	*	*	3.5	*
Quail (1966)	Growing	13,000	*	*	*	—	*	*	*	*	*	*	*	*	*
Turkey (1966)	Growing 0-8 weeks	4,000	900	*	0.7	—	*	1,900	3	0.9	70	11	3	3.6	*
	Growing 8-16 weeks	4,000	900	*	*	—	*	*	*	*	*	*	*	*	*
	Breeding	4,000	900	*	*	—	*	*	*	0.8	*	16	*	3.8	*
Swine (1968)	Growing 5-10[d]	2,200	220	*	*	—	*	1,000	22	0.5-1	22	13	1.5	3	1.3
	Growing 10-20	1,750	200	*	*	—	*	900	15	0.5-1	18	11	1.5	3	1.1
	Growing 20-35	1,300	200	*	*	—	*	*	11	*	14	11	1.1	2.6	1.1
	Growing 35-60	1,300	125	*	*	—	*	*	11	*	10	11	*	2.2	1.1
	Growing 60-100	1,300	125	*	*	—	*	*	11	*	10	11	*	2.2	1.1
	Breeding 110-160	4,100	275	*	*	—	*	*	13.8	*	22	16.5	*	4.4	1.4
	Lactating 140-200	3,300	220	*	*	—	*	*	11	*	17.6	13.2	*	3.3	1.1
	Boars 110-180	4,100	275	*	*	—	*	*	13.8	*	22	16.5	*	4.1	1.4

Compiled by J. C. Bauernfeind and E. DeRitter.

[a] AA, Ascorbic acid; Bi, Biotin; Ch, Choline; Cy, Cyanocobalamin; FA, Folic acid; Ni, Niacin; Pa, Pantothenic acid; Py, Pyridoxine; Ri, Riboflavin; Th, Thiamine.

[b] Required but specific needs not established. AA only a dietary essential for monkey and guinea pig.

[c] NAS-NRC publications also exist for the vitamin requirements of large animals.

[d] Kilograms

[e] Not a dietary essential.

[f] From 7-31 weeks of age, male body wt. goes from 690 to 2,070 g with consumption of 27 to 75 g of dry food daily; female body wt. goes from 560 to 1,110 g with consumption of 32 to 60 g of dry food daily.

[g] From 7-31 weeks of age, male body wt. goes from 1.4 to 5.7 kg with consumption of 59 to 160 g of dry food daily; female body wt. goes from 1.3 to 4.7 kg with consumption of 36 to 118 g of dry food daily.

[h] Growing.

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J C Bauernfeind
E. De Ritter

Miscellaneous

K-49

1849

FINAL

Company Code: [redacted]

FPC No: 0246

1849

[redacted]

FORM 6

[redacted]

NUMBER: [redacted]
DATE: SEPT. 30, 1970
CANCELS: AUG. 4, 1970

INGREDIENT: VITAMIN D_3 - USED FOR EVAPORATED MILK ONLY

ALL DELIVERIES OF VITAMIN D_3 SHALL COMPLY IN EVERY RESPECT TO THE REQUIREMENTS OF THE FEDERAL FOOD, DRUG AND COSMETIC ACT, AS AMENDED AND REGULATIONS PROMULGATED THEREUNDER, AS WELL AS ANY OTHER STATE, FEDERAL FOOD REGULATORY AGENCIES. IN ADDITION, THE PRODUCT SHALL COMPLY TO THE FOLLOWING SPECIFICATIONS AND ALL LOTS ARE SUBJECT TO THE APPROVAL OF THE QUALITY CONTROL DEPARTMENT OF [redacted]

		PROCEDURE
COLOR & FORM:	WHITE TO CREAM COLOR-FREE FLOWING POWDER	VISUAL
ODOR:	NONE	
IDENTIFICATION OF VITAMIN D_3:	MUST MEET CODEX EXCEPT NEED NOT BE CRYSTALLINE	CODEX - FIRST EDITION PAGES 715-716
+POTENCY:	1) 200 MILLION USP UNITS/LB. 2) 440,917 USP UNITS/GRAM	AS ESTABLISHED BY BIOASSAY TWO LEVELS OF STANDARD AND ONE OF UNKNOWN.
SOLUBILITY:	DISPERSIBLE IN WATER	
PARTICLE SIZE:	100% THRU NO. 20 80% THRU NO. 80 MIN.	TYLER ROTAP U.S.S. SIEVES
COMPONENTS:	VITAMIN D_3 DEXTRIN BHA BHT LECITHIN	
LABEL COLOR:	ORANGE BACK-GROUND WITH BLACK PRINT.	
ASSAY REPORT:	LABORATORY BIOASSAY REPORT TO BE FURNISHED FOR EACH LOT WHICH IS PREPARED AND SENT TO: DR. A. Z. HODSON CHEMISTRY DEPARTMENT PRODUCT DEVELOPMENT SERVICES GREENVILLE, ILLINOIS 62246	
STORAGE:	KEEP IN A COOL, DRY AREA PROTECTED FROM LIGHT.	
SHIPMENT:	EACH SHIPMENT SHALL NOT CONTAIN MORE THAN TWO LOT OR BATCH NUMBERS. LOT OR BATCH NUMBER TO BE INDICATED ON LABEL.	
PERFORMANCE:	MUST BE COMPARABLE IN PERFORMANCE BY USAGE TEST TO PRODUCT PREVIOUSLY APPROVED.	

+LATEST CHANGE MARKED WITH A PLUS.

VITAMIN D_3

Table of Contents

Rehm 8/11/67 Manuscript "Vitamin A and Male Fertility."

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Pediatric Clinics of North America
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A New Look at Infantile Hypercalcemia

DONALD FRASER, M.D.

B. S. LANGFORD KIDD, M.D.

SANG WHAY KOOH, M.D.

LUC PAUNIER, M.D.

Historical Aspects and Terminology

In adults the finding of hypercalcemia brings to mind a fairly extensive list of diagnoses among which are primary hyperparathyroidism, excessive vitamin D intake, sarcoidosis, malignant disease (especially with skeletal metastases), immobilization, the "milk-alkali" syndrome, and others. Hypercalcemia in infancy can almost never be attributed to any of these conditions.* Thus, in 1952, when hypercalcemia was first observed in infants with failure to thrive, Lightwood[40] and Payne[43] were unable to establish a specific cause for the condition, and the malady was appropriately called "idiopathic hypercalcemia." In the same year Fanconi and Schlesinger[18] described two children in whom chronic hypercalcemia was associated with other abnormalities. These authors ruled out the possibility of excessive intake of vitamin D in their patients, since it had long been known that this agent, if administered in massive doses, could produce severe hypercalcemia in children and adults. Yet in the course of the next few years no other etiologic factor could be discovered to account for the appearance of a considerable number of infants with this condition, particularly in Great Britain. Accordingly, the postulate gradually became accepted that the disease might be attributable to individual hypersensitivity to vitamin D or to its abnormal

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* Familial neonatal hyperparathyroidism has recently been reported in a single family.[31]

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metabolism. Certain metabolic studies tended to substantiate this hypothesis, and recent epidemiologic data from Britain are thought by many to provide strong circumstantial evidence that the prime cause of the hypercalcemic syndrome in infants (at least the mild form) is excessive sensitivity to vitamin D. Thus the condition is now usually referred to as "infantile hypercalcemia." The new terminology implies considerable confidence that the cause has now been established. In our opinion, it is still open to some question whether such confidence is justified.

This article reviews the clinical aspects of infantile hypercalcemia, discusses the present evidence regarding causation, estimates the magnitude of the problem and comments upon suggested prophylactic measures.

Clinical Features of Infantile Hypercalcemia

From the earliest reports it was evident that the clinical manifestations showed wide variations from case to case, and it became customary to refer to the "mild" form and the "severe" form of infantile hypercalcemia. Some authors[24] consider that the two forms represent opposite ends of a spectrum of the same pathologic process; others[7] tend to disagree with this suggestion. The present authors have not been able to reach a definite decision whether two separate disease entities exist or not. In practice, there is no question that intermediate manifestations are seen. Indeed, it is often difficult to classify a given case into one or other category with certainty. It has recently been estimated that in Britain at least 8 per cent of cases were of the severe form,[9] but what additional proportion of infants had stigmata which might eventually give rise to long-term consequences (i.e. renal or cardiac lesions, mental defect) is impossible to state. From a prognostic standpoint it is probable that intermediate cases should be included along with the severe syndrome. In a later section of the paper we point out that the late stage of the severe form of infantile hypercalcemia must be regarded as one aspect of the disease.

The Mild Form of Infantile Hypercalcemia. The baby with the mild form of infantile hypercalcemia does well until three to seven months of age, at which time symptoms suddenly develop. The primary signs are gastrointestinal: anorexia, vomiting, constipation. Failure to thrive soon becomes obvious. Thirst, secondary to polyuria, is usually a feature, and there may be clinical dehydration and bouts of fever. The child tends to be alert but irritable. From experience with other hypercalcemic syndromes it is known that all these features can be produced by hypercalcemia per se.

On reviewing the history of pregnancy, delivery and neonatal progress, no unusual features have been noticed. Although the number of adequately documented cases is small (24 published cases), birth

weights of patients with the mild form of infantile hypercalcemia fall within the normal range (Fig. 69). An increased incidence of prematurity has not been observed. There is no mention in the literature of excessive maternal intake of vitamin D during pregnancy.

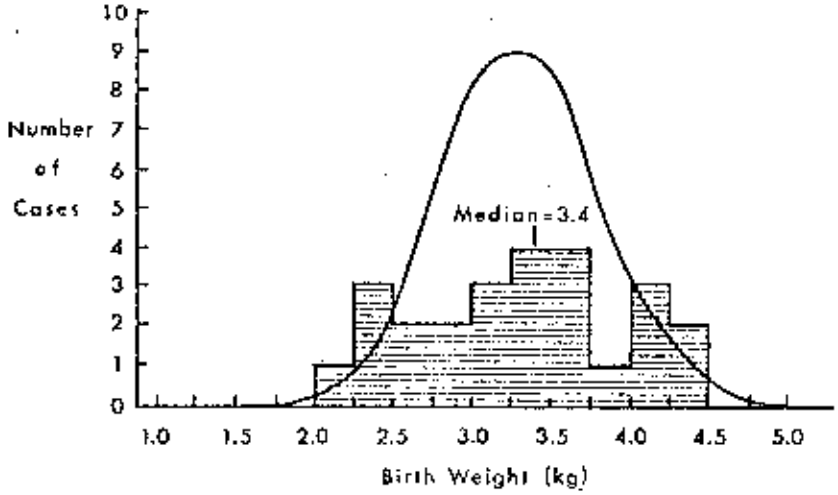

Figure 69. Birth weights in the mild form of infantile hypercalcemia (24 cases collected from the literature plus our case 8). Median weight is 3.4 kg. and is not different from that of a normal population.[2]

There is an equal sex distribution. A familial incidence, though reported, is uncommon. Illig and Prader[15] reported binovular twins with the mild form of infantile hypercalcemia and observed hypercalcemia in a third sibling. Forfar[25] has observed two sets of affected siblings in 11 families.

On physical examination the infant is usually thin, often dehydrated and "marasmic," but if the condition is diagnosed shortly after the onset of symptoms, he may still be well nourished and plump. The face looks normal, and there is no strabismus and no mental defect (Fig. 70). Typically, the musculature is hypotonic, but hyperreflexia has been observed in a high proportion of the cases. A cardiac murmur has been reported in approximately 10 per cent of cases.[10, 13] Blood pressure is usually normal, but may be elevated during the hypercalcemic phase.

In summary, patients with the mild form of infantile hypercalcemia have no distinctive features that separate them on physical examination alone from any other infant with failure to thrive. By the same token, infantile hypercalcemia must be considered in the differential diagnosis of any infant who presents with failure to thrive as a symptom.

The Severe Form of Infantile Hypercalcemia. The onset of the symptoms of the severe form of infantile hypercalcemia tends to occur somewhat earlier; sometimes symptoms can be dated back to birth. Other infants may have progressed normally until three to six months of age

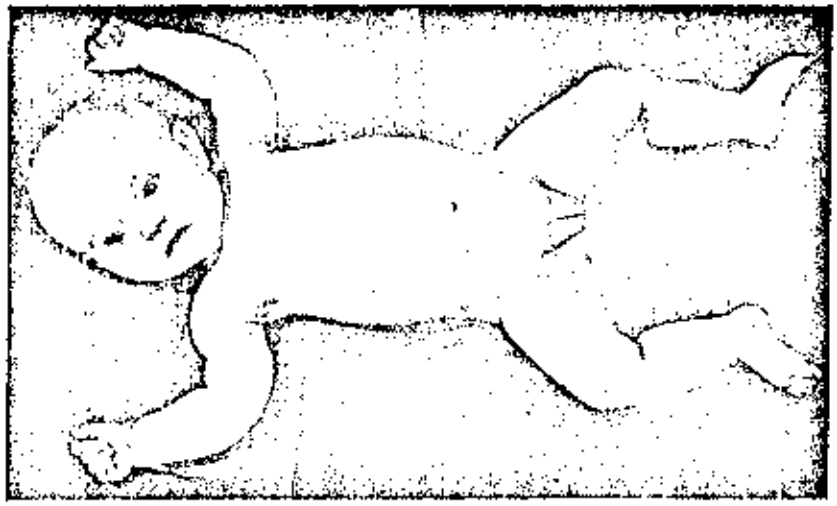

Figure 70. Case 3 (F.C.), 9-month-old infant with the mild form of infantile hypercalcemia.

before the sudden onset of failure to thrive. As with the mild form, the presenting symptoms are usually referable to the digestive system: e.g. anorexia, vomiting, constipation. Difficulty in getting the infant to swallow and retain food is often conspicuous, and weight loss may occur rapidly. On questioning, polyuria is prominent, and dehydration is a frequent problem.

As in the mild form of the disease, no abnormalities of pregnancy have been reported. Until recently, the vitamin D intake of the mother during pregnancy has not been systematically recorded. In recent reports[6, 37, 39] the vitamin D intake was stated to have been normal. The total vitamin D intakes of the mothers of two severely affected patients seen at this hospital did not exceed 1000 I.U. per day during pregnancy.

There is no sex preponderance, and familial incidence is rare. Kenny et al.[30] reported a patient with asymptomatic hypercalcemia who had a severely affected sibling.

One feature of patients with the severe form of infantile hypercalcemia which has not been stressed in the past is the birth weight. In Figure 71 we have plotted the birth weights of 36 infants with severe infantile hypercalcemia, including two of our own. These are compared with the birth weight distribution of live-born infants in the general population.[2] There appears to be a definite trend toward low birth weights in the severely affected group.

In the full-blown picture of the severe form there are a number of physical signs which, when all are present, are distinctive enough to suggest the diagnosis before biochemical confirmation. The sign which immediately comes to mind on mention of the severe form of infantile hypercalcemia is the "elfin facies." This consists of full, pouting cheeks

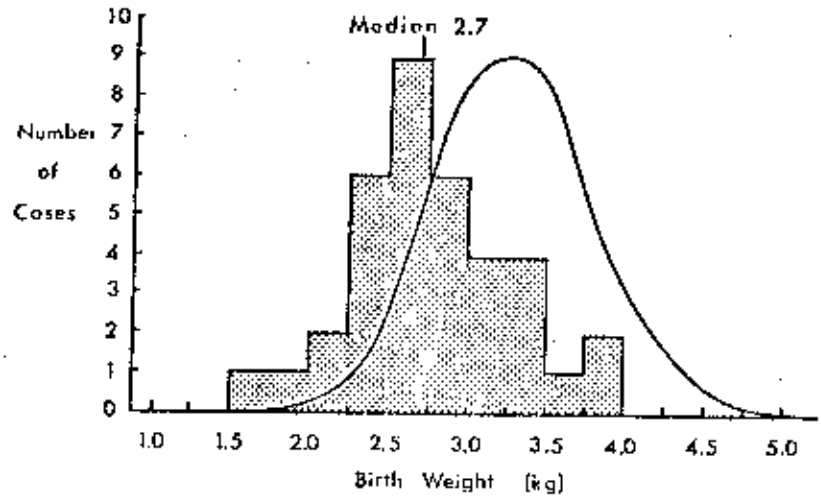

Figure 71. Birth weights in 36 cases of the severe form of infantile hypercalcemia (34 cases collected from the literature plus our cases 1 and 2). The median weight is 2.7 kg., and compared to that of a normal population there appears to be a definite trend toward low birth weight.

and a broad mouth with a prominent upper lip shaped in a "cupid's bow." The chin is recessive. The bridge of the nose is flat, and the nose is short and up-turned. Often there is hyperteloism and an epicanthic fold. Sometimes there is convergent strabismus. The ears tend to be low placed and to stand out prominently from the head. There are so many published illustrations of the classic facial appearance that real gnomes and elves, wherever they may reside, need have no fear that they will go unrecognized by mortal man, should they choose to appear among us.

We too are publishing pictures of patients with the "elfin facies" (Figs. 72, 73). In our opinion, however, this feature deserves special comment. There is no question that a characteristic facies is prominent in some cases. Nevertheless some infants who otherwise fit the "severe" category cannot be considered to have the facies. Of the two severely affected patients diagnosed at this hospital, it must be stated that the elfin facies was not noticed at the time of admission in either case, but only some days later, immediately after hypercalcemia had been reported by the laboratory. On the other hand, one of these patients was stated by the obstetrician to have a "peculiar" face some 18 months before the actual diagnosis was established. Although an unusual facial appearance has been remarked upon at birth in a few other patients in the literature, the typical elfin facies does not normally appear so early. The development of this feature tends to be insidious; it is rarely possible to state the age at onset. The "elfin facies" is sometimes a useful sign, particularly for clinicians with previous experience. We believe, however, that the sign is often not characteristic enough to attract attention and therefore may be of no help in arriving at the diagnosis.

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Figure 72. Case 1 (J.T.). Severe form of infantile hypercalcemia with supravalvular aortic stenosis and peripheral pulmonary artery stenoses.

Figure 73. (G.C.). Two-year-old boy with late normocalcemic stage of infantile hypercalcemia. He has a supravalvular aortic stenosis, peripheral pulmonary artery stenoses and mental retardation.

Craniosynostosis is another feature present at the time of diagnosis in a small proportion of infants with the severe form.[12, 17, 18] The time of suture fusion is not known.

By the time patients with the severe form of infantile hypercalcemia are old enough for significant psychometric assessment, they are found to be moderately to severely mentally retarded; usually the intelligence quotient is between 40 and 70. At the time of diagnosis, when hypercalcemia is prominent, youth and general debility usually make it difficult to assess mental function; thus there is a question at what stage in development the mental defect first arises.

At the time of diagnosis, patients with the severe form of hypercalcemia usually have had a systolic murmur in the upper parasternal region. This has usually been sufficiently loud and coarse to justify the diagnosis of a ventricular outflow stenosis, and, until recently, the murmur was assumed to be evidence of valvular calcinosis.[47] In the past two years, however, some five infants with the severe form of infantile hypercalcemia have been studied by catheter and angiocardiographic techniques, and a definitive conclusion has been reached about the significance of the murmur. In these patients the lesion consisted of supravalvular stenosis of the aorta or peripheral pulmonary artery stenoses, sometimes both. This aspect is discussed at greater length in a later section (see Cardiovascular Lesions in Infantile Hypercalcemia).

Like other features of the severe form, it is usually impossible to state the time of onset of the cardiovascular lesions with accuracy. It is known that in some patients a suspicious murmur had been heard at birth or shortly after. In two recent cases[47] the murmurs were detected at eight months of age and at birth, respectively, at a time when the serum calcium levels were normal; the hypercalcemia did not appear in one case until six months later, and in the other the delay was even longer. In several cases the murmur either disappeared or became less marked with time.[42, 50]

Systemic blood pressure is often considerably elevated in patients with the severe form of infantile hypercalcemia. The electrocardiogram may show left ventricular hypertrophy, right ventricular hypertrophy or combined ventricular hypertrophy.

Other physical features have been noted. Usually, muscular hypotonia associated with increased deep tendon reflexes is present. A peculiar raucous voice, and occasionally transient facial nerve paresis (thought to be due to cranial bone pressure) have been reported.[39]

Laboratory Findings in Infantile Hypercalcemia (Mild and Severe Forms)

The laboratory features of the mild and severe forms of infantile hypercalcemia are similar, but those observed in the severe form are much more prominent.

The essential feature is, of course, hypercalcemia, but the degree of

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elevation above normal is variable from case to case. In the literature, levels of total serum calcium have been between 12 and 18 mg. per 100 ml., sometimes higher. Obviously, however, the lower limit which defines a case is dependent upon the analytical method and its reproducibility in a given laboratory. A feature not usually emphasized in the past is the wide fluctuations in the serum calcium which undoubtedly occur spontaneously in some cases of infantile hypercalcemia during the acute phase of the disease. Using reproducible methods for measuring serum calcium, we have observed variations of 2 to 3 mg. per 100 ml. within a few days in a given hypercalcemic patient. Thus, in a confirmed case, single determinations may occasionally be within the normal range. The finding of normocalcemia may also raise false hopes for a given therapeutic procedure which, in actual fact, may have transient value. The ultrafiltrable and ionized calcium fractions have not been systematically studied. It has been reported that the cerebrospinal fluid calcium level (which normally tends to mirror the ultrafiltrable calcium) is normal.[13] The serum inorganic phosphorus level is normal, and alkaline phosphatase activity is normal or slightly low, features which are helpful in ruling out hyperparathyroidism. Histologic studies of the parathyroid glands are reported to be normal.[45, 47]

Leukocytes and granular casts may be present in the urine, and in almost all patients the blood urea nitrogen level is elevated during the acute phase. In mild cases this is transient, but in severely affected patients renal signs may persist. Reduction of glomerular filtration rate, renal plasma flow and tubular secretory function has been demonstrated.[19] The total serum protein is often increased as a result of increased alpha-2 and gamma globulin fractions.[13, 43] Serum electrolytes are rarely abnormal; in a few cases hypokalemia was reported. Cholesterol levels were often found to be elevated. Forfar et al.[23] reported a positive correlation between the serum calcium level and both the total and free cholesterol levels in 19 cases of infantile hypercalcemia of the mild form. Cholesterol esters were normal. Fyfe[26] reported an elevation of vitamin A levels in eight cases of infantile hypercalcemia. Citrate levels were found by some to be low;[23] by others to be elevated during the acute phase of the disease.[19]

Radiographic Findings

In the mild form of infantile hypercalcemia, radiographs of the long bones are usually normal, but sometimes transverse bands of increased density are seen in the metaphyses similar to those observed in heavy metal poisoning.[13]

In the severe form the x-ray changes tend to be more widespread,[17, 48] although in some instances radiographic changes are not detected. There is usually an increase in the density at the metaphyses of long bones and of the skull, most often seen at the base and in the

orbital regions. Faulty tubulation of the femora and sometimes osteoporosis of the metaphyses have been described. Deposits of calcium occur particularly in the kidneys, but also in blood vessels, brain, intramuscular planes and bronchi. Craniosynostosis is occasionally found.

Cardiovascular Lesions in Infantile Hypercalcemia

It has been recognized since the earliest case reports that a high proportion of children with the severe form of infantile hypercalcemia have a systolic murmur, maximal at the upper left sternal border. Rashkind et al.[44] reviewed the clinical cardiovascular findings in 53 published cases of infantile hypercalcemia, mostly of the severely affected form. Thirty-three patients had a significant cardiac murmur.

Originally it had been thought, on the basis of the physical signs, that the murmur was due to aortic stenosis caused by thickening of the aortic valve cusps. Rashkind et al.[44] reviewed the autopsy data on 11 patients with infantile hypercalcemia. In three of these, all mild, no murmur had been noted and no cardiovascular lesions were found. Eight patients had loud parasternal systolic murmurs. One of these had myxedematous degeneration of the aortic valve. Three had thickened and calcified atrioventricular valves, and two had coronary subintimal proliferation. Four cases also exhibited myocardial calcification, and four had left ventricular hypertrophy. One case had fibrous thickening of the aorta. True aortic valve stenosis was not reported in any instance.

In the early cases of severe infantile hypercalcemia, angiocardiography was not undertaken, and although clinical signs pointed to an obstruction of the outflow from the left ventricle, the anatomic site of the lesion had never been demonstrated definitively. In 1961 Garcia et al.[27] carried out angiocardiography in a nine-month-old infant who had an aortic systolic murmur, hypercalcemia, elfin facies and probable mental retardation. The studies demonstrated that the murmur was produced by supravalvular aortic stenosis (i.e. a narrowing of the aorta a few centimeters distal to the aortic valve, which itself was not stenosed). The child also had stenoses in the peripheral pulmonary arteries.

Since the report of Garcia et al.,[27] Jue et al.[37] have also demonstrated supravalvular aortic stenosis in a child who presented at eight months of age with most of the features of severe infantile hypercalcemia. A second patient had typical infantile hypercalcemia, but the angiocardiographic studies demonstrated stenoses of the peripheral pulmonary arteries only. This child's systolic murmur was noticed in the neonatal period, and the typical facial signs and failure to thrive were noted at nine months.

Two children with the full-blown severe form of infantile hypercalcemia and one child with the mild form have been carefully assessed from the cardiologic viewpoint at this hospital.

CASE 1. J.T. was a full-term infant with a peculiar facies and a birth weight of 2.3 kg. She failed to thrive since birth, and was first diagnosed as having a severe form of infantile hypercalcemia at 18 months (Fig. 72). Both the mother, during pregnancy, and the infant received 1000 I.U. of vitamin D daily. She had hypercalcemia (15.3 mg. per 100 ml.), a typical facies and an injection systolic murmur with thrill, and an electrocardiogram showed right ventricular hypertrophy. A cardiac catheterization at that time demonstrated an elevated pressure in the main pulmonary artery (50 mm. of mercury) and a gradient of 45 mm. between the main and the left pulmonary arteries, suggesting pulmonary artery stenosis. With a low calcium vitamin D-free diet, her serum calcium level became normal, and when she was re-examined recently, at the age of six years, she was normocalcemic (9.8 mg. per 100 ml.), moderately mentally retarded and very short. She still had an ejection systolic murmur in the second left intercostal space. At cardiac catheterization the systolic pressure in the main pulmonary artery had fallen to near normal (26 mm. of mercury), and there was only a small gradient of 7 mm. into the left pulmonary artery. Angiocardiograms did not show clear-cut stenoses in the pulmonary artery. There was no pressure gradient across the aortic root, but the descending aorta was minimally but definitely narrowed. This case, therefore, appears to demonstrate an improvement in the cardiovascular lesion with time and is consistent with the clinical reports in which murmurs have been noted to diminish or disappear.[48, 50]

CASE 2. D.M. was first seen recently at the age of eight months. Pregnancy and neonatal progress had been normal, but his birth weight at full term was only 2.3 kg. The mother received 1000 I.U. of vitamin D daily during the last half of her pregnancy. He had a sudden onset of weight loss at four months of age and when referred to this hospital was hypercalcemic (serum calcium 15 mg. per 100 ml.), with a fairly clear-cut elfin facies and failure to thrive. He was considered to have the severe form of infantile hypercalcemia. His vitamin D intake had not exceeded 800 I.U. per day. He had a loud ejection systolic murmur in the aortic area and left ventricular hypertrophy on the electrocardiogram. Systolic blood pressure was 110 mm. of mercury. At cardiac catheterization no gradient was detected across the aortic valve region, but on angiocardiography there appeared to be some narrowing of the aorta just above the sinus of Valsalva. The pulmonary artery branches appeared to be normal. This child is now 18 months old and normocalcemic on a low calcium vitamin D-free diet. He is still far below the third percentile for length and weight. He is being followed up closely to determine whether his cardiovascular status gets better or worse.

CASE 3. F.C. was considered at eight months to have the mild form of infantile hypercalcemia (Fig. 71). He had been well and was thriving until a few days before admission. His vitamin D intake had not exceeded 800 I.U. of vitamin D per day. At the time of diagnosis he had a left sternal systolic murmur but this was not thought to be definitely organic. When reviewed recently, at 10 years of age, he had no murmurs and had a normal electrocardiogram and chest radiograph. His height is reasonably good (tenth percentile), and his weight is in the fiftieth percentile. He does not exhibit the typical facies which the other two do.

Whether future evidence will establish that vascular, rather than valvular stenoses (particularly supravalvular aortic stenosis) are invariable features of the severe form of infantile hypercalcemia is not known. At present it can be said that a large proportion of infants with the severe form of infantile hypercalcemia have had systolic murmurs which

could represent aortic or pulmonary stenoses. The present authors are aware of only these five severely affected infants who have had definitive cardiovascular studies. Three patients had demonstrable supravalvular aortic stenosis, one of whom also had stenoses of the peripheral pulmonary arteries. The other two infants had peripheral pulmonary artery stenosis, one with a probable supra-aortic lesion in addition.

The Late Normocalcemic Stage of the Severe Infantile Hypercalcemia Syndrome

The severe form of infantile hypercalcemia presents a characteristic and striking picture to the extent that it had been considered unlikely that patients with this syndrome would escape diagnosis, especially in the larger centers of Europe and the English-speaking world where clinical awareness of the condition was well established.

It is now probable that this assumption was incorrect. Within the last five years papers have appeared from New Zealand,[34] Germany,[4] Belgium[32] and Britain,[6] each describing groups of older children and adolescents who had not been carefully investigated previously, and all of whom presented practically identical features. These children were not hypercalcemic at the time of study, but all the other aspects of the severe form of hypercalcemia were present: supravalvular aortic stenosis (Fig. 74) and/or peripheral pulmonary artery stenoses,[6] mental deficiency and a facies remarkably reminiscent of the elfin facies of the infantile syndrome (Figs. 73, 75). Sometimes there were dental abnormalities; hypoplasia, notching or absence of one or more teeth.[6, 32] Systemic hypertension 6 and increased bone density were occasional additional features. Since it is established that the hypercalcemia of the infantile syndrome disappears spontaneously after some months or years, since the cardiovascular lesions are so similar in both conditions, and since

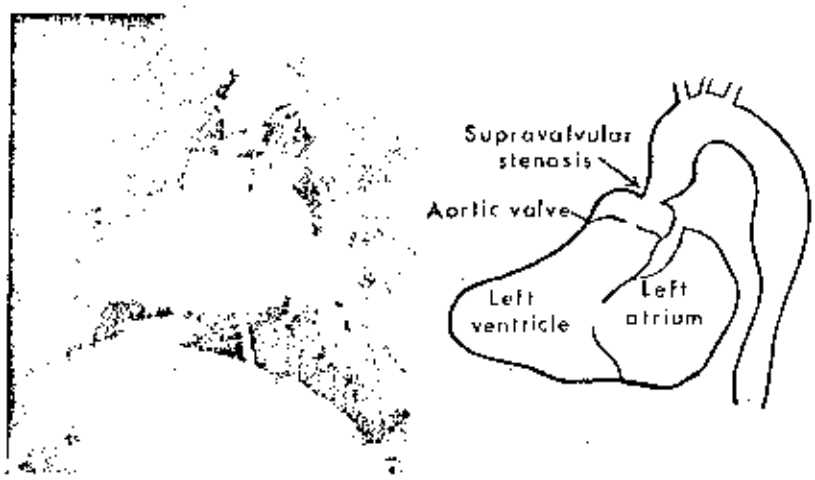

Figure 74. Angiocardiogram in C.C. (Fig. 73) demonstrates supravalvular aortic stenosis.

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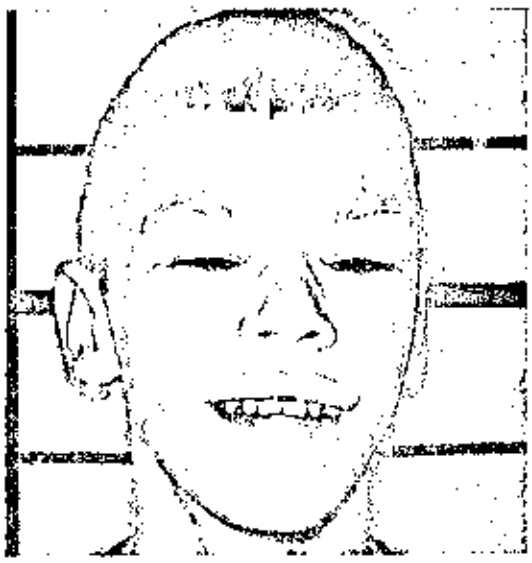

Figure 75. (R.E.). Eleven-year-old boy with presumed late normocalcemic stage of infantile hypercalcemia. He has a supravalvular stenosis of the aorta and mental retardation.

some of the older children are reported to have had low birth weights and to have failed to thrive,[4, 6, 54] there can be no question, as several authors had previously postulated, that some, possibly all, of these older children represent previously unrecognized cases of severe infantile hypercalcemia.

In the light of present evidence it is now reasonable to make the presumptive diagnosis of the late normocalcemic stage of unrecognized severe infantile hypercalcemia in any patient with the complete triad of supravalvular and/or pulmonary artery stenosis, severe mental defect and typical facies. On the other hand, it is becoming evident that other teratogenic factors, in particular the fetal rubella syndrome, may cause identical pulmonary artery stenoses[16] and probably supravalvular aortic stenosis,[13a] sometimes along with mental defect. Hence, to qualify for inclusion in the late stage infantile hypercalcemia group, typical facies and, desirably, other less constant features (low birth weight, small stature, dental abnormalities) are prerequisites, in addition to mental defect and supravalvular stenosis of the aorta or pulmonary arteries.

By reviewing the records of the Toronto Heart Registry and the Cardiological Service of The Hospital for Sick Children during the past 16 years, it has been possible to identify 11 children who had proved severe infantile hypercalcemia or who appear to fit the criteria of the late infantile hypercalcemia syndrome. All the 11 patients had loud systolic ejection murmurs at the sternal border, mental retardation, and all had the typical peculiar facial appearance. Supravalvular aortic stenosis has been established in seven patients by appropriate special tests. In the remaining four, the definitive studies have not yet been carried out. Peripheral pulmonary artery stenoses were demonstrated in five of the confirmed cases.

The birth weights of the group averaged 2.7 kg. (range 2.3 to 3.0 kg.), and at present the heights are at the third percentile or below in two thirds of the patients. The series of 11 patients includes the two established cases of severe infantile hypercalcemia mentioned previously. With the exception of these two infants, there is no clear-cut evidence that the others had symptoms of hypercalcemia in infancy, although there was sometimes a history of ill-defined malaise.

It should be emphasized that the cardiovascular lesion was not stenosis of the aortic valve, but the much less common lesion first described by Denie and Verheugt[15] in 1958 in which the stenosis is in the aorta a few centimeters distal to the aortic valve. It is now known that there are at least two separate conditions with supravalvular aortic stenosis as a feature. The syndrome described above is unrelated to the second condition, in which the stenosis is an isolated, sometimes familial lesion without associated facial signs or mental defect.

Recently Beuren et al.[6] have reported a study of 11 older children with the presumed late stage of severe infantile hypercalcemia. They observed that the stenosis may take three forms: (1) a localized narrowing at the upper margin of the sinus of Valsalva, usually with plication of the inner aortic wall, (2) the aortic valve leaflets fused to the stenotic area, and (3) the ascending aorta narrowed for some distance up into the aortic arch. They also observed peripheral pulmonary artery stenoses, mostly multiple in seven of their 11 cases.

Prognosis

As a general rule, the prognosis of the mild form of infantile hypercalcemia is good. Mitchell's follow-up survey[42] of 13 infants who had had the mild form some three to six years previously found all the children in good health. Weight gain and linear growth were satisfactory, intelligence was normal, and renal function was normal. On the other hand, occasional deaths have been reported.[14, 45] These deaths have occurred during acute hypercalcemia. Presumably, if the patient can be carried through this dangerous period, the outlook is excellent. Our mildly affected case is a boy 10 years of age, whose height is in the tenth percentile and his weight in the fiftieth percentile. He is not mentally defective.

By contrast, the prognosis of patients with the severe form of infantile hypercalcemia is bad. One quarter of the 44 patients reviewed by Hovels and Stephan[33] in 1961 had died between the ages of 11 months and 3½ years, and presumably additional deaths have occurred since the study was terminated. Renal insufficiency is the usual cause of death.

Patients who do not die are left with serious sequelae. The most tragic is severe mental defect. Renal and cardiac sequelae may cause serious complications. The hypercalcemia may persist for as long as four

years.[35] Elevated serum cholesterol levels may persist considerably longer than hypercalcemia.[32, 47] It should be stressed, however, that hypercalcemia always disappears eventually as the patient grows older, even without specific therapy.

Growth of severely affected patients remains retarded after the disappearance of hypercalcemia. In our series only one patient has attained the twenty-fifth percentile for height, and two thirds are at or below the third percentile. Hooft,[32] however, reported that growth in one severe case returned to normal after correction of hypercalcemia.

Treatment

Dietary Management. The most important aspects of the treatment of infantile hypercalcemia are elimination of vitamin D intake and provision of a diet of minimal calcium content. Both measures are necessary. In practice, it has been shown that withholding vitamin D alone, although beneficial, was not sufficient to control the serum calcium. Similarly, we have observed that drastic reduction of dietary calcium without restriction of vitamin D may lower the serum calcium level significantly within a few days, but the improvement has been neither complete nor permanent.

In treating patients with infantile hypercalcemia, it is important to keep the calcium intake below 100 mg. per day, and preferably as low as 25 to 35 mg. It is also necessary virtually to eliminate vitamin D from the diet, and to protect the infant from exposure to direct sunlight.

Since milk and milk products contain high concentrations of calcium, these must be eliminated from the infant's diet. There are three practical ways of obtaining a suitable dietary regimen: (*a*) use of milk from which the calcium has been removed by passage through an ion exchange column,[16] (*b*) use of a proprietary milk substitute (e.g. Locasol* or special soybean protein formulas to which supplemental calcium has not been added), or (*c*) use of meat, instead of milk, to cover protein requirements.

In our experience the meat-base formula has proved to be the most feasible. This diet is usually well accepted, and it contains minimal amounts of calcium and vitamin D. In addition, it can be easily and inexpensively made up in the home. Our formula is as follows:

Strained lamb	121 gm.
Dextro-maltose	44 gm.
Sucrose	6 gm.
Distilled water	622 gm.
	793 gm.

One hundred grams of formula provides 45 calories, 2.0 gm. of protein, 1.3 gm. of fat, 6.2 gm. of carbohydrate and 1.1 mg. of calcium.

This formula is supplemented with fruits (applesauce, peaches,

* Trufood Ltd., London, England.

pears, bananas), vegetables (potatoes, carrots, peas, beets) and low-calcium cereals, without exceeding the daily allowance for calcium. In making up the formula in areas where only hard water is available, it is preferable to use distilled water for dilution of the formula.

There has been little study of how long to continue the special dietary management. Our procedure has been to maintain the low calcium, low vitamin D intake for at least nine months after serum calcium has finally become normal. Thereafter, gradual increases in calcium and exposure to sunlight may be permitted, provided the serum calcium level is carefully monitored. None of our cases have developed rickets, despite prolonged therapy of up to two years' duration.

Cortisone. Used in conjunction with a low calcium, vitamin D-free diet, administration of cortisone (25 to 50 mg. orally) to patients with infantile hypercalcemia may sometimes reduce serum calcium in the matter of a few hours.[42] Thus this agent may occasionally be useful in initiating the therapy of acutely hypercalcemic infants. After a few days, when the diet has produced its full effect, cortisone should be discontinued.

In the few cases which have been refractory to the low calcium, vitamin D-free diet, steroid therapy has proved of considerable advantage. The dangers of long-term cortisone therapy must be borne in mind.

Pathogenesis

Although infantile hypercalcemia has been recognized since 1952, and although the pathogenesis has been investigated from numerous angles, there are still many unsettled aspects. It is not yet certain whether the mild and severe forms of infantile hypercalcemia are different diseases or whether they represent differing manifestations of the same underlying metabolic defect. Vitamin D has been implicated in the pathogenesis of both forms of the disease; although there is evidence in support of this thesis, the present authors believe that data bearing upon this question tend to be inconclusive or sometimes contradictory. Recent clinical observations lend support to the hypothesis that the severe form of infantile hypercalcemia has its inception in utero, but whether this is true in all cases is not yet clear. All these questions have a bearing upon theories of causation and, by the same token, upon suggested means of prevention.

It is not the purpose of this article to review the specific data which bear upon these and other questions in detail, but rather to summarize the present state of knowledge and to present our own views of the situation.

Of the several hypotheses advanced to explain the pathogenesis of infantile hypercalcemia, the one most seriously considered involves vitamin D. Evidence concerning the possible role of this factor derives

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from two kinds of study: epidemiologic data and the investigation of vitamin D concentrations and metabolism in individual patients with the disease. On the basis of the evidence there has been, on the whole, a strong tendency to accept the hypothesis that vitamin D is a primary factor in the causation of infantile hypercalcemia. In the opinion of the present authors, this important point has not yet been rigorously proved.

There are certain features about the toxic action of vitamin D which make it logical to explore the relation of this substance to infantile hypercalcemia. Excessive intakes of vitamin D are well known to produce hypercalcemia in animals and human beings.[30] In rabbits[11, 29] and immature rats[28] large doses of vitamin D (25,000 to 125,000 I.U. per day for periods up to three weeks) produce various vascular lesions, which are similar in certain respects to those observed in human patients. Of great interest is the finding that the aorta (in particular the proximal segment of the aorta) is especially susceptible to damage, and an analogy has been drawn to the supravalvular lesions in the aortas of patients with severe infantile hypercalcemia.[27] In connection with these interesting observations, it should be noted that the experimental animals on such high doses of vitamin D would undoubtedly have been hypercalcemic. The question is therefore not settled whether the vascular lesions in the animals were produced by vitamin D directly or, as seems more likely, by the ensuing hypercalcemia.

There is no question that vitamin D can produce hypercalcemia, and sometimes renal and vascular lesions in human beings, but hypercalcemia does not ordinarily ensue unless the vitamin D intake is of the order of 10,000 to 20,000 I.U. per day,*[3, 30] an amount approximately 25 times the recommended prophylactic dose of 400 I.U. per day and far in excess of intakes known to have been ingested by infants with the hypercalcemic syndrome. A considerable number of patients with infantile hypercalcemia, including our three patients, are known to have ingested less than 1000 I.U. of vitamin D per day. Accordingly, in proposing vitamin D as an etiologic factor,[31] hypersensitivity to vitamin D,[41] defective cholesterol metabolism,[32] and defective removal of vitamin D[15, 42] have been invoked as additional contributory factors.

The epidemiologic studies so widely quoted in connection with the interrelation of vitamin D and infantile hypercalcemia were commenced by the British Paediatric Association Committee on Hypercalcaemia in the early 1950's, when it was apparent that infantile hypercalcemia had become a problem of national concern in Great Britain. Nutrition surveys conducted at this time indicated that the normal British infant, consuming fortified powdered milk, infant cereals and specific

* In the opinion of the present authors the often-cited study of Jeans and Stearns[16] purporting to show that the growth of normal infants was significantly reduced by vitamin D intakes of 1800 I.U. per day or greater does not stand up to modern tests of significance. Other investigators have failed to confirm the conclusions.[21]

vitamin D supplements, might readily ingest as much as 4000 I.U. of vitamin D per day. Accordingly, when it was observed that more than 200 cases, mainly of the mild form, had been diagnosed between 1953 and 1955, measures were taken to reduce the vitamin D intake of British infants. By 1957 to 1958, it is estimated that the over-all intake of infants under one year had been approximately halved.

The British Paediatric Association Committee[9] recently reported three nation-wide surveys of the incidence of infantile hypercalcemia (all forms) in Great Britain. The first, conducted in 1953–55, showed an incidence of 7.2 cases per month; the second, carried out in 1959, 1½ to two years after changing the food enrichment policies, still showed an incidence of 6.8 cases per month. The third survey, conducted in 1960–61, recorded an incidence of only 3.0 cases per month.

These data have been accepted by many as evidence that vitamin D is a prime factor in the causation of infantile hypercalcemia. Nevertheless the British Paediatric Association Committee's interpretation of the results was guarded. To quote the Committee's conclusions:[9] "The marked decline in hypercalcaemia (1960–61) does not correspond in time with the major reduction in vitamin D allowances which took place in 1957–58, but followed it after an interval of two to three years. . . . Unfortunately, the results of these surveys do not provide a positive answer to the question of the part played by excessive intake of vitamin D in the pathogenesis of infantile hypercalcaemia. . . . It remains speculative whether the decrease in hypercalcaemia noted from 1960 is a consequence of reduced vitamin D intake."

Two additional aspects should be borne in mind in interpreting the results of the surveys. Firstly, the studies were conducted before practitioners were aware of the late manifestations of the severe form of infantile hypercalcemia; hence this group is not included. Secondly, the British series was comprised mainly of the mild form of infantile hypercalcemia. The majority of cases diagnosed in North America appear from the literature to have been of the severe form, and it is not yet proved that the two forms have the same cause.

Evidence obtained by biochemical study of the vitamin D concentrations and metabolism in individual cases tends to be contradictory or inconclusive.

The serum vitamin D concentrations of eight mildly affected infants were found in each case to be normal.[14, 53] When mildly affected infants were challenged with vitamin D, four appeared to have an exacerbation of the hypercalcemia,[8, 22, 39] and four had no rise in serum calcium after large doses of vitamin D for suitable periods of time.[13, 22, 34]

In the severe form of the disease, vitamin D levels were elevated five to 10 times above normal in five infants,[13, 27, 49] a finding which points to a defect in vitamin D metabolism. Yet in four other infants the level was normal.[14] Apparently, patients with the severe form of

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infantile hypercalcemia have not been studied after a vitamin D challenge. Vitamin D in excess has been shown to produce hypercalcemia, and in animals it produces vascular lesions which are to some extent comparable to those observed in infants. Whether vitamin D (or hypercalcemia) can account for the other features of the severe form of infantile hypercalcemia—in particular whether it can cause mental defect and the characteristic facies—has not yet been established. Infants born to mothers with hyperparathyroidism are known not to have any features similar to those seen in infantile hypercalcemia.[53]

In surveying these data there is no question that a certain amount of evidence has been adduced which implicates vitamin D in the causation of infantile hypercalcemia. On the other hand, it is the opinion of the present authors that a direct causal relation between vitamin D and infantile hypercalcemia has not yet been proved.

In an earlier section we outlined the evidence which suggests that the mild form of infantile hypercalcemia does not develop until after birth. On the other hand, we consider that there is good support for the hypothesis that the severe form of the disease frequently, perhaps always, has its inception in utero. Evidence for this statement comes from several directions. A number of patients,[27, 37] including one from this hospital (case 1), have had clearly audible parasternal systolic murmurs in the neonatal period. The demonstration of definite anatomic lesions of the cardiovascular system by Garcia et al.[27] in an infant of nine months supports the contention of these authors that lesions of such a nature and extent may have been present at the time of birth. The severity of the mental defect, the extent of the facial abnormalities, the occasional recorded observation of a peculiar facial appearance at birth and the tendency to low birth weight support this thesis.

We are still unable to decide whether the mild and severe forms of infantile hypercalcemia should be considered different entities with different causes, or whether they are widely differing manifestations of the same pathologic process. It is clear that any theory about pathogenesis of the severe form must allow for the development of lesions in utero, whether that theory happens to implicate vitamin D, some as yet unknown teratogenic factor or a genetic mechanism.

There is no question that the vitamin D intake of pregnant North American women varies widely. Undoubtedly, many women ingest considerably more than the recommended 400 I.U. daily allowance which is considered by United States, Canadian and British advisory committees to be sufficient to meet the vitamin D requirements of the pregnant woman and her fetus during the second half of pregnancy. On the other hand, the estimated intakes of several mothers who have borne severely affected infants, including two carefully evaluated patients of this hospi-

tal, have not exceeded 1000 I.U. of vitamin D per day,[6, 27, 30] dosages which must be considered within the normally safe range.

Incidence of Infantile Hypercalcemia

Infantile hypercalcemia has been observed in various parts of the northern and southern hemispheres and in persons of many racial extractions. On the other hand, during the 14 years since the condition was first delineated, it has become apparent that infantile hypercalcemia is very rare, even when one considers the relative prominence given to the condition by the various British surveys, and even when one realizes that the late normocalcemic stage of the disease has gone unrecognized until recently.

By using figures obtained from the most recent survey of the British Paediatric Association Committee on Hypercalcaemia,[9] a case rate of 35 patients per year was recorded for all forms of infantile hypercalcemia in 1960–61. Since in 1960 there were 785,000 births in Great Britain, the incidence of all forms of infantile hypercalcemia becomes approximately 0.05 case per 1000 births, or, roughly, one case for every 20,000 births.

Approximately 10 per cent of the cases reported to the British Paediatric Association Committee were considered to be severely affected. It can therefore be estimated that the incidence of this prognostically tragic form of the disease was probably not more than one case for every 100,000 to 200,000 births.

It is unlikely that one can transpose data on the incidence of this disease in Great Britain to the North American situation. There is no evidence that the mild form of infantile hypercalcemia has occurred on this continent in the epidemic manner experienced in the British Isles. Indeed, of the approximately 50 cases of infantile hypercalcemia reported from the United States, only a few have been classified as mildly affected.

No attempt has been made in North America to estimate the incidence of infantile hypercalcemia within a population group of known size. Yet it occurred to us that a rough estimate of the prevalence of the severe form of infantile hypercalcemia might be obtained by starting from a study of the incidence of supravalvular aortic stenosis. From a survey of the cases of supravalvular aortic stenosis and of confirmed infantile hypercalcemia included in the records of the Toronto Heart Registry and the Cardiological Service of The Hospital for Sick Children, a total of 11 presumptive cases of severe infantile hypercalcemia have been diagnosed in the course of studying 8000 consecutive cases of congenital heart disease over a period of 16 years. Taking six cases per 1000 births as an estimate of the incidence of congenital heart disease in the pediatric age group,[38] one derives an estimate of 0.008

case of severe infantile hypercalcemia per 1000 births or, roughly, one case per 150,000 births.

There are obvious criticisms of any estimate of incidence based on such assumptions as we have made. Nevertheless the two remarkably similar estimates confirm the general impression that infantile hypercalcemia, especially in its more serious form, is an extremely rare disease.

Public Health Considerations

Of the various etiologic factors that have been implicated in the causation of infantile hypercalcemia to date, only vitamin D has any public health connotations.

It has been known for over 40 years that vitamin D is essential for maintenance of normal calcium and phosphorus metabolism in human beings, and over the years efforts have been directed toward establishing an adequate intake of vitamin D throughout the population. National advisory committees have arrived at recommended daily allowances of vitamin D for the various age groups, education programs have stressed the need for the vitamin, and vitamin D has been made available to the population at large through provision of vitamin supplements and enrichment of commonly used commercial foodstuffs. As a result, the incidence of rickets has been dramatically reduced.

It had long been recognized that vitamin D was potentially dangerous in excessive dosage, and this knowledge was taken into consideration by the United States Food and Nutrition Board and the Canadian Council on Nutrition in arriving at the present recommended dietary allowances (a total intake of 400 I.U. of vitamin D per day for prematures, infants, children, adolescents, during the last half of pregnancy and during lactation; no specific supplementation required by normal adults). But only since the discovery and investigation of infantile hypercalcemia has there been any serious question that intakes slightly greater than the presently recommended dietary allowances might have deleterious effects.

For some years there has been concern in both the United States and Canada over the widespread and rather indiscriminate enrichment of foods with vitamin D, since it is clear that many persons now consume considerably more than the recommended dietary allowance. Certain specific measures, such as adjustment of proprietary vitamin D products to contain 400 I.U. of vitamin D per dose, and discontinuance of vitamin D supplementation of all foodstuffs except milk, have been urged by responsible organizations[1] with a view to promoting an intake of vitamin D which more closely approximates the recommended dietary allowance and minimizes the likelihood of excessive intakes by the population at large. Regulatory action has been taken or is being contemplated with respect to some of these recommendations.

The principle of sensible moderation in vitamin D consumption is supported by the still equivocal evidence implicating vitamin D in the

pathogenesis of infantile hypercalcemia. The same general principle holds, on theoretical grounds, for all age groups. By this token, the total vitamin D intake of the pregnant woman should be limited to the recommended allowance of 400 I.U. per day, a measure which is in accord with the observation that the severe form of infantile hypercalcemia may have its inception during fetal development.

The question has been raised whether recent findings concerning infantile hypercalcemia consitute a reason to revise further the national policies concerning vitamin D in the United States and Canada. It is our opinion that the recommendations cited above embody the principles of good nutrition and offer suitable public health measures for protection of the population at large, at the same time minimizing the risk of undue vitamin D excess. We believe that the incidence of infantile hypercalcemia is too low and the relation to vitamin D intake too tenuous to justify consideration of infantile hypercalcemia as an important reason for modifying current American public health policies involving vitamin D.

ADDENDUM

Since completion of this manuscript, a 12-year-old boy has been reported[55] who fits the description of the late stage of the severe infantile hypercalcemia syndrome. It is of interest that this patient had excessive tortuosity of the retinal and cerebral arteries, as well a supravalvular aortic stenosis and peripheral pulmonary artery stenoses.

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THE INFLUENCE OF DISODIUM ETHANE-1-HYDROXY-1,1-DIPHOSPHONATE (EHDP) ON THE DEVELOPMENT OF EXPERIMENTALLY INDUCED URINARY STONES IN RATS

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SUMMARY

1. Bladder stones composed of calcium hydrogen phosphate dihydrate, calcium oxalate mono- and di-hydrate and magnesium ammonium phosphate hexahydrate (struvite) were successfully induced in rats by various dietary manipulations and by implanting zinc pellets in the bladder.

2. The effect of a diphosphonate, disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP), given in the drinking water at concentrations of 0·0025, 0·05 and 0·5% (w/v), on the size and composition of these stones was examined.

3. All the concentrations of EHDP decreased the weight of the calcium oxalate calculi. In contrast, only the highest concentration of EHDP inhibited calcium hydrogen phosphate stone formation and the magnesium ammonium phosphate stones were unaffected.

4. The difference between the effects on calcium oxalate and magnesium ammonium phosphate stones is consistent with the finding that EHDP inhibited the precipitation of calcium oxalate from solution *in vitro* but had only a slight effect on magnesium ammonium phosphate precipitation.

5. It is suggested that EHDP might be of use in the prevention of some types of urinary stones in man.

Key words: bladder stones, disodium ethane-1-hydroxy-1,1-diphosphonate, urinary stones, calcium oxalate.

Previous experiments have shown that diphosphonates inhibit the crystallization of hydroxyapatite *in vitro* (Fleisch, Russell, Bisaz, Casey & Mühlbauer, 1968; Fleisch, Russell, Bisaz, Mühlbauer & Williams, 1970; Francis, 1969; Francis, Russell & Fleisch, 1969) and prevent D-induced calcification of aorta and kidney *in vivo* (Fleisch *et al.*, 1968, 1970; Francis *et al.*, 1969). Most studies have been done with the diphosphonate, disodium ethane-1-hydroxy-1,1-

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diphosphonate (EHDP, disodium etidronate). In the rat, only about 3% of this compound is absorbed from the intestine after oral administration and about half is then excreted in the urine (W. R. Michael, W. R. King & J. M. Wakim, unpublished work).

These observations suggest that EHDP given by mouth might inhibit the formation of urinary stones. In this study, we have shown that EHDP inhibits the precipitation of calcium oxalate but not of magnesium ammonium phosphate *in vitro*. We have also shown that EHDP fed to rats decreases the growth of urinary stones composed of calcium hydrogen phosphate dihydrate and calcium oxalate but not those composed of magnesium ammonium phosphate.

METHODS

Effect of EHDP on the crystallization of calcium oxalate in vitro

The crystallization of calcium oxalate was studied *in vitro* by the technique of Fleisch & Bisaz (1964). Solutions of constant ionic strength (0·3) buffered at pH 6·1, and with a calcium concentration of 1·7 mM and oxalate concentrations varying between 0·1 and 0·6 mM, were incubated for 20 h at 37°. After incubation they were filtered through Millipore filters (HA, pore size 0·45 μm) to remove any crystals formed. Measurement of calcium and total oxalate in the filtrate allowed the determination of the minimum ion product ([Ca] × [total oxalate]) necessary to induce crystal formation. The ability of EHDP, at 0·1 mM, 10 μM and 1 μM concentrations, to increase this minimum ion product was tested.

Effect of EHDP on the crystallization of magnesium ammonium phosphate hexahydrate

This was also investigated by a technique described previously (Fleisch, Bisaz & Russell, 1967). In brief, increasing equimolar concentrations (between 8·3 and 25 mM) of phosphate and ammonium ions were incubated at constant ionic strength (0·3), pH 7·5, and constant magnesium concentration (4·14 mM) for 3 days at 37°. After incubation any crystals formed were removed by filtration through Millipore filters. Measurement of magnesium and phosphate in the filtrate allowed the determination of the minimum product ([Mg] × [P]) necessary to induce crystal formation. The effect of addition of EHDP at 0·1 M and 10 μM on this minimum ion product was tested.

Effect of EHDP on formation of bladder stones in rats

Animals. Female Wistar rats weighing between 140 and 190 g and bred in this laboratory were allocated at random to cages, initially seven animals per cage. All animals in a single cage received one of five stone-forming regimens, and one of four treatments, according to a block design as shown in Table 1.

Stone-forming regimens. The following stone-forming regimens were chosen in an attempt to produce stones of various composition.

Regimen A. Normal rat chow (Diet 194, manufactured by Nafag AG, Gossau, Switzerland) which contained 1·0% (w/dry wt) of calcium and 0·85% (w/dry wt) of phosphorus.

Regimen B. As A, plus 10000 i.u. (0·25 mg) of vitamin D_3 (from Wander AG, Bern, Switzerland) once a week by stomach tube.

Regimen C. As A, plus 1% (v/v) ethylene glycol in the drinking water, to produce stones of calcium oxalate (Vaille, Debray, Martin, Souchard & Roze, 1964; Lyon, Borden & Vermeulen, 1966).

Regimen D. As A, plus 0·25% (v/v) ethylene glycol in the drinking water.
Regimen E. As A, plus 0·8% (w/v) $NaHCO_3$ in the drinking water.

Treatments. The four treatments were as follows:

1. Control, no treatment (water).
2. 0·0025% (w/v) EHDP (as the disodium salt, mol. wt. 250) in the drinking water.
3. 0·05% (w/v) EHDP in the drinking water.
4. 0·5% (w/v) EHDP in the drinking water.

These doses correspond approximately to 0·8, 16 and 160 mg of EHDP as P day^{-1} kg^{-1}, respectively, by using as an estimation that rats of average weight 200 g consume an average of 25 ml of water per day.

TABLE 1. Block design of experiments

Treatment	No. of cages				
	A*	B	C	D	E
1. Control	2	2	2	2	2
2. 0·0025% EHDP	1	1	1	1	1
3. 0·05% EHDP	1	1	1	1	1
4. 0·5% EHDP	1	1	1	1	1

* Stone-forming regimen.

All treatments were given for 45 days. Animals received food and distilled water *ad libitum*. In addition to the substances listed above, the drinking water contained nitrofurantoin (60 mg/l) [*N*-(5-nitro-2-furfurylidene)-1-aminohydantoin from Boehringer, supplied by Laevosan AG, Zurich, Switzerland] to decrease urinary infection rate (Vermeulen & Goetz, 1954).

Operation, handling of animals and killing. Stones were produced in the rats by implantation of zinc pellets into the bladder, as described by Vermeulen, Grove, Goetz, Ragins & Correll (1950). On the third day after starting the stone-forming regimen and the treatment, each animal was weighed and anaesthetized with trichloroethylene. The bladder was opened by a short transverse incision using aseptic techniques. A sterilized 4 mm diameter zinc disc weighing approx. 23 mg was inserted and the bladder incision closed by a single 5·0 polyethylene suture.

Rats from each cage were weighed weekly as a group. On day 45, 6 weeks after insertion of the zinc disc, each rat was weighed individually. Urine, obtained by aspiration into a sterile syringe, was cultured on nutrient agar and the zinc disc, on which the stone was deposited, was removed. Plasma was obtained from control animals and those receiving the 0·5% EHDP treatment for determination of P_i by the method of Fiske & Subbarow (1925) and of calcium by atomic-absorption spectrophotometry (Perkin-Elmer, model 290 B).

Analysis of the stones

The stones were dried for 24 h at room temperature. These mild conditions were used to avoid transformation of any amorphous calcium phosphate into crystalline hydroxyapatite. The total weight of the stone together with the underlying zinc disc was then recorded. Since the zinc pellets can dissolve to some extent during the experimental period, the true stone

weight could only be determined after taking into account the zinc remaining in the pellet. Therefore, as much of the deposit as possible was scraped off the zinc disc, taking care not to remove any of the metal. The powder was analysed by X-ray diffraction in a Debye–Scherrer camera. The remaining zinc, with adherent traces of the stone, was dissolved in 10 ml of 0·1 M-HCl and the Zn determined quantitatively by atomic-absorption spectrophotometry (Perkin Elmer, model 290 B). The weight of the mineral deposit was then calculated as the difference between the residual zinc and the total weight of the stone. In certain cases where the X-ray diffraction did not allow an exact identification, as in mixed stones or when the material was in an amorphous form, an i.r. analysis was performed in KBr pellets (i.r. spectrograph Perkin Elmer 457) (Sucker, 1963; Tsay, 1961).

Analysis of results

Although the experiment employed a block design and each cage initially contained seven animals, the final numbers in each cage varied because some of the operated animals died. The entire block was repeated three times in the order A, B, C, D, E, A, B, . . . , etc. Because of the considerable mortality it was not possible to employ a block design to analyse the results statistically. Therefore the results from animals receiving a particular regimen and treatment were pooled and compared with control animals by Student's *t* tests.

RESULTS

Experiments in vitro

The effect of EHDP on the precipitation of calcium oxalate is shown in Fig. 1. EHDP at concentrations between 5 and 100 μM increased the minimum product ([calcium]×[total oxalate]) necessary to induce crystal formation. In contrast, EHDP at 10 μM did not inhibit significantly the precipitation of magnesium ammonium phosphate and 100 μM only slightly (Table 2).

Experiments in vivo

The survival rates of rats receiving the various treatments and the various stone-forming regimens are summarized in Table 3. The survival rates of all successfully operated rats under the various stone-inducing regimens, but receiving 0·5% or 0·05% (w/v) EHDP were somewhat lower than the rates of animals receiving no EHDP or 0·0025% (w/v). Similarly, rats that received ethylene glycol at 1% or 0·25% (v/v) had somewhat lower survival rates than animals that received the other stone-forming regimens. However, the body weight gains over the 6 weeks of the experiment were unaffected by the EHDP. Thus the mean±SE final body weights were 225±3·0 g for all rats receiving 0·5% EHDP compared with 230±1·2 g for all rats receiving distilled water only.

Bacteriological culture of the urine at the time of death was positive in approx. 10% of the rats. The presence of bacteriuria had no discernible effect on the size or composition of the renal calculi.

Table 4 shows the numbers and types of stones produced with each of the stone-forming regimens. The type of each stone was determined from the X-ray-diffraction pattern. All rats surviving to the end of the experiment produced a stone and the study therefore involved a total of 438 stones of various types. In animals receiving rat chow alone (regimen A), the calculi

were principally magnesium ammonium phosphate hexahydrate (struvite, $MgNH_4PO_4,6H_2O$). Addition of vitamin D_3 (regimen B) promoted formation of calcium hydrogen phosphate dihydrate ($CaHPO_4,2H_2O$) calculi. Addition of 1% (v/v) ethylene glycol to the drinking water

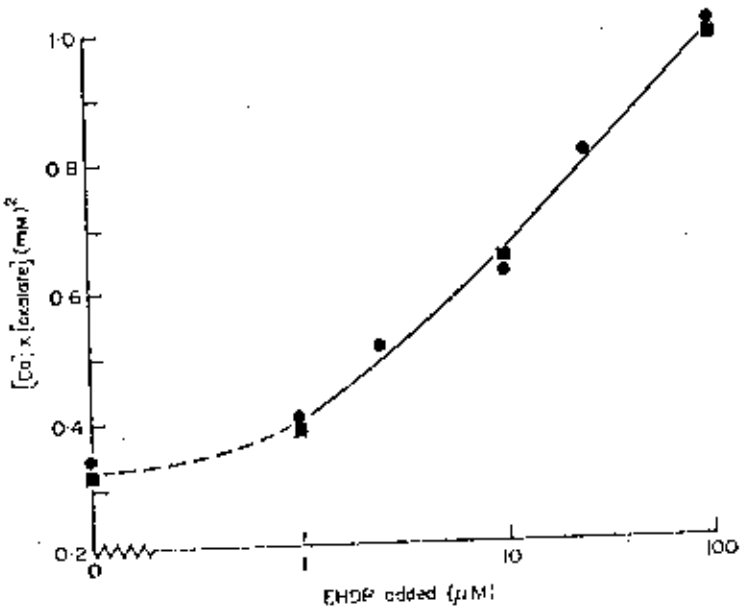

FIG. 1. Effect of EHDP on the minimum product ([Ca] × [total oxalate]) required to induce precipitation of calcium oxalate from solution. The experimental conditions are described in the text and each point represents one determination of the minimum product.

TABLE 2. Influence of EHDP on the formation of magnesium ammonium phosphate hexahydrate crystals *in vitro*. Minimum products ([Mg] × [total phosphate]) required to induce crystal formation are shown as mean ± SE of the number of experiments given in parentheses.

Addition	Minimum product at which crystals form ([Mg] × [total phosphate]) (mM)^2
None	67·2 ± 3·7 (6)
EHDP 10 μM	69·5 ± 1·0 (5)
EHDP 100 μM	78·4 ± 0·5 (5)

Differences between EHDP and control significant at 5% level.

(regimen C) favoured deposition of calcium oxalate stones. Of the seventy-eight stones, sixty-one were composed of calcium oxalate dihydrate, eight of calcium oxalate monohydrate and nine of a mixture of the two types. With addition of 0·25% (v/v) ethylene glycol to the

drinking water (regimen D) the predominant stone reverted to magnesium ammonium phosphate and very few calcium-oxalate calculi were produced. Magnesium ammonium phosphate stones were also the predominant type in animals receiving bicarbonate in the drinking water (regimen E). This regimen produced a moderately alkaline urine. None of the stone-forming regimens caused deposition of calcium hydroxyapatite calculi. The composition of a number of the stones could not be identified. When the deposit was very small in amount, as occurred in the case of several of the zinc discs from animals receiving regimens A, D and E, it was also

TABLE 3. Survival rates of rats

(a) Operative survival rate

No. submitted to operation	No. surviving operation	Survival rate (%)
508	481	95

(b) Effect of various concentrations of EHDP on survival of successfully operated animals (all stone-forming regimens, i.e. A, B, C, D, E combined)

Treatment	Concn. of EHDP in water (%, w/v)	No. successfully operated	No. surviving until killed	Survival rate (%)
1	0	181	170	94
2	0·0025	101	95	94
3	0·05	99	85	86
4	0·5	100	88	88
Overall		481	438	91

(c) Effect of various stone-forming regimens on survival of successfully operated animals (all concentrations of EHDP combined)

Stone-forming regimen	No. successfully operated	No. surviving until killed	Survival rate (%)
A No addition	90	85	95
B Vitamin D_3 10000 i.u./week	96	91	95
C 1% (v/v) Ethylene glycol	98	86	88
D 0·25% (v/v) Ethylene glycol	93	81	87
E 0·8% (w/v) $NaHCO_3$	104	95	91
Overall	481	438	91

not possible to carry out an X-ray diffraction examination (see column designated 'too small to examine').

Table 5 shows the influence of various concentrations of EHDP on the mean weights for the predominant stone-type produced by each stone-forming regimen. The table records the values for stones whose composition has been established by X-ray diffraction, and also the values when the mean weights of the stones too small to be examined by X-ray diffraction were included in the analysis. It is assumed that these small stones were of the same composition as the

TABLE 4. Number of stones of particular types produced by the various diets. Information pooled from control and treated (EHDP) groups

Regimen	$MgNH_4PO_4$, $6H_2O$	$CaHPO_4$, $2H_2O$	Calcium oxalate, $2H_2O$ and $1H_2O$	Calcium hydroxy-apatite	Amorphous calcium phosphate	$MgNH_4PO_4$ + calcium hydroxy-apatite, $6H_2O$	$CaHPO_4$, $2H_2O$ + calcium oxalate	X-ray pattern not identifiable	Too small to examine	Total number of stones (all types)
A. No addition	56	3	0	0	1	2	1	1	21	85
B. + vitamin D_3 10 000 i.u./week	4	81	1	0	1	0	0	0	4	91
C. + 1% Ethylene glycol	6	0	78	0	0	0	0	1	1	86
D. + 0·25% Ethylene glycol	40	0	7	0	0	1	11	7	15	81
E. + 0·8% $NaHCO_3$	68	0	0	0	6	0	0	9	12	95
Total number of stones of each type	174	84	86	0	8	3	12	18	53	438

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predominant stone-type. For the calcium oxalate stones, the results on all three types, dihydrate, monohydrate and mixture of the two, were pooled since they were not significantly different.

EHDP did not affect the formation of magnesium ammonium phosphate stones produced by regimens A, D and E. In contrast, growth of calcium hydrogen phosphate calculi, the predominant stone type with regimen B, was markedly inhibited when the drinking water contained the highest concentration of EHDP (0·5%). The formation of calcium oxalate, the predominant stone type produced with regimen C, was inhibited by all concentrations of EHDP.

TABLE 5. Influence of the dose of EHDP on the weight of the predominant stone type for each stone-forming regimen. Values are given as means ± SEM of the numbers of stones given in parentheses.

Regimen	Predominant stone type	Dose of EHDP (% of the drinking water)	Weight of stones of proven composition (mg)	Weight of stones proven to be of predominant type plus stones too small to identify by X-ray diffraction (mg)
A	$MgNH_4PO_4,6H_2O$	0	19·0 ± 4·0 (25)	15·6 ± 3·5 (31)
		0·0025	23·4 ± 3·5 (11)	18·0 ± 3·5 (15)
		0·05	33·1 ± 9·2 (12)	25·5 ± 7·6 (16)
		0·5	20·4 ± 4·6 (8)	13·5 ± 3·7 (13)
B Vitamin D_3 10000 I.u./week	$CaHPO_4,2H_2O$	0	72·8 ± 9·0 (33)	70·9 ± 8·9 (34)
		0·0025	70·1 ± 8·8 (18)	70·1 ± 8·8 (18)
		0·05	63·0 ± 8·7 (19)	63·0 ± 8·7 (19)
		0·5	22·1 ± 5·3 (11)**	17·9 ± 4·7 (14)**
C 1% Ethylene glycol	Calcium oxalate, H_2O and $2H_2O$	0	24·8 ± 1·8 (32)	24·8 ± 1·8 (32)
		0·0025	19·1 ± 1·5 (17)*	18·5 ± 1·5 (18)*
		0·05	18·2 ± 1·7 (15)*	18·2 ± 1·7 (15)*
		0·5	15·6 ± 1·3 (14)**	15·6 ± 1·3 (14)**
D 0·25% Ethylene glycol	$MgNH_4PO_4,6H_2O$	0	29·0 ± 6·4 (15)	20·8 ± 4·8 (23)
		0·0025	27·8 ± 4·8 (8)	17·0 ± 4·4 (14)
		0·05	28·6 ± 3·4 (11)	26·6 ± 3·7 (12)
		0·5	16·8 ± 2·5 (8)	16·8 ± 2·5 (8)
E 0·8% Na HCO_3	$MgNH_4PO_4,6H_2O$	0	22·8 ± 2·8 (27)	21·5 ± 2·7 (29)
		0·0025	22·7 ± 3·9 (20)	20·9 ± 3·8 (22)
		0·05	31·7 ± 9·6 (10)	24·3 ± 7·5 (14)*
		0·5	16·8 ± 2·3 (11)	13·1 ± 2·3 (15)

* and ** denote difference from control (no EHDP) significant at 5% and 1% level, respectively.

The interpretation of all the findings was uninfluenced by inclusion of the small unexamined calculi in the analysis.

The mean plasma calcium and phosphate concentrations at the time of death are shown in Table 6 for rats receiving control treatment and 0·5% EHDP treatment for each of the five stone-forming regimens. The only significant effect on plasma calcium was the increase over control observed in rats receiving EHDP with regimens A (no addition) and B (vitamin D_3). This effect was not observed in animals receiving EHDP with the other regimens. Plasma phosphate concentrations were lower in rats on regimen D (0·25% ethylene glycol) than in

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those receiving regimens A (no addition) and B (vitamin D). For regimens B (vitamin D) and C (1% ethylene glycol), plasma phosphate concentrations of animals receiving 0·5% EHDP were higher than those of the corresponding control animals.

DISCUSSION

These results show that EHDP inhibited the growth of calcium oxalate stones when given at concentrations of 0·5%, 0·05% and 0·0025% (w/v) in the drinking water. It also inhibited formation of calcium hydrogen phosphate stones when given at a concentration of 0·5% (w/v). In contrast, EHDP did not inhibit the formation of magnesium ammonium phosphate stones. This is consistent with the observation that EHDP has little or no effect on growth of magnesium ammonium phosphate crystals *in vitro*. Unfortunately, no calcium hydroxyapatite stones were produced by any of the dietary manipulations used in this experiment.

TABLE 6. Plasma calcium and phosphate concentrations for control and 0·5% (w/v) EHDP treatment groups under each stone-forming regimen

Regimen	Treatment	Plasma Ca (mg/100 ml)	Plasma P (mg/100 ml)
A	Control	9·95 ±0·10 (24)	8·10 ±0·19 (24)
	0·5% EHDP	10·33* ±0·16 (17)	8·48 ±0·33 (16)
B	Control	10·72 ±0·19 (29)	7·91 ±0·21 (29)
	0·5% EHDP	11·61** ±0·26 (18)	8·92** ±0·32 (18)
C	Control	10·53 ±0·10 (25)	7·09 ±0·21 (25)
	0·5% EHDP	10·73 ±0·19 (16)	8·16** ±0·36 (16)
D	Control	10·33 ±0·14 (17)	6·56 ±0·50 (17)
	0·5% EHDP	10·53 ±0·30 (16)	6·89 ±0·27 (16)
E	Control	10·52 ±0·12 (18)	7·79 ±0·43 (18)
	0·5% EHDP	9·82 ±0·51 (21)	6·73 ±0·34 (21)

* and ** denote results of 0·5% (w/v) EHDP treatment significantly different from control at 5% and 1% level, respectively. Values are given as means ±SEM of the number of animals given in parentheses.

In rats a small but significant fraction (1–5%) of ingested EHDP is absorbed from the intestine and about half of this is excreted in the urine (W. R. Michael, W. R. King & J. M. Wakim, unpublished work). With the simplified assumption of a mean absorption of 3% and a daily water intake and urinary excretion of 25 ml by rats, the approximate concentrations of EHDP in the urine of these rats were calculated to be approx. 1·5 μM, 30 μM and 300 μM at doses of EHDP of 0·0025%, 0·05%, 0·5% (w/v) respectively. Unfortunately, these calculated concentrations could not be checked by direct analysis because no specific method was available at this time for measurement of EHDP. Nevertheless, the ability of the lowest dose of EHDP to inhibit calcium oxalate stones correlates well with the ability of 4 μM-EHDP to inhibit calcium oxalate precipitation *in vitro*. The most attractive explanation for the ability of low concentrations of EHDP to inhibit calcium oxalate urolithiasis is therefore that the EHDP in urine directly inhibits precipitation of the salt from solution. Some of the observed

effect, particularly with the higher doses of EHDP, may be due to other factors, e.g. binding of calcium by EHDP in the urine. Since no calcium hydroxyapatite stones could be induced in this study, the effect of EHDP on this stone type could not be tested. However, it is likely that the diphosphonate would inhibit its formation, since EHDP is a very efficient inhibitor of hydroxyapatite crystal formation (Fleisch *et al.*, 1968, 1970; Francis, 1969; Francis *et al.*, 1969).

In North America at least, calcium oxalate lithiasis is the most common form of urinary calculus in the human (Prien & Frondel, 1947; Prien, 1963). There is some evidence that precipitation of calcium oxalate is the first step in formation of most renal stones (Robertson, Peacock & Nordin, 1969). EHDP may therefore prove useful in preventing the formation of stones in man. Our results suggest that diphosphonates deserve a clinical trial, especially since they have been shown to inhibit ectopic calcification in two other human conditions, myositis ossificans progressiva (Bassett, Donath, Macagno, Preisig, Fleisch & Francis, 1969; Weiss, Fisher & Phang, 1971) and dental calculus disease (Mühlemann, Bowles, Schait & Bernimoulin, 1970; McCune & Tucker, 1970; W. W. Briner & M. D. Francis, unpublished work; M. D. Francis & W. W. Briner, unpublished work).

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PATHOGENESIS OF HEREDITARY VITAMIN-D-DEPENDENT RICKETS

An Inborn Error of Vitamin D Metabolism Involving Defective Conversion of 25-Hydroxyvitamin D to 1α,25-Dihydroxyvitamin D

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Abstract Requirements of vitamin D_2, vitamin D_3, 25-hydroxyvitamin D and 1α,25-dihydroxyvitamin D, were studied in five patients with vitamin-D-dependent rickets, a recessively inherited form of vitamin-D-refractory rickets. Massive doses of vitamin D_2 (1.25 to 2.50 mg per day), vitamin D_3 (1.25 mg per day) and 25-hydroxyvitamin D (0.4 to 0.9 mg per day) were required to heal the rachitic lesions, but minute doses of 1α,25-dihydroxyvitamin D (1.0 μg per day) promptly initiated healing. The dosage of 1α,25-dihydroxyvitamin D was probably in the physiologic range for the human infant — evidence against target-cell unresponsiveness.

Our data suggest that conversion of vitamin D to 25-hydroxyvitamin D was normal in our patients, but that a block was present in subsequent conversion to 1α,25-dihydroxyvitamin D, the active metabolite. We postulate, therefore, that vitamin-D-dependent rickets is an inborn error of vitamin D metabolism due to a genetic defect in 25-hydroxycholecalciferol-1-hydroxylase, the enzyme responsible for the production of 1α,25-dihydroxyvitamin D. (N Engl J Med 289:817-822, 1973)

HEREDITARY vitamin-D-dependent rickets is one of the vitamin-D-refractory syndromes. Despite intakes of vitamin D that would ordinarily prevent rickets, patients have all the clinical and biochemical features of the advanced stage of vitamin D deficiency, including early onset of hypocalcemia, hypophosphatemia, elevated serum alkaline phosphatase activity, generalized aminoaciduria and very severe rachitic bone lesions. Treatment is with very large doses of vitamin D. In contrast with familial hypophosphatemia and the other types of vitamin-D-refractory rickets, this therapy brings about complete remission of the disease; hence the name "vitamin-D-dependent rickets."* The inheritance is autosomal recessive. The incidence of the condition is higher than once suspected.

Several possibilities have been suggested to explain the vitamin-D-dependent state. Defective intestinal absorption of dietary vitamin D has been ruled out because normal levels of antirachitic activity were demonstrable in serum of untreated patients by the rat tibia line test,[1] and because greatly elevated levels were observed in serum of patients receiving large doses of vitamin D.[1,2]

There are two remaining theories that could explain the vitamin-D-dependent state in this disease: defective conversion of vitamin D to the metabolically active derivatives[1-7]; and defective ability of the target organs to respond to physiologic concentrations of antirachitic agents.[1,7] The recent advances in understanding of vitamin D metabolism offer the possibility of resolving which of these two hypotheses is correct.

Vitamin D_3 must undergo two enzymatic hydroxylations before it can function at the target cells (Fig. 1). The first metabolic step, which takes place in the liver, is the conversion of vitamin D_3 to 25-hydroxyvitamin D_3 (25-OHD_3). This reaction is catalyzed by the specific liver enzyme cholecalciferol-25-hydroxylase.[9,10] 25-OHD_3 then undergoes hydroxylation in the kidney to 1α,25-dihydroxyvitamin D_3 (1α,25-$[OH]_2D_3$),[11-13] a reaction that is catalyzed by another enzyme, 25-hydroxycholecalciferol-1-hydroxylase.[14,15] Presumably, vitamin D_2 is metabolized in the same manner as vitamin D_3 by the same hydroxylating enzymes.[16] In rachitic animals, 1α,25-$(OH)_2D_3$ promotes growth, elevates serum calcium and phosphorus levels, and heals the bone lesions.[15,16] It promotes calcium absorption in the isolated intestinal loop of the rachitic rat[17] and stimulates calcium resorption from fetal-rat bone in vitro.[17,18] In most respects, 1α,25-$(OH)_2D_3$ is more potent than either of its precursors,[17-19,20] and it acts more rapidly.[21-24] 1α,25-$(OH)_2D_3$

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*Synonyms are pseudo vitamin-D-deficiency rickets,[2] and hypophosphatemic vitamin-D-refractory rickets with aminoaciduria.[3]

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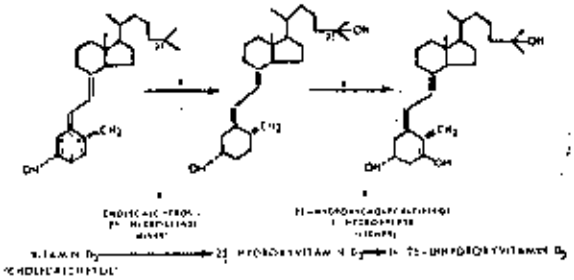

Figure 1. Metabolism of Vitamin D, and the Enzymes Involved.

is now considered to be the metabolically active, hormonal form of vitamin D.[10-14,19]

By studying the responses of patients with vitamin-D-dependent rickets to vitamin D_2, vitamin D_3, 25-OHD_3 and 1α,25-$(OH)_2D_3$, the present series of investigations makes use of these recent developments to evaluate our hypothesis that vitamin-D-dependent rickets is caused by a genetically determined block in vitamin D metabolism.

Materials and Methods

Patients

Five patients ranging in age from 30 months to 12 years were investigated; 3 were sibs. All presented initially in the actively rachitic phase of the disease, each showed the characteristic clinical and biochemical features, and each failed to respond in the hospital to a 2-month trial of 0.1 mg (4000 IU) of vitamin D_2 per day, a diagnostic test that initiates healing in vitamin-D-deficiency rickets.

Protocols for Assessing Efficacy of Vitamin D and 2 Metabolites*

Maintenance requirements. Maintenance requirements of vitamin D_3 were established in 4 patients (Cases 1-4), of vitamin D_2 in 1 (Case 1) and of 25-OHD_3 in 4 patients (Cases 2-5). The requirement of these 3 agents was established according to a protocol used at the Hospital for Sick Children.[5] Starting with a daily oral dose less than the predicted requirement for the drug in question, the dosage was raised by small increments at 2-month intervals until levels of serum calcium, serum inorganic phosphorus and alkaline phosphatase activity became normal, generalized aminoaciduria disappeared and rachitic lesions were completely healed.

Response to 1α,25-$(OH)_2D_3$. Response to 1α,25-$(OH)_2D_3$ was assessed in 1 patient (Case 5). At 9 months of age, this infant had begun to show gradual improvement in his rachitic state while receiving 2.0 mg of vitamin D_2† per day. However, the parents neglected to give vitamin D regularly, and at 28 months of age, he again had hypocalcemia, hypophosphatemia, increased alkaline phosphatase and recurrence of rachitic lesions in the skeleton. He was admitted to the Clinical Investigation Unit of the Hospital for Sick Children, Toronto, for studies of his response to 1α,25-$(OH)_2D_3$. During the 6-week period before the studies with 1α,25-$(OH)_2D_3$ were carried out, the patient received only a prophylactic intake of vitamin D provided by his diet.

The extremely limited supply of 1α,25-$(OH)_2D_3$ necessitated a different protocol from that described above. The patient's response was assessed on 2 dosages of the agent. In the 1st investigation, he received daily intravenous injections‡ of 1.0 μg of 1α,25-$(OH)_2D_3$ for 8 successive days. Response to this vitamin was assessed by the following measurements: serial determinations of serum calcium, serum inorganic phosphorus and alkaline phosphatase; successive 3-day external calcium and phosphorus balance studies before, during and after 1α,25-$(OH)_2D_3$ treatment; oral ^{47}Ca intestinal absorption studies carried out before treatment and on the 3d day of therapy; and periodic radiographic examinations of the skeleton.

In the 2d trial, the dosage of 1α,25-$(OH)_2D_3$ was 0.1 μg per day, given intravenously for 8 days. This study began 19 days after the 1st series of doses was completed, when the patient had again become hypocalcemic and somewhat hypophosphatemic. Response was assessed on the basis of serial determinations of serum calcium and inorganic phosphorus.

Starting on the 35th day, the patient was placed on 25-OHD_3 by mouth. He responded satisfactorily to this vitamin, and, during the succeeding 4 months' observation in the hospital, his maintenance requirement of 25-OHD_3 was determined according to the protocol previously described.

Therapeutic Agents

Vitamin D_2 was given by mouth as a proprietary solution§ containing 2.5 mg of ergocalciferol per milliliter. Vitamin D_3 was given as a solution containing 2.5 mg of cholecalciferol§ per milliliter in peanut oil. 25-OHD_3 was given as a solution containing 0.1 mg of 25-hydroxycholecalciferol per milliliter dissolved in vegetable oil. Initially, this substance was chemically synthesized in the Department of Biochemistry, College of Agriculture and Life Sciences, University of Wisconsin, Madison[6]; subsequently, it was generously supplied‖ by Philips-Duphar Company, Weesp, Netherlands, and later by the Upjohn Company, Kalamazoo, Michigan. Individual doses of the 3 orally administered vitamins were measured volumetrically.

1α,25-$(OH)_2D_3$ was chemically synthesized at the University of Wisconsin.[7] Doses were administered intravenously as sterile propylene glycol solutions containing either 1 μg of 1α,25-$(OH)_2D_3$ per milliliter or 0.2 μg per milliliter to provide individual doses of 1.0 μg and 0.1 μg respectively.

Laboratory Methods

Serum calcium levels were determined by an automated micro-EDTA titration,[8] serum inorganic phosphorus by the Gomori method,[9] adapted by us to the AutoAnalyzer, and serum alkaline phosphatase activity by the method of Bowers and McComb.[10] Calcium in urine and in ashed samples of feces and diet was measured by flame photometry.[11] Fecal determinations were corrected for timing errors and losses during collection by the method of Kerr et al.[12] in which an unabsorbed fecal marker, $^{51}CrCl_3$, diluted in 90 ml of whole milk, was administered 4 times per day with the meals starting 3 days before and continuing throughout the balance study. The method for estimating intestinal calcium absorption followed the protocol of Harrison et al.[13]: a single oral dose of 2 μCi of $^{47}CaCl_2$ diluted in 90 ml of whole milk was administered, and the percentage absorption of this dose was calculated from measurements of whole-body retention and urinary output of ^{47}Ca. The normal range for calcium absorption in the adult is 15 to 40 per cent of the administered ^{47}Ca dose.

Antirachitic substances in fractions of plasma were measured as follows: the plasma from 20-ml to 30-ml samples of heparinized blood was extracted by methanol and chloroform and chromatographed on silicic acid columns as previously described.[14,15] The individual samples of eluate were pooled into 2 fractions, the 1st containing the so-called Peaks I to III, which consist of unaltered and esterified vitamin D, and the 2d containing Peak IV, which consists mainly of 25-OHD_3.[16] The level of bioassayable antirachitic substances was estimated in each fraction by a rat-tibia line test.[16]

*These research protocols were reviewed by the Committee on Human Experimentation, University of Toronto, and the implications of the procedures were discussed in detail with the parents and, when appropriate, with the child.

†1.0 mg of vitamin D_2 or D_3 is equivalent to 40,000 IU.

‡Tanaka et al.[17] have recently shown that, in the rat, 1α,25-$(OH)_2D_3$ is more active when given intravenously than when given by mouth.

§Radiostol — BDH Pharmaceuticals Ltd., London, England.

‖Generously supplied by Philips Roxane Laboratories, Inc., Columbus, O.

Results

Maintenance Requirements of Vitamin D and 25-OHD$_3$

Table 1 shows the daily dosages of vitamin D_2, and D_3 and 25-OHD$_3$ required to maintain biochemical and radiographic healing in the patients, and the dosage of 1α,25-$(OH)_2D_3$ that was effective to heal the rickets in Case 5. All required massive doses of vitamin D_2, the requirements ranging from 1.25 mg per day (Case 1) to 2.50 mg per day (Case 3). The requirements of individual patients were not correlated with age or with body weight. Vitamin D_2 and vitamin D_3 had equal therapeutic effect in the one patient tested with both (Case 1). The maintenance requirements of 25-OHD$_3$ were also very large, ranging in the four patients tested (Cases 2-5) from 0.2 to 0.9 mg per day. In the three in whom a comparison is possible, the potency of 25-OHD$_3$ was 2.6 to 3.8 times greater than that of vitamin D_3.

Table 1. Dosages of Vitamin D_2 (D_2), Vitamin D_3 (D_3), 25-OHD and 1α,25-$(OH)_2D_3$ to Heal Biochemical and Radiographic Abnormalities in Patients with Vitamin-D-Dependent Rickets.

Case No.	Body Wt (Kg)	Dosage (Mg/Day)				Antirachitic-Activity Ratios		
		D_2	D_3	25-OHD$_3$	1α,25-$(OH)_2D_3$	D_2:D_3	25-OHD$_3$:D_3	1α,25-$(OH)_2D_3$:25-OHD$_3$
1*	23	1.25	1.25			1.0:1		
2	25	1.50		0.40			3.8:1	
3*	32	2.50		0.90			2.6:1	
4	50	1.40		0.50			2.8:1	
5*	11			0.20	0.001			200:1
Average of patients tested		1.66		0.50	0.001			

*Sibs.

Table 2 shows the levels of bioassayable antirachitic substances in the silicic acid column fractions of plasma from Cases 2, 3 and 4 obtained when rickets was under satisfactory control. The antirachitic activities were several times greater than those of normal children (normal value for whole plasma, <2.0 U per milliliter; for the Peak I-III fraction, <1.0 U per milliliter, and for the Peak IV fraction, <1.0 U per milliliter). The results confirm previous observations[1,3] that vitamin D_2 is well absorbed in vitamin-D-dependent rickets, and show that some of it is converted to polar metabolites.[17] The high value of Peak IV activity in Case 4 during treatment with 25-OHD$_3$ indicates that this agent was well absorbed.

Table 2. Therapeutic Requirements of Antirachitic Agent — Vitamin D (D_2) or 25-OHD$_3$ — in Three Patients with Vitamin-D-Dependent Rickets, with Corresponding Antirachitic Activities of Plasma Fractions (Silicic Acid Column Fractionation.)

Case No.	Dose of Antirachitic Agent (Mg/Day)		Antirachitic Activity in Plasma Fractions (U/Ml)		
			Peak I-III Fraction	Peak IV Fraction	Totals
2	D_2	1.50	5.0	5.6	10.6
3	D_2	2.50	8.3	6.7	15.0
4	D_2	1.40	7.6	6.1	13.7
	25-OHD$_3$	0.50	1.1	6.4	7.5

Responses to 1α,25-$(OH)_2D_3$

The responses of Case 5 to intravenously administered 1α,25-$(OH)_2D_3$ are shown in Figures 2, 3 and 4. Administration of 1 μg per day for eight days caused a prompt increase in serum calcium from a mean value of 7.5 mg per 100 ml before treatment to 9.6 mg per 100 ml on the eighth day (Fig. 2). This effect was apparent after the second dose, and the serum calcium continued to increase until medication was stopped on the eighth day. The serum inorganic phosphorus increased by 1.0 mg per 100 ml of plasma. The product of calcium times phosphorus, expressed as serum calcium times inorganic phosphorus (in milligrams per 100 ml)[2], increased from less than 30 before treatment to 50 on the eighth day. Serum alkaline phosphatase, which was much elevated before treatment was initiated, had decreased considerably by the eighth day.

Administration of 1α,25-$(OH)_2D_3$ also caused definite increases in the calcium and phosphorus balances (Fig. 3). During the four periods before treatment the patient was, inappropriately for a growing infant, in zero balance for calcium and nearly so for phosphorus. While he was receiving 1.0 μg of 1α,25-$(OH)_2D_3$ per day, the calcium balance became strongly positive (up to 52 mEq for Period 7). The improvement in calcium balance was due to diminution of fecal calcium excretion. The phosphorus balance became progressively more positive during treatment with 1α,25-$(OH)_2D_3$. This result was attributable to a decrease in fecal phosphorus output; there was no appreciable change in urinary phosphate excretion. After treatment was stopped, calcium balance became progressively less strongly positive; phosphorus balance remained positive for 12 days after cessation of treatment.

^{45}Ca absorption increased from a value of 12.6 per cent of the administered dose before treatment to 18.0 per cent on the third day of therapy. This improvement in calcium absorption is consistent with the diminution observed in the fecal calcium output.

A small amount of newly deposited mineral was apparent at the zones of provisional calcification on radiographs of the epiphyseal plates at the knees and wrists by the eighth day, and these changes were more readily seen by the 19th day (Fig. 4).

When treatment was discontinued on the eighth day, serum calcium began to diminish. By the 26th day the patient was irritable, and Trousseau and Chvostek tests were positive. Figure 2 shows that the serum calcium had decreased below pretreatment values. Serum inorganic phosphorus had also decreased somewhat; alkaline phosphatase and the skeletal radiographs were unchanged.

Treatment with 0.1-μg doses of 1α,25-$(OH)_2D_3$, which took place during the 26th to 33d days, resulted

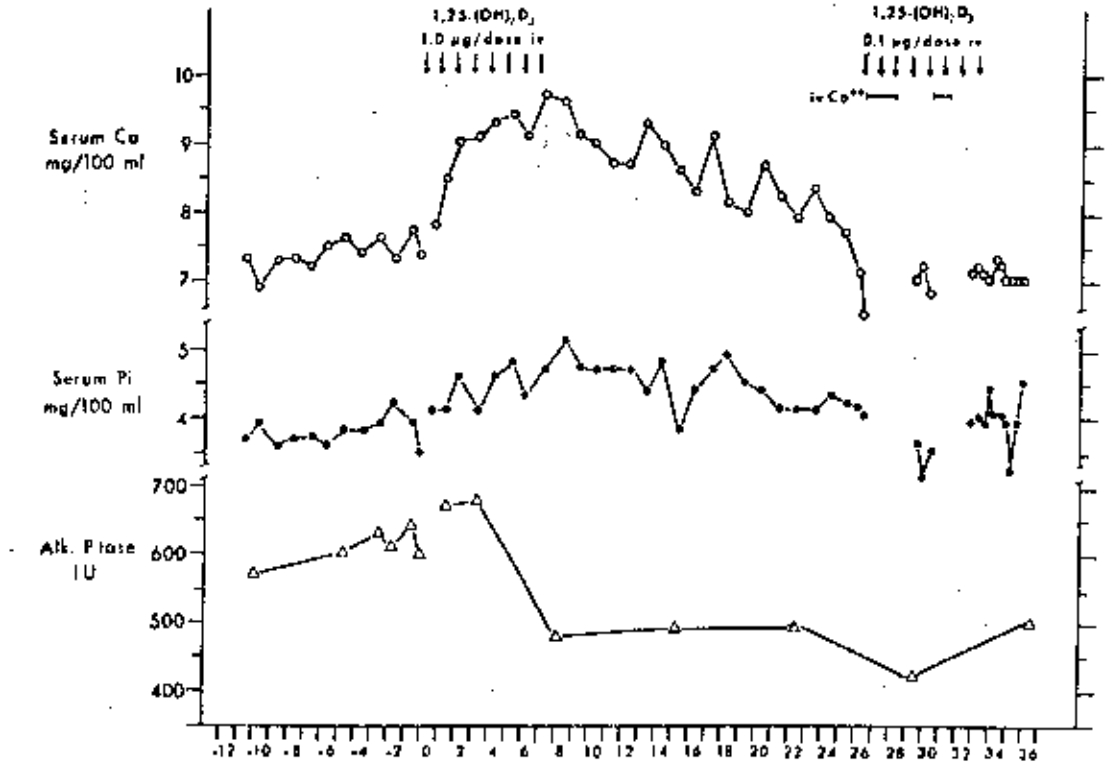

Figure 2. Serum Calcium (Ca) and Inorganic Phosphorus (Pi) Concentrations and Alkaline Phosphatase (Alk. P'tase) Activities in Case 5, Treated with α,25-(OH)₂D₃, 1.0 μg per Day on Zero to Seventh Days and 0.1 G per Day on 26th to 33d Days.

in the serum calcium inorganic phosphorus and alkaline phosphatase values shown in Figure 2. Because of symptomatic hypocalcemia, calcium gluconate was administered intravenously from the 26th to 28th and the 30th to 31st days. During the intervening periods, the serum calcium averaged 7.1 mg per 100 ml. Thus, the daily administration of 0.1 μg of 1α,25-(OH)₂D₃ was ineffective in raising the serum calcium during the eight-day period of treatment, although the possibility cannot be excluded that this dose would have prevented an even greater degree of hypocalcemia and caused gradual healing if a longer course of treatment had been feasible. No changes were observed in serum inorganic phosphorus, alkaline phosphatase or radiographs.

Therapy with 25-OHD₃, which was started after the trials with 1α,25-(OH)₂D₃ were completed, required 0.2 mg per day to maintain healing (Table 1).

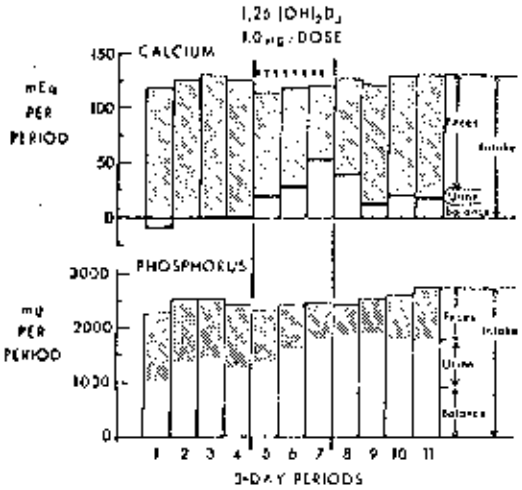

Figure 3. Calcium and Phosphorus Balance Studies in Case 5, Treated with 1α,25-(OH)₂D₃, 1.0 μg per Day for Eight Doses during Periods 5, 6 and 7.

Discussion

Soon after the discovery of vitamin D, it was evident that there were children in whom rickets was present in spite of satisfactory intakes of vitamin D; at least 10 clinically distinguishable types of vitamin-D-refractory rickets have now been identified.[5-18] With elucidation of the normal pathway of vitamin D metabolism, the possibility immediately arose that one or more of the rachitic syndromes was due to faulty conversion of vitamin D to 1α,25-(OH)₂D, the active metabolite. Although this suggestion has received support in the chronic renal insufficiency[19-21] and in anticonvulsant-induced rickets and osteomalacia,[11] no convincing evidence has yet been presented for defective vitamin D metabolism in any of the genetically determined vitamin-D-refractory conditions.

Because the biochemical features of vitamin-D-dependent rickets are so similar to those of vitamin D deficiency, and because all the biochemical and radiologic lesions are completely reversed by pharmacologic

doses of vitamin D, it seemed logical that this recessively inherited form of refractory rickets might be caused by a block in the metabolism of vitamin D to 1α,25-$(OH)_2D$. An essential corollary to this hypothesis is that the target tissues should be fully responsive to physiologic concentrations of the active metabolite.

The availability of synthetic 25-OHD_3 and 1α,25-$(OH)_2D_3$ provided us with a possibility to test our hypothesis. By substituting these two metabolites for vitamin D in our patients, we ascertained the dose of each agent required to reverse the rachitic lesions, and thus obtained estimates of the relative antirachitic activity of the three compounds in the condition. As expected, massive doses of vitamins D_2 and D_3 (1.25 to 2.50 mg per day) were required to heal the rachitic lesions. Although somewhat smaller doses of 25-OHD_3 were necessary, the requirements of this metabolite were also very large (0.2 to 0.9 mg per day). By contrast, a minute dose of 1α,25-$(OH)_2D_3$ (0.001 mg per day) promptly initiated healing in the one patient tested.

A reasonable interpretation of these findings is that a block exists in the metabolism of vitamin D to 1α,25-$(OH)_2D$. To verify this supposition requires knowledge of the efficiency with which vitamin D is hydroxylated both in vitamin D dependency and in normal subjects. This information is not yet available. However, the relative amounts of vitamin D, 25-OHD_3 and 1α,25-$(OH)_2D$ required for healing provide indirect measures of the conversion of vitamin D to the active metabolites. In our patients, the ratios of dosages of vitamin D:25-OHD_3:1α,25-$(OH)_2D$ were, on the average, 1700:500:1. Unfortunately, the corresponding ratios are not yet known for the normal human being, but, for the rat,[20] the ratios are approximately 10:3:1. Assuming that the latter ratios are applicable to the normal human being, our data support the hypothesis that the mechanism for conversion of vitamin D to 25-OHD is intact in vitamin-D-dependent rickets, but that the subsequent synthesis of 1α,25-$(OH)_2D$ is defective.

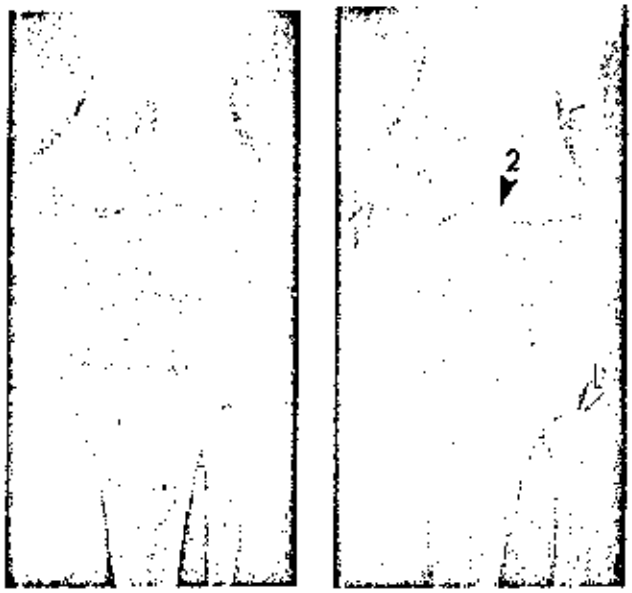

Figure 4. Radiograph of the Left Knee of Case 6 on Zero Day (A) and on 19th Day (B).

The patient received 1.0 μg of 1α,25-$(OH)_2D$ on zero to seventh days. In B, arrows 1 indicate new mineral deposits, and arrow 2 narrowing of the space between the metaphysis and the epiphysis.

This explanation of the cause of vitamin-D-dependent rickets implies that healing of the rachitic lesions should occur with physiologic doses of the active metabolite of vitamin D. The physiologic requirement of 1α,25-$(OH)_2D$ in the human subject is not yet known. However, assuming in the human being, as has been shown for the rat, that 1α,25-$(OH)_2D$ is 10 times more potent than vitamin D_3, a dose of 10 μg (i.e., 400 IU) of vitamin D, long established to be a satisfactory daily intake for prevention of rickets,[14] would have the same antirachitic activity as 1 μg of 1α,25-$(OH)_2D$. Daily injections of this amount of 1α,25-$(OH)_2D$ caused rapid healing of the biochemical and radiographic lesions in our patient. We believe from these considerations that the doses of 1α,25-$(OH)_2D$ given were probably in the physiologic range for human beings.

Our data imply that vitamin-D-dependent rickets is due to an inborn error of vitamin D metabolism caused, we suggest, by a recessively inherited defect in 25-hydroxycholecalciferol-1-hydroxylase in the kidney. Final proof of the exact nature of the defect awaits measurement of enzyme activities in patients with the condition.

Our investigations were not designed to evaluate the practical therapeutic value of 1α,25-$(OH)_2D$. At present, this agent is not available in sufficient quantity for long-term therapy; furthermore, there is a question whether it is effective when administered by mouth.[18] In the absence of appropriate clinical trials of 1α,25-$(OH)_2D$ or of 1α-OHD_3, a more readily utilizable analogue,[43] massive doses of vitamin D, 25-OHD_3 or dihydrotachysterol[8,11,13] remain the practical therapy for vitamin-D-dependent rickets. If treatment is administered assiduously, all the physical and biochemical abnormalities can be controlled, the growth rate becomes normal, and excellent long-term results are achieved.

If our hypothesis is correct, an explanation must be found for the excellent response of patients to pharmacologic doses of vitamin D. It is possible that massive doses of vitamin D produce concentrations of 25-OHD_3 high enough to cause synthesis of the minute amounts of 1α25-$(OH)_2D$ required for healing. Alternatively, because of its high concentration in the cytosol, 25-OHD_3 may become bound to nuclear membrane in sufficient amounts to exert direct physiologic action.

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INFLUENCE OF VITAMIN D ON THE VAGINAL EPITHELIUM OF THE MENOPAUSE AND POSTMENOPAUSE*

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VITAMIN D affects the estrous cycle of various animals. It increases their prolific capacity[1]; but toxic doses will inhibit ovulation.[2] When given to mature rats, recently oophorectomized, the resulting vaginal smears may be early estrous[3] or full estrous.[4] Reed, Struck, and Steck[5] noted that the vitamin could alter the menstrual cycles of women, and in one case they were able to restore a normal cycle to a patient with secondary amenorrhea. They re-established menstruation in one menopausal woman of 52 with amenorrhea of 31 months' standing. Simpson and Mason[6] employed cod-liver oil or haliver oil in senile vaginitis and found relief of subjective complaints and healing of the vaginal mucosa. Swift[7] noted the influence of cod-liver oil on vulvovaginal dystrophies at the menopause and later. These last three authors attribute results to vitamin A action solely, and completely ignore any possible contributing influence of the vitamin D in the oil.

Present Study

This is a study of vaginal changes in 27 women who were given vitamin D at the menopause and postmenopause. Observations on changes of subjective symptoms are incidentally noted. They were all private patients whose ages varied from 39 to 68 years: 16 were between 45 and 55 years old. The women were divided into three groups.

The first group consisted of 5 women who had undergone artificial castration five to twenty-one years previously. Four were surgical castrates and one was a radium sterilization case. The second group included 7 women who had not menstruated for three years or more. One of these women had a hysterectomy performed seven years previously, but the ovaries were left in situ. The last group of 15 patients had menstruated within three years. Several of these were in their early menopause.

After an initial vaginal smear the majority of the women received a course of 2,250,000 to 2,550,000 units of vitamin D. This was given in divided doses in the form of capsules,† each containing 50,000 units of vitamin D, one orally three times a day for 15 to 17 days. Smears and vaginal mucous were then studied for possible stimulating effects.

In some cases much smaller amounts (600,000 to 1,200,000 units) sufficed to give results. Occasionally there was a delayed effect after the last dose of vitamin taken. The smears were stained according to the Papanicolaou technique. Occasionally the Shorr, Fletcher, or Fuller stains were used in addition.

*Read before the Montreal Clinical Society, Dec. 18, 1949.

†The capsule Ostoforte made by Charles E. Frosst & Co., Montreal, was employed.

Menopausal Smear Grading

There is considerable variation in the initial vaginal smears of menopausal patients before treatment. These may show ranges from advanced to slight degrees of estrogenic deficiency. One must take cognizance of the fact that local conditions such as cervicitis or vaginitis can produce false estrogenic pictures due to the increased cornified cell output. Mechanical stimulation,[5,6] or chemical irritation[8] of the vaginas of spayed rats can elicit estrus-like changes.

An arbitrary grouping of the menopausal vaginal smear into four stages is here presented. The criteria considered are the amount, appearance, and arrangement of (1) the large superficial cornified cells, with pyknotic nuclei and acid stain, (2) large and smaller superficial basophilic non-cornified cells with either pyknotic nuclei (karyopyknotic) or larger nuclei possessing a loose chromatin network, and (3) the deeper basal cells of the vaginal wall. Microscopic changes in white blood cells, mucus, and bacteria are not considered. This is because of the prevalence and variability of these elements in the group under study; also the amount of vitamin D used was not potent enough to stimulate the smears to a typical full-blown preovulatory stage with concomitant elimination of white blood cells. However, definite study is given to the macroscopic appearance of mucus at the vaginal introitus, if occurring in excessive amounts from the previously dry or slightly moist vagina.

Stage 0 presents the most atrophic smear. It shows an occasional large noncornified superficial cell with lower basal cells in profusion. The cells are closely packed in mucus, bacteria, and debris. The nuclei are relatively large and contain a finely meshed chromatin network. The edges of the cells are irregular and poorly defined. Clumping of cells is noted; also a tendency to curl or fold over. The whole slide is unclean and takes the basic stain rather poorly. White blood cells are abundant.

Stage 1 exhibits more of the superficial type cells, several of which are quite large. They are all uncornified, with large nuclei showing a lacy chromatin pattern, and take the basic stain. However a rare basophilic uncornified cell may be seen with a smaller pyknotic nucleus (uncornified karyopyknotic cell). There are still many lower type basal cells in view. In this stage the cells attempt to separate themselves; and clumping, although in evidence, is less prominent. Cell edges are more sharply defined. Many of the large cells are folded. White blood cells, bacteria, and mucus are present.

Stage 2. Here one notes a preponderance of large uncornified basophilic cells of the superficial layers, and many of them are karyopyknotic. A few of these pyknotic nuclei appear in acidophilic cells (cornified). The cells tend to be arranged loosely and appear sharply. Some lower basal type cells may still be present. Very little curling or folding is seen. White blood cells do not necessarily disappear. Bacteria and mucus may persist.

Stage 3 has an estrogenic picture resembling the early preovulatory phase of the menstrual cycle. There are abundant large discrete acidophilic cornified cells visible, but some uncornified karyopyknotic cells are also present. The odd superficial cell with a larger nucleus and loosely arranged chromatin may still be seen. Basal cells are not in evidence. White blood cells may persist, as well as mucus and bacteria.

Results

A positive result is achieved after vitamin D treatment if the vaginal smear rises to a higher estrogenic stage, or a profuse mucoid discharge is produced in the dry or slightly moist vagina. Small increases in mucus even when visible macroscopically are not considered.

As several of the women had repeat tests the 27 patients received 37 examinations in all. The 5 women of the castrate group underwent 6 tests. Two

of these subjects showed positive vaginal smears, one smear rising from Stage 0 to 2 and the other from Stage 1 to 2. Another woman was positive upon exhibiting a white mucus discharge in the vagina which was previously only moist. This woman's smear remained at the same level, Stage 1, but she is classified as belonging to Stage 3. The 3 negative smears were not elevated from their original position, Stage 1. Interestingly, another castrate of eight years' standing who had no estrogenic response demonstrated a healing effect of the vitamin on labial ulcers.

The second group of 7 women, who had not menstruated for three years or more, took 9 tests and there were 4 positive vaginal smears. One smear rose from Stage 0 to 1, three from Stage 1 to 2; and four remained stationary at Stage 1. One woman showed a positive result, Stage 3, when she had a mild milky discharge developing from a moist vagina. However, her vaginal smear did not rise, persisting at Stage 1. One of the positive cases in addition to a raised vaginal smear received therapeutic benefits to labial excoriations, with partial relief of an intertriginous itch.

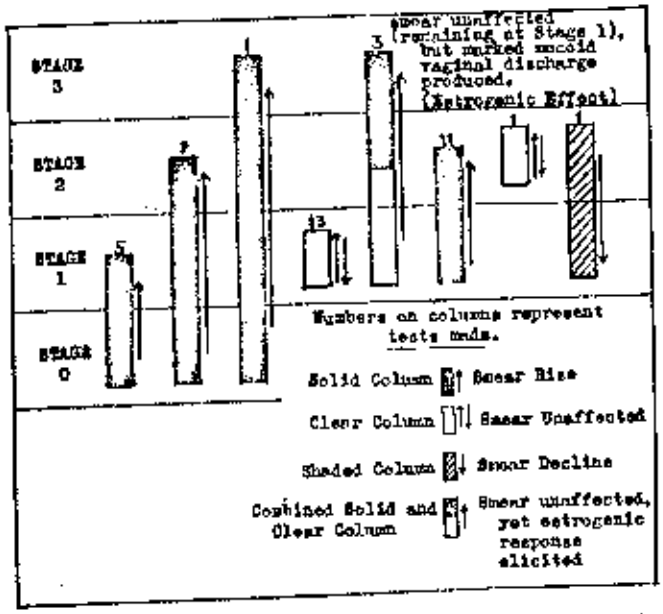

Fig. 1. Estrogenic response to oral vitamin D at the menopause and postmenopause. Twenty-seven women received 37 tests. Effects on vaginal smears classified under Stages 0 to 4. The majority received doses of 2,050,000 to 2,550,000 units of vitamin D.

The last group of 15 women, menstruating less than three years previously, had 22 tests. The results showed 13 positive vaginal smears. Four were elevated from Stage 0 to 1, one from Stage 0 to 2, one from Stage 0 to 3, and seven from Stage 1 to 2. Unaffected were six smears which remained at Stage 1, and one which was stationary at Stage 2. One smear dropped from Stage 2 to 1. One patient had such a profuse mucus flow developing on a moist vagina that it evoked complaints. Her grading was therefore Stage 3, even though her smear remained at Stage 1. This woman's smears, upon repetition of the test on two more occasions, went from Stage 0 to 1; but there was no significant outpouring of mucus. These last two smear elevations are included in the total of vaginal smear rises as given above.

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When the three groups are taken together the results show that out of 37 tests performed on 27 women there were 22 positive. Nineteen of these were vaginal smear elevations and three vaginal mucus rises.

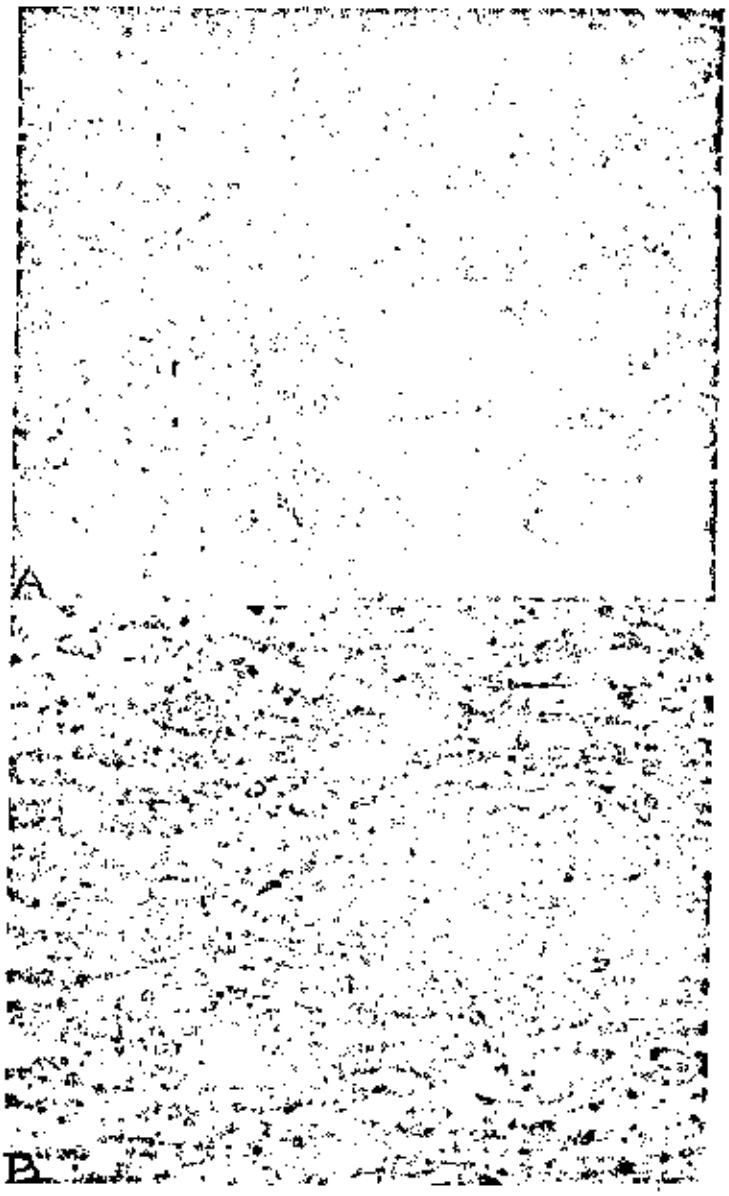

Fig. 2 (Case 1). E. D., aged 50 years, had a subtotal hysterectomy with bilateral salpingoovariectomy and appendectomy 15 years previously. Her initial smear of grading 0 (see A) rose to 2 (see B) after she received 3,300,000 units of vitamin D. Note the transformation of the small basal cells in A to the larger more superficial type in B. An occasional acidophilic cornified cell is seen among the basophilic cells prevailing. An excess of mucus interferes with the clarity of this last picture. There was no clinical improvement. This case demonstrates the direct action of vitamin D on the vagina of a patient with no ovaries. (Shorr stain. ×325.)

It should be noted that the patient giving a positive result did not always perform in the same fashion upon repetition of the test. At times there would be no stimulating effect in the next experiment (Fig. 1).

The main individual symptoms of the menopause were partially affected. Best results were obtained with the complaint of hot flushes. Here there was a cure or improvement in 18 out of 27 instances. A majority of the patients with rheumatoid pains were helped (Table 1).

Changes in menopausal symptoms after vitamin D treatment are not included as positive results in this paper.

Three typical clinical cases demonstrating the effects of vitamin D on the vaginal smear are briefly presented (Figs. 2, 3, and 4).

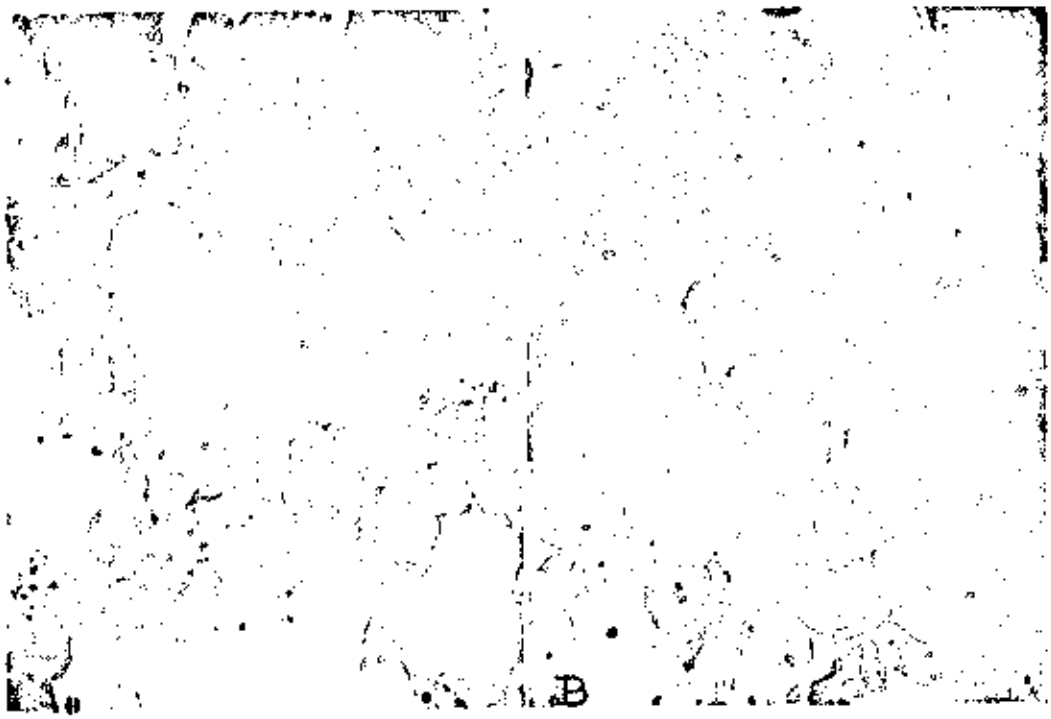

Fig. 3 (Case 2).—C. L., aged 34 years, with history of subtotal hysterectomy and bilateral oophorectomy 21 years before. After taking 2,100,000 units of vitamin D, her initial smear of grading 1 (see *A*) became elevated to 2 (see *B*).

This case resembles Case 1 but the cells are sharper and clearer after treatment. It too shows how vitamin D can act on the vagina directly when the ovaries are absent. There was no clinical improvement. (Shorr stain. ×225.)

Comment

The estrogenic response elicited in three of the castrate group seems to point to a direct action of vitamin D on vaginal epithelium and not necessarily through ovaries.

The profuse vaginal mucoid discharge appearing after vitamin D treatment, which is also taken as a criterion of estrogenic stimulation, is an interesting phenomenon. The same reaction is also noted in castrated rats receiving this vitamin.[3]

Bennett[12] treated menopausal patients with small oral doses of stilbestrol and observed a change from the scanty, cellular, tenacious mucus of the cervix

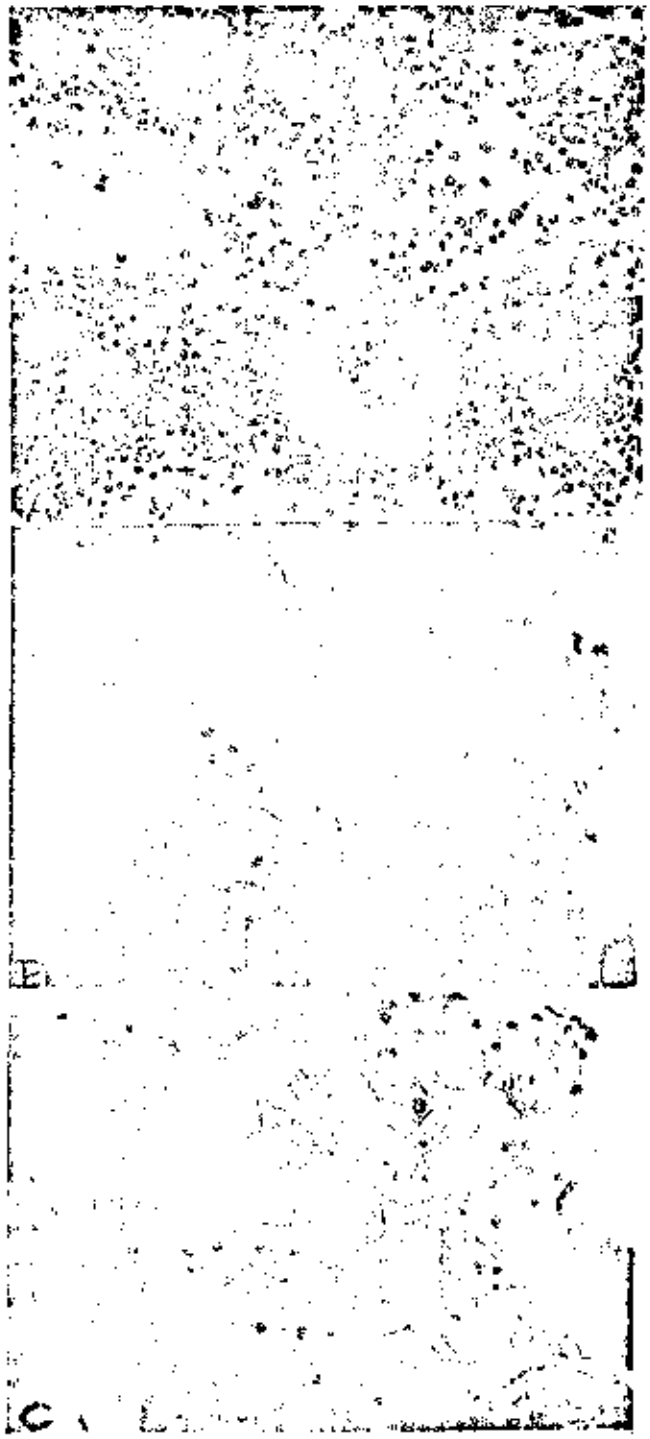

FIG. 4.

(For legend see opposite page.)

to increased amounts of mucus with higher pH and fewer cells. Those patients receiving 1.0 mg. or more of stilbestrol per day and those who had subcutaneous implantation with estrogenic pellets noticed an abundance of mucus which was glairy and transparent, with a high pH, and almost no cellular elements.

It is also noteworthy that the three patients receiving vitamin D showing this excessive mucus did not simultaneously exhibit estrogenic changes in the vaginal smear. This expected correlated smear picture might have been missed because of insufficient smears done.

TABLE I. RELIEF OF INDIVIDUAL SYMPTOMS AFTER VITAMIN D ADMINISTRATION*

SYMPTOMS	NO. OF INSTANCES	CURED	IMPROVED	UNAFFECTED
Hot flashes	27	2	16	9
Perspiration, cold sweats	8		3	5
Dizziness	7		2	5
Nervousness	6		2	4
Headaches	5		1	4
Weakness	4		3	1
Palpitation	3	1		2
Sleeplessness	3			3
Drowsiness	2		2	
Rheumatoid pains	16		12	4

*Twenty-seven patients underwent thirty-seven tests. The majority received 2,250,000 to 2,550,000 units of vitamin D.

The patients who obtained relief of the subjective symptoms of the menopause can be compared with the number usually improved or cured under standard estrogenic treatment. If one uses the relief of hot flashes as an index of treatment, the report of Coulton and Sewell[11] with Mepiane therapy will be found to claim 91.7 per cent success. After vitamin D therapy the result is 66.7 per cent.

Summary and Conclusions

Large doses of vitamin D were administered orally to 27 women at the menopause and postmenopause. Five of the patients were artificial castrates. The majority of the women received 2,250,000 to 2,550,000 units. Vaginal smears and mucus were then studied for signs of estrogenic stimulation.

The smears were graded according to an arbitrary estrogenic smear scale. The most atrophic smear appeared in Stage 0, and there was a gradual rise of estrogenic influence through Stages 1 and 2 until the highest type was reached in Stage 3. There the estrogenic picture resembled the early preovulatory phase of the menstrual cycle.

Thirty-seven tests were performed.

In nineteen instances the vaginal smear showed an estrogenic response.

Eight smears were initially in Stage 0, and five of these rose to Stage 1, two to Stage 2 and one to Stage 3. Eleven smears were elevated from Stage 1 to Stage 2.

Fig. 4 (Case 3).—A. D., aged 46 years. Last menstrual period 36 days previously. Complaining of hot flashes, perspiration, dizziness, tiredness, and palpitation. *A* is her menopausal smear, with a grading of 0. Most of the cells are small and belong to the outer basal layer of the vagina. *B* is the smear resulting after 2,100,000 units of vitamin D were taken. Grading is now 2. Note the large basophilic superficial cells, many showing pyknotic nuclei (uncornified karyopyknotic cells). A few of these take the acidophilic stain (cornified cells). There is less clumping and curling noted. A few white blood cells are still present. The patient had fewer flashes and less perspiration, and was not so tired and sleepy. *C* is the smear 28 days after the last dose of vitamin was taken. Lower type vaginal cells are now returning, and there is more clumping and curling in evidence (Stage 1). Clinically the patient was still improved. She had not menstruated as yet. (Shorr stain. ×226.)

In three other tests the estrogenic response was manifested solely by the production of a profuse, glairy, and free flowing vaginal mucoid discharge, with no cornified cell changes developing.

There was some relief of menopausal symptoms, especially hot flashes.

Rheumatoid pains were relieved in a fair number of patients.

There is no certain correlation between the rise in vaginal cellular elements, the vaginal mucus increase, or the amelioration of subjective symptoms.

Vitamin D may influence menopausal vaginal epithelium even when ovaries are absent.

Vitamin D would appear to have a role in the maintenance of proper vaginal tone at the menopause and postmenopause.

My appreciation is tendered to the Director of the Department of Obstetrics and Gynecology, Dr. George J. Stream, who has been very generous with advice and encouragement.

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Vitamin D and the Supravalvar Aortic Stenosis Syndrome

The Transplacental Effects of Vitamin D on the Aorta of the Rabbit

By William F. Friedman, M.D., and William C. Roberts, M.D.

RECENT REPORTS have indicated that idiopathic infantile hypercalcemia, a disease related to deranged vitamin D metabolism,[1-4] may be a feature of the nonfamilial, congenital supravalvar aortic stenosis syndrome.[5-8] Other manifestations of this syndrome include mental retardation, a peculiar "elfin" facies, narrowing of peripheral pulmonary and systemic arteries, strabismus, inguinal herniae, and dental abnormalities.[9] Congenital supravalvar aortic stenosis and peripheral pulmonary arterial stenosis also occur in a familial and a sporadic form unassociated with the other features of the syndrome.[10-13] It is not known if the multiple-system involvement in the supravalvar aortic stenosis syndrome is genetically determined or whether some or all of the features are related to a derangement of maternal or fetal vitamin D metabolism, or a combination of the two.

A paucity of information exists regarding the effects on the fetus of induced maternal hypervitaminosis D, and there appears to be no direct evidence for transplacental passage of vitamin D.[14-16] Studies concerning hypervitaminosis D in the nonpregnant animal emphasize the vascular toxicity of the vitamin.[17-23] Administration of excessive vitamin D to animals has been shown to produce dose-related vascular lesions, ranging from subendothelial edema to calcification, which are most pronounced in the ascending aorta just above the aortic sinuses. The pathogenesis of these vascular abnormalities is unclear.[24]

The present study was undertaken to determine whether vitamin D crosses the placenta, and to explore the relationship between hypervitaminosis D in the mother and the development of supravalvar aortic stenosis in the offspring. Blood levels of vitamin D from female rabbits that received excessive amounts of vitamin D and from their offspring were therefore determined, and the proximal aortae of these animals were examined anatomically.

Methods

Adult, white, New Zealand rabbits were bred so that each female was mated with a different male. Eight females fed a stock diet* were designated as controls. In addition, three females were fed the stock diet especially prepared without vitamin D supplements, and these are referred to as "vitamin D-deficient mothers." Eight females were fed the stock diet and, starting on the day after observed copulation and continuing until delivery, were given intramuscular vitamin D (activated ergosterol in cotton seed oil†) in divided doses every other day for a total dose of 1.5 million units. These animals are referred to as "mothers given vitamin D" (1.5 million units).

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*Stock diet: Protein, 18.0%; fat, 2.5 to 4.0%; fiber, 13.5%; ash, 9.0%; calcium, 1.3 to 1.6%; phosphorus, 0.7 to 0.9%; iodine, 0.0030; salt, 0.75 to 1.0%; carotene, 40 gamma/g, pantothenic acid, 8.4 g/lb; niacin, 15.75 mg/lb; thiamin, 2.0 mg/lb; riboflavin, 3.2 mg/lb; biotin, 0.09 mg/lb; vitamin C, 0.70 mg/lb; vitamin D, 768 mg/lb; vitamin E, 30 mg/lb; choline, 350 mg/lb.

†Hi-Derated injectable, Brewer and Co., Inc., Worcester, Massachusetts.

Three groups of five females each were fed the stock diet and in similar fashion were given intramuscular vitamin D for 30 days (the length of the rabbit's gestational period) beginning the day after observed copulation, for total amounts of 2.5, 3.5, and 4.5 million units, respectively.

Five control mothers and five given vitamin D (1.5 million units) were sacrificed with their progeny shortly after delivery. Prior to sacrifice blood was drawn from each mother for determination of calcium, phosphorus, alkaline phosphatase, cholesterol, total protein, albumin, lipoprotein phenotype,[21] and vitamin D. Similarly, blood for calcium, lipoprotein phenotype, and vitamin D determinations was obtained from each neonate. To obtain an adequate amount of serum for the vitamin D assay, samples were pooled from as many as three newborns from the same litter. The vitamin D bioassay[22] measured the response of rachitic rats to serum fed by stomach tube with the standard line test, as modified by Shute and associates.[24] The rachitogenic diet and methods described in the A.O.A.C. methods of analysis were employed.[27] Four or more rats were used for each assay.

Autopsy was performed on each of the five control and on each of the five mothers given vitamin D (1.5 million units) and on their offspring. The tissues were fixed in 10% Formalin, dehydrated in alcohol, embedded in paraffin, and sectioned serially at 6 μ intervals. The sections of heart and great arteries of each of the offspring were stained and examined histologically. In addition to hematoxylin and eosin, the Reinhart (for mucopolysaccharides), elastic van Gieson (for elastic tissue), Sudan black (for fat), and periodic acid-Schiff (for glycogen) stains were employed.

To evaluate anatomic changes in growing offspring given small daily quantities of vitamin D, three control mothers, three vitamin D-deficient mothers, and three mothers given vitamin D (1.5 million units) were not bled or sacrificed after delivering their offspring. After parturition the three vitamin D-deficient mothers were fed the stock diet and permitted to nurse their offspring. To avoid the consumption of large amounts of vitamin D from maternal milk, the offspring of the mothers given vitamin D (1.5 million units) were nursed by control mothers. The litter offspring as well as those of the controls were thereafter fed 250 units of vitamin D per day.[9] Whenever death occurred spontaneously in one of the offspring of the mothers given vitamin D (1.5 million units), a rabbit of the same age born to a control mother and to a vitamin D-deficient mother was sacrificed. All of the remaining offspring were sacrificed at 3 months of age. Gross and microscopic examinations of the hearts and great vessels were carried out in each rabbit.

Results

The results of the analyses of the serum of the mothers and offspring are presented in table 1. The levels of vitamin D were strikingly different in the various groups (table 1). The levels in the mothers given vitamin D (1.5 million units) and in their offspring were 7 and 9 times greater than the controls, respectively. Other than the levels of vitamin D there were no statistically significant differences in any of the other measurements between the groups of mothers (table 1). The calcium levels were somewhat lower and the phosphorus values somewhat higher in the mothers given vitamin D (1.5 million units) when compared to the controls. Significantly higher serum calcium levels were observed in the offspring of the mothers given vitamin D (1.5 million units) when compared to the controls. The high serum calcium levels noted in this study are in the same general range as those reported for the normal rabbit by others.[28] Cholesterol values were not significantly higher in the mothers given vitamin D (1.5 million units), and there were no qualitative differences in the electrophoretic lipoprotein phenotypes. The control mothers and the mothers given vitamin D (1.5 million units) had chylomicron, beta (β-), and alpha (α-) lipoprotein bands of equal staining intensity. All of the offspring showed less intense β- and α-lipoprotein bands than their parents, but there were no differences in staining intensity between the two groups of offspring.

Anatomic Observations

Adult Females

There were no gross or microscopic abnormalities in the hearts or aortae of the control mothers. All of the females given 2.5, 3.5, and 4.5 million units of vitamin D died spontaneously within 65 days after their first injection of the vitamin, and all that con-

[9] Crystalline vitamin D_2 in propylene glycol (Dihydrol).

Table 1

Results of Analysis of Serum of the Mothers and Offspring

	Mothers		Offspring	
	Controls	Given vitamin D (1.5 million units)	Controls	Mothers given vitamin D (1.5 million units)
Calcium (mEq/L)	7.34 ± 0.24 (SD)	6.88 ± 0.26 (SD)	4.8 ± 0.11 (SD)	5.55 ± 0.24 (SD)
Phosphorus (mg %)	4.5 ± 0.73	5.7 ± 0.52	$P < 0.05$	
Alkaline phosphatase (King-Armstrong units)	1.76 ± 0.55	1.15 ± 0.16		
Cholesterol (mg %)	47.3 ± 3.4	56.3 ± 3.9		
Total protein (g %)	4.3 ± 0.77	4.8 ± 0.33		
Albumin (g %)	0.80 ± 0.27	0.87 ± 0.30		
Vitamin D assay (units/100 ml)	310 ± 268	2190 ± 382	150 ± 138	1290 ± 359
	$P < 0.001$		$P < 0.001$	

ceived either aborted during the first 12 days of pregnancy or delivered macerated fetuses. The entire aorta of each of these adult rabbits showed advanced changes, including irregular depressions in the intimal wall, focal calcium deposits, and foci of degeneration and necrosis of the aortic media. The aortae of the mothers given vitamin D (1.5 million units) showed similar but less striking changes. The alterations were most pronounced in the proximal portion of the aorta.

Offspring

Control Newborns Sacrificed after Delivery

The aortic roots of the 23 control neonates were histologically normal. The normal invagination or plica at the upper margin of the sinuses of Valsalva[29,30] was observed in some sections of each of these aortae, indicating that the plica does not extend around the entire inner circumference. The medial thickening that accounted for this invagination appeared moderately exaggerated (fig. 1 right) in five of these 23 aortae, but in none was it believed to be of sufficient proportion to narrow the aortic lumen significantly.

Older Control Offspring

With the exception of a ventricular septal defect in one, no other abnormalities of the heart or aorta were found in these 12 rabbits. The normal supravalvar aortic plica did not appear unusually prominent in any of these animals.

Offspring of Vitamin D-Deficient Mothers

No abnormalities were observed in these nine rabbits. The supravalvar aortic plica was present in each, but in none did it appear to be exaggerated.

Newborns of Mothers Given Vitamin D (1.5 Million Units)

The aortae of 14 of the 18 newborns in this group were normal, and 11 of these 14 aortae showed an exaggeration of the supravalvar plica similar to that observed in the five controls mentioned above (fig. 1 right). The higher incidence of this finding in this vitamin D group when compared to the controls is significant ($P < 0.001$). Four newborn rabbits,

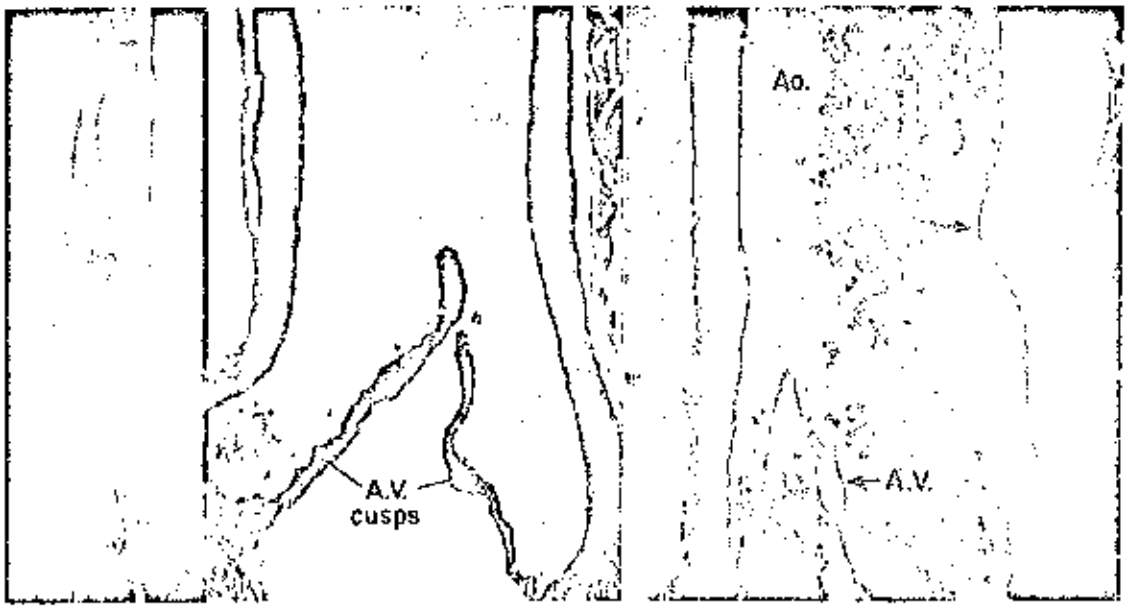

Figure 1

Gross and microscopic appearance of aortic roots of control newborns. (Left) The left ventricle (L. V.) and aorta (Ao.) of a typical animal is shown. (Center) The aorta is smooth-walled beyond the sinuses of Valsalva. Aortic valve (A. V.); × 79. (Right) An exaggerated plica (arrow) at the superior margin of the sinuses of Valsalva is present in this animal. A similar degree of prominence of this structure was seen in five controls and 11 rabbits born to mothers given vitamin D (see text). Hematoxylin and eosin; × 30.

each from a different litter, had abnormal aortae. In each animal there was a prominent annular protrusion at the superior margin of the sinuses of Valsalva resulting in significant narrowing of the luminal circumference at this level (fig. 2). The protrusion was caused entirely by proliferation of the media; the intima was normal. There was widening of the spaces between the elastic fibrils in the inner media. Stains for glycogen, fat, calcium, and mucopolysaccharides did not reveal abnormal deposits of these substances in the medial lesions.

Older Offspring of Mothers Given Vitamin D (1.5 Million Units)

Ten rabbits died spontaneously at 2 to 20 days of age. The cause of their deaths was not apparent at autopsy. There were no cardiac or aortic abnormalities in the four that lived less than 10 days and in two that died at 16 and 20 days of age, respectively. Supravalvar aortic abnormalities were noted in the four remaining rabbits, representing different litters. The aorta of a 7-day-old rabbit appeared normal on gross examination, but on microscopic examination an area of medial thickening and early degeneration just above the aortic sinuses was apparent. A 10-day-old rabbit had a nonobstructing fibrous band stretched across the lumen of the ascending aorta at the superior margin of the sinuses of Valsalva and a particularly prominent plica of the wall at this level. The aortae of the other two rabbits, a 20 day-old (fig. 3) and a 14-day-old (fig. 4), were severely narrowed in the region immediately above the sinuses of Valsalva by triangular-shaped protrusions or hillocks, and the aortic lumina distal to these sites were widened. These protrusions into the lumen resulted from localized thickening of the aortic media. In addition to an apparent increase in the elastic fibers and smooth muscle cells in the media, small foci of degeneration and round-

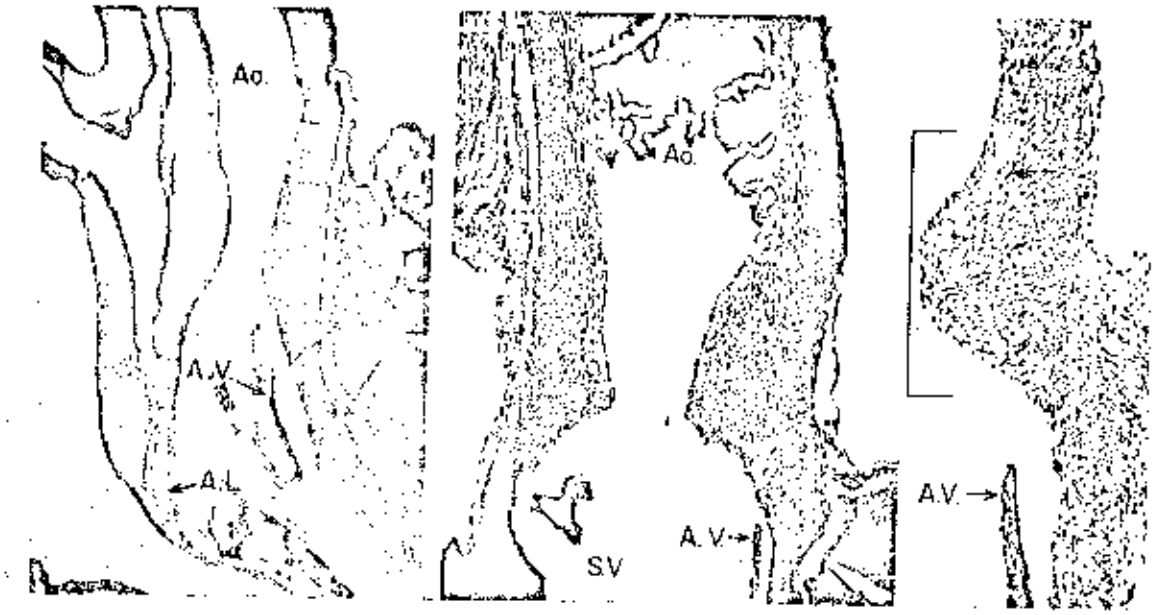

Figure 2

The aortic roots of two newborns (left and center) whose mothers were given vitamin D (1.5 million units) show prominent annular protrusions impinging on the lumen at the superior margin of the sinuses of Valsalva. A higher magnification of the lesion (brackets) in a third newborn is shown on the right. The narrowing of the lumina results entirely from proliferation of the media. S. V., sinus of Valsalva; Ao., ascending aorta; A. V., aortic valve; A. L., anterior leaflet of mitral valve. Hematoxylin and eosin; × 59 (left); × 63 (center); × 97 (right).

cell infiltration also were present. The arrangement of the elastic fibers and smooth muscle cells in the hillock produced an irregular pattern (fig. 3). A focal aneurysmal protrusion of the ascending aorta was observed in one animal (fig. 3). In the other animal, calcium deposits also were present in the media (fig. 4). The intima in each rabbit appeared normal, but the adventitia in one was focally thickened and invaginated into the media in a wedgelike fashion (fig. 3).

The remaining six rabbits in this group were sacrificed at 3 months of age. The changes in their aortae resembled quite closely the findings in the mothers given vitamin D (1.5 million units). The proximal aorta in these six animals showed generalized irregularities of the wall with degeneration and calcification of the media. The supravalvar area was not narrowed or disproportionately thickened, however, when compared to the rest of the ascending aorta.

Discussion

Three specific anatomic types of supravalvar aortic stenosis (SAS) have been described in the human.[29, 31] The hour-glass type is the most common and is characterized by extreme thickening of the aortic media producing a constricting annular ridge. The membranous type is produced by a semicircular diaphragm with a small central opening stretched across the lumen of the aorta. The hypoplastic type is characterized by uniform hypoplasia of the ascending aorta. A fourth anomaly, of no functional significance, consists of a nonobstructing band or cord stretched across the lumen of the aorta at the level of aortic leaflets.

According to Perou,[29] the most common basic lesion is an angulation and exaggerated infolding of the wall with a thickened and focally disorganized medial layer capped by a zone of intimal thickening. The infolding may be shaped like a hillock or triangle

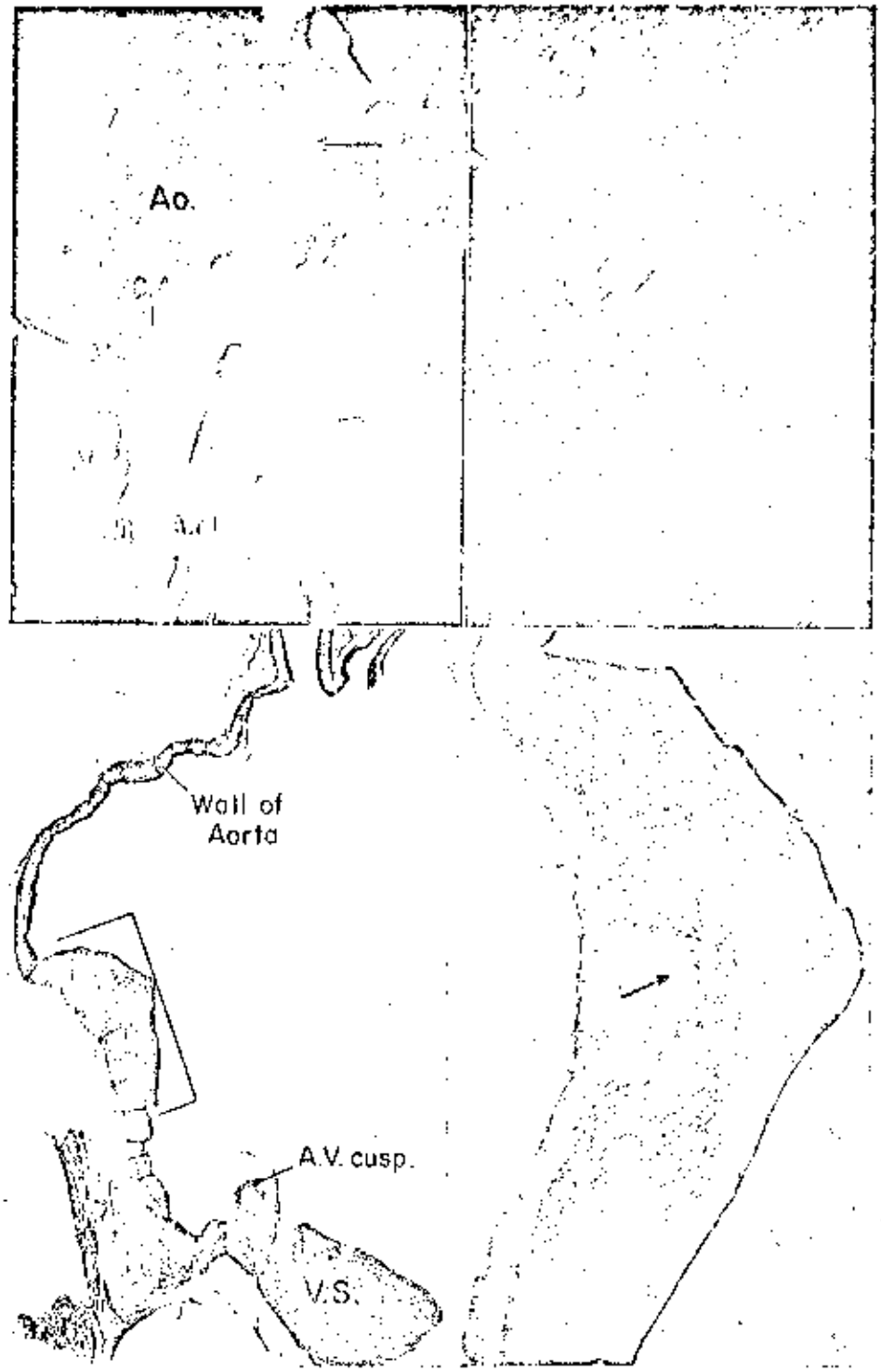

Figure 3

Heart and aorta in a 20-day-old rabbit whose mother had received vitamin D. (Upper left)

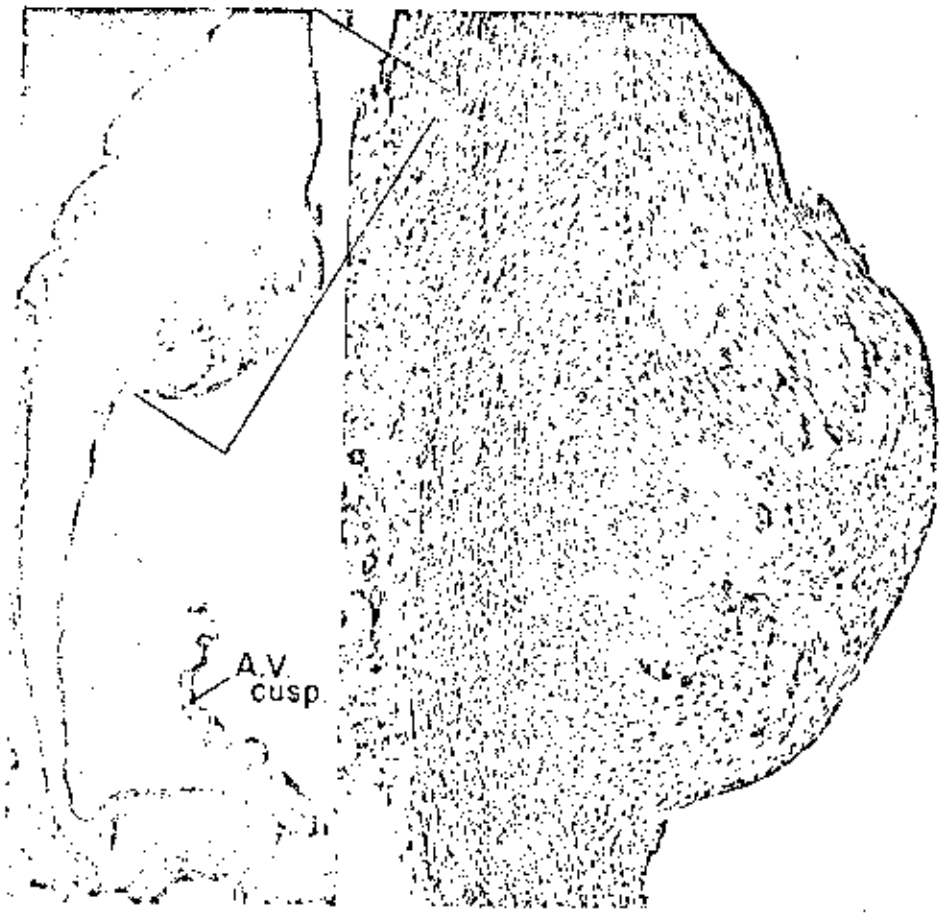

Figure 4

The aortic root in a 14-day-old rabbit whose mother was given vitamin D. (Left) The wall of the aorta just above the sinuses of Valsalva is markedly thickened (brackets) and bulges into the lumen. A close-up of this area is shown in the photomicrograph on the right. Areas of necrosis and focal deposits of calcium are apparent. A. V., aortic valve. Hematoxylin and eosin; × 25 (left); × 160 (right).

whose base is formed by the abnormal media and whose apex points toward the lumen. Foci of necrosis, round-cell infiltration, and calcification also have been noted in the hillock. Similar to the vascular changes in the animal given excessive vitamin D, the intrin-

Figure 3 (continued)

The anterolateral wall of the left ventricle (L. V.) and left atrium has been removed, and the aorta (Ao.) opened. The lumen immediately above the aortic valve (A. V.) is narrowed and the aortic wall is thickened. The ostium of the right coronary artery (C. A.) is dilated. A focal protrusion or aneurysm (arrow) also is present in the ascending aorta. (Lower left) Section of wall of ascending aorta, aortic valve cusp (A. V.), and ventricular septum (V. S.). The prominent supravalvar aortic lesion (brackets) is triangular shaped, with the apex pointing toward the lumen. The aortic valvar cusp is folded back toward the heart. Hematoxylin and eosin; × 7. (Upper right) Close-up view of the irregular pattern of the smooth muscle cells in the supravalvar area of thickening. Hematoxylin and eosin; × 107. (Lower right) View of another region of the supravalvar lesion. At this site the adventitia has invaginated in a wedgelike fashion (arrow) into the thickened media. Elastic van Gieson; × 25.

sic pathology involves the inner half of the media.[17] In SAS the elastic fibers are broken and disorganized. There are focal increases in interstitial fibrous tissue and an irregular increase in smooth muscle. The adventitia is usually normal, but in one of Peron's patients it invaginated into the media in a wedgelike fashion.[21]

The aortic wall of many normal human newborns exhibits an incomplete transverse infolding or plica at the upper margin of the sinuses of Valsalva that is conspicuous in some normal aortae but practically absent in others.[29, 30] SAS is thought to be a developmental exaggeration of this normal structure.[29, 30] The present study demonstrates that this mild angulation and infolding of the aortic wall occurs in normal rabbits with varying degrees of prominence. Exaggerated prominence of the normal plica occurred without apparent narrowing of the lumen in a significantly higher percentage of rabbits born to mothers given vitamin D during pregnancy when compared to the controls. The aortae of the offspring whose mothers were fed a diet lacking vitamin D, however, also showed a supravalvar plica, albeit of lesser prominence, suggesting that the presence of the invagination does not depend solely on some action of vitamin D.

In the present study, eight offspring exposed *in utero* to high levels of antirachitic substances, presumably vitamin D, and to the maternal biochemical products of excessive administration of the vitamin revealed pathological abnormalities confined to the supravalvar aortic wall. In six instances the aortic lesions seriously impinged on the lumen and demonstrated essentially all of the histological features of SAS as seen in man.[29] The aneurysmal protrusion of the aorta of one rabbit has been observed in a similar location in SAS in man.[32] A nonobstructing delicate fibrous band crossing the proximal aorta was present in one rabbit. In another rabbit a medial lesion was microscopically evident although no gross abnormality was detected. The six offspring which were sacrificed at age 3 months showed the pathological changes associated with vasculotoxicity in the adult animal,[17-23] although they received a cumulative oral dose of only 22,000 units of vitamin D from birth, leading one to question if they were predisposed to an increased tissue sensitivity to the toxic effects of the vitamin.

From this study it may be concluded that antirachitic substance crossed the hemochorial placenta of the rabbit and that the vascular toxicity of vitamin D can be transmitted across the placenta. There are definite similarities between SAS in the human and the lesions of the aortae in the offspring whose mothers received vitamin D. From these findings it seems reasonable to suggest that an *in utero* derangement in vitamin D metabolism on the part of mother or fetus or of both may be responsible for SAS, especially when the latter is associated with infantile hypercalcemia. It is necessary, however, to be cautious in drawing an analogy between the experimental animal model and the clinical disease. Because of the poorly understood and highly complex interaction between environmental and genetic factors that are involved in the genesis of human malformations, it must be stressed that our study does not supply a simple explanation for the syndrome of SAS and infantile hypercalcemia, but rather raises many questions. No emphasis has been placed on the prophylactic and therapeutic implications of the experimental findings. Further investigations will necessarily be stimulated by such considerations and hopefully will clarify the basic genetic, metabolic, and pathological mechanisms involved.

Summary

Pregnant rabbits were given high doses of vitamin D to determine whether the vitamin crossed the placenta and to explore the relationship between maternal hypervitaminosis D and congenital supravalvar aortic stenosis. The blood levels of antirachitic substance in the mothers given vitamin D and their offspring were 7 and 9 times greater than in the control mothers and offspring, respectively, indicating that transplacental passage oc-

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curred. Serum calcium levels in the offspring whose mothers received vitamin D were significantly higher when compared to control values. A total of 14 abnormalities of the aorta were noted in the 34 offspring whose mothers received vitamin D. Aortic lesions that appeared similar anatomically to supravalvar aortic stenosis in man were noted in six rabbits. Also, one rabbit showed a supravalvar fibrous band and another had an abnormality of the proximal portion of the aorta observed only on microscopic examination. Six additional offspring at age 3 months showed generalized vitamin D vasculotoxicity, without supravalvar narrowing of the aorta, of an advanced type commonly seen in the adult animal given massive doses of the vitamin. Thirty-five control offspring and nine rabbits born to mothers on vitamin D-deficient diets showed no abnormalities of the aorta. The results suggest that an *in utero* derangement in vitamin D metabolism on the part of mother or fetus, or of both may be responsible for supravalvar aortic stenosis, especially when the latter is associated with infantile hypercalcemia. The questions raised by the experimental findings are emphasized.

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Vitamin D as a cause of the supravalvular aortic stenosis syndrome

Before 1958, most textbooks on cardiovascular disease either did not mention supravalvular aortic stenosis (SAS) or gave the entity only passing mention. In that year, Denie and Verbeulat[1] reviewed the 12 previously reported cases of autopsied patients with SAS and described the case of another patient. Many of these patients actually had nonobstructing bands or cords stretched across the lumen of the supravalvular aorta, and 3 of these patients were probably retarded mentally. In 1961,

SAS and a parathyroid adenoma were described in a mentally retarded boy who died of nephrocalcinosis and uremia.[3] The same year, Wooley and his associates[5] described SAS in several otherwise normal members of one family, and a familial incidence was subsequently confirmed by others.[4] When the anomaly occurs in a familial form, it is thought to be transmitted as an autosomal dominant trait with variable expression. Williams and coworkers, also in 1961, described SAS in a group of unrelated patients with mental retardation and a peculiar facies.[5] Beuren[6,7] then extended the features common to the latter type of patient to include stenosis of peripheral pulmonary arteries, dental abnormalities, and strabismus. Subsequently, narrowing of peripheral systemic arteries was added to the already impressive list of features of the sporadic form of this disease, which has come to be called the "supravalvular aortic stenosis syndrome." Chromosome studies, with one exception, have consistently revealed normal karyotypes.[7,8] One patient with peculiar facies and mental retardation showed a 46/47 mosaic pattern, with an extra chromosome resembling the 19/20 group.

In 1963, Black and Bonham-Carter[9] astutely recognized that the elfin facies observed in patients with severe idiopathic infantile hypercalcemia resembled the peculiar facies observed in patients with the SAS syndrome. Shortly thereafter, Garcia and co-workers[10] documented the occurrence of idiopathic hypercalcemia in an infant with SAS, peripheral pulmonary stenosis, mental retardation, and elfin facies in whom the blood level of vitamin D was elevated. The importance of recognizing the association between hypercalcemia and the abnormalities of multiple systems during infancy was later stressed because of the therapeutic implications of early diagnosis and the difficulties encountered in trying to establish a retrospective clinical diagnosis of idiopathic hypercalcemia in the older patient.[11] Recently, SAS, mental retardation, and peculiar facies was observed in twins,[12] and a mentally normal child with congenital hepatic cholesterol-ester storage disease, hypercholesterolemia, and SAS has been studied and operated upon at the National Heart Institute.[13] The latter patient is of particular interest because of the postulated interaction between cholesterol and vitamin D metabolism in the etiology of idiopathic infantile hypercalcemia.[11]

Studies with large doses of vitamin D in nonpregnant animals have emphasized the vascular toxicity of the vitamin. Although the pathogenesis of the abnormalities of the blood vessels remains unclear, it has long been appreciated that excessive vitamin D may produce dose-related vascular lesions, ranging from subendothelial edema to calcification.[14-17] A paucity of information exists in regard to the effects on the fetus of induced maternal hypervitaminosis D, and until recently there was no direct evidence for transplacental passage of vitamin D.[18]

Because it was not known whether the involvement of multiple systems in the SAS syndrome was genetically determined, or whether some or all of the features were related to deranged maternal or fetal vitamin D metabolism or a combination of genetic and in utero factors, large amounts of vitamin D were administered to pregnant rabbits in a preliminary exploration of the relationship between hypervitaminosis D in the mother and the development of SAS in the offspring.[19] A total of 14 abnormalities of the aorta were noted in 34 offspring whose mothers had received vitamin D. Aortic lesions that resembled those of SAS in man were noted in 8 rabbits. Six additional offspring that were sacrificed at the age of 3 months showed generalized vitamin D vasculotoxicity of an advanced type commonly seen in the adult animal given massive doses of the vitamin, but did not exhibit narrowing of the supravalvular aorta. Thirty-five control offspring and 9 rabbits born to mothers on diets deficient in vitamin D showed no abnormalities of the aorta. These results suggested that a derangement of vitamin D metabolism during pregnancy on the part of mother or fetus, or both, may be responsible for SAS, especially when the latter is associated with infantile hypercalcemia. Most recently, studies were undertaken to explore the relationship between the characteristic facial and dental features of the SAS syndrome and exposure to vitamin D during pregnancy. Abnormalities of the craniofacial complex have been observed in 70 per cent of the offspring of rabbits given large amounts of the vitamin during pregnancy.[20] The findings resemble anatomically the skeletal and orthodontic manifestations of the SAS syndrome and suggest that the peculiar facies and abnormal dentition, like the aortic lesion, are related to deranged vitamin D metabolism during pregnancy. Because of the poorly understood and highly complex interaction between environmental and genetic factors that are involved in the genesis of human malformations, it should be stressed that the studies in rabbits do not provide a simple explanation for the syndrome of SAS and infantile hypercalcemia, but rather raise many questions. It is still not clear why varying sensitivities to vitamin D exist between different individuals of the same species and between different species. Also, the differences in sensitivity of various organ systems to the vitamin have not been clarified. Requiring elucidation are the precise role of vitamin D in the etiology of infantile hypercalcemia; the role of prenatal mineral balance and the manner in which excessive vitamin D or a lowered threshold to the toxic effects of vitamin D may alter metabolic processes during pregnancy; the time during gestation that vitamin D may interfere with the normal development of the aortic wall, jaws, and teeth; and the effects on these lesions of postnatal exposure to vitamin D. In particular, the mechanism by which vitamin D produces vascular damage still requires definition. It is not clear whether vitamin D has a local action on vascular tissue or whether it exerts its effects by altering calcium or fat metabolism or in some other undefined manner.

Emphasis on the prophylactic and therapeutic implications of the findings in experimental animals has been deferred until the basic mechanisms involved are clarified further. Spontaneous healing and complete repair of the cardiovascular lesions induced by vitamin D have been reported to occur in experimental animals, a finding which raises the

question of whether prompt diagnosis and treatment of the metabolic disorder in infancy may retard the progression of the cardiovascular lesions. Ultimately, it is possible that a means of deterring pregnancies susceptible to the teratogenic effects of vitamin D or related steroids may lead to the prevention of the SAS syndrome. The implications are sufficient to warrant a major effort to obtain epidemiologic, genetic, metabolic, and pathologic information relating to the disease.

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THE RELATIONSHIP BETWEEN VITAMIN D AND THE CRANIOFACIAL AND DENTAL ANOMALIES OF SUPRAVALVULAR AORTIC STENOSIS SYNDROME

1950

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ABSTRACT. There is evidence that a derangement in vitamin D metabolism on the part of the mother, the fetus, or both may be responsible for supravalvular aortic stenosis, especially when the latter is associated with infantile hypercalcemia. It has been shown that the offspring of rabbits given large amounts of parenteral vitamin D throughout pregnancy may be born with aortic lesions resembling supravalvular aortic stenosis as seen in man. The present study was designed to explore experimentally the relationship between exposure to excessive amounts of vitamin D during pregnancy and the development of the craniofacial complex and dentition because children with the multiple system abnormalities of the supravalvular aortic stenosis syndrome (SASS) share a characteristic craniofacial appearance and exhibit, in common, many abnormalities of dentition. Examination of their jaws and teeth reveals hypoplasia of the mandible, congenital absence of teeth (usually the lateral incisors and second premolars of the maxilla), microdontia, dysgnathia, enamel hypoplasia, and a narrowed occlusal table. In the present study, analogous findings were observed in rabbit offspring whose mothers received large amounts of vitamin D during pregnancy. The most marked functional accompaniment of these abnormalities was severe malocclusion of the teeth. Peculiar facies, premature closure of the cranial bones, strabismus, odd shaped ears, and a low birth weight were additional features of SASS that were noted in many test animals. Buphthalmos was also noted occasionally, probably related to maldevelopment of the bony ocular orbits. Thus, these experimental observations suggest that the cranial, facial, and dental peculiarities as well as the aortic lesion of the supravalvular aortic stenosis syndrome may be related to a derangement in vitamin D metabolism during pregnancy. *Pediatrics*, 43:12, 1969, IDIOPATHIC HYPERCALCEMIA, CRANIOSYNOSTOSIS, MALOCCLUSION, FETUS, CONGENITAL MALFORMATIONS, VITAMIN D.

In the past 10 years there has been a growing interest among pediatricians and cardiologists in a relatively uncommon condition called supravalvular aortic stenosis (SAS). This cardiovascular anomaly appears to occur in three clinical settings. SAS may be noted sporadically as an isolated lesion in otherwise normal patients.[1] More often, the aortic anomaly, associated with peripheral pulmonary arterial stenosis, occurs in familial aggregates suggesting transmission as an autosomal dominant trait with variable expression.[2,3] The sudden interest in SAS stems from recent recognition that, in its third setting, the lesion may be associated with idiopathic infantile hypercalcemia,[4,5] a disease probably related to deranged vitamin D metabolism.[6,7]

The designation "supravalvular aortic stenosis syndrome" (SASS) has been applied to the distinctive clinical picture produced when the cardiovascular anomaly and idiopathic infantile hypercalcemia coexist.[8] The features comprising the full expression of SASS include a characteristic "elfin" facies, psychomotor and growth retardation, strabismus, anomalies of dental development, craniosynostosis, narrowing of peripheral pulmonary and systemic arteries, inguinal hernias, cryptorchidism in males, and early development of secondary sexual characteristics in females.[8-10]

Whether none, some, or all of these features may result from a derangement in maternal or fetal vitamin D metabolism is a question of current concern. In a previous

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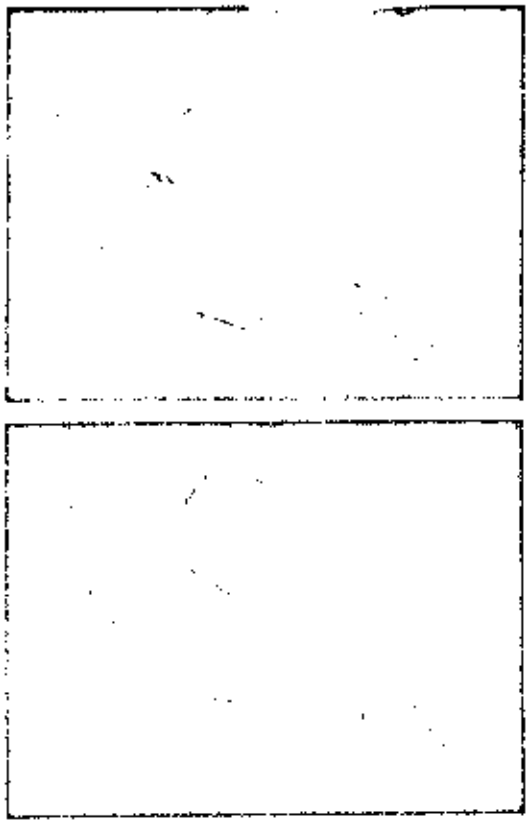

FIG. 1. Cranial view of the frontal fontanel and the coronal and sagittal sutures in representative 1-day-old neonates (top, test animal; bottom, control). Note the premature closure of the fontanel and bossing of the paired frontal bones and paired nasal bones, and somewhat flattened contour of the zygomatic arches in the test animal.

study from this laboratory it was shown that the offspring of rabbits given large amounts of parenteral vitamin D throughout pregnancy were born with aortic lesions that seriously impinged on the lumen of the aorta and demonstrated essentially all of the histological features of SAS as seen in man.[11] Maternal intoxication with vitamin D has also produced similar lesions in rats.[12] Because children with SASS share a characteristic craniofacial appearance and exhibit, in common, many abnormalities of dentition, the present study was designed to explore experimentally the relationship between exposure to excessive amounts of vitamin D during pregnancy and the development of the craniofacial complex and dentition. The results of this investigation were reported in preliminary form.[13]

MATERIALS AND METHODS

Fifteen pregnant, white New Zealand rabbits, designated "test" animals, were fed a stock diet* and given divided doses of intramuscular vitamin D_2† (activated ergosterol in cotton-seed oil) every other day, starting on the second day after insemination and continuing until delivery. The total dose administered was 750,000 units. The offspring of six pregnant females who received injections of the cotton-seed oil only and of 15 pregnant females given no supplement served as controls.

Whenever a test offspring was sacrificed or died spontaneously, a rabbit of the same size born to a control mother and selected by a random number technique was sacrificed. Each animal was decapitated, and after several days fixation in 80% ethyl alcohol, the soft tissues were removed to expose the bony cranio-facial complex. A three-dimensional orthodontic calipers was employed for measuring dental arch dimen-

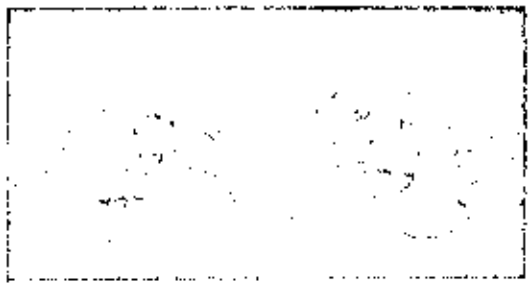

FIG. 2. Lateral view of the articulated jaws in representative 1-day-old neonates (left, test animal; right, control). Note the significant anterior dental crossbite in the test animal. The head of the mandibular condyle is maximally posterior in the glenoid fossa in both. The angle of the ramus is more obtuse in the test rabbit.

* Purina Lab Chow.

† Hi-Dematol injectable, Brewer and Co., Inc., Worcester, Massachusetts.

FIG. 3. Representative occiput views of 1-day-old littermates (left, test animal; right, control) showing flattened skull contour in test rabbits. In the test offspring the external occipital protuberance was sharper and more prominent and the nuchal surface roughened. The occipital fontanel, at the union of the lambdoidal and sagittal sutures, closed prematurely in test rabbits.

sions, and radiographs, casts, and histological sections of the skull and jaws were examined.

RESULTS

Control Offspring

The 6 females that had received cottonseed oil injections delivered 54 young and the 15 females given no supplement delivered 140 young. The average litter for both groups contained nine kits with a mean birth weight of 43.7 ± 1.1 gm (*SEM*) and crown-rump length of 9.9 ± 0.2 mm. Within 7 days of parturition, 30 (16%) of the control offspring died spontaneously. The external appearance, the contour of the craniofacial bones, and the dental anatomy were remarkably similar in all control offspring.

Test Offspring

The test offspring numbered 110, an average of seven kits per litter. The average weight of each test newborn was significantly less than control offspring (35.3 ± 0.4 gm, $P < 0.01$), as was the crown-rump length (7.5 ± 0.3 mm, $P < 0.01$). Within 7 days of parturition 95 (86.4%) of the test offspring died spontaneously. While the occurrence of spontaneous death was significantly greater than in the control group ($P < 0.001$), no attempt was made in this study to define its etiology. The remaining 15 rabbits were sacrificed at age 90 days.

Offspring Less Than 7 Days of Age

Characteristic malformations were noted upon exposure of the bony parts of the test skulls. Premature closure of the saggital, coronal, and lambdoidal sutures was a constant finding (Fig. 1) and provided the most striking difference in the external appearance of the skull when compared with control offspring. The skulls of the test animals, matched chronologically or by equiv-

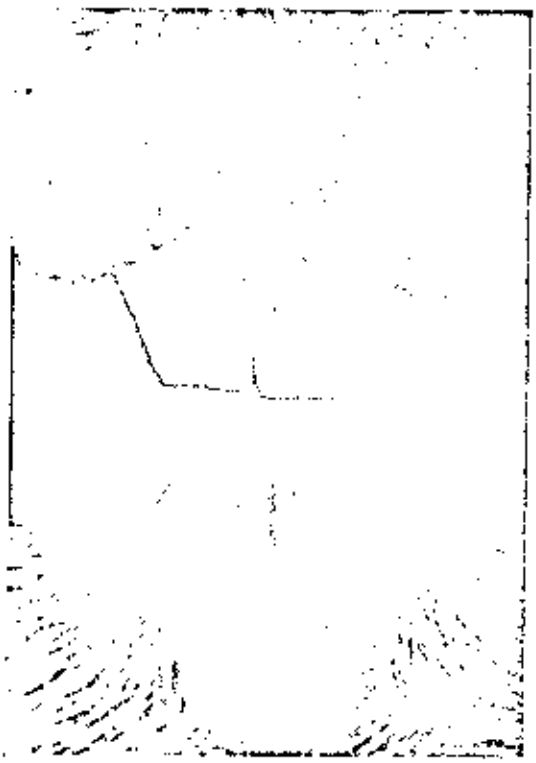

FIG. 4. Labial view of the maxillary central incisors in representative 1-day-old offspring. The incisal third of the central incisors of the test rabbit in the lower portion of the photo show characteristic grooving and pitting associated with enamel hypoplasia. The upper portion of the photo shows normal incisors.

alent size with the controls, were smaller in the anteroposterior plane and more narrow in the coronal plane (Fig. 1). The body of the mandible was narrower, the angle of the ramus was more obtuse, and the premaxillary and nasal bones were smaller and somewhat compressed in appearance (Fig. 2).

The paired nasal bones displayed bossing near their posterior borders and the paired frontal bones exhibited marked bossing slightly posterior to the supraorbital ridges. The paired parietal bones appeared flattened in test animals, creating a level, rather than a raised and rounded, appearance posteriorly (Fig. 3). Caudally, the occipital bones were smaller and their nuchal surface were rougher when compared to controls. The external occipital protuberance was particularly prominent in test offspring (Fig. 3).

Striking differences in dentition were noted between the groups. In the test sample, the maxillary and mandibular central incisors exhibited severe enamel hypoplasia of the incisal one third of the tooth (Fig. 4). This finding occurred in 95% of the test progeny. A prominent anterior crossbite was noted in 66% of the test offspring (Fig. 2).

Ninety-Day Offspring

Additional abnormalities were noted in the 15 surviving test offspring (Fig. 5 and 6). Strabismus was present in 80% and buphthalmos was present in 30%. The ears of all of the test animals were 8 to 10 mm shorter than controls, while a deep notch of the apical portion of the pinna of one or both ears was noted in half of the former animals. In addition to the abnormalities in dentition noted in the neonates, partial anodontia and an altered palatal contour was observed in the older test rabbits. The maxillary second premolars were absent bilaterally in 60% (Fig. 7). Moreover, the shortening of the premaxillary and maxillary bones, and the acute angle of inclination of the alveolar process of the maxilla resulted in a higher, "cathedral-type," palatal vault (Fig. 8). The arch breadth, measured at the level of the first molar, averaged 25.7 ± 1.0 mm in the control rabbits and was significantly less in the test group (22.3 ± 1.1 mm, $P < 0.05$). The length of the maxillary arch was also diminished in test animals (29.5 ± 1.1 mm for controls, 21.7 ± 1.1 mm for test rabbits, $P < 0.01$). In the control rabbits mandibular

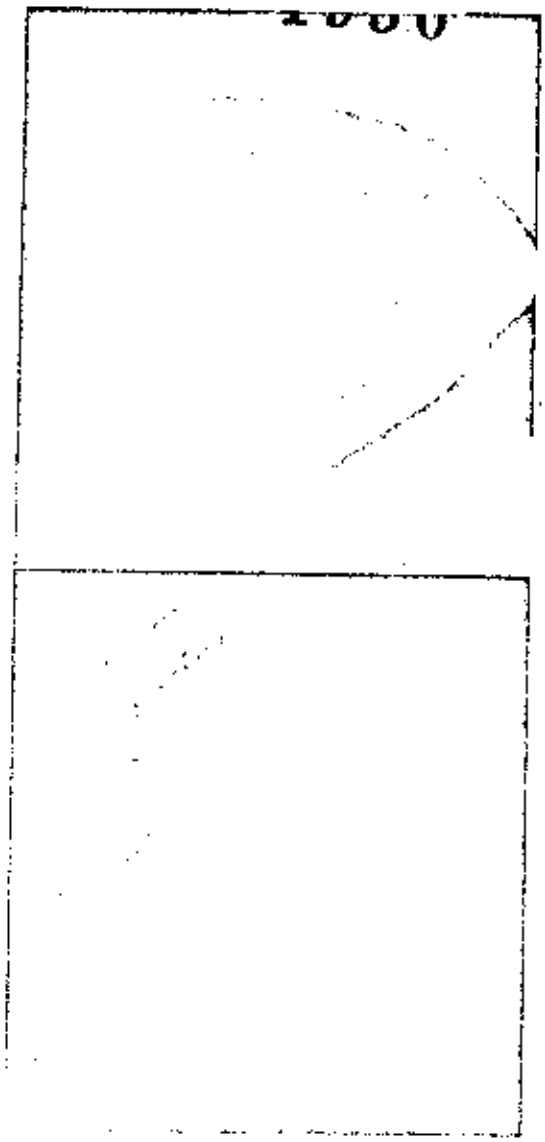

FIG. 5. Representative views of the ears in 90-day-old normal (top) and test (bottom) offspring. Nontraumatic notching of the apical portion of the pinna occurred in 50% of the test surviving progeny.

breadth and length, measured in a similar way, averaged 20.3 ± 1.0 mm and 24.3 ± 1.1 mm, respectively, and were significantly less in the test animals (19.3 ± 1.0 mm and 20.0 ± 1.2 mm, $P < 0.02$).

DISCUSSION

Since the recognition of infantile hypercalcemia in 1952, an abnormality of vitamin D metabolism has been assigned a causal role in the disorder.[14] However, it is clear that ingestion of a large amount of the vitamin is not an etiologic prerequisite in all patients, and metabolic studies have provided strong circumstantial evidence that the disease may, in some instances, be attributable to individual hypersensitivity to vitamin D or its abnormal metabolism.[5,7,15] Unfortunately, there are no systematic metabolic studies of the mothers or of the pregnancies resulting in afflicted infants. Furthermore, very few experimental studies in animals exist concerning the effects on the fetus of maternal exposure to vitamin D, and direct evidence of passage of the vitamin transplacentally has only recently been obtained.[11] However, it is interesting that one mother of two children with the severe form of infantile hypercalcemia has been observed to develop symptoms associated with hypercalcemia after vitamin D provocation, while the father did not.[16]

It seemed reasonable from preliminary experimental results in rabbits[11] to suggest that a derangement in vitamin D metabolism on the part of the mother, the fetus, or both may be responsible for supravalvular aortic stenosis, especially when the latter was associated with infantile hypercalcemia. The present study was designed in a similar was as a preliminary exploration of the relationship between hypervitaminosis D in the mother and the development in the offspring of the craniofacial and dental abnormalities observed in SASS. Examination of the jaws and teeth of children with the SASS reveals hypoplasia of the mandible, congenital absence of teeth (usually the lateral incisors and second premolars of the maxilla), microdontia, dysgnathia, enamel hypoplasia, and a narrowed occlusal table.[8] In the present study, analogous findings were observed in the rabbit offspring whose mothers received large amounts of vitamin D during pregnancy. The most marked functional accompaniment of these abnormalities is severe malocclusion of the teeth. Peculiar facies, premature closure of the cranial bones, strabismus, odd shaped ears, and a low birth weight were additional features of SASS that were noted in many test animals. Buphthalmos was also noted occasionally, probably related to maldevelopment of the bony ocular orbits.

Thus, these experimental observations suggest that the cranial, facial, and dental peculiarities as well as the aortic lesion may be related to a derangement in vitamin D metabolism during pregnancy. Moreover, the ease of recognition and the higher incidence of skeletal and orthodontic malformations (70 to 95%) than cardiovascular lesions (40%)[11] in test neonates indicates that the former findings may be the most useful

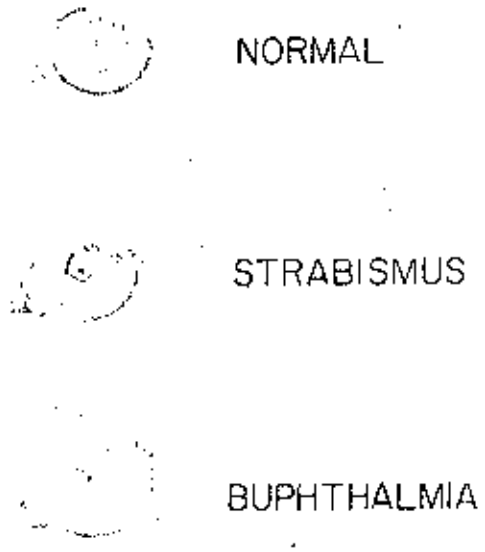

FIG. 6. Representative views of the eyes in 90-day-old offspring. Strabismus and buphthalmia were noted only in test animals.

markers for future testing of vitamin D teratogenicity and may facilitate efforts directed at relating dose and time of administration to the appearance of congenital abnormalities. Not studied in the present investigation was the specific relationship between the abnormalities of bone and dental development and the known effects of vitamin D on calcium and phosphorus metabolism. In a previous study[11] significant hypercalcemia was seen in offspring of mothers given large amounts of the vitamin.

It is important to interpret cautiously the specific relevance of the descriptive animal findings and the etiology of SASS as it appears in man. Most congenital malformations appear to result from complex interactions between multifactorial genetic and environmental systems. Although the rabbit studies suggest that excess vitamin D produces structural malformations either by a direct damaging effect or by creating in the mother in adverse environment for normal

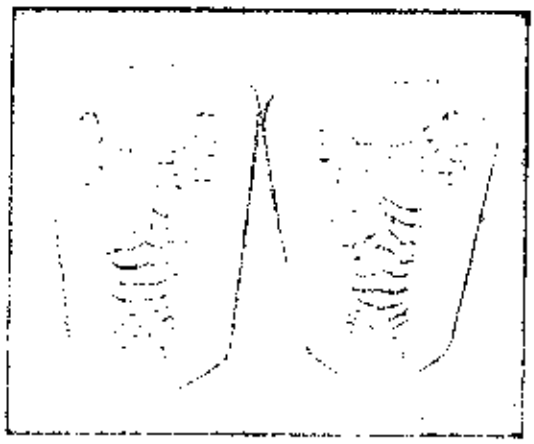

FIG. 7. Representative dental casts with occlusal view of the permanent maxillary dentition at 90 days of age (left, control; right, test animal). Three premolars and three molars are present in the control whereas the maxillary second premolars were congenitally missing bilaterally in the test animals, significantly shortening the occlusal table. In addition, the distance from the mesial surface of the first premolar to the labial surface of the central incisors was significantly less in the test animals.

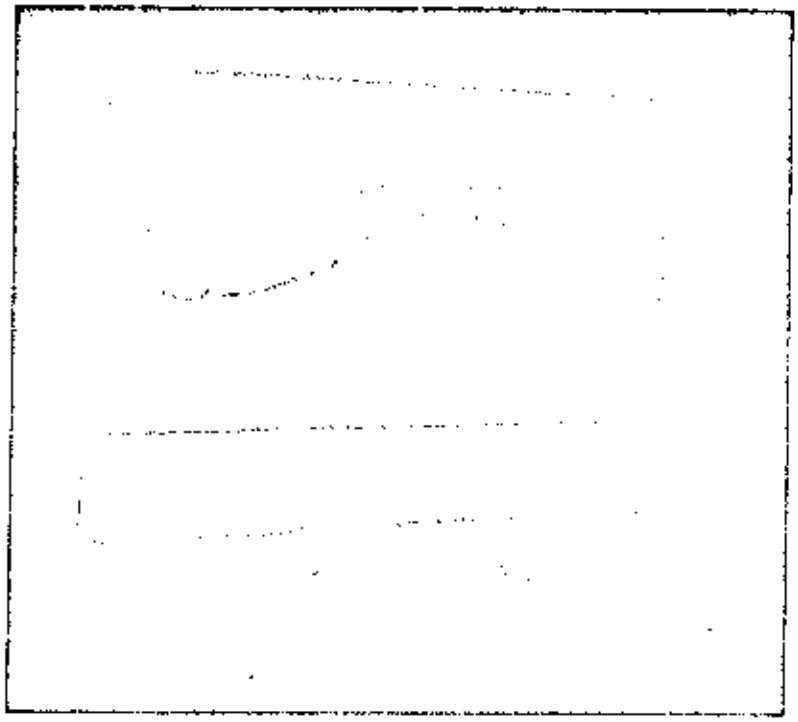

FIG. 8. Lateral view of the maxilla in representative 90-day-old animals (top, test animal; bottom, control) showing the more inclined plane of the alveolar process and the foreshortening of the premaxilla in the test animal that results in a high "cathedral-like" palatal vault.

fetal development, the extent and manner in which genetic predisposition may be the mediating factor in pathogenesis is unclear.

In the human it has become evident that the typical elfin facies may be observed in the familial form of SAS[17] and that abnormal dental anatomy and varying degrees of mental subnormality may occur in patients with SAS and normal facies.[20] Retrospective surveys have failed to disclose excessive intakes of vitamin D in either the mothers or infants with SASS[10] and vitamin D tolerance testing has provided varying results.[10,18] An explanation for the differences in sensitivity to vitamin D between different individuals of the same species, between different species, and between different organ systems is still forthcoming. Requiring elucidation is the precise role of vitamin D in the etiology of infantile hypercalcemia—the role of prenatal mineral balance and the manner in which excessive vitamin D or a lowered threshold to the toxic effects of vitamin D may alter metabolic processes during pregnancy. The time during gestation that vitamin D or its effects may interfere with the normal development of the aortic wall, dental and skeletal structures, and the effects on the various lesions of postnatal exposure to vitamin D is not known. Furthermore, the mechanism by which vitamin D produces pathologic damage still requires definition.

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GIANT FOOD INC.

PETE MISLER
VICE PRESIDENT
DELICATESSEN & DAIRY OPERATIONS

BOX 1804 • WASHINGTON, D.C. 20013

April 17, 1974

Humphrey F. Sasson, Ph.D.
Tracor Jitco, Inc.
1300 East Gude Drive
Rockville, Maryland 20851

Dear Dr. Sasson:

During 1969, when we opened our Dairy operation, we manufactured both the plain homogenized and Vitamin D milk. There was a 5¢ spread in retails between the two milks.

In 1971, the retails were the same and sales dropped on the plain homogenized making it no longer economically possible to continue to process plain homogenized milk.

Sincerely yours,

Pete Misler

Pete Misler,
Giant Food, Inc.

PM/ms

Exp. Med. Surg. 14 (136-143) (1956)

Calcium, Phosphorus and Vitamin D as Factors Regulating the Integrity of the Cardiovascular System

By JOSEPH GILLMAN and CHRISTINE GILBERT

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Almost 30 years ago, observations made in Johannesburg suggested that many forms of cardiovascular disease, including thrombosis, affecting the white population occurred only infrequently amongst the Africans and other economically-undeveloped peoples (1, 2, 3). Since that time greater factual characterisation of cardiovascular disease in our city confirmed this first impression (4, 5, 6, 7).

The growing appreciation that cholesterol and its protein complexes may be causally related to atherosclerosis has stimulated surveys among ethnic groups in various parts of the world to ascertain whether or not a correlation exists between the frequency of atherosclerosis and dietary habits. The results of such surveys have indicated that in many instances a positive correlation exists between the fat intake and the frequency of atherosclerosis. However, in some circumstances, the co-existence of atherosclerosis and a high fat consumption has not always been established. Moreover, in some diseases hyperlipaemia may establish itself even when the diet is low in fat. A trend is now appearing away from dietary fat to the more complex lipoproteins and their derivatives obtained either through the diet or through some endogenously-related metabolism (8).

The deposition of lipid in organs and tissues is dependent upon a complex biochemical process as is evident from the extensive literature even on a limited aspect of the problem as in the case of hepatic steatosis. While it would be satisfying and also important in clinical practice to establish a direct relationship between one factor, like the

consumption of cholesterol or fat and a complex reaction like atherosclerosis, our experience over the last 20 years has shown the remarkable infrequency of such a one-one relation in biological processes. The absence of this one-one relationship in the case of atherosclerosis is well demonstrated by the peculiar localisation of atheroma even where hyperlipaemia is present. Further investigation of the pathogenesis of atherosclerosis has led to the suspicion that some injury to the arterial wall may produce a locus minoris resistentiae permitting in some instances the early deposition of fat. In other words injury to the vessel wall may still be a primary event (9 to 27). Offset against atherosclerosis is the other pattern of cardiovascular disease accompanying hypertension. In the latter, injury to the vessel wall can be consistently established and such injury may eventually lead to one form of arteriosclerosis.

The demonstration that in particular circumstances severe kidney disease can initiate a syndrome having as one of its manifestations widespread vascular disease has provided a new approach to one form of vascular disease, the fruits of which are manifest in the contributions of *Goldblatt* and his co-workers (28, 29), *Braun-Menendez* (30), *Grollman* (31) and others. The experimental data have disclosed thus far that in particular circumstances, not fully understood, severe injury to the kidney can be followed by extensive lesions in the heart and in the arterial tree. Some of the lesions produced experimentally simulate those described in the rheumatic and pararheumatic diseases. Furthermore, it is common knowledge that extensive calcification of the vascular tree may occur secondarily to kidney disease (32, 33).

It is current practice to regard as separate entities, atherosclerosis and those vascular lesions which are not associated with any lipid infiltration in the intima or media of the affected arteries, although it is accepted that both kinds of processes can lead to sclerosis. In approaching experimentally the problem of vascular disease, which may be accompanied by necrosis and lipid infiltration and which may terminate in sclerosis, we gave consideration first to the fact that a defined stimulus, like carbon tetrachloride, could produce in the liver, for example, a fatty change, necrosis or a combination of both reactions, either of which could result in restitution or fibrosis, depending on the nutritional and endocrine status of the animal (34). That is to say, cell injury, induced by the same stimulus, could assert itself in seemingly different ways although the end picture could be similar, namely sclerosis. Secondly, we accepted provisionally, with others, that

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lipid infiltration of the wall of an artery was not an automatic response to chronic hyperlipaemia, whatever its nature, but rather that chronic lipaemia was a manifestation of some underlying metabolic disorder which could produce injury to the vessel wall and only thereafter, and in special circumstances still to be defined, would result in lipid infiltration. Thirdly, we recognised that the relative infrequency and mildness of atherosclerosis in the African, while correlated with a low fat intake, could also be correlated with a low calcium and a high phosphorus intake in a dietary context low in animal proteins but containing abundant cereals. Indeed, on reviewing the published information from those regions where atherosclerosis is prevalent and those areas where it is infrequent, it seemed that the intake of calcium and phosphorus was also a significant variable. Fourthly, severe cardiovascular injury could be consistently produced by methods known to influence in some way the metabolism of phosphorus and calcium, namely, excess vitamin D and parathormone against a dietary background of a high calcium intake. In this connection we appreciated from the fundamental work of *Burger* and *Schlenker* (35) and *Lansing* and his co-workers (36) that mineralisation of the aorta was part of an age process but that such mineralisation was probably dependent on the calcium intake and on those factors, such as vitamin D and animal proteins, which promoted the absorption of calcium. Furthermore, it was clear that the present-day emphasis on milk as a food, supplemented with vitamin D might not only have delayed repercussions but also might be responsible in part for acute episodes in children, manifest as hypercalcaemia (37, 38, 39) as well as contribute to the almost universal occurrence of varying degrees of nephrocalcinosis in the adult inhabitants of the United Kingdom (40). Indeed, *Pyrah* (40) demonstrated that in 10% of apparently normal kidneys examined radiologically and in 22% examined histologically, there was evidence of calcification. Finally, we entertained the possibility that the frequently reported occurrence of hypertension in the African might be related in some way to the high phosphorus-low calcium intake as well as to an insufficiency of animal proteins.

Having regard to all these possibilities, as part of an enquiry into the factors regulating the integrity of the cardiovascular system, we decided to reinvestigate the effects of vitamin D on the cardiovascular system. We were fully aware that in the past, numerous investigators had established that diffuse calcification could be produced by excess vitamin D (41). However, it was equally apparent that apart from the calcifying lesions there was little information about other vascular effects of this vitamin. Even where calcification supervened, the deposition of calcium was ascribed to the ensuing hypercalcaemia.

In this preliminary study, it will be our purpose, first, to re-examine the sequential changes observed in the aorta, coronary arteries and in the heart following the administration of varying amounts of vitamin D; secondly, to show that it is possible to uncouple vascular injury from calcification, thirdly, to draw attention to the possibility that the effects of excess vitamin D may be dependent on or complicated by impairment of kidney function and of other tissues and organs, thus linking the arterial lesions produced by vitamin D with those known to follow severe kidney disease, induced by techniques required for the study of experimental hypertension and, fourthly, to emphasise the remarkable regenerative capacities of the aorta, coronary arteries and myocardium. In this connection it will be indicated that extensive necrosis of the myocardium need not necessarily be followed by fibrosis, revealing that in the heart, no less than in the liver, special conditions need to be fulfilled before necrosis is followed by the elaboration of fibrous tissue. Finally, mention will be made of the fact that despite extensive destruction of the wall of the aorta, of the coronary arteries and of the endocardium, repair may be accomplished without the supervention of thrombosis.

Material and Methods

A series of 300 rats, consisting of 220 males and 80 females, ranging in age from 3 to 6 months, were used in the present investigation. Vitamin D, in the form of re-crystallised, highly purified calciferol was dissolved in destearinated peanut oil. The rats were divided into 5 groups. The first group received a single dose of vitamin D, the second group, two doses at daily intervals, the third group, three doses, the fourth group four doses and the fifth group, five doses. In all cases, vitamin D was administered orally by dropper at daily intervals.

The rats were killed at standard times after the last administration of vitamin D, namely at 2, 4, 8, 10, and 21 days. In the fifth group, which received five doses of 25,000 units vitamin D, rats were killed in addition to the times mentioned above at 12-day intervals after the 21st day until the 200th day after the last administration of vitamin D.

The tissues were preserved in neutral formalin and paraffin sections

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prepared for histological examination. Histochemical tests for iron, mucopolysaccharides, calcium and lipids were performed by standard techniques on selected tissues.

Observations

For convenience of description, it has been decided to examine the lesions first in the aorta, then in the coronary arteries and later those in the myocardium and in the valves. The large range of reactions encountered in our experimental animals led us to present the information in the form of a pathogenesis rather than as an inventory of the kind of responses that are to be anticipated after the administration of vitamin D. Reconstruction of the pathogenesis of the various lesions was greatly facilitated by killing animals at the intervals specified above. While it is realised that not all rats behave in the same way to the same stimulus, nevertheless, the amount of material examined allowed us to be reasonably confident at least of the trends in the pathogenesis of the main lesions.

Reactions in aorta

The reactions, evoked in the aorta by excess vitamin D depend upon whether (a) the initial injury is confined to the endothelium and sub-endothelial tissues (b) the superficial part of the media is injured in conjunction with the endothelium with or without a variable degree of calcification and (c) the endothelium remains intact anatomically but the media becomes extensively calcified.

(a) Damage to the endothelium and sub-endothelial tissues when it occurs in the aorta is confined to the sinus of Valsalva and the first part of the aortic arch. Mild sub-endothelial oedema is accompanied by lifting of the overlying endothelial cells, a variable degree of metachromasia and the accumulation of Schiff-positive material (Fig. 1). Local emigration of a few leucocytes and slight swelling of the endothelial cells associated with rounding of the nucleus results in a small 'bleb' similar to that described in rheumatic endocarditis of the aorta (42). The 'bleb' may become detached or undergo resorption.

More frequently, sub-endothelial oedema is associated with increased "stickiness" of the endothelium and a tendency for glycogen-laden polymorphs to become agglutinated, reminiscent of the leukergic phenomenon described by *Fleck* and *Lille-Szyrkowicz* (43). The clusters of polymorphs adhere to the endothelium forming small vegetations

studding the wall of the aorta over some distance (Figs. 2 and 3). The vegetations are encapsulated by endothelium in a manner similar to that described by *Harrison* (44) and by *Duguid* (45, 46). By narrowing of the pedicle, the vegetation may eventually be separated from the intima and cast into the lumen of the aorta.

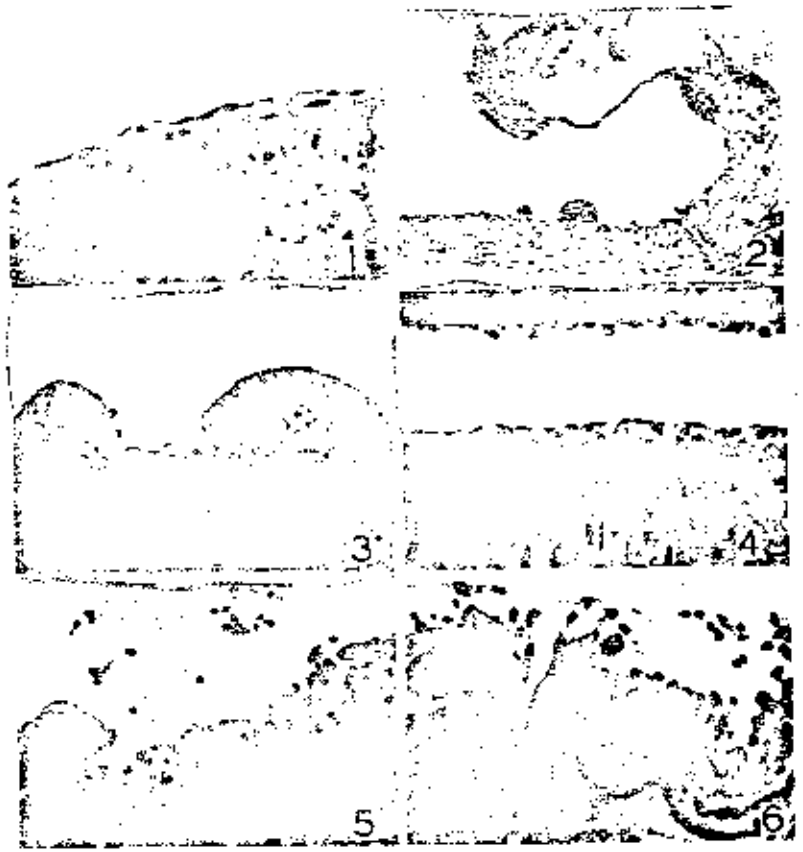

Fig. 1. Sub-endothelial oedema in the root of the aorta.
Fig. 2. Vegetations above the sinus of Valsalva and sub-endothelial cellularity above and below the attachment of the aortic valve.
Fig. 3. High power of the sinus shown in Fig. 2 showing the cellular vegetations on the inner lining of the aorta.
Fig. 4. Sub-endothelial cellularity in the root of the aorta with marked vacuolation of the media.
Fig. 5. Multinucleated giant cells lying in the cellular intima of the aorta.
Fig. 6. Calcification of the elastic lamina, degeneration of the smooth muscle, infolding of the intima and intense, sub-endothelial oedema, cellularity and giant cells.

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When injury to the endothelium is more extensive, the sub-endothelial region is invaded mainly by cells derived from the proliferating endothelium and to a lesser extent by lymphocytes and monocytes which have migrated from the lumen of the vessel (Fig. 4). The cellular plaque thus formed is covered by an irregular layer of rounded cells which merge imperceptible with the flattened cells of the neighbouring endothelium. Not infrequently, the cellular plaque contains multinucleated giant cells (Fig. 5).

(b) *Endothelial and sub-endothelial damage with or without some medial calcification*

Oedema of the intermuscular tissue of the media accompanied by the presence of Schiff-positive material and metachromasy, sometimes emphasised by the accumulation of lipid, is one of the mildest forms of medial injury. These changes are usually preceded by swelling of the cells, marked hyperchromasy of the nuclei or even by frank degeneration of the endothelium. Transitory calcification of the related elastic laminae is followed by degeneration of the muscle cells, the liquefied remains of which contrast with the sharply outlined, robust elastic fibres. However, calcification can occur rapidly in which case it involves many laminae. Calcium may be resorbed initially without any visible participation of cells. As a consequence, the affected elastic fibres become increasingly metachromatic and large clear, fluid-filled spaces replace the degenerated muscle fibres and widely separate the elastic fibres. At this stage the inner part of the wall is thrown into folds with some bulging of the outer wall, the latter being easily appreciated with the naked eye when the chest is opened in the anaesthetised animal. Slight infolding of the intima produces numerous shallow furrows (Fig. 6), from the margins of which cells proliferate and migrate into the depths of the furrows and between the laminae, thereby enhancing the cellularity of the superficial part of the media. The small cells with large, chromatic nuclei and intensely basophilic cytoplasm, thus formed soon acquire endothelioid features. Thereafter, the connective tissue cells below and at the edges of the injured site also enlarge, proliferate and migrate to meet the cells derived from the endothelium. A cellular plaque (Fig. 7) is thus fashioned with the long axes of the cells arranged at right angles to the endothelium (Fig. 8) resembling very much the plaque developing spontaneously in the aorta of dogs (47). Migration of numerous endothelioid cells into the spaces between the laminae, together with the invasion of fibroblasts and possibly of some

muscle cells, still further deform the remnants of the elastic fibres. At this stage, the flat, endothelial cells become spheroidal and elaborate an abundant, semi-solid matrix. Finally, the continuity of the inner

Fig. 7. Cellular plaque on the root of the aorta showing very wavy elastic fibres and proliferated endothelium.

Fig. 8. High power of cellular plaque shown in Fig. 7 showing flat cells and abundant matrix occupying the re-constituted intima.

Fig. 9. "Chondrification" of the reconstituted intima and media of the aorta, with progressive displacement of the calcified plaque towards the adventitia.

Fig. 10. High power of luminal surface of "chondrified" wall of aorta shown in Fig. 9. Note the abundant matrix, cartilage-like cells and fibrils in the matrix.

aspect of the intima is restored but the underlying media is occupied by a cellular tissue not unlike foetal cartilage. In appropriately stained sections a meshwork of delicate elastic fibres, derived from the unaffected laminae, is embedded in the abundant ground substance.

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Intense sub-endothelial and medial calcification may lead to the formation of solid plates which usually approach the endothelium at one point. Sometimes, these plates may erode the endothelium to project freely into the lumen of the aorta without producing any clots. Repair of this kind of lesion is slow and may take several months, but even after it is repaired the vessel wall remains permanently abnormal. Although functionally efficient, having regard to the gross disorganisation induced by vitamin D, the endothelial cells are again mainly responsible for covering the calcified spicule. Calcium is at first partially resorbed without any obvious cellular activity. The proliferating endothelial cells spread over the surface of the spicule which is then separated from the lumen. Some of the endothelial cells invade the calcified spicule facilitating the resorption of calcium. In the process, giant cells, resembling osteoclasts, may appear in considerable numbers. The calcified plates are progressively covered by an increasing number of layers of endothelial cells and the calcium plate gradually becomes reduced and more deeply embedded in the much thickened wall of the aorta (Fig. 9).

Once the spicule is covered by endothelial cells and as the calcium is removed, an abundant matrix is again elaborated. This matrix is separated from the lumen of the aorta by a flat layer of cells similar to that commonly encountered over the free surface of articular cartilage (Fig. 10). In contrast to articular cartilage, the matrix in the aorta is feebly Schiff-positive and stains faintly metachromatically.

At the end of 150 days after the last administration of vitamin D, the original calcified part of the aorta may be replaced by a thick, cartilage-like material with calcified tissue forming an irregular band in the deeper layers of the artery and sometimes reaching to the adventitia (Fig. 9).

Calcification of the media, with minimal involvement of the intima, occurs mainly in the lower thoracic and abdominal segments of the aorta. Calcium is deposited on the elastic laminae often spreading to involve the entire width of the wall of the artery. This reaction may be unassociated with visible change in the endothelium or adventitia and may persist unmodified for several weeks. At first the calcium is slowly resorbed without any obvious cellular response, thereby exposing the "ghosts" of the non-nucleated muscle cells as described by *Duguid* (48). Neighbouring, "unaffected" cells proliferate and gradually replace the "ghost" cells. As a consequence, the interlaminar regions become more widely separated. The irregular arrangement of the elastic laminae in the injured region may be the only vestige of previous damage to the vessel.

Cellular response following medial calcification of the aorta is only provoked, either when the endothelium is injured or when the calcified bar fractures and damages the intima or the adventitia. If the endothelium alone is damaged, the mode of resorption of the calcium is similar to that described above in the aorta except that the reaction is less intense and only rarely is repair effected by the formation of pseudo-cartilage. When the calcified bar fractures, a remarkable series of events is enacted as can be appreciated from an examination of Figs. 11 to 17.

In Fig. 11, the broken spicule is dislodged and the endothelium is replaced by several layers of flattened cells. Connective tissue cells immediately related to the spicule are activated, erode the calcium and for a while are lodged in lacunae. Neighbouring connective tissue cells fill the gap between the splintered ends of the calcified bar and may even cause bulging of the endothelium. At this stage, repair may be arrested with the formation of a false joint between the broken ends of the calcified bar while connective tissue cells occupy the gap between the calcified bar and the endothelium (Fig. 12). On the other hand, the connective tissue may increase steadily between the separated ends of the bar of calcium. The fragments of calcium are steadily removed, accompanied by an overgrowth of connective tissue derived mainly from the media and adventitia (Figs. 13 and 14).

When the calcified bar is extensively fragmented over a restricted part of its length and the wall of the aorta is grossly deformed (Fig. 16), an intense fibroblastic reaction is excited and is accompanied by the formation of new capillaries which communicate with the adventitia and with the lumen of the aorta (Fig. 17). Calcium is steadily resorbed from the spicules and the injury is healed by fibrous tissue, the end picture often resembling that portrayed in Fig. 15.

Coronary artery

Two main patterns of response to vitamin D have been observed in the coronary arteries, namely, (a) a variable degree of local or diffuse calcification which in extreme forms may express itself as calcification of the entire thickness of the vessel wall, (b) injury to the endothelium, sub-endothelial tissues, media and even the adventitia, implicating either a circumscribed part of the wall or the entire

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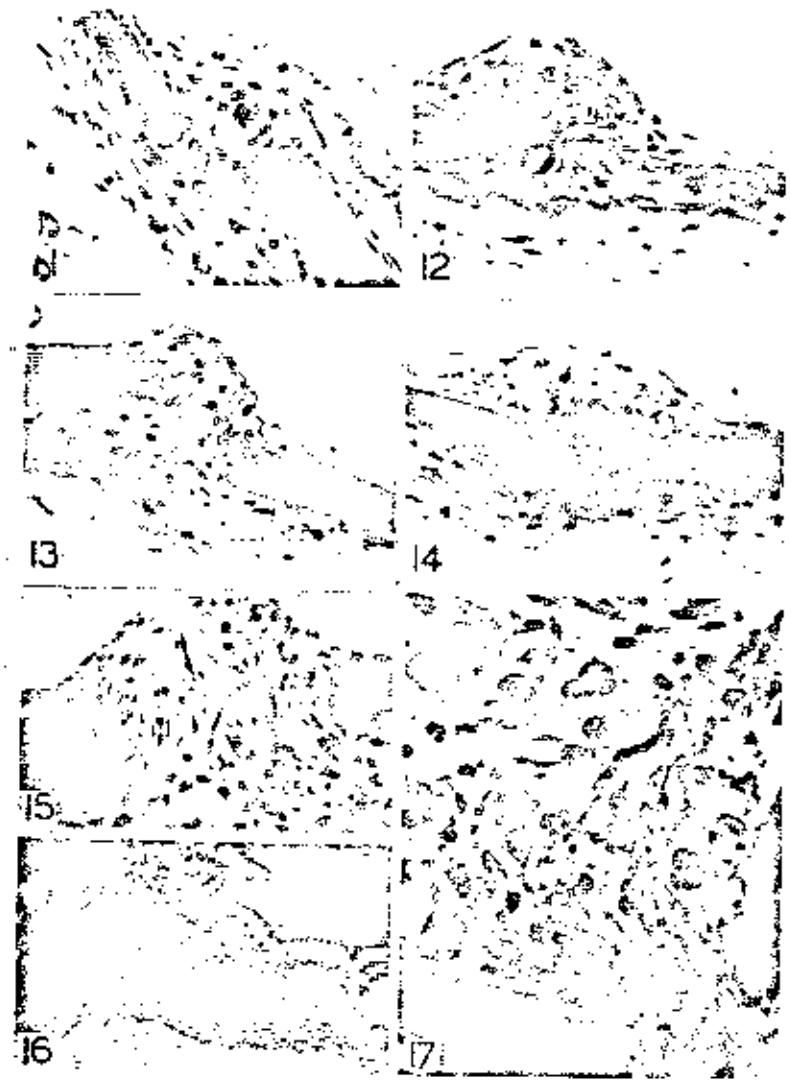

Fig. 11. Fracture of the calcified plate in the thoracic aorta showing bulging on the intimal surface produced by proliferating connective tissue cells and abundant matrix. Note mitotic figure in endothelium and absence of clotting.
Fig. 12. Formation of pseudo-arthrosis between the ends of the calcified bars in the wall of the thoracic aorta. Note the sub-endothelial cellularity and abundant matrix.
Fig. 13. Fracture of the calcified plate in the thoracic aorta showing resorption of fragments and repair of the wall of the artery without evidence of thrombosis.
Fig. 14. Vascularisation of the intima of the aorta at the level of fracture of

circumference of the vessel over a restricted segment, unaccompanied for the most part by calcification.

(a) Calcification of the coronary artery apparently occurs with great rapidity and may be extensive involving the entire wall, the soft tissues being replaced by a solid bar of calcium extending from the endothelium to the adventitia (Fig. 18). It would seem that just prior to precipitation of calcium, the vessel dilates, the thickness of the wall becoming correspondingly reduced. Connective tissue cells, muscle and elastic fibres, are all embedded in the calcified matrix, but, as will be indicated below, at least some of these cells retain their vitality. Calcification of the order shown in Fig. 18 is by no means irreversible.

Resorption occurs in two stages. During the first stage, the intense basophilia is progressively reduced until eventually the staining reaction with haematoxylin and eosin changes from a deep purple to pale blue. The von Kossa reaction is steadily reduced. Thereafter, the cellular phase of calcium resorption is initiated as a preliminary to the later reconstruction of the vessel wall. The cells are derived from three sources, namely, from endothelium, from those embedded in the calcified bar and from connective tissue cells of the adventitia. The relative contribution of each source depends on local conditions prevailing in the artery. As the endothelial cells proliferate and invade the media, they erode and fragment the calcified tissue, a process which is facilitated by invasion of cells from the adventitia and by release of cells hitherto entrapped within the calcified matrix.

Some of the cells from the adventitia, after penetrating the calcified bar, move circumferentially in the direction of the lumen with the result that their axes are placed at different angles in respect of the lumen of the vessel. The calcified bar, now represented as a thin lamina, is progressively displaced towards the adventitia.

As the calcium disappears, the matrix becomes abundant and

a calcified plate. In serial section, the capillaries communicated with the lumen of the aorta.
Fig. 15. Fibro-cellular repair of almost the entire wall of the artery between the fractured ends of the calcified plates. Note the direction of the fibrocytes.
Fig. 16. Multiple fractures of the calcified plate with distortion of the wall of the artery. Note the absence of thrombosis.
Fig. 17. High power of the intima of artery shown in Fig. 16 illustrating vascularisation, numerous large endothelioid-like cells and formation of multinucleated giant cells.

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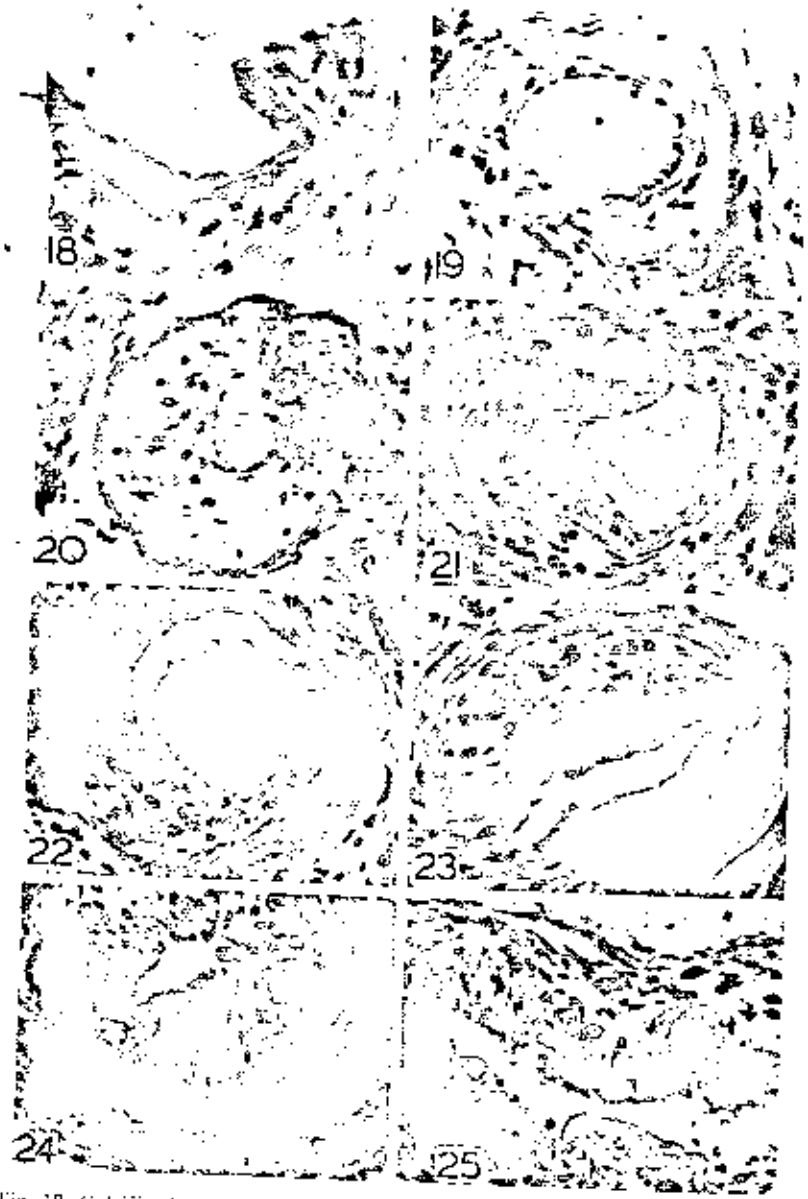

Fig. 18. Calcification of the entire wall of the myocardial segment of a coronary artery.

Fig. 19. Section of coronary artery showing an advanced stage in resorption of calcium. Note the gelatinous, cellular tissue interposed between the endothelium and the fissured, calcified bar near the adventitia.

assumes a fluid or gelatinous appearance in much the same fashion as occurs in the medium-sized coronary artery (Fig. 20). The lumen of the vessels may be greatly reduced. Thereafter, the matrix is slowly removed, the cells become more tightly packed and their outlines sharply defined. A new internal elastic lamina is differentiated and the fragmented remains of the old lamina are dispersed at irregular depths in the media.

In the medium-sized and small coronary arteries, massive calcification of the wall may initiate a process which may culminate in the formation of a thick, muscular media and re-duplication of the internal elastic lamina (Figs. 19 to 24). As in the larger vessels, the first stage in reconstruction is heralded by reduction in the basophilia of the site of calcification. It is apparent, after appropriate staining, that the internal elastic lamina is discontinuous. Proliferation of cells, either from the endothelium or related media, results in broadening of the intima and localised dissolution of the related calcium (Figs. 19 and 20). The widely-separated cells are embedded in a semi-solid matrix which may now be invaded to some extent by cells from the adventitia (Figs. 19 and 20). As is evident from Figs. 21 to 23 the reaction occurs asymmetrically in the vessel wall. In some regions, the calcium is in close contact with the endothelium and in other parts widely separated from it (Figs. 19, 20, and 21). Where proliferation has occurred, the cells become elongated and fine fibrils course through the cytoplasm (Fig. 21). Immediately beneath the endothelium

Fig. 20. Medium-sized coronary artery surrounded by a sheath of calcium located near the adventitia. Note resorption of calcium in one part of wall and replacement of calcium by gelatinous material. The lumen of the vessel is greatly narrowed.

Fig. 21. Asymmetric reconstruction of the wall of a medium-sized coronary artery. Note increase in cellularity, and metallaxis of cells in the intima. Vestiges of the internal elastic lamina separate the intima from the cellular media and adventitia.

Fig. 22. Further stage in the reconstruction of the intima. Note great reduction in gelatinous matrix and increase in cellularity of intima enclosed by the calcified, internal elastic lamina.

Fig. 23. Still further stage in the reconstruction of the wall of the artery. The lumen is markedly excentric.

Fig. 24. Almost completely reconstructed wall of coronary artery with formation of a new, internal elastic lamina. Vestiges of the original elastic lamina can still be recognised. Note marked thickening of wall of artery.

Fig. 25. Necrotis of the inner half of a medium-sized coronary artery with disruption of the internal elastic lamina and formation of giant cells from the endothelium.

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the cells are concentrically arranged whereas in the deeper regions, there is great variation in the direction of the long axes of the cells (Figs. 21, 22, and 23). The internal elastic lamina is reconstituted immediately beneath the endothelium, and may become reduplicated, the laminae being separated by one or more layers of cells. The media itself is greatly thickened and is separated from the adventitia by the original internal elastic lamina which has been displaced to a peripheral position (Fig. 24).

Although for a while the lumen of the vessel may remain eccentric (Figs. 21, 22, and 23), eventually by proliferation of cells and remodelling of the intima and media, the lumen once again assumes a central position (Fig. 24). The outstanding difference between the original and the reconstructed vessel is to be found in the great hypertrophy of the media. The artery assumes an appearance similar to that described in hypertrophied arteries in experimentally-produced hypertension. Without a knowledge of the pathogenesis, the mode of production of such an hypertrophied coronary artery could not be suspected. Perhaps the feature distinguishing the artery described above from that found in hypertension resides in the peculiar, modified tissue located in the outer aspect of the media and representing the original calcified internal elastic lamina.

b) Although large concentrations of vitamin D tend to cause precipitation of calcium in the coronary arteries, in some circumstances, dependent on the amount of vitamin D and upon nutritional and endocrine factors, to be fully described elsewhere, the artery may be injured in a diversity of ways, unaccompanied by calcification. The reactions excited may simulate those described in the rheumatic and para-rheumatic diseases, in lupus erythematosus, in allergic conditions produced in rabbits by injection of horse serum (49) and in the arteries of rats and dogs used for the experimental production of hypertension. In its mildest form, vitamin D may produce only sub-endothelial oedema, accompanied by swelling of the endothelial cells and agglomeration of polymorphs which become adherent to the injured endothelium, such as has been described and depicted in the aorta (Fig. 4). In time, this lesion may resolve completely.

In its most severe form, the internal elastic lamina is destroyed, sub-endothelial oedema is intense, associated with marked proliferation of the endothelial and connective tissue cells and hyalinisation and oedema of the muscle with a variable degree of periarteritis. Eosinophils, monocytes and a few neutrophils and lymphocytes tend to accumulate

below the endothelium. All gradations exist between the mild lesion, confined mainly to the endothelium and the media and the severe lesion which involves almost the entire wall of the artery.

One of the commonest reactions observed after vitamin D administration is mild damage to the endothelium accompanied by migration of monocytoid elements and polymorphs into the superficial part of the media with lifting of the overlying endothelium. In some instances, even red cells may be found in the sub-endothelial space. As a consequence, two or three rows of cells may separate the media from the endothelium. In such instances, the internal elastic lamina remains intact. In the course of time, the numbers of cells in the sub-endothelial tissue are reduced and several multinucleated cells are found in the endothelium or lodged just deep to it.

Necrosis of the intima associated with "blurring" on the inner aspects of the vessel wall and intense metachromasy usually implicates a small part of the vessel wall (Fig. 25). In such instances, the internal elastic lamina is disrupted and the free ends become swollen in a manner similar to that described in an aorta treated with elastase (50, 51). The underlying media is not infrequently oedematous and metallaxis is in evidence. Such a picture is sometimes encountered in the coronary arteries of cases dying from rheumatic fever (52) or in lupus erythematosus. Repair of this lesion is usually a very slow process and all layers usually contribute to the reconstruction of the wall. In the first place, small folds of surviving endothelium invade the hyalinised media and thus remove the necrotic material. As a consequence, the intimal and medial complex becomes spongy and cellular (Fig. 26). The surviving connective tissue and muscle cells in the media also enlarge and, with the accompanying oedema, the wall of the coronary artery is greatly thickened. Metallaxis is a characteristic phenomenon (Fig. 26). As the débris is removed and with the subsidence of the oedema, the cells in the intima are reduced in size, the capillaries which communicate with the lumen of the coronary artery are steadily obliterated but the endothelial cells may fuse to form small giant cells which may persist for months. Eventually, a new internal elastic lamina is elaborated but the intima is more cellular than that of the original coronary artery.

Repair of the media is a more tardy affair. Oedema persists in the media for a very much longer period and, even when resorbed, the formation of collagen fibres which has occurred in the interim may result in sclerosis of a segment of the vessel wall. It would seem that

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Fig. 26. Repair of the necrotic lesion displayed in Fig. 25. Note the appearance of monocytoid multinucleated giant cells and absence of thrombosis.

Fig. 27. Intense localized accumulation of eosinophilic material beneath endothelium and loss of structure in underlying segment of the wall of medium-sized coronary artery.

Fig. 28. Repair of lesion shown in Fig. 27. Note intense sub-endothelial oedema, leucocytic infiltration and cells derived from endothelium.

Fig. 29. Disruption of the intima with exposure of a cellular granular mass to the circulating blood in the absence of thrombosis.

Fig. 30. Resorbing degenerated cardiac muscle cells and replacement by proliferating endothelial and perithelial cells.

Fig. 31. Stage later than Fig. 30 showing repair of damaged muscle by fibrocellular tissue.

Fig. 32. Localised necrosis of the sub-endocardial tissue with very early cellular reaction.

Fig. 33. Intensification of the cellular reaction in the subendocardium with partial disruption of the endothelium.

Fig. 34. Cellular nodule at the root of the aortic valve.

the initial fibrinoid degeneration of the intima and related media favours dehydration of the necrotic fibres and cells and thus hampers repair. Such injury is much more serious than that produced by massive calcification of the entire wall of the coronary artery as is evident from the remarkable repair in the latter instance (Figs. 18 to 24).

The accumulation of large amounts of granular, eosinophilic material in the superficial part of the media with loss of stainability of the connective tissue and muscle cells can occur without disrupting the continuity of the endothelium. This reaction may be confined to one sector of the wall of the coronary artery and when fully developed causes marked local thickening of the affected artery (Fig. 27). Later, monocytoid cells, lymphocytes and even polymorphs may penetrate the granular material from the lumen, adding to the thickness of the intima and enhancing the medial oedema (Fig. 28). Even red cells may add to the cellularity of the intima. At this stage, part of the intima may be disrupted exposing a cellular granular mass to the circulating blood. Despite this free contact of an extensively damaged intima (Fig. 29), thrombosis was characteristically absent. Repair of the lesion portrayed in Figs. 28 and 29 takes a long time and is usually accompanied by the formation of a small verruca, as carefully illustrated and described for the human aorta (53).

Noteworthy too is the resemblance of the sub-endothelial eosinophilia and granularity of the early lesion (Fig. 27) to that reported in human arteritis, ascribed by *Gore* (54) to some abnormality in the platelets whereby they accumulate in the intima of the artery and in the capillary wall. It would seem that in our rats, such a lesion is due to seepage of plasma through damaged endothelium and is not obviously

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due to platelets. Nevertheless, it needs to be emphasized that the administration of vitamin D to rats can lead to a wide range of reactions in the coronary arteries, with extensive calcification at one extreme and necrotising lesions at the other.

Reactions in the myocardium in response to vitamin D

It has been repeatedly stated that myocardial degeneration following excess vitamin D is primarily due to precipitation of calcium in the walls of the small arterioles and on the muscle fibres themselves (55). The coexistence of calcification and myocardial degeneration has also been observed repeatedly in our own experiments. However, by the graded administration of vitamin D, it has been possible to dissociate myocardial degeneration from calcification.

Myocardial injury is foreshadowed by swelling and intense eosinophilia of the muscle, followed by dilatation of the related capillaries. Soon thereafter, swelling of the perithelial and endothelial cells, accompanied by proliferation, emphasizes the cellularity of the affected region. The degenerated muscle cells undergo liquefaction and disappear leaving behind the cellular framework of proliferating endothelial cells (Fig. 30). The fluid in the interstices of the cellular meshwork is then absorbed, the neighbouring muscle cells hypertrophy and the capillary network is re-established. In the course of time, all trace of previous injury is obliterated. A singular feature of this low grade damage to the myocardium is the absence of polymorph infiltration.

When degeneration of the myocardium is extensive and occurs rapidly, a mild fatty change precedes destruction of the myofibrils; the sarcoplasm shrinks, drawing together the nuclei into a compact mass. In this way, discrete nodules of varying size consisting of giant cells derived from the muscle, numerous plasma cells and a variable number of polymorphs are formed throughout the myocardium. As the sarcoplasmic débris is removed, the cellular nodule may decrease in size and eventually disappear. On the other hand, when the nodule is large, connective tissue may be formed and contribute to the repair of the injured site (Fig. 31). Only in exceptional cases, and in those instances where there has been excessive destruction of tissue does a residual scar persist. But, even in these instances, in the course of time, the scar is progressively reduced leaving little if any trace of previous injury. When scars persist, they are usually

found in the septum and in the base of the heart where fibrous tissue is normally more abundant than elsewhere in the myocardium.

Myocardial injury occurs most commonly in the septum, in the base of the heart, immediately below the epicardium, in the left ventricle, and in the endocardium and subendocardium of both ventricles near the apex. In the ventricles, injury is often localised around the arteries.

Injury to the valves

Lesions may occur in the base of the aortic and pulmonary valves and on the surface of the mitral and tricuspid valves as well as along the chordae tendineae and especially at the sites of attachment of the tendinous chords to the papillary muscles or their counterpart in the rat. The earliest manifestation of damage is recognised as subendothelial oedema and intense metachromasy similar to that described in the aorta (Fig. 1). At a later stage, the site of injury is accentuated by the accumulation of a few monocytoid elements over the endothelium and slight enlargement of the fixed connective tissue cells (Fig. 32). When the lesion progresses, the cellular aspect of the reaction is emphasized while the continuity of the endothelium is seemingly disrupted (Fig. 33). Proliferation of the fixed cells as well as the accompanying oedema may lead to the formation of small nodules projecting beyond the free surface. In Fig. 2, the cellular excrescences on the aorta as well as on the aortic and ventricular surface of the aortic valve are displayed. The nodule on the aortic surface is clearly the larger (Fig. 34) and resembles in structure the nodule mentioned by *Apitz* (49) in the rabbit after injection of horse serum. It is significant that in the valves as elsewhere, the initial pathological change is confined to the endothelium and to the immediately underlying tissue. Calcifying lesions of the valves are uncommon, indicating once again that vitamin D has effects other than those characterised by the deposition of varying amounts of calcium.

Consideration of the mechanism underlying the vasculotoxic effect of vitamin D

In the light of his own investigations, *Duguid* (48) crystallised the views held prior to 1930 concerning the way vitamin D produced its toxic action on the aorta. *Duguid* was of the opinion that calcification of the elastic laminae and degeneration of the smooth

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muscle represented responses of two different tissues to vitamin D. While recognising that calcification of the aorta had been previously evoked by adrenalin (56) and local trauma (57), *Duguid* nevertheless ascribed the changes in the aorta after excess vitamin D primarily to hypercalcaemia. The excess of calcium in the blood presumably passed through the intima into the media where it was automatically bound by the elastic fibres. On *Duguid's* hypothesis, therefore, an elevated blood calcium was a sine qua non of aortic calcification.

Vitamin D only inconsistently increases the level of calcium and phosphorus in the blood. Indeed, *Reed, Struck* and *Steck* (41) were led to make the following comment: "Quite commonly one finds statements to the effect that the action of vitamin D is to produce hypercalcaemia or hyperphosphataemia or both. And yet there is abundant evidence that either may occur independently or that neither may follow the administration of large amounts of vitamin D." Similarly, *Dowling* et al. (58, 59) and *Debré* (60) have disclosed that serum calcium need not be raised even though vitamin D produces toxic effects in the patient.

At one stage in our own experiments we have observed a drop in the serum calcium and when the tissues were shown to be mineralising rapidly. On the other hand, extensive metastatic calcification has been described in human infants with hypercalcaemia. Then too, in many of our rats developing a phaeochromocytoma, the serum calcium may be of the order of 17 mg.% and yet metastatic calcification could not be detected histologically. In other words, serum calcium estimation at a particular moment may afford little information of the toxicity of vitamin D nor need it reflect the presence of metastatic calcification. However, *Auning* et al. (61) claim to have demonstrated an increase in the ionised calcium in 30 cases of vitamin D intoxication. This observation is seemingly in conflict with that of *Reed, Struck* and *Steck* (41) who could detect no change in the ratio of filtrable to total calcium of serum and plasma even after massive doses of vitamin D. It still remains to be established firmly whether or not the factor determining metastatic calcification is a function of the amount of ionisable calcium in the plasma or of some other condition. For the present, at any rate, it would seem that hypercalcaemia *per se* is neither attended necessarily by severe constitutional reactions nor is it automatically accompanied by the deposition of calcium in the tissues.

Despite the uncertain relationship of vitamin D to hypercalcaemia there is, nevertheless, more than adequate evidence that excess vitamin

D may be followed by extensive calcification of the soft tissues and that such calcium in the initial stages at least is derived from the bones. Even in rats on a low calcium diet, provided sufficient vitamin D is administered, the aorta calcifies. At the same time, calcium is withdrawn from the skeleton and the skull in particular may become paper thin and almost transparent.

Since extensive calcification of the aorta and of other tissues can occur without any marked rise in the blood calcium, it would seem that at least two conditions need to be satisfied before calcification is possible, namely, an increased permeability of the endothelium of the large arteries and of the capillaries and some secondary change in the elastic fibres and in the muscle cells. While it is well known that the elastic tissue in the aorta mineralises progressively with age (26), the speed of such mineralisation cannot be compared with that occurring after excess vitamin D. Since in the latter instance the blood calcium need not necessarily be markedly elevated, the elastic tissue must have undergone some change to allow for the binding of the large amounts of calcium in the face of an almost normal level of calcium in the blood. The fact that both the endothelium of the aorta and the muscle may show some evidence of functional change or even degeneration before calcification supervenes suggests that excess vitamin D directly or indirectly alters the various tissues of the aorta, large arteries, capillaries, myocardium and valves before calcification supervenes. Increased permeability of the endothelium is suggested by the sub-endothelial oedema in the aorta (Fig. 1), in the valves (Fig. 32) and also in the coronary arteries (Fig. 32). It would thus appear that vitamin D induces two serial processes not necessarily dependent upon one another, namely, tissue damage followed by calcification. These two processes may be uncoupled so that tissue damage, that is, the necrotising lesions of arteries and endothelial injury may occur without calcification. The kind of arterial and myocardial lesions occurring in rats after excess vitamin D are reminiscent of those described in rats, rabbits and dogs used for the investigation of hypertension by techniques aimed at severely disorganising kidney function (28, 29, 30, 31).

The fact that vitamin D also deranges kidney function and structure leads to the suggestion that the arterial and cardiac lesions, observed in vitamin D-treated rats, may be in large measure dependent on partial disorganisation of kidney function. This possibility is further strengthened, first, by the investigations of *Lehr* and *Churg* (62) that necrosis of the arteries attended in many instances by extensive calci-

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fication can be produced in rats by damaging the kidney by the intraperitoneal injection of sparingly soluble sulphonamides; secondly, by the oft-repeated findings in man that in circumstances not yet clearly understood, kidney disease can be followed by extensive calcification implicating also the cardiovascular system (32, 33); thirdly, by the fact that nutritional techniques in rats can produce severe kidney disease which may be accompanied by extensive cardiovascular injury including calcification (unpublished data). All these facts taken in conjunction with those reported (63 to 69, 29, 31) reveal that the kidney plays a crucial role in regulating the integrity of the cardiovascular system. Furthermore, there is every indication that in this regulation, bone, connective tissues, the alimentary canal, lung, smooth and skeletal muscle at least are also directly or indirectly implicated, while the liver is rarely drawn into the pathological process.

It is significant that excess vitamin D can initiate the kind of kidney damage which is rapidly followed by profound changes in the cardiovascular system, in smooth and skeletal muscle, bones etc. There is adequate evidence that amongst its various actions, vitamin D directly or indirectly influences the metabolism of calcium and phosphorus and that both these ions in some circumstances can derange kidney function. Once drawn into the picture, the kidney may initiate a new series of pathological processes culminating in one or more of the vascular and cardiac lesions described above. But there may be additional complications. Towards the end of the 5-day course of vitamin D treatment the animals begin to lose weight precipitously, as much as 40 g. being lost within 2 or 3 days. Apart from depletion of the fat depots, there is obvious wasting and even frank degeneration of the skeletal muscle of the kind observed in vitamin E deficiency. This degeneration of the skeletal muscle occurs in association with necrosis of the myocardium and of the smooth muscle in the arteries and in the alimentary canal. Such extensive breakdown of muscle may contribute toxic metabolites which in turn may still further derange kidney function leading to a modified crush syndrome superimposed on an already inefficient kidney and an injured arterial tree. In the latter circumstances, it is possible that phosphate-containing compounds, either adenosine triphosphate or pyrophosphate, are liberated from the damaged muscle and that such compounds may produce other side effects of the kind described by *Green* and *Stoner* (70).

Although it is becoming increasingly evident that the kidney plays a part in regulating the integrity of the arteries and of the myocardium,

kidney disease is by no means followed invariably by hypertension or by arterial disease (31). Apparently, special conditions need to be satisfied before such pathological reactions in the cardiovascular system will declare themselves. Some of these conditions can be created experimentally by the administration of desoxycorticosterone acetate as well as by a high salt intake against a background of severe kidney disease (64), while other conditions may be dependent on kidney damage induced by methylandrostenediol (68, 69).

Similarly in the case of vitamin D, not all individuals (human or animal) will develop toxic reactions to the same dose of the vitamin. As a consequence, the concept of vitamin D resistance or sensitivity has been frequently used in the literature, implying thereby that special conditions of metabolism are required before any individual will develop resistance or sensitivity to a defined amount of the vitamin. Hitherto, the intake of calcium phosphorus and of alkalis has been suspected as playing some role in modifying the effects of massive doses of vitamin D. We shall bring forward evidence elsewhere that both the adrenal and the thyroid glands as well as the intake of calcium and phosphorus not only modulate in part at least the intensity of the lesions induced by excess vitamin D but also determine the kind of reactions which will declare themselves in the experimental animal. At this stage, it can be asserted that some of the arterial lesions are in part reversible (Figs. 18 to 24). Such reversibility is associated with recovery of the kidney. In the case of experimental hypertension, the kidney injury is permanent and the arterial lesions accordingly are progressive and eventually undermine the survival of the animal. It is not difficult to anticipate a situation where short episodes of kidney disease may eventually lead to widespread cardiovascular disease and yet, at autopsy, the extent of the cardiovascular disease may be out of proportion to the degree of kidney damage. It is in the investigation of such kinds of cardiovascular disease that further experiments with vitamin D may prove useful.

Relevance of experiments to the understanding of human and experimental vascular disease

The information presented above demonstrates that vitamin D can produce a wide range of reactions affecting the capillaries and one or more layers of the larger and smaller arteries. It remains therefore to comment on the relevance of the information derived from rats for

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understanding the pathogenesis of human and experimentally-induced diseases of the cardiovascular system.

The endothelial and subendothelial reactions (Figs. 1, 3) are similar to those reported in the human aorta in cases of rheumatic fever (52). The necrotising lesions of the artery (Figs. 25, 26, and 29), for reasons already mentioned, compare with those described in rats (52, 64) and in dogs (21) and in the rheumatic and pararheumatic diseases in man (71, 72).

Mention needs to be made of the fibrinoid degeneration of the subendothelial tissues of the valves (Fig. 72) and of the later intense concentration of connective tissue cells, monocytoid elements and mast cells (Figs. 33 and 34). Such reactions are reminiscent of those occurring after the injection of horse serum into rabbits (73) and in human rheumatic fever (52, 53). Periarteritis may frequently be associated with valvular disease, suggesting either that a similar end-picture may be produced in different ways or that severe kidney injury inflicted by one or other of the techniques such as horse serum, used to study experimentally-produced hypertension, all act on a basically similar mechanism. As far as the aorta is concerned, it is evident that vitamin D produced lesions in those sites in which atheroma develops spontaneously in man. Furthermore, the epicardial segment of the coronary artery in the rat is particularly vulnerable to vitamin D indicating that, as in man, some mechanical or biochemical factors create a locus minoris resistentiae in this part of the coronary artery.

The mode of response of the rat's aorta to varying grades of injury discloses unambiguously the commanding role played by the endothelium in regenerative processes even in arteries as large as the aorta. Removal of the calcium from the damaged intima, not only promotes extensive endothelial hyperplasia which assists in part in removing the calcium but also results later in the production of an abundant matrix (Fig. 8) and in the re-arrangement of the cells in very much the same way as has been described in spontaneously-developing aortic lesions in the dog (47). After vitamin D, extensive calcification of the coronary arteries of the rat initiates a series of processes which culminate in the reconstruction of the entire wall with the formation of a thickened intima and media and of new elastic fibres (Figs. 19 to 24). The possibility is still to be entertained that the muscular hypertrophy reported in dogs (74, 75) after vitamin D may be secondary to calcification as we have shown in the coronary arteries of rats. Despite the fact that these phenomena in the calcified artery of the rat were described over

20 years ago (55, 76), repair of such a degree of injury in the human coronary artery arising "spontaneously" is not generally believed possible.

In hyperparathyroidism secondary to nutritional kidney disease in rats, such regenerative processes as described above do not occur and the pathological processes ultimately lead to a fatal outcome (unpublished data). From this latter observation, taken in conjunction with those described in rats after a short course of vitamin D treatment, it can be inferred that repair of a damaged artery is prevented not so much by the extent of the injury as it is by the persistence of the noxious stimulus. If this stimulus can be removed, even well-advanced coronary disease, developing spontaneously, is potentially reversible. These observations warrant a measure of optimism that it may be possible to improve the functional efficiency of diseased coronary arteries by physiological procedures. In this connection it should be recalled that myocardial necrosis produced by vitamin D can also be repaired without the formation of scar tissue. The formation of scar tissue in the myocardium, as in the liver, is not automatically dependent on necrosis. It would seem that the connective tissue needs to be in a state of heightened reactivity in the myocardium, as in the liver, before necrosis will stimulate the proliferation of the fibroblasts and reticulum cells and the elaboration of collagen fibres. Moreover, our studies in the rat have shown that fibrous tissue will more easily follow damage to the cardiac muscle in those sites like the interventricular septum where fibrous tissue normally abounds. Again, the conclusion is inescapable that necrosis of the myocardium, within limits, can be repaired effectively without leaving any evidence of the extent of the antecedent injury.

A steadily increasing literature indicates that in atherosclerosis, the deposition of lipid may be dependent on antecedent injury to the wall of the artery (26). According to *Handler* et al. (77) the formation of the atheromatous plaque proceeds in three stages. During the first stage, enacted usually in the first three decades of life, the artery shows progressive, symmetrical thickening of the layers deep to the endothelium. Thereafter, the artery thickens asymmetrically due in part to an increase in elastic fibres. Only after the stage of asymmetrical thickening do lipids appear at varying rates and of varying intensity. The fibrous plaque may either accumulate lipid or it may calcify (18).

Some investigators are of the opinion that the hypercholesterosis is of secondary importance in the pathogenesis of atherosclerosis and that intimal or even medial injury is the primary event. Some change

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in the elastic fibres seems to occur early in most forms of arterial disease. Except in the case of syphilis, damage to the elastic fibres is usually accompanied by mineralisation (77). *Lansing* and his associates (56) have demonstrated that in early atheromatous lesions, the calcium content is elevated. It would seem that mineralisation of the elastic fibres in the aorta or elsewhere is dependent upon some injury to the elastic fibres. Indeed, *Gilman* et al. (78) have emphasized the importance of elastotic degeneration as a precursor of calcification in the arteries, no less than in the dermis. In this connection it should be mentioned that *Kelly* et al. (27) were able to produce in their cholesterolized rabbits atheromatous lesions similar to those occurring in man only if they traumatised the vessel wall.

If injury to the wall of the artery coupled with excess and possibly premature mineralisation predisposes to the various degenerative change in the vascular system, then the observations reported above may become meaningful. Vitamin D directly or indirectly, produces those disturbances in metabolism which undermine the integrity of the vascular endothelium, promote degeneration of the smooth muscle and facilitate mineralisation of the elastic fibres. A disorder in calcium and phosphorus metabolism can also lead to widespread disease of the cardiovascular system without frank calcification. These observations, taken in conjunction with those of *Lansing* and others would suggest that further investigation of the factors regulating the mineralisation of the aorta and of the coronary arteries may be a fruitful approach to the problem of cardiovascular disease. Certainly, the low incidence of atherosclerosis in the African can be correlated not only with the lowered consumption of fats by the African but also with the low calcium intake and the unfavourable calcium-phosphorus ratio in a dietary context, low in animal proteins and rich in maize (79). It may well be the case that the prevalence of hypertension in the African (80) may be attributable in part at least to the high phosphorus, low calcium, low animal-protein containing diets. It should be recalled that vitamin D may injure the vascular system without any marked elevation in the blood calcium or phosphorus. The attack on the human problem may not be very fruitful if conventional procedures are followed whereby the metabolism of calcium and phosphorus is investigated only in terms of these ions in the blood. We have already indicated that extensive calcification, induced by vitamin D, may neither be preceded nor accompanied by any elevation in the blood calcium. Although we have emphasized the importance of vitamin D and calcium and phosphorus,

we are aware that this approach may provide only a small link in a complicated mechanism, derangement of which in many different ways may lead to the end-picture so well known by students of cardiovascular pathology.

At this stage, attention should be drawn to the infrequency of thrombosis in the aorta, ventricle and coronary arteries even when the free surfaces were grossly injured. Even the great narrowing of the coronary artery at the position where it arises from the aorta or where it branches in the myocardium did not promote any thrombus formation despite the disorganisation of the endothelium. Although such narrowing of the coronary artery could obviously interfere with the blood supply to the myocardium, particularly when a load is placed on the cardiovascular system, nevertheless, the narrowing or even injury to the coronary artery does not in itself promote clotting. Furthermore, even when major repair was being effected to the aorta immediately above the valves where a measure of turbulence is known to occur normally, thrombus formation was not achieved. The inference is inescapable either that the damaged endothelium secretes an anti-clotting factor or else some change had occurred in the circulating blood which inhibited clotting. Preliminary observations disclose that the clotting time of the blood is not significantly altered. It can only be assumed therefore, that the endothelium secretes some anti-clotting factor. In this connection it should be recalled that *Chambers* and *Zweifach* (81) have demonstrated that the cement substance of the capillary endothelium is liberated continuously into the circulation and that the stickiness of the cement substance is in part a function of the level of calcium in the perfusing blood. An increase in the stickiness of the endothelium could of course account for the agglutination of the leucocytes and the fixation of such leucocytes to the aortic endothelium (Fig. 3). However, during this process, neither platelets nor red cells were deposited on the injured endothelium of the aorta or of other arteries. The possibility needs to be excluded that the secretion of some substance from the endothelium or the liberation of the cement substance may determine whether or not thrombus formation will be initiated. On the other hand, we have drawn attention to the increase in the mucopolysaccharides below the endothelium of the aorta and the coronary arteries. Such mucopolysaccharides, particularly of the sulphated variety, may act as a local anticoagulant and thus prevent thrombosis. The latter possibility is suggested firstly by the reactions depicted during repair of the abdominal aorta (Figs. 11 to 16) and

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secondly, by those reactions occurring in the coat of the aorta during the reconstruction of a new intima and media (Figs. 19 to 24).

Finally, it is noteworthy, that despite extensive injury to the arteries and even where massive calcification occurred in the intima and media, the veins remained singularly free from observable injury. Such a phenomenon raises a number of questions which, if answered, would throw some light not only on the mechanism of arterial calcification but also on those processes which promote damage to the endothelium, elastic fibres and smooth muscle in the walls of the aorta and other arteries.

From the foregoing, it is evident that while damage to an artery or narrowing may create a focus favouring thrombosis, injury to the vessel wall *per se* is not the cause of thrombosis. Some other conditions need to be satisfied before injury to the intima will be attended by thrombus formation. These other conditions may be determined by a change in the circulating blood but most probably by the presence of some anti-clotting substance elaborated over the site of injury.

Summary

A wide range reactions in the cardiovascular system has been described in rats receiving an intensive but short course of treatment with massive doses of vitamin D. These reactions range from calcification at the one extreme to subendothelial oedema and small cellular vegetations on the endothelium of the aorta, in the ostia of the coronary arteries and on the valves, at the other. Extensive necrotising arteritis may supervene without histological evidence of calcification.

The sequential stages in the repair of the various kinds of vascular lesions have been described. The participation of the endothelium in reparative processes is noteworthy. Attention was drawn to the remarkable regenerative capacity of all the arteries, including the aorta. Although, in some circumstances, the architecture of the vessel wall was markedly deformed, nevertheless the functional efficiency of the vessel was maintained.

In the coronary and medium-sized arteries, healing could be so effective that metaplasia and residual calcification of the internal elastic lamina were the only signs of previous injury. Even extensive destruction of the myocardium could be effectively repaired with a minimal fibrous tissue response. It was evident that special conditions required to be fulfilled before necrosis of the myocardium would be followed by fibrosis.

The infrequency of thrombosis, in the face of severe injury to the endocardium and to the intima of the aorta, coronary and other large arteries indicated that injury to the artery *per se*, even when severe, will not automatically promote thrombosis.

From a consideration of relative data it was suggested that the gravity of the cardiovascular lesions depended upon or was complicated by impairment of kidney function. The vitamin D-induced arterial lesions were linked with those known to follow severe kidney disease induced by other techniques.

Importance was attached to the role of vitamin D, calcium and phosphorus as one link in a complicated mechanism responsible for regulating the structural integrity of the cardiovascular system. Moreover, it was suggested that the differential incidence of cardiovascular disease reported in the European and African in this country might be attributable in part to fundamental differences in calcium and phosphorus metabolism determined by diet.

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ARTERIAL INJURY, REPAIR AND DEGENERATION

SIR,—The tendency today is to attribute arterial diseases, in young adults, to some relatively sudden degeneratory change. Hence the search for recent causes and the hope placed in such measures as regulating the intake of fats. There is reason to think, however, that these diseases are often the result of arterial injuries early in life.

Previously my colleagues and I have pointed out that, though single acute injuries to arteries heal directly like cutaneous wounds, their repair is much slower (Poole, Sanders, and Florey [1] have recently shown that stripped rabbit aortic endothelium is not completely regenerated even after 7 months). Consequently scar tissue may produce arterial rigidity, stenosis, and contracture long after the initial insult,[2,3] and we have come to believe that some forms of arterial disease may originate in a single

injury, whose results are detectable clinically only 10–15 years later.

Our studies of vascular lesions in lathyrus intoxication in rats [4,5,6] have indicated (1) that the aorta is maximally sensitive to this toxin when growing at its greatest rate (in rats about 25–30 days after birth) and (2) that the greater sensitivity of the aorta at this time is due to the remodelling of the elastic membranes essential for their growth in length and diameter. It seems possible that the arteries of man, too, may likewise be maximally sensitive to injury during periods of greatest growth with its associated remodelling of arterial components—especially of the more internal elastic membranes which seem, in rats, to lyse more rapidly and frequently.

There seem to be three periods of rapid body growth, and therefore arterial growth in man: namely, the last trimester of pregnancy, the first year or two after birth, and then (greatest of all perhaps) between the ages of 12 and 20.[7] On this basis it might be expected: (1) that early and mild arterial lesions would be found in the very young, increasing in incidence during adolescence; (2) that malnutrition during such active vascular growth and remodelling would favour greater or more frequent injuries; and (3) that true fibrosis of these early lesions would supervene only 10–15 years later. Mason [8] has found that intimal lesions are quite common under the age of 20, and thinks that they are "identical with the early nonlipid phases of arterial sclerosis in adults".

Now Holman et al.[9] find (1) that the incidence of aortic lesions increases between the ages of 13 and 18 more than at any other age they studied, and (2) that the lesions in adolescents are mainly intimal "fatty streaks" without fibrosis. They believe that it probably takes 10–15 years for the early lesions of adolescence to fibrose. Their data, taken in conjunction with our own findings,[1,3,4] indicate that injuries to vessels in adolescents may culminate in localised fibrosis, hardening, and subsequently in further deterioration, perhaps in the manner outlined by us.[2,3,10] Mucopolysaccharides which accumulate in injured sites (before collagen synthesis) may have an important role both in precipitating circulating lipids and in binding them in the sites of repair.[10,11,12] The initial injury to vessels during their periods of active growth could result from deficiencies in nutrition, from physical overexertion, from any acute illness, or from metabolic derangement.

All the above facts together suggest the need for a reorientation of studies of the aetiology and pathogenesis of degenerative arterial diseases. In particular, the role of postnatal vascular growth and remodelling, and the injuries caused by derangements thereof, deserve more attention. It now seems desirable to discover, first, how the intimal lesions (which may be attendant on growth changes) can be prevented or at least lessened, and secondly how to retard if not prevent the deterioration of these early lesions into atheromatous plaques, intimal erosions with complicating fibrin deposits, and mural thrombi which may culminate in occlusions.

Appreciation of the amazing capacity of uterine vessels to recover from endarterial thromboses (after the onset of

another pregnancy)[13] and the possible susceptibility of the aorta to remodelling and even to dissecting aneurysms during pregnancy,[14] as well as the subtle effects of endocrine glands on intermediate fat metabolism, plasma fibrin, and fibrinolysins,[11,15,16] may perhaps throw light on at least two apparently distinct aspects of vascular diseases—namely, (1) mural injury leading to sclerosis and later to lipoidal degeneration of the scars, and (2) intimal fibrin deposits with mural thrombi, progressing to stenosis and ultimately to occlusive episodes.

The approach here suggested may permit an explanation of the paradoxical frequency of arterial lesions in Africans, as described by Laurie and Woods [17] (and others quoted by them), and the rarity of coronary occlusive episodes among these people.

The African is probably at least as susceptible as the European to the early arterial injuries attendant on deranged remodelling and vascular injury during active growth. However, the deterioration of such injuries, and especially intramural lipid accumulations, intimal fibrin deposits, and mural thrombi might be expected to proceed more slowly in Africans, while the separate thrombotic process (e.g., in coronaries), with occlusions, could also be expected to be rarer though coronary lesions are common. For the Africans' diet is low in fat; moreover, we have now confirmed our previous finding [18] that adult African males have more active circulating fibrinolysins than European males, and this might decrease their susceptibility to intimal deposits and even perhaps to thrombotic episodes generally. On the other hand, the early (adolescent) lesions in Europeans might be expected to undergo fatty deterioration and culminate in thrombotic episodes more often, and at an earlier age, by virtue, respectively, of the Europeans' higher and more regular fat intake and their generally less active plasma fibrinolysins. This may be partly due to the full metabolic effects of uninhibited masculinity expressed in greater growth and physical activity, and perhaps, as in rats, in less efficient handling of ingested fats and lower circulating plasma fibrinolytic capacities than in females.[11,12] Similar factors may explain the differences in the incidence of advanced lesions and occluding thrombi in the coronary arteries in young Americans compared with Japanese.[17]

As for the deterioration in the aged, leading to the vascular sclerosis characteristic of senility, this (as has been shown elsewhere [2]) seems to be an entirely different process—namely, the gradual deterioration, with ageing, of vascular regeneration into repair.[6,10]

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Loss of a Parathyroid Hormone–Sensitive Component of Phosphate Transport in X-Linked Hypophosphatemia

Abstract. *Mutant hemizygotes with X-linked hypophosphatemia lack a parathyroid hormone-sensitive component of inorganic phosphate transport in kidney; female heterozygotes retain a variable proportion of this type of transport. The residual mechanism for reabsorption in affected males allows inorganic phosphate efflux from the kidney to urine so that net "secretion" is sometimes observed; the latter is directly proportional to the serum concentration of inorganic phosphate. Calcium acts on the kidney tubule to enhance net reabsorption by this component of inorganic phosphate transport.*

The primary defect of X-linked hypophosphatemia has eluded clarification since the first descriptions of this form of vitamin D-resistant rickets (*1, 2*). Because of the impaired clinical responsiveness to vitamin D, and because of the discovery that intravenous calcium infusion could suppress the elevated renal clearance of inorganic phosphate (P_i) which accompanies hypophos-

phosphate (2, 3), attention was originally focused on vitamin D–dependent mineral metabolism in this trait. The primary defect was thought to involve impairment of calcium absorption in the intestine, or abnormal vitamin D metabolism, and secondary hyperparathyroidism was invoked as the basis for the renal loss of P_i (2, 4). These hypotheses are improbable because untreated patients with X-linked hypophosphatemia have normal concentrations of parathyroid hormone (PTH) in the blood (5).

An alternate hypothesis emphasizes a primary disturbance of P_i transport in kidney, and perhaps also in other tissues (2, 6). hypophosphatemia is the single constant phenotypic trait in carriers of this X-linked mutant allele (2), suggesting that the allele primarily influences P_i metabolism. Replacement of P_i is particularly effective in correcting the mutant clinical phenotype (7), as would be expected if P_i metabolism is mainly affected in the disease.

We present evidence that the defect in X-linked hypophosphatemia concerns a PTH-sensitive component of P_i transport that is responsible for about two-thirds of the total net reabsorption of P_i in human kidney; this component is partially absent in female patients and completely absent in male patients. A residual component of P_i transport, capable of being saturated, is retained in male patients. This permits P_i flux from tubular lumen to plasma and also allows a flux in the reverse direction. Although this transport is insensitive to PTH it can be modulated by calcium ion. The net reabsorption of P_i is enhanced immediately upon intravenous infusion of calcium.

Tubular transport of P_i is saturable in normal human subjects (8). We examined the tubular reabsorption rate [TRP_i, micromoles per 100 ml of glomerular filtrate (GF)] and the maximum reabsorption rate (TmP_i, micromoles per 100 ml of GF, at saturation) at various concentrations of serum P_i (9) in six males (all children) and nine females (three children, six adults) with X-linked hypophosphatemia. Subjects with bone disease (six male and three female children) were on treatment regimens with P_i (1 to 3 g/day) and vitamin D_2 (10,000 to 50,000 unit/day) prior to investigation; each patient had a normal glomerular filtration rate (GFR).

The tubular reabsorption rate is lower in mutant hemizygotes than in female heterozygotes, and below the normal mean in all patients (Fig. 1); this finding confirms many earlier reports (2). The TRP_i in female heterozygotes overlaps the normal range, as predicted by the Lyon hypothesis (which implies random inactivation of one X chromosome early in development) if P_i transport in the kidney is closely coupled to the effect of an X-linked mutation. However, we could find no constant relation between the TRP_i and the degree of bone disease in heterozygotes.

A saturable component of P_i reabsorption is present in both hemizygotes and heterozygotes but is much lower

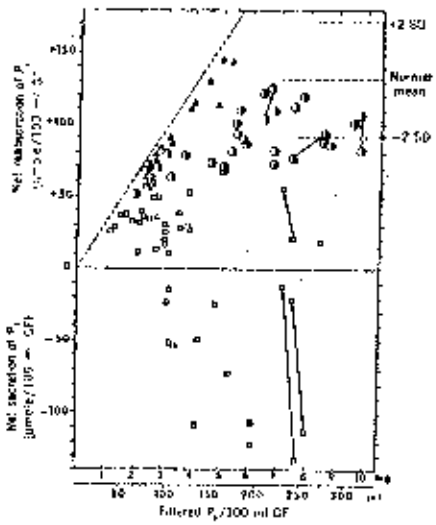

Fig. 1. The in vivo kinetics of net reabsorption of inorganic phosphate (P_i) by the kidney of normal subjects and those with X-linked hypophosphatemia. The substrate concentration (P_i) is varied in the glomerular filtrate by an intravenous infusion method (8). Reabsorption rate is calculated as described (9). Mutant hemizygotes are represented by squares, heterozygotes by half-filled circles and normal subjects by triangles. The mean ± 2 standard deviations (S.D.) for the normal maximal rate of tubular reabsorption (TMP_i) in man (2) is shown. The interrupted diagonal line (upper graph) indicates complete reabsorption of filtered phosphate; points falling below this line indicate incomplete net tubular reabsorption of phosphate. The net reabsorption rate (TRP_i), the difference between filtered and excreted P_i, is plotted upward as a positive value when the excreted P_i is less than the filtered P_i. Negative reabsorption (net secretion), found in several hemizygotes (excreted P_i > filtered P_i), is plotted downward. Arrows indicate the change in P_i reabsorption 30 to 60 minutes after intravenous infusion of calcium (1 mg/kg) over a 15-minute period; this dose was sufficient to raise total calcium in serum by 2 mg/100 ml. A single patient is represented by the two studies of the effect of calcium on P_i secretion.

in the former (Fig. 1). [illegible] previous reports (2, 10). There is no unusual "splay" in the graph of P_i reabsorption, indicating no change in binding of P_i by the available transport system. The residual P_i transport, in mutant hemizygotes, is virtually saturated even at endogenous concentrations of plasma phosphate. This suggests the presence of more than one form of P_i transport, the residual form being unaffected by the X-linked mutation.

The renal excretion of P_i in mutant hemizygotes was often greater than its filtered load; this was observed over a threefold range of P_i concentration in serum (lower part of Fig. 1). These observations were monitored by inulin clearance, and the rate of net tubular secretion was found to be directly proportional to the P_i concentration in serum. When net reabsorption of P_i occurred, the serum calcium concentration in these subjects was 9.9 ± 0.9 mg/100 ml (mean ± standard deviation, 27 samples) and when there was net secretion of P_i, it was 9.9 ± 0.4 mg/100 ml (14 determinations).

We examined the effect of bovine PTH (11) on the endogenous P_i excretion in normal, heterozygous, and mutant hemizygous phenotypes. Because of prior phosphate therapy, the endogenous serum immunoreactive PTH (12) (IPTH) was above normal in one male and one female patient before bovine PTH infusion (5); the concentration was normal in the remaining subjects. Renal clearance of P_i relative to creatinine increased sharply in the four normal subjects after infusion of bovine PTH (Fig. 2). The GFR was not changed significantly by this procedure in any patient. The P_i excretion in three heterozygotes overlapped the normal response. On the other hand, each of the three mutant hemizygotes were less responsive to PTH than were the heterozygotes, and one male actually excreted less P_i after the PTH infusion. One of the hemizygotes (second from right, Fig. 2) was studied when his serum P_i had been raised to 8 mg/100 ml by the infusion method (9). His response to PTH was like that of the other two male patients who were studied when their concentrations of serum P_i were low (< 3 mg/100 ml). We reported previously (5) that a tenfold increase in endogenous serum IPTH does not influence TRP_i in mutant hemizygotes. We conclude, from the

present and the early studies, that the residual P_i transport, unmasked in the hemizygote with X-linked hypophosphatemia, is virtually insensitive to PTH.

Phosphaturia may not be a satisfactory index of the renal tubular response to PTH. Urinary excretion of adenosine 3',5'-monophosphate (cyclic AMP) is said to be a more reliable index (*13*), and for this reason we measured cyclic AMP in the urine of the patients infused with bovine PTH (*14*). Urinary cyclic AMP was normal in all patients before infusion. The excretion rate of cyclic AMP in female heterozygotes, mutant hemizygotes, and normal subjects increased by 19 to 80 nmole/min in the first 30 minutes after bovine PTH injection; the response for mutant and normal subjects overlapped each other and all were in the normal range (*13*). We also measured the change in calcium excretion relative to creatinine, before and after PTH infusion. Calcium excretion dropped by 30 to 70 percent after PTH infusion in normal, heterozygous, and hemizygous subjects. Therefore, PTH acts in a normal manner on the renal tubule in X-linked hypophosphatemia but the hormone is unable to exert its usual effect on P_i transport.

Intravenous infusion of calcium enhances P_i transport in the mammalian kidney (*2, 15*). The effect is mediated both through a parathyroid-dependent mechanism and by a direct effect upon the tubule (*15*). The latter can be the only mode of action of calcium in male patients with X-linked hypophosphatemia who respond to calcium infusion with improvement in the net tubular reabsorption of P_i (*2, 3*), because P_i reabsorption is insensitive to PTH, and serum IPTH is not abnormally elevated in the untreated mutant hemizygote (*5*). Studies of the change in P_i reabsorption after calcium infusion in X-linked hypophosphatemia, often were performed with large amounts of calcium infused over several hours (*1*). We examined five patients (three female, two male) at 30 to 60 minutes after a calcium infusion (4 mg/kg over 15 minutes) that was sufficient to raise serum calcium by 2 mg/100 ml. Tubular transport of P_i was studied at concentrations of serum P_i sufficient to saturate the transport. Tubular reabsorption of P_i improved under these conditions in each subject (Fig. 1). Whether net tubular reabsorption or net secretion of P_i was present initially. Although serum immunoreactive PTH (cell

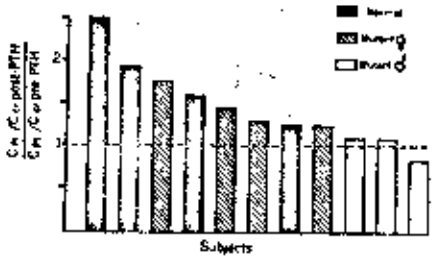

Fig. 2. Effect of an intravenous infusion of purified bovine parathyroid hormone (PTH) on phosphate excretion by mutant hemizygotes (open bars) and heterozygotes (hatched bars) with X-linked hypophosphatemia, and normal subjects (black bars). The ordinate is the ratio (C_{Pi}/C_{cr}) after PTH infusion to the ratio (C_{Pi}/C_{cr}) before PTH infusion, where C is the endogenous renal clearance rate of inorganic phosphate (P_i) and creatinine (cr) averaged for three 30-minute periods before and after PTH infusion.

by about 20 percent in all subjects after the calcium infusion, this response would not account for the change in TRP_i in hemizygotes.

Familial hypophosphatemia is a dominantly expressed X-linked disease (*2*) and, according to the Lyon hypothesis, such a disease will be expressed fully in male hemizygotes and variably in the females. We must conclude, however, that the loss of P_i transport in kidney is not complete even in mutant hemizygotes with X-linked hypophosphatemia. A component of P_i transport, with a saturable capacity for net reabsorption at about one-third the normal value, is still active in the male patient. Transport of P_i in females is between normal and hemizygous values; this is compatible with partial retention of the transport that is absent in males. We propose, therefore, that P_i transport in human kidney involves at least two components (*16*). One component accounts for about two-thirds (about 100 μmole per 100 ml of GF) of the total net reabsorptive capacity; this transport is modulated by endogenous parathyroid hormone. A second component accounts for all or most of the remaining uptake; its capacity is about 50 μmole per 100 ml of GF, and its P_i transport can be directly modulated by the calcium ion. These findings suggest that calcium may regulate P_i efflux (plasma to lumen) relative to net influx (lumen to plasma) by the second type of P_i transport. It is possible that whatever benefit has been achieved by massive vitamin D treatment in X-linked hypophosphatemia was actually derived from the effect of vitamin D on calcium metabolism, which in turn modulates P_i transport by the calcium-sensitive system.

It is still not known whether the two (or more) components of P_i transport are separate binding sites (proteins) with different capacities and sensitivities to PTH and calcium, functioning in parallel at the brush border, or whether they function in series, perhaps at opposite poles of the tubular epithelial cell. The latter hypothesis implies a mediated brush border transport with high capacity, which is sensitive to PTH and calcium, and an antiluminal carrier capable of transporting P_i from cell to plasma and also from plasma to cell. The X-linked mutation presumably influences only one mode of transport in the parallel hypothesis and there is precedence for this type of membrane transport mutation (*17*). In the series hypothesis the mutation concerns transport on the brush border site which allows P_i binding but not a coupled transport to allow accumulation of P_i in the cell; there is also precedence for mutations which "uncouple" substrate-specific transport (*18*). The X-linked mutation may affect the membrane system directly or it may affect another cell function which modulates membrane transport of phosphate.

We do not know whether other cells and organs in man have similar mechanisms for P_i transport. A number of the clinical features of X-linked hypophosphatemia, particularly in the female heterozygote, cannot be explained solely on the basis of an hereditary defect in P_i transport in kidney. Moreover, some nonsaturable but relatively inefficient P_i transport in cell membrane (*19*) is likely to be present since high concentration of P_i improves phosphate retention (*7*). We anticipate that mediated P_i transport may be impaired in other tissues in this disease; there is evidence for this in other inborn errors of membrane transport (*17*). The X-linked hypophosphatemia may be genetically heterogeneous and different types of defective P_i transport may be identified; such genetic heterogeneity has been found in the other inborn errors of membrane transport (*17*).

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UKRAINIAN TRANSLATION

Effect of Large Doses of Vitamins A, D and B_1 On the Copper Content of the Animal Organism

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In medical practice, the "hypervitaminosis state" (symptomatic therapy) is induced with large doses of vitamins. However, vitamins are not at all "indifferent", and clear clinical symptoms of hypervitaminosis A and D have been noted.

During the past 10 years, there has been an increasing number of data showing the interrelationship of vitamins with trace elements (1-9). Attesting to this fact is the redistribution of trace elements noted with a lack (or shortage) of vitamins A, D, C, B_1, E, etc. An increased amount of certain trace elements in the food ration affects the vitamin requirements: in some cases, these requirements are greater, in others lower.

In connection with this fact, it was of interest to study the effect of large doses of vitamins A, D and B_1 on the content of trace elements in the animal organism. In this study, data are given on the distribution of copper in the organism of white rats when these vitamins are present in excess amounts.

Method

In the first series of tests, the effect of large doses of vitamin A on the copper content of the animal organism was studied. For this purpose, 40 young rats weighing 40-60 g were divided into 2 groups. Animals in the first group (20 rats) were kept on a diet consisting of 76% wheat meal (flour),

10% lard, 10% dry baker's yeast and 4% salt mixture (10). The Cu content was 1.8 microgram per gram ration. Each animal received daily 50 I.U. (international units) of vitamin A in the form of an oil solution added to the ration. Animals in the second group received, in addition to the basic ration, 50,000 I.U. of the vitamin A oil solution during 24 hours.

Animals in both groups were kept for 15 days on the above ration, and were then killed by decapitation; the amount of copper was determined in the liver, heart, bone tissue, kidneys, brain, spleen, eye tissue, muscles and small intestine.

In another series of studies, the effect of large doses of vitamin D was determined on 40 young rats, divided into 2 groups. Animals in the first group (20 rats) were maintained on a ration consisting of 90% wheat meal, 5% brewers yeast, and 5% salt mixture (10). The amount of copper was 2 microgram/g ration. To the ration, 300 I. U. vitamin D per rat and per day was added. Rats in the second group were kept on the same ration, but received a large dose of vitamin D (10,000 I. U.) per rat during 24 hours.

In a third series of tests, the effect of large doses of vitamin B_1 was studied on 40 rats, weighing 40-60 g and divided into 2 groups. Rats in the first group were kept on a vitamin B_1-free ration consisting of 17% casein, 58% starch, 8% cream, 4% salt mixture no. 1, 10% twice autoclaved yeast and 2% cod liver oil; to this ration vitamin B_1 was added in an amount of 20 micrograms per rat per day. The rats of the second group were kept on the same diet as these in the first group, but a large amount of vitamin B_1 was added, equal to 2 mg per rat per day.

Animals of the second and third group were observed for 20 days, after which they were killed; the amount of copper was determined in the liver, bone tissue, the wall of the small intestine, spleen, heart, kidneys, brain, lungs

and adrenal glands. The organs and tissues were dried to constant weight and calcined in a muffle furnace at 350-400°C, followed by a 3-fold treatment during calcination with distilled concentrated nitric acid. Such organs as the spleen, kidneys and adrenal glands, which give a small yield of ash, were calcined once by using 2-3 organs together. The quantitative determination of copper was done colorimetrically by the dithizone method, as modified by V.T. Chuyko (11).

Results

Animals receiving high doses of vitamins A and D became sluggish already after 10 days, while those receiving excess vitamin B_1 became aggressive. An index of the hypervitaminous state of the rats was the change in the body weight, namely a much smaller weight gain than in the controls. Thus, in control animals of the first and third test series, the body weight increased by 20% during the first 10 days, while the weight gain of test animals was only 10%. In rats of the control group, after 20 days, the weight gain was nearly 50%; the hypervitaminous rats started to lose weight during the first week of the test, and on the 15th day their weight dropped to 80% of the initial weight.

Approximately 20% of the animals which received large amounts of vitamins started to die on the 10-15th day of the experiment.

As the results of these tests show, large doses of all the vitamins studied exerted an effect on the copper content in the organism of white rats (see Tables 1 and 2). Changes under the effect of vitamins D and B_1 were particularly significant. Large doses of vitamin D resulted in an accumulation (increae) of copper in bone tissue (a 2.5-fold increase), in the spleen (60% increase), in the heart (30% increase), and brain (30% increase). A decrease in the copper content was noted in the liver and intestines.

TABLE 1

Copper content in organs and tissues of rats receiving large doses of vitamins A and D (in mg % of fresh tissue).

Орган, тканини	Кількість дослідів	Вітамін А			
		Контроль	Досліл	Контроль	Досліл
Печінка (1)	18	0,14	0,65	0,48	2,60
Кісткова тканина (2)	17	0,13	0,13	0,08	3,20
Кишки (3)	9	0,35	0,20	0,35	0,11
Селезінка (4)	7	0,19	0,20	0,17	0,36
Серце (5)	7	0,51	0,67	0,51	0,69
Нирки	8	0,44	0,41	0,34	0,35
Мозок (7)	7	0,30	0,32	0,21	0,27
Тканини ока (8)	5	0,06	0,04	—	—

1-Liver
2-Bone tissue
3-Intestines
4-Spleen
5-Heart
6-Kidneys
7-Brain
8-Eye tissues
9-Organ, tissue
10-No. of tests
11-Vitamin A
12-Controls
13-Test animal
14-Vitamin D
15-Controls
16-Test animals

TABLE 2

Copper content in organs and tissues of rats receiving large doses of vitamin B_1 (in mg % of fresh tissue).

Орган	Кількість дослідів	Контроль	Досліл
Мозок (1)	9	0,35	0,42
Печінка (2)	20	0,31	0,51
Серце (3)	9	0,52	0,72
Селезінка (4)	7	0,51	0,41
Легені (5)	6	0,19	0,23
Нирки (6)	8	0,52	0,54
Надниркова залоза (7)	5	1,20	0,71

1-Brain
2-Liver
3-Heart
4-Spleen
5-Lungs
6-Kidneys
7-Adrenal gland
8-Organ
9-No. of Tests
10-Controls
11-Test animals

An excess of vitamin B_1 caused an accumulation of copper in the liver and a decrease of the copper content of the intestine, spleen (by 20%), lungs (by 40%), kidneys (by 16%) and adrenal glands (by 40%); no changes in the copper content were noted in the heart and brain.

An excess of vitamin A caused the smallest changes: the copper content decreased in the intestines (by 40%) and in eye tissues (by 40%), and increased in the liver (by 41%) and in the heart (by 30%). No changes were noted in the other organs tested.

Thus, an excess of the vitamins studied here affected the distribution of copper in the animal organism. It was of interest to investigate to what extent these changes are specific for each vitamin. This is important not only from the standpoint of establishing the interrelationship between vitamins and trace elements. Apparently, the copper content undergoes extensive changes in the blood and various organs during different physiological and especially pathological conditions, such as pregnancy, diabetes, radiation sickness, anemia, tumors, hepatitis, dysentery, avitaminoses, starvation, etc. Most authors consider that an excessive copper content of the blood (hypercuprenemia) in pathological conditions is one of the protective reactions of the organism. One could expect that the character of quantitative changes of the copper content in the organism of hypervitaminous animals would be the same for different vitamins. However, the data obtained by us did not confirm this assumption.

In comparing the copper content in various hypervitaminoses, it was found that the distribution of copper in the heart, liver and kidneys is the same for vitamins A and D; it is true that in both hypervitaminoses the copper content in the wall of the small intestine is reduced, and that the rate of this decrease is also different. However, there are some organs in which

the copper distribution is radically different (opposed) for these two vitamins. An example of such a phenomenon is the bone tissue, in which an excess of vitamin D causes a considerable increase of the copper content (2.5 times), whereas hypervitaminosis A does not affect the amount of this trace element present in bone tissue. A similar picture is noted in the spleen and brain.

With an excess of vitamin B_1, an accumulation of copper is observed in the liver and a decrease of the copper content is noted in the spleen, kidneys, lungs and adrenal glands, in which an excess of vitamins A and D causes an increase in the amount of this trace element.

Hence, it follows that each of the vitamins studied here is characterized by its own and particular distribution of copper in the organism.

Conclusions

1. An excess of vitamins A, D and B_1 in the animal organism has a negative (unfavorable) effect: it slows down growth, causes a loss of weight and shortens the duration of life.

2. An excess of vitamins A, D and B_1 in the feed ration of animals leads to a redistribution of copper in organs and tissues, which possibly is specific for each vitamin.

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Вплив великих доз вітамінів A, D і B_1 на вміст міді в організмі тварин

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У медичній практиці «гіпервітамінозний стан» (симптоматична терапія) викликають з допомогою великих доз вітамінів. Проте вітаміни зовсім не «байдужі». Відомі чіткі клінічні прояви гіпервітамінозів A і D.

В останні десятиріччя все більше з'являється даних про взаємозв'язок вітамінів з мікроелементами [1—9]. Про це свідчить перерозподіл мікроелементів при нестачі вітамінів A, D, C, B_1, E та ін. Збільшення кількості деяких мікроелементів в раціоні впливає на потребу у вітамінах: в одних випадках підвищує її, в інших — зменшує.

З огляду на це становило інтерес вивчити вплив великих доз вітамінів A, D і B_1 на вміст мікроелементів в організмі тварин. В роботі викладено дані про розподіл міді в організмі білих щурів при надлишку досліджуваних вітамінів.

Методика

В першій серії дослідів вивчали вплив великих доз вітаміну A на вміст міді в організмі тварин. Для цього 40 молодих щурів вагою 40—60 г розділили на дві групи. Тварини першої групи (20 штук) перебували на раціоні, що складався з пшеничного борошна (76%), лярду (10%), пекарських сухих дріжджів (10%), суміші солей (4%) [10]. Вміст міді складав 1,8 *мкг* на 1 *г* раціону. Щоденно кожна тварина одержувала 50 І. О. вітаміну A у вигляді масляного розчину, який добавляли до раціону. Тварини другої групи, крім основного раціону, одержували по 50000 І. О. масляного розчину вітаміну A протягом доби.

Тварини обох груп перебували на зазначеному раціоні 15 днів, після чого їх декапітували; кількість міді визначали в печінці, серці, кістковій тканині, нирках, мозку, селезінці, в тканинах ока, м'язах і тонких кишках.

В другій серії дослідів вивчали вплив великих доз вітаміну D. 40 молодих щурів розділили на дві групи. Тварини першої групи (20 штук) пе-

реб[illegible] на раціоні, що містив пшеничне борошно (90%), пивні дріжджі (5%) [illegible] солей (5%) [16]. Кількість міді — 2 *мкг* на 1 *г* раціону. До раціону добавляли 300 І. О. вітаміну D на щура за добу. Щури другої групи перебували на тому ж раціоні, але одержували великі дози вітаміну D — 10000 І. О. на щура протягом доби.

В третій серії експериментів досліджували вплив великих доз вітаміну B_1. 40 тварин вагою 40—60 *г* розділили на дві групи. Щури першої групи перебували на B_1-авітамінозному раціоні, що складався з казеїну (17%), крохмалю (53%), вершкового масла (8%), суміші солей № 1 (4%), двічі автоклавованих дріжджів (10%) і риб'ячого жиру (2%); до раціону добавляли вітамін B_1 з розрахунку 20 *мкг* на щура на добу. Щури другої групи перебували на тому ж раціоні, що й першої, але їм добавляли велику кількість вітаміну B_1 — 2 *мг* на тварину протягом доби.

Тварини другої і третьої груп перебували в досліді 20 днів, після чого їх забивали; кількість міді визначали в печінці, кістковій тканині, стінці тонких кишок, селезінці, серці, нирках, мозку, легенях і надниркових залозах. Органи і тканини висушували до постійної ваги і озолювали в муфельній печі при температурі 350—400° С з наступною триразовою обробкою в процесі спалювання перегнаною концентрованою азотною кислотою. Такі органи, як селезінка, нирки, надниркові залози, що дають малий вихід золи, брали в кількості двох-трьох і спалювали разом. Кількісне визначення міді проводили колориметрично дитизоновим методом, модифікованим В. Т. Чуйко [11].

Одержані результати

Тварини, які одержували великі дози вітамінів A і D, уже через 10 днів ставали млявими, а у тих, що одержували надлишок вітаміну B_1, проявлялась агресивність. Показником гіпервітамінозного стану щурів була зміна ваги тіла — значно менший приріст ваги, ніж у контрольних. Так, у тварин контрольної групи першої і третьої серії експериментів за перші 10 днів вага тіла збільшилась на 20%, а у дослідних — тільки на 10%. Щури контрольної групи через 20 днів набули ваги майже на 50%; гіпервітамінозні почали втрачати її (ті, що одержували надлишок вітаміну B_1, під кінець досліду втратили 5% початкової ваги). Щури, які одержували надмір вітаміну A, почали втрачати вагу на першому ж тижні досліду, і на 15-й день вона впала до 80% порівняно з початковою.

Близько 20% тварин, що одержали велику кількість вітамінів, на 10—15-й день почали гинути.

Як показали результати експериментів, великі дози всіх досліджуваних вітамінів впливали на вміст міді в організмі білих щурів (табл. 1, 2). Особливо значними були зміни під впливом вітамінів D і B_1. Великі дози вітаміну D приводили до нагромадження міді в кістковій тканині (у 2,5 раза), селезінці (на 60%), серці (на 30%) і мозку (на 30%); в печінці та кишках кількість міді зменшувалась.

Надлишок вітаміну B_1 зумовлював нагромадження міді в печінці і зниження її вмісту в кишках, селезінці (на 20%), легенях (на 40%), нирках (на 16%) і надниркових залозах (на 40%); в серці і мозку змін не відмічено.

Надлишок вітаміну A викликав найменші зміни: вміст міді

Таблиця 1

Вміст міді в органах і тканинах щурів, які одержували великі дози вітамінів A і D
(в *мг*% на свіжу тканину)

Содержание меди в органах и тканях крыс, получавших большие дозы витаминов A и D
(в *мг*% на свежую ткань)

Орган, тканини	Кількість дослідів	Вітамін A		Вітамін D	
		Контроль	Дослід	Контроль	Дослід
Печінка	18	0,44	0,65	0,48	0,40
Кісткова тканина	17	0,13	0,13	0,08	0,20
Кишки	9	0,35	0,29	0,35	0,11
Селезінка	7	0,19	0,20	0,17	0,28
Серце	7	0,51	0,67	0,54	0,69
Нирки	8	0,44	0,41	0,54	0,55
Мозок	7	0,30	0,32	0,21	0,27
Тканини ока	5	0,06	0,04	—	—

Таблиця 2

Вміст міді в органах і тканинах щурів, які одержували великі дози вітаміну B_1
(в *мг*% на свіжу тканину)

Содержание меди в органах и тканях крыс, получавших большие дозы витамина B_1
(в *мг*% на свежую ткань)

Орган	Кількість дослідів	Контроль	Дослід
Мозок	9	0,35	0,32
Печінка	20	0,31	0,51
Серце	9	0,52	0,52
Селезінка	7	0,51	0,41
Легені	8	0,40	0,27
Нирки	8	0,52	0,44
Надниркова залоза	5	1,20	0,71

зменшувався в кишках (на 40%) і тканинах ока (на 40%), збільшувався — в печінці (на 41%) і серці (на 30%). В інших досліджуваних органах змін не виявлено.

Таким чином, надлишок досліджуваних вітамінів впливав на розподіл міді в організмі тварин. Цікаво було прослідкувати, наскільки ці зміни специфічні для кожного з вітамінів. Це було важливим не тільки з точки зору вивчення взаємозв'язку між вітамінами і мікроелементами. Відомо, що вміст міді зазнає великих змін у крові і різних органах при різноманітних фізіологічних і особливо при патологічних станах (вагітність, діабет, променева хвороба, анемії, пухлини, гепатити, дизентерія, авітамінози, голодування та ін.). Більшість авторів розцінює гіперкупреїнемію при патології як одну із

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захисних реакцій організму. Можна було б чекати, що характер кількісних змін міді в організмі гіпервітамінозних тварин буде однаковим для різних вітамінів. Проте одержані дані не підтвердили цього припущення.

При порівнянні вмісту міді при різних гіпервітамінозах виявилось, що в серці, печінці і нирках розподіл її однаковий для вітамінів A і D; правда, в стінці тонких кишок при обох гіпервітамінозах знижується вміст міді, але ступінь цього зниження також різний. Проте є органи, в яких розподіл міді діаметрально протилежний для цих двох вітамінів. Прикладом такого явища є кісткова тканина, в якій надлишок вітаміну D приводить до значного підвищення вмісту міді (в 2,5 раза), а гіпервітаміноз A на кількість мікроелементу не впливає; аналогічна картина спостерігається в селезінці та мозку.

При надлишку вітаміну B_1 відбувається нагромадження міді в печінці і зменшення її вмісту в селезінці, нирках, легенях, надниркових залозах, в яких надлишок вітамінів A і D викликає збільшення кількості мікроелементу.

Звідси випливає, що кожному з досліджуваних вітамінів властивий свій, особливий розподіл міді в організмі.

Висновки

1. Надлишок вітамінів A, D і B_1 в організмі тварин впливає негативно: сповільнює ріст, викликає втрату ваги, скорочує тривалість життя.

2. Надлишок вітамінів A, D і B_1 в раціоні тварин приводить до перерозподілу міді між органами і тканинами, можливо, специфічно для кожного вітаміну.

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Влияние больших доз витаминов A, D и B_1 на содержание меди в организме животных

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Резюме

Изучалось влияние больших доз витаминов A, D и B_1 на содержание меди в организме животных. Опыты проводились на молодых белых крысах. Опыт продолжался 15—20 дней. Затем животных забивали и определяли количество меди в ряде органов и тканей.

Установлено, что избыток витаминов A, D и B_1 оказывает неблагоприятное влияние на состояние животных и приводит к перераспределению меди между органами и тканями, возможно, специфичному для каждого из витаминов.

Effect of Large Doses Vitamins A, D and B_1 on the Copper Content in the Animal Organism

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Summary

The authors studied the effect of large doses of vitamins A, D and B_1 on the copper content of the animal organism. The experiments were conducted on young albino rats and lasted 15—20 days. Then the animals were sacrificed, and the quantity of copper was determined in a number of organs and tissues.

It was established that an excess of vitamins A, D and B_1 has an unfavourable effect on the state of the animals and leads to redistribution of copper between the organs and the tissues, which is possibly specific for each of the vitamins.

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Placental transfer of vitamin D_3 and 25-hydroxycholecalciferol in the rat

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Tritium-labeled vitamin D_3 (D_3-^{3}H) and its biologically potent metabolite 25-hydroxycholecalciferol were injected into pregnant and nonpregnant rats. Pregnant animals' plasma contained more water-soluble metabolites than their nonpregnant controls 48 hours following intravenous D_3-^{3}H. Silicic acid chromatograms of lipid-soluble extracts from maternal plasma and whole fetuses were strikingly similar 12 and 48 hours following maternal dosing with D_3-^{3}H. Similar maternal/fetal ratios of vitamin D_3 and 25-hydroxycholecalciferol were observed 1 hr. after maternal administration of these compounds. Prior bioassay evaluation of maternal-fetal transfer of antirachitic sterols is confirmed and extended to include 25-hydroxycholecalciferol.

Although it has been fairly well established that mineralization of the fetal skeleton and subsequent bone maturation is dependent on maternal mineral metabolism,[1-3] the direct fetal effects of physiologic vitamin D supplements during the gestation period are still in doubt. Neonatal rickets has been demonstrated in the fetuses of women with osteomalacia,[4] and vitamin D supplements to pregnant rats have been shown to delay the time of onset of rickets in their pups placed on rachitic rations at weaning.[5] These and other accumulated observations[4-6] suggest that antirachitic activity can be transferred across the placenta. Despite these observations there is to date no direct confirmation of the transplacental passage of vitamin D. Previous studies concerned with maternal-fetal transfer of vitamin D have employed nonphysiologic vitamin D dosages and the rachitic rat tibial bone bioassay system.[5, 7] Reportedly, pharmacologic

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doses (> 40,000 International Units [IU]) not only damage the placenta but also alter the normal physiologic transfer mechanisms of the placental barrier.[8] Moreover, as it has been well established that inorganic phosphate supplementation can in itself heal vitamin D-lack rickets,[9] the use of dried tissue and milk vehicles might well promote cartilage nucleation in the tibial line bioassay test system independent of any vitamin D effect.

Recent advances in understanding the metabolism of vitamin D reveal that the ingested vitamin undergoes intestinal absorption, rapid hepatic uptake, and enzymatic conversion to a biologically potent metabolite, 25-hydroxycholecalciferol.[10] These observations, as well as the availability of high specific activity preparations of vitamin D_3 and 25-hydroxycholecalciferol, prompted an evaluation of the metabolism and placental transfer of these substances in the rat during late gestation.

Materials and methods

Female Sprague-Dawley rats at approximately 18 days of gestation and their nonpregnant female litter mates were anesthetized lightly with ether. ^3H-vitamin D_3* (D_3-^3H) (2.9 or 5.8 U.) or ^3H-25-hydroxycholecalciferol† (25OHD_3-^3H) (1.7 U.) in 50 to 100 μl of ethanol was injected into a jugular vein, and the rats were again allowed free access to Purina rat chow and tap water. At varying intervals, the nonpregnant and pregnant rats were reanesthetized and killed to death from the abdominal aorta. The fetuses were separated, rinsed with cold 0.15M saline, weighed, minced, and homogenized in isotonic saline 2:1 (vol./wt.) in a Virtis homogenizer. Heparinized blood from the adult rats was centrifuged immediately at 4° C., and duplicate hematocrits were obtained. Forty eight-hour urine samples were collected under toluene and refrigerated at 5° C. The blood volume of nonpregnant animals was considered to be 6.5 per cent of body weight,[11] and that of pregnant animals, 33 per cent higher.[12] Fetal weight of 4 grams or more indicated near full-term maturity.

Extraction procedure. Aliquots of plasma and fetal homogenates were extracted with chloroform-methanol (2:1 v/v) as described previously.[13] Following overnight separation at 5° C., the chloroform and methanol layers were measured and aliquoted for quantitation of radioactivity content. The lipid layers were dried under a stream of nitrogen and stored in the frozen state at −20° C. until chromatographic analysis. Aliquots of urine were combusted‡ for total radioactivity estimation; the remaining urine was extracted into a chloroform-methanol (2:1 v/v) mixture.

Column chromatography. Chloroform extracts of plasma and fetuses were chromatographed in 38 by 1.5 cm. glass columns packed with activated silicic acid§ in n-hexane. Five hyperbolic gradients of n-hexane → diethyl ether → methanol[14] were used, and the eluates were collected in 10 ml. fractions.

Measurement of radioactivity. Aqueous samples were assayed in Bray's solution,[15] and dried lipid extracts and eluates were dissolved in a liquid scintillation toluene phosphor solution.‖ For combusted samples, a different scintillation fluid was utilized.¶ Observed counts per minute were converted to disintegrations per minute (d.p.m.) by reference to ^3H-toluene standards. All samples were counted in an automatic Packard Tri-Carb liquid scintillation counter, Model 314X#.

*[1, 2-^3H]-Cholecalciferol with a specific activity of 75,000 disintegrations per minute (d.p.m.) per unit from New England Nuclear Co., Boston, Mass.

†[26, 27-^3H]-25-Hydroxycholecalciferol with a specific activity of 175,000 d.p.m. per unit was supplied kindly by Dr. Hector F. DeLuca.

‡Thomas Oxy Safety Igniter (A. H. Thomas Co., Philadelphia, Pa.).

§Biosil (Laboratories, Richmond, Calif.

‖PPO (2, 5-diphenyloxazole) (5.0 Gm.), and di-methyl-POPOP (1,4-bis[5-phenyl-2 oxazolyl]-benzene) (100 mg.) per liter of toluene.

¶Dimethyl POPOP (1, 4-[5-phenyl-2 oxazolyl]-benzene) (40 mg.), PPO (2,5-diphenyloxazole) (4.0 Gm.), absolute ethanol (200 ml.), and toluene (800 ml.).

#Packard Instrument Co., Inc., Downers Grove, Ill.

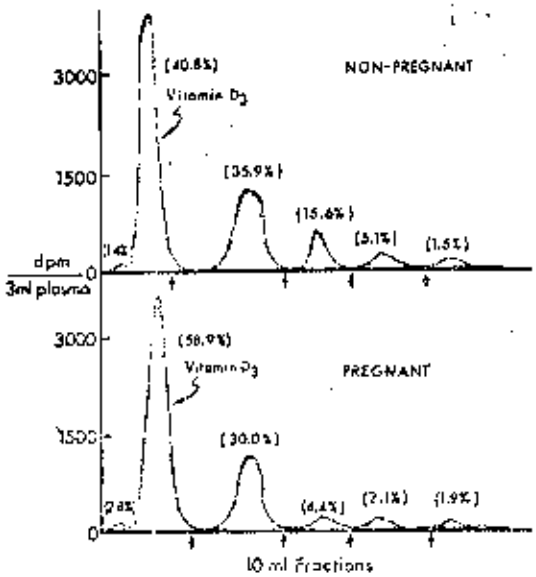


Fig. 1. Silicic acid column chromatography for the separation of ^3H-labeled metabolites in lipid extracts of nonpregnant and pregnant plasma. The radioactive peak to the right of vitamin D_3 corresponds to the elution characteristics of 25OH cholecalciferol. Arrows depict the initiation of hyperbolic gradients ranging from hexane/ether to pure methanol (far right).

Earlier eluted materials less polar than D_3-^3H (Figs. 2 and 3) were also noted in the fetuses.

Fig. 2 illustrates the distribution of lipid-soluble radioactivity in maternal blood and fetuses 12 hours following the intravenous administration of D_3-^3H to the pregnant animal. There was no apparent failure of transfer to the fetus or different metabolites in the fetal lipid layer. The chromatographic profiles of each group 48 hours after the D_3-^3H was administered are also quite similar (Fig. 3). Whereas peaks less polar than D_3-^3H (Peaks I and II) represent an insignificant portion of the plasma D_3-^3H metabolites (2.8 per cent) in maternal plasma, they account for 12.2 per cent of the chromatographed lipid-soluble material of fetal homogenates. The contribution to the lipid-soluble fraction by material migrating as $25OHD_3$-^3H on the chromatograph has increased in both maternal blood and fetuses by 48 hours.

Experiments with $25OHD_3$-^3H. One hour following the maternal administration of $25OHD_3$-^3H, fetuses contained 2 per cent of the dose as material chromatographically identical to $25OHD_3$-^3H (Table II). The maternal blood/fetal ratios at one hour were almost identical for both D_3-^3H and $25OHD_3$-^3H. The fact that a smaller percentage of the dose of D_3-^3H than $25OHD_3$-^3H remained in maternal plasma at one hour is consistent with the previously demonstrated early rapid plasma clearance of D_3-^3H,[10] and the relatively longer plasma half-time of $25OHD_3$-^3H.[16] Fig. 4 illustrates the distribution of maternal and fetal lipid radioactivity one hour following $25OHD_3$-^3H. By one hour, the fetal compartment had already accumulated metabolites more polar than $25OHD_3$-^3H.

Table I. Per cent injected dose at 48 hours following intravenous ^{3}H-vitamin D_3

	Water-soluble	*Peak I and II*	*D_3-^{3}H*	*25OHD_3-^{3}H*	*Polar metabolites**
Blood					
Nonpregnant	0.59	0.04	1.46	1.28	0.80
Pregnant	0.97	0.08	1.69	0.88	0.24
Fetal compartment	12.83	0.80	3.68	1.54	0.52
	Water-soluble metabolites†			*Lipid-soluble metabolites†*	
Urine					
Nonpregnant	1.08			0.09	
Pregnant	0.40			0.09	

*As defined by material which was more polar than substance(s) in the peak IV or 25OH-D_3 region on the silicic acid chromatogram.[8]

†As defined following the separation of the phases during the chloroform-methanol extraction.[8]

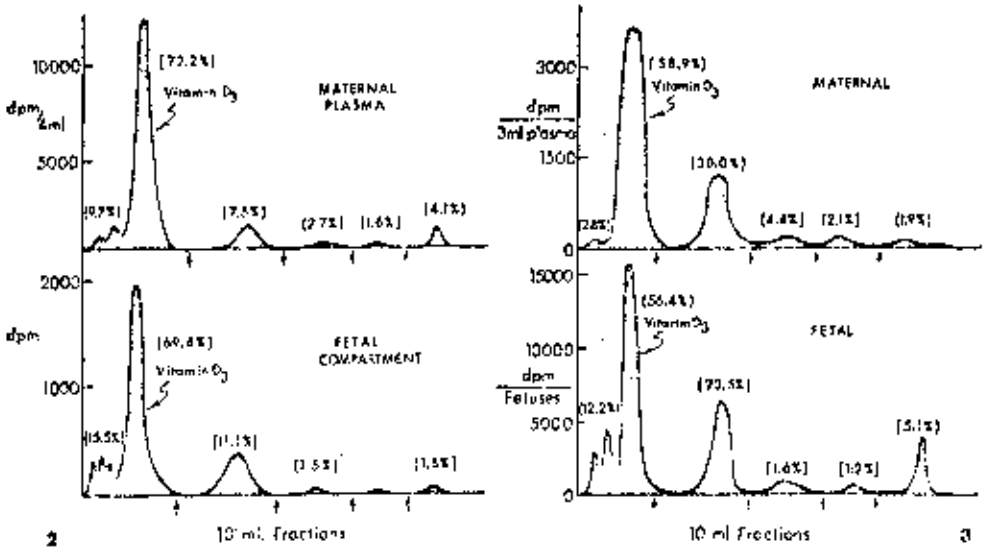
Results

Pregnant and nonpregnant plasma. The distribution of radioactivity as percentage of injected dose in nonpregnant and pregnant plasma is shown in Table I. Forty-eight hours following intravenous D_3-^{3}H, 4 per cent of the injected radioactivity was detected in the vascular compartment of both the nonpregnant and pregnant animals. Compared to nonpregnant blood, the blood of pregnant animals contained more D_3-^{3}H and water-soluble metabolites thereof. In addition, 48 hours after the injection of D_3-^{3}H, nonpregnant plasma contained 50 per cent more 25OHD_3-^{3}H and 3.5 times the amount of lipid-soluble metabolites more polar than 25OHD_3 observed in plasma of pregnant animals. As noted in Table I, aqueous D_3-^{3}H metabolites constituted 25 and 14 per cent, respectively, of the total circulating radioactivity in pregnant and nonpregnant animals.

Fig. 1 emphasizes further the difference in the adult animals. The column profile characterizes only the plasma lipid-soluble metabolites of D_3-^{3}H. It is readily apparent that maternal plasma contains a greater percentage of unaltered D_3-^{3}H. These plasma data suggest a change to enhanced formation and/or decreased excretion of aqueous-soluble conjugates in the pregnant animals. As noted in Table I, nonpregnant urines contained twice as much radioactivity as did maternal urines. In both groups, however, the major portion of urinary radioactivity was water soluble.

Maternal-fetal transfer.

EXPERIMENTS WITH VITAMIN D_3-^{3}H. By 48 hours, almost 20 per cent of the injected dose was found in the fetuses and 12.8 per cent of the fetal radioactivity was water soluble (Table I). The very similar maternal/fetal (M/F) ratios of D_3-^{3}H (25.1) and 25OHD_3-^{3}H (26.4) suggest that the placental transfer for vitamin D_3 and its active hydroxylated metabolite proceeds at comparable rates. The amount of water-soluble metabolites in the full-term fetus also suggests that these vitamin D_3 metabolites cross the placenta rapidly and/or are produced by the fetuses. Although urine obtained from the pregnant animals contained less radioactivity than that of nonpregnant animals, it is obvious that the fetal accumulation of aqueous metabolites cannot be explained fully on this basis.



Figs. 2 and 3. (Fig. 2) Silicic acid column chromatography for the separation of ^{3}H-labeled metabolites in the lipid extracts of maternal plasma and fetal tissues 12 hours following maternal administration of ^{3}H-vitamin D_3. *(Fig. 3)* Chromatographic profiles of lipid-soluble radioactivity 48 hours following maternal administration of ^{3}H-vitamin D_3.

Table II. Distribution of ^{3}H25OHD_3 or ^{3}H-D_3 in maternal blood and fetuses one hour following intravenous dose (% dose)

Origin of blood	D_3		25OHD_3	
	Lipid soluble	*As Peak III*	*Lipid soluble*	*As Peak IV*
Maternal	10.34	10.05	59.08	56.72
Fetal compartment	0.43	0.40	2.42	2.15
Maternal / Fetal		25.1		26.4

use

Discussion

Previous work in rats has indicated that fetal mineralization is not independent of maternal mineral metabolism.[1-5] Pregnancy and lactation are associated with an acceleration of calcium kinetics, and fetal bone accretion has been shown to be markedly higher than resorption.[17] Recent studies suggest also that either a calcium "pump" operates in the placenta supplying a higher concentration of calcium to the fetal blood supply than is found in the maternal circulation, or that the calcium homeostatic mechanism operates at different levels in maternal and fetal compartments.[18] It has been shown that high doses of vitamin D_3 to the gravid rat are associated with a decrease in rat weight, ash weight, and in calcium and phosphorus content of the whole fetus and of pooled fetal bones.[19] Our studies indicate that vitamin D_3, as well as its hydroxylated active metabolite, crosses the rat placenta. It should be noted that the similar M/F ratios of ^{3}H-D_3 and ^{3}H25OHD_3 observed (Table II) at one time point might represent similar placental transfer rates for these substances and/or a balancing out of

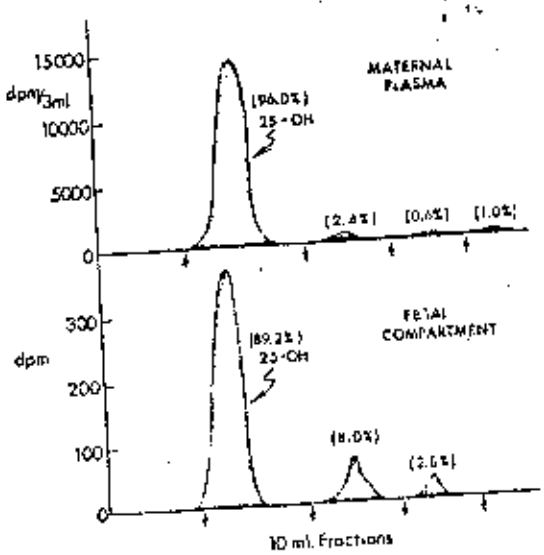


Fig. 4. Chromatographic profiles of lipid soluble radioactivity one hour following maternal administration of ^{3}H-25OH cholecalciferol.

more rapid transfer but more rapid utilization of one substance than the other. 25-Hydroxycholecalciferol in the fetus might contribute to fetal bone maturation and turnover as this metabolite, unlike vitamin D_3, has a direct effect on bone resorption.[20]

It seems likely that cartilage nucleation occurring in the fetus would be largely dependent on the $Ca \cdot PO_4$ mole product. The maternal-fetal exchange of calcium and phosphorus has been described as a dynamic, 2 way process,[21] and there is evidence that fetuses have a higher circulating calcium and phosphorus than the mother.[16] The largest accumulation of mineral in the rat fetus occurs in the last trimester,[21] but marked increases in mineralization do not begin until late suckling and weaning periods of development.[17] The vitamin D content of mammalian milk is thought to be predominantly in a sulfate form.[22] Seelig[23] has reviewed the evidence that a milk vehicle potentiates the vitamin's biological efficacy, but this may be due in part to the high phosphorus content of this fluid.

The finding of increased water-soluble vitamin D_3-^3H metabolites in the pregnant animals was unexpected, as Quinn and colleagues[24] have shown that rat hepatic microsomal systems were suppressed by estrogen treatment. It has been suggested previously that idiopathic hypercalcemia and supravalvular aortic stenosis of the newborn might be the result of the effects of a vitamin D metabolite produced in maternal or placental tissues during pregnancy.[25] In addition, resistance to the development of hypervitaminosis D has been recognized in pregnant rats.[8] As the effects of water-soluble metabolites of vitamin D on fetal bone metabolism are unknown, further characterization of these substances would be of considerable interest.

note for Metab.

Our results are consistent with earlier bioassay evaluations of the fetal

content of antirachitic sterols. Studies in man,[5,6] rabbits,[25] and calves[27] have indicated the liver, skin, and blood to be major fetal sources of vitamin-D-like material. However, there is to date no evidence that vitamin D plays a direct physiologic role in fetal bone maturation. Fetal livers are capable of metabolizing steroids other than vitamin D,[28] and adult rat liver homogenates have been shown to be capable of transforming vitamin D_3 to 25OHcholecalciferol in vitro.[29] The possibility exists, therefore, that some of the vitamin D_3 metabolites isolated from the fetal compartment may be of fetal origin. Although our data (Table I) indicate a large amount of water-soluble and less polar material in the fetuses, these findings are probably more consistent with their ready placental transfer rather than fetal production. A better understanding of possible fetal contributions to vitamin D metabolism must await studies designed specifically to analyze maternal and fetal compartments concurrently and dynamically.

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The effect of vitamin D in rats maintained on diets with different mineral content but with the same calcium to phosphorus ratio of unity

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1. Eight groups of ten weanling rats were maintained for 60 d on diets containing calcium and phosphorus in the ratio 1:1 (w/w) at four different levels of mineral, namely 0·08, 0·12, 0·24 and 0·36% Ca, with and without the addition of ergocalciferol.

2. Provision of vitamin D increased final body-weight except at the highest level of mineral intake. Similarly, increasing the mineral intake increased final body-weight except at the highest level.

3. Vitamin D raised the serum Ca concentration at all levels of mineral intake but in the experiment as a whole had no significant effect on serum P concentration. Serum Ca concentration was significantly higher at the two higher levels of mineral intake than at the two lower levels. Increasing dietary mineral caused a progressive increase in serum P concentration, except at the highest level of intake.

4. In general, the addition of vitamin D to the diet had no effect on the fresh or dry weights of the femur; there was an indication, however, that at the two lower levels of mineral intake, vitamin D reduced the fresh weight of bone. The addition of the vitamin caused a reduction in bone volume, and an increase in bone length, bone ash and the ratio of the weight of ash to the weight of organic matter in dry, fat-free bone. With increasing dietary mineral there was an increase in the values of all the bone measurements except bone volume, which was decreased.

5. The addition of vitamin D and increasing dietary mineral both caused an increase in tooth mass and ash content.

6. The ratios of tooth mass to bone mass and tooth ash to bone ash were unaffected by vitamin D, but decreased with increasing mineral intake.

7. Histological examination of the bones showed that the animals receiving 0·08% Ca and P were markedly rachitic although the presence of vitamin D ameliorated the condition. When the dietary mineral intake was raised to 0·12% Ca and P the animals were less severely rachitic and in the presence of vitamin D the bones appeared normal.

8. The incisor teeth were abnormal at the two lower levels of mineral intake, but when vitamin D was provided, the histological appearance was improved. When the Ca and P level in the diet was 0·24%, the incisal dentine appeared normal but the apical predentine was slightly wider than normal; additional vitamin D produced teeth of normal appearance. The incisor teeth were normal in appearance in the remaining groups.

9. These results are compared with those obtained in two previous studies where the Ca to P ratios were 1:10 and 10:1 (Harrand & Hartles, 1968, 1969).

The effect of vitamin D on the bones and teeth of the rat varies with the state of calcium and phosphorus nutrition of the animal. We have reported on the effect of the vitamin at differing levels of mineral intake at two extremes of Ca to P ratio, namely 1:10 and 10:1 by weight (Harrand & Hartles, 1968, 1969). The experiments now reported involved the maintenance of weanling rats on diets with and without vitamin D, at four different levels of dietary minerals and with a constant Ca to P (w/w) ratio of 1:1. The four levels of dietary Ca were 0·08, 0·12, 0·24 and 0·36%. The effects of these variations in mineral intake and of adding vitamin D were determined by

measuring final body-weight, serum Ca and P, weight, volume, length and ash content of the humeri, and weight and ash content of the mandibular incisor teeth. Histological examinations were made of the femurs and maxillary incisors.

EXPERIMENTAL

Animals. Eighty black and white weanling rats (24–28 d old) from our own colony were distributed in ten blocks of eight animals according to weight and sex. There were, therefore, five blocks of each sex, the weights of the animals within each block being as similar as possible.

Treatments. One animal from each block was distributed at random to each of the eight treatment groups designated 1, 1D, 2, 2D, 3, 3D, 4, 4D. Each group consisted of five male and five female rats.

Diets. The basal diet contained sucrose (1340 g), egg albumen (400 g) and groundnut oil (100 g). This mixture contained 0·026% Ca and 0·06% P. The Ca and P contents of the diets were adjusted to the required levels by the addition of appropriate amounts of AR grade salts. In all diets, to each 1840 g basal diet were added 80 g of a salt mixture containing neither Ca nor P (Harrand, Green & Hartles, 1968). To each 1920 g batch of diet 1·75 g $CaCO_3$ were added, raising the Ca content to 0·06%, the same level as for P.

The following salts were added to each batch of diet to achieve the differing levels of Ca and P at a ratio of 1:1.

Groups	$CaHPO_4$ (g)	KH_2PO_4 (g)
1 and 1D	1·37	0·40
2 and 2D	4·08	1·18
3 and 3D	12·26	3·54
4 and 4D	20·16	5·83

To each batch of diet were added 40 ml water-soluble vitamin solution (Hartles & Leaver, 1961) and 40 mg biotin. In groups 1–4 the groundnut oil contained vitamins A, E and K; in groups 1D, 2D, 3D, 4D the oil also contained ergocalciferol (Hartles & Leaver, 1961).

The main features of the eight dietary treatments were as follows:

Group	Ca (%)	P (%)	Ergocalciferol (μg/kg diet)
1	0·08	0·08	None
1D	0·08	0·08	208
2	0·12	0·12	None
2D	0·12	0·12	208
3	0·24	0·24	None
3D	0·24	0·24	206
4	0·36	0·36	None
4D	0·36	0·36	205

Husbandry and techniques. The experimental regimen and analytical techniques were as described by Harrand & Hartles (1968). The animals were maintained on the diets for 60 d.

Histological examinations. The femurs and maxillary incisors were treated and examined as described by Ferguson & Hartles (1963, 1964).

RESULTS

The terminal body-weights and results of serum and bone analyses are given in Table 1. The results for the incisor teeth are presented in Table 2.

The results were subjected to an analysis of variance in which the total treatment variation was divided into that due to treatments, that due to the blocks and the residual or error variation. The treatment variation was then further divided into that due to the addition of vitamin D to the diet, that due to alteration in dietary Ca and P, and that due to the interaction of these two factors.

The following comparisons were made:

C_1	No added vitamin D (groups 1–4)	$v.$	Added vitamin D (groups 1D, 2D, 3D, 4D)
C_2	Lower mineral intake (groups 1, 1D, 2, 2D)	$v.$	Higher mineral intake (groups 3, 3D, 4, 4D)
C_3	0·08% Ca and P (groups 1, 1D)	$v.$	0·12% Ca and P (groups 2, 2D)
C_4	0·24% Ca and P (groups 3, 3D)	$v.$	0·36% Ca and P (groups 4, 4D)

Consequently, the following interactions were determined: C_1 *v.* C_2; C_1 *v.* C_3; C_1 *v.* C_4. The significance levels for all comparisons are shown in Table 3.

Table 1. *Effect of vitamin D and increasing mineral intake at a calcium to phosphorus ratio of 1:1 on the composition of serum and bone of rats*

Ca content of diet (%) ...	0·08		0·12		0·24		0·36		
Group* ...	1	1D	2	2D	3	3D	4	4D	SE
Serum Ca (μg/ml)	96·1	116·7	82·1	119·1	134·5	109·9	108·0	113·6	3·79
Serum P (μg/ml)	34·8	32·2	49·1	65·0	73·7	72·7	72·6	75·4	5·61
Fresh wt of humeri (mg)	414·6	395·4	434·4	399·9	460·9	470·4	488·0	459·0	11·07
Volume of humeri (μl)	321·9	297·0	300·8	276·7	277·1	276·1	280·2	280·0	5·51
Length of humerus (mm)	21·2	23·1	22·1	24·6	24·7	24·7	24·9	24·7	0·42
Fresh wt/volume (g/ml)	1·29	1·34	1·41	1·45	1·67	1·71	1·75	1·76	0·06
Dry wt of humeri (mg)	171·0	170·0	205·6	201·3	286·0	299·2	317·3	323·0	22·58
Wt of ash in humeri (mg)	62·5	70·6	98·5	108·2	178·1	189·8	203·6	209·1	19·10
Ash as % fresh humeri	15·3	19·6	23·6	27·4	38·7	40·4	41·9	42·8	3·43
Ash as % dry humeri	36·6	41·6	48·3	53·9	62·4	63·4	64·2	64·8	3·48
Ratio D:R†	0·70	0·77	0·92	1·04	1·61	1·76	1·88	1·94	0·16
Ratio A:R‡	0·59	0·73	0·93	1·18	1·66	1·74	1·80	1·86	0·16
Final body-wt (g)	133·5	172·4	175·0	214·8	214·4	218·5	219·1	217·2	9·91
Fresh wt of humeri/body-wt (mg/g)	3·15	2·30	2·50	1·88	2·19	2·18	2·26	2·30	0·12
Dry wt of humeri/body-wt (mg/g)	1·30	0·99	1·19	0·96	1·36	1·39	1·47	1·52	0·07
Wt of ash in humeri/body-wt (mg/g)	0·49	0·41	0·57	0·52	0·85	0·88	0·93	0·98	0·07

* D denotes addition of vitamin D to the diet.

† Ratio of dry weight to weight of fat and water in fresh bone.

‡ Ratio of weight of ash to weight of organic matter in dry, fat-free bone.

Table 2. *Effect of vitamin D and increasing mineral intake at a calcium to phosphorus ratio of 1:1 on the composition of the mandibular incisor tooth*

(Mean values with standard errors for groups of five ♂ and five ♀ rats)

Ca content of diet (%)	0·08		0·12		0·24		0·36		
Group*	1	1D	2	2D	3	3D	4	4D	SE
Dry wt of incisor tooth (mg)	58·9	58·4	53·5	60·6	65·3	66·6	68·9	6[illegible]·6	1·6[illegible]
Wt of ash in incisor tooth (mg)	44·3	42·8	38·9	44·7	48·6	49·8	51·2	50·7	1·5
Ash as % of dry tooth	75·6	73·3	71·6	73·7	74·5	74·8	74·5	75·0	[illegible]
Ratio A:R†	2·70	2·75	2·45	2·09	2·02	2·07	2·07	2·07	0·[illegible]
Dry wt of tooth/body-wt (mg/g)	0·15	0·34	0·31	0·29	0·34	0·32	0·35	0·33	0·[illegible]
Wt of ash in tooth/body-wt (mg/g)	0·33	0·25	0·22	0·21	0·24	0·24	0·24	0·25	0·0[illegible]
Dry wt of tooth/dry wt of humerus (mg/mg)	0·63	0·62	0·52	0·60	0·56	0·55	0·44	0·43	0·04
Wt of ash in tooth/wt of ash in humerus (mg/mg)	1·41	1·24	0·98	0·83	0·55	0·53	0·51	0·40	0·[illegible]

* Denotes addition of vitamin D to the diet.
† See footnote to Table 1.

Table 3. *Significance levels (P) for the effects on bones and teeth of rats of vitamin D, increased dietary calcium and phosphorus content at a constant ratio of 1:1 and their interactions (I)*

	Vitamin D	Groups 1, 2 v. groups 3, 4	I	Group 1 v. group 2	I	Group 3 v. group 4	I
Serum Ca	0·001	0·05	0·001	NS	0·05	NS	NS
Serum P	NS	0·001	NS	0·001	0·025	NS	NS
Fresh wt of humeri	NS	0·001	0·025	NS	NS	0·025	NS
Volume of humeri	0·01	0·001	0·01	0·025	NS	NS	NS
Length of humerus	0·001	0·001	0·001	0·001	NS	NS	NS
Fresh wt/volume	0·001	0·001	NS	0·001	NS	0·001	NS
Dry wt of humeri	NS	0·001	NS	0·001	NS	0·001	NS
Wt of ash in humeri	0·025	0·001	NS	0·001	NS	0·001	NS
Ash as % fresh humeri	0·001	0·001	0·01	0·001	NS	0·001	NS
Ash as % dry humeri	0·001	0·001	0·001	0·001	NS	0·05	NS
Ratio D:R*	0·001	0·001	NS	0·001	NS	0·001	NS
Ratio A:R*	0·001	0·001	0·001	0·001	0·05	0·001	NS
Final body-wt	0·001	0·001	0·001	0·001	NS	NS	NS
Fresh wt of humeri/body-wt	0·001	0·001	0·001	0·001	NS	NS	NS
Dry wt of humeri/body-wt	0·001	0·001	0·001	0·025	NS	0·001	NS
Wt of ash in humeri/body-wt	NS	0·001	0·001	0·001	NS	0·001	NS
Dry wt of incisor tooth	0·05	0·001	0·05	NS	0·01	0·05	NS
Wt of ash in incisor tooth	0·01	0·001	0·025	0·05	0·001	0·05	NS
Ash as % dry tooth	0·05	0·001	NS	0·05	0·01	NS	NS
Ratio A:R*	0·025	0·001	NS	NS	0·01	NS	NS
Dry wt of tooth/body-wt	0·001	0·01	0·001	0·001	0·001	NS	NS
Wt of ash in tooth/body-wt	0·01	0·025	0·001	0·001	0·001	NS	NS
Dry wt of tooth/dry wt of humerus	NS	0·001	0·01	0·001	0·01	NS	NS
Wt of ash in tooth/wt of ash in humerus	NS	0·001	NS	0·001	0·001	NS	NS

NS, not significant.
* See footnote to Table 1.

Histological examination

Femurs. In groups 1 and 1D (0·08% Ca and P) the bones were markedly rachitic. The epiphyseal cartilage was wide and irregular. There was much osteoid in the shafts and trabeculae. The cortex of the shaft and the trabeculae were thin. At this level of mineral intake, addition of vitamin D caused an improvement in the appearance of the bones; the epiphyseal disc was only slightly wider than normal but there was still much osteoid present. Raising the mineral intake to 0·12% Ca and P (group 2) did not prevent rachitic signs, the epiphyseal cartilage was still wider than normal, osteoid was present but the trabeculae and the cortex of the shaft were thicker than in groups 1 and 1D. When vitamin D was added to the diet (group 2D) the bone appeared normal. The bones in all other groups were normal in appearance.

Incisor teeth. The teeth from group 1 animals were severely affected. In the apical region the predentine was twice the width of the dentine, which contained many unfused calcospherites; this lack of fusion was also present in the incisal dentine. When vitamin D was added to the diet (group 1D) there was some improvement to the apical region, the predentine was now only one-third as wide as the dentine, and the incisal dentine, although the pattern of mineralization was irregular, showed fewer unfused calcospherites.

The teeth from group 2 animals (0·12% Ca and P) were similar in appearance to those from group 1. Addition of vitamin D to the diet (group 2D) caused an improvement in appearance similar to that in group 1D.

Raising the mineral intake to 0·24 Ca and P (group 3) resulted in a dramatic improvement; the apical dentine was almost normal and the incisal region was normal in appearance. Addition of vitamin D (group 3D) produced an almost normal tooth. The teeth in groups 4 and 4D were normal in appearance.

DISCUSSION

These experiments complete a series of three studies in the rat designed to provide information concerning the effects of vitamin D and the level of dietary Ca and P on the serum, bones and teeth. We have already reported on the effects of vitamin D at four different dietary levels of mineral at constant Ca to P ratios of 1:10 and 10:1 (Hurrand & Hartles, 1968, 1969). We have now obtained similar results when the Ca to P ratio was 1:1. The diets used ranged from those grossly deficient in Ca and P (0·08%) to those containing 'adequate' amounts (0·36%).

Growth of animals

Comparison of the final body-weights of animals receiving vitamin D with those of the animals denied the vitamin revealed a significant increase in weight attributable to the addition of vitamin D to the diet. However, inspection of the results (Table 1) shows that this increase was confined to the two lower levels of mineral intake (groups 1, 1D, 2 and 2D). The animals receiving the two higher levels of mineral were significantly heavier than those receiving the diets containing less mineral.

Serum Ca and P

The addition of vitamin D to the diet caused a significant increase in serum Ca concentration, an effect which was greatest at low levels of mineral intake. Comparison of groups 1 and 2 with groups 3 and 4 shows that the increasing mineral intake caused an increase in serum Ca but at a lower level of significance ($P < 0.05$). This effect is due to the action of the increased mineral in the absence of vitamin D. In the presence of the vitamin, increasing the mineral in the diet did not increase the serum Ca concentration. This is reflected in the highly significant interaction (Table 3).

The effect of the addition of vitamin D on serum P concentration was not significant, whereas increasing dietary mineral content had a highly significant effect. Thus at a Ca to P ratio of 1:1, serum Ca concentration was unaffected by increased dietary mineral, provided that the diet contained vitamin D. Serum P concentration on the other hand increased with increasing dietary mineral both in the presence and absence of the vitamin.

Bones and incisor teeth

A comparison of the results for all groups with and without vitamin D reveals several interesting effects of the vitamin. In general, vitamin D had no significant effect on the fresh weight or dry weight of the humeri, but examination of Table 1 shows that at the two lower levels of dietary mineral the vitamin reduced the fresh weight of the humeri and at the two higher levels of mineral the fresh weight was increased. This effect almost disappeared for the dry weight of the humeri.

The weight of ash deposited in the humeri was increased in the presence of vitamin D by a small but significant amount. The percentage of ash, the ratio of ash to dry fat-free organic matter, the ratio of dry fat-free mass to water plus fat in the fresh bone and the length of the humeri were all significantly increased in the presence of vitamin D. The volume of the humeri was significantly reduced when vitamin D was added to the diet, but examination of Table 1 shows that this effect was confined to the two lower levels of dietary mineral. Consequently, the crude density of the bone (fresh weight/volume) was significantly raised by the addition of the vitamin, but this increase at the lower levels of mineral intake was largely due to a decrease in water and fat content and not merely to an increase in the deposition of bone salts. The ratios of bone mass (both fresh and dry fat-free) to body-weight were significantly lower in the presence of vitamin D, but again the effect was confined to the two lower levels of dietary mineral, showing that in these circumstances the vitamin increased body-weight more than bone weight.

Increasing the dietary mineral content caused significant increases in fresh weight, dry weight, weight of ash, percentage of ash, ratio of ash to dry fat-free organic matter and ratio of dry fat-free mass to water + fat in the fresh bone in the humeri. The volume of the humeri was significantly reduced with increasing mineral intake and the length significantly increased except at the highest level of intake.

The relative effect of increasing dietary mineral is of interest. When the two higher levels of mineral are compared with the two lower, there is a significant fall in the ratio

of fresh weight of bone to body-weight, indicating that the increase in total bone mass was less than the increase in final body-weight. On the other hand, a similar comparison of dry weight and weight of ash with body-weight shows a significant rise in the ratio, showing that with increasing mineral intake the absolute amounts of fat-free dry bone and bone ash were increased to a greater extent than that of body-weight. This is a reflection of the fact that when dietary mineral is in short supply much of the bone mass is fat and water.

The provision of vitamin D had a small effect of low significance in increasing dry weight, weight of ash, percentage of ash and ratio of ash to dry fat-free organic matter of the incisor teeth. Inspection of Tables 2 and 3 shows that these effects were mainly confined to those animals receiving 0·12% Ca and P (groups 2, 2D). Increasing the dietary mineral resulted in an increase in total mass and weight of ash. The percentage of ash and A:R ratio increased with increasing mineral intake up to 0·24% Ca and P.

Comparison of results obtained at three different Ca to P ratios

It is of great interest to compare the results obtained with those from earlier studies carried out under similar conditions and experimental design (Harrand & Hartles, 1968, 1969).

In order of ascending dietary Ca to P ratio, the three series of experiments were as follows:

	Ca % in diet			
Ca to P ratio 1:10	0·08	0·12	0·23	0·32
1:1	0·08	0·12	0·24	0·36
10:1	0·8	1·2	2·3	3·3

At the lowest levels of dietary intake there is an absolute deficiency of Ca at a Ca to P ratio of 1:10, and of P when the ratio is 10:1.

Growth. When the Ca to P ratio is 1:10 vitamin D enhances growth at all levels of mineral intake studied; when the ratio is 1:1 the vitamin enhances growth only when there is a deficiency of dietary mineral. When the diet contains adequate Ca but is deficient in P (Ca to P ratio of 10:1) addition of vitamin D causes a reduction in body-weight; increasing the absolute dietary mineral at the same Ca to P ratio reverses this effect (see Fig. 1).

In relation to mineral intake an excessive amount of P in the diet (Ca to P = 1:10) appears to be toxic and has a deleterious effect on growth, whereas an abundance of Ca (Ca to P = 10:1) does not have this effect. Although a statistical comparison between the three studies would not be valid, certain inferences can be drawn. Fig. 1 indicates that the greatest final body-weight was achieved when the diet contained 0·36% Ca and P. At low levels of dietary mineral a Ca to P ratio of 10:1 results in a lower final body-weight than when the Ca to P ratio is 1:10. With increased mineral intake the converse is true. If weight gains during the 60 d experimental period, rather than final body-weights, are compared, the patterns are almost identical.

Serum Ca and P. Fig. 2 shows in diagrammatic form the effect of vitamin D and increasing dietary mineral intake on serum Ca and P concentrations in the three series

of experiments. There is a marked rise in serum Ca concentration as the dietary Ca to P ratio changes from 1:10 through 1:1 to 10:1, accompanied by a fall in serum P concentration.

Vitamin D exerts its greatest effect on raising serum Ca concentration (calcemic effect) when there is a deficiency in absolute terms of either Ca or P; the effect of the vitamin on Ca concentration is minimal when the dietary Ca or P content rises to 0·24 or 0·32 %, irrespective of the Ca to P ratio.

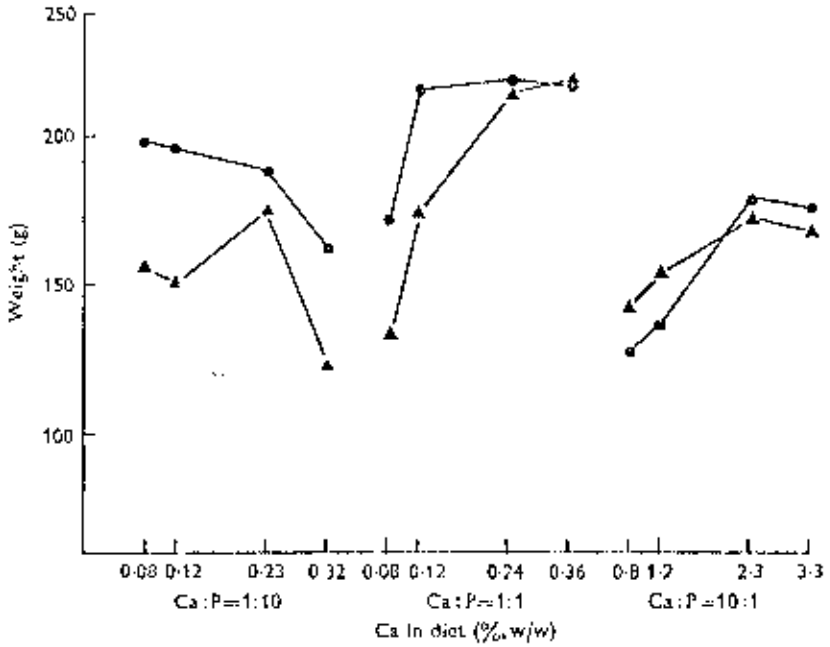

Fig. 1. Effect of vitamin D on the final body weight of rats at different levels of mineral intake and with a calcium to phosphorus ratio of 1:10 (left), 1:1 (centre) and 10:1 (right). ▲- ▲, without vitamin D; ●-●, with vitamin D. In this and all Figures, values for the Ca:P ratio 1:10 and 10:1 are derived from Harrand & Hartles (1968, 1969).

The apparent effect of the vitamin on serum P concentration is quite different; at a Ca to P ratio of 1:10 the serum P is lowered, at a ratio of 1:1 it is not significantly changed, and at a ratio of 10:1 the serum P level is raised by provision of vitamin D.

When the dietary mineral content is increased, there is no corresponding increase in serum Ca concentration provided that the animals are receiving vitamin D. The very low serum Ca concentrations occur only when there is a deficiency of both Ca and vitamin D. In contrast, serum P concentration rises with increasing dietary intake of P at each Ca to P ratio. In general terms, therefore, it can be stated that serum Ca concentration is affected more by vitamin D than by dietary Ca content at each of the three dietary Ca to P ratios. On the other hand, serum P concentration is affected to a much greater degree by dietary intake of P and less by the presence of vitamin D. Harrison & Harrison (1960) administered vitamin D to rats fed on a diet of normal

mineral composition but lacking vitamin D; they observed a persistent rise to normal in serum Ca and only a transient rise above normal in serum P. They attributed this latter effect to vitamin D, and its transient nature suggests that it was due to its mobilization along with Ca from the bone.

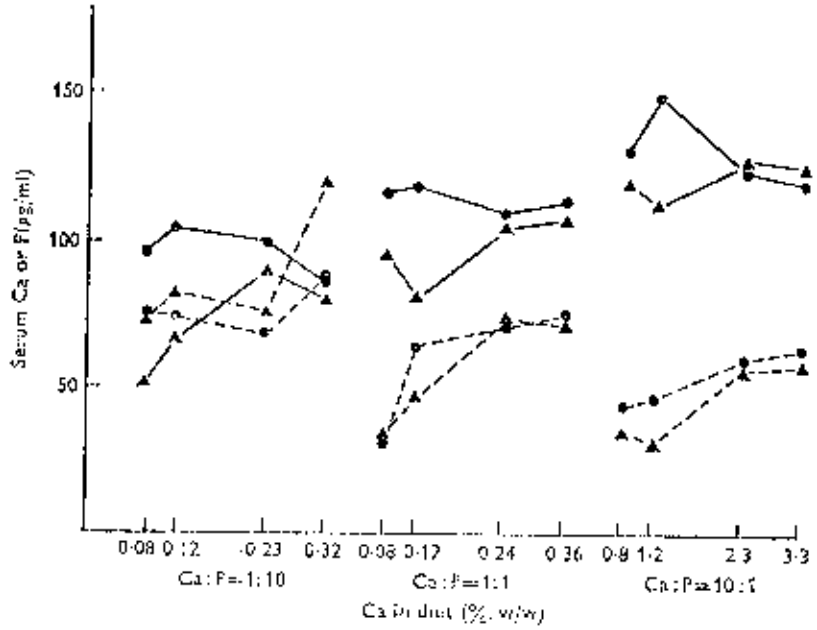

Fig. 2. Effect of vitamin D on the serum calcium and phosphorus of rats at different levels of mineral intake and with a Ca to P ratio of 1:10 (left), 1:1 (centre) and 10:1 (right). ▲---▲, serum Ca without vitamin D; ●—●, serum Ca with vitamin D; ▲---▲, serum P without vitamin D; ●—●, serum P with vitamin D.

Bone formation, mineral intake and vitamin D. Fig. 3 shows the effect of vitamin D and increasing dietary mineral on the fresh weight of the humeri at the different Ca to P ratios. Fig. 4 shows comparable values for dry weight and weight of ash in the humeri. When the Ca to P ratio in the diet is 1:10, provision of vitamin D increases the fresh weight, dry weight and weight of ash in the bone, although in the latter two the effect vanishes when the absolute intake of mineral is lowest (0·08% Ca). When the Ca to P ratio is 1:1 or 10:1 and there is a deficiency of P in absolute terms (0·08 or 0·17%) the provision of the vitamin reduces the fresh weight of bone; when the intake of P is increased this effect is reversed (Fig. 3). There is some indication that the provision of vitamin D also reduces the dry weight of bone formed when dietary P is very low at a Ca to P ratio of 10:1, but the weight of ash is not significantly changed by the vitamin (Fig. 4).

When the Ca intake is very low and the Ca to P ratio also very low (e.g. 1:10) in the absence of vitamin D the bones formed in the young growing rat are osteomalacic (Harrison & Fraser, 1960; Ferguson & Harries, 1963). Animals on a similar mineral

intake but provided with vitamin D, i.e. animals subject to a simple Ca deficiency, develop a condition that appears closer to osteoporosis than to osteomalacia. Harrand & Hartles (1968) confirmed these findings, and showed that the addition of vitamin D to a diet low in Ca (0·08%) but adequate in P (0·8%) caused a shift from osteomalacia

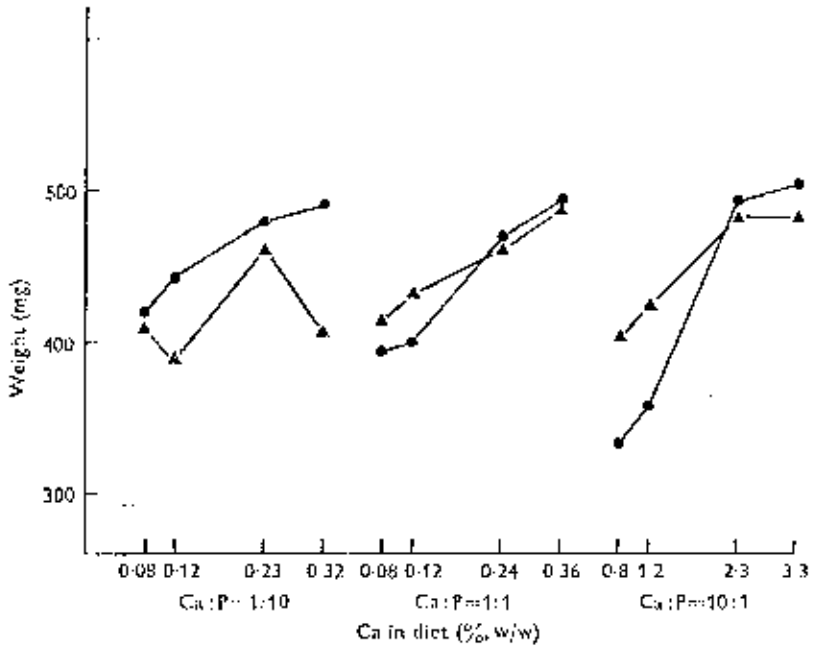

Fig. 3. Effect of vitamin D on the fresh weight of the femur of rats at different levels of mineral intake and with a calcium to phosphorus ratio of 1:10 (left), 1:1 (centre) and 10:1 (right). ▲—▲, without vitamin D; ●—●, with vitamin D.

to a porotic condition of the bone with demonstrably less osteoid. There was a concomitant large rise in serum Ca concentration, but no corresponding rise in the amount of ash deposited in the bone (Figs. 3 and 4). It seems reasonable to conclude, therefore, that in these circumstances, where the only mineral deficiency is of Ca, the function of vitamin D is to potentiate the action of parathormone in maintaining Ca homeostasis by mobilizing the skeletal mineral. That there is no demonstrable change in the mass of mineral ash in the bones is understandable, since the total Ca circulating in the blood of a rat is of the order of 1 mg. When the Ca intake is increased at the same Ca to P ratio of 1:10, the vitamin has a decreasing effect on raising the serum Ca concentration, but significantly increases the actual amount of mineral deposited in the bone especially at the highest level of intake (Fig. 4). Thus, with increasing mineral intake at a Ca to P ratio of 1:10 the calcaemic effect of vitamin D decreases, whereas the effect on deposition of bone mineral increases.

When the dietary Ca to P ratio is 1:1 and the diet contains 0·08% Ca, vitamin D raises the serum Ca concentration to normal levels but is without effect on the low serum P (Fig. 2). The weight of mineral (ash) deposited in bone is very low (Fig. 4).

and is raised slightly but significantly by vitamin D. At the higher levels of mineral intake both serum Ca and P levels are raised, bone mineral increases rapidly and the calcaemic effect of vitamin D becomes less. At low levels of mineral intake the fresh weight of the bone is decreased (Fig. 3).

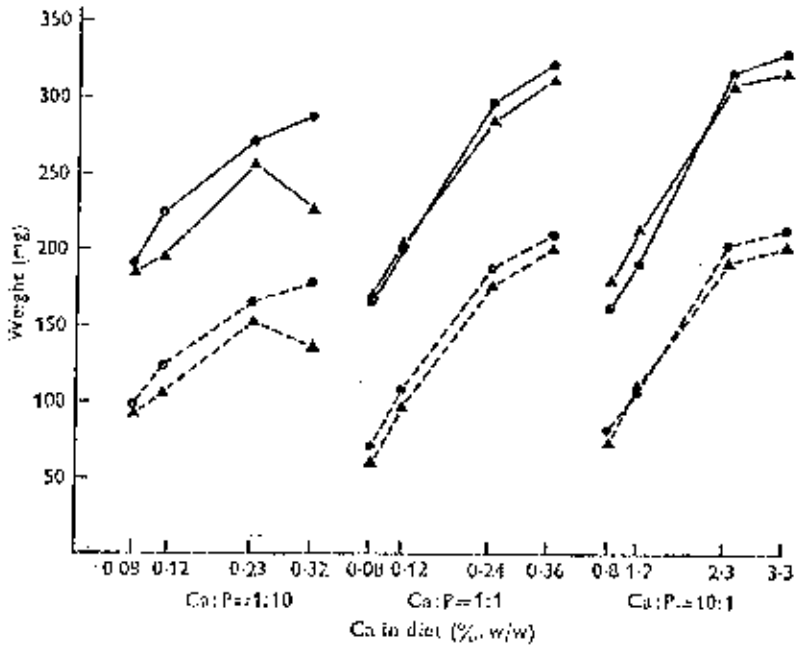

Fig. 4. Effect of vitamin D on the dry weight and weight of ash of the femora of rats at different levels of mineral intake and with a calcium to phosphorus ratio of 1:10 (left), 1:1 (centre) and 10:1 (right). ▲--▲, dry weight without vitamin D; ●—●, dry weight with vitamin D; ▲- - -▲, weight of ash without vitamin D; ●- - -●, weight of ash with vitamin D.

When the dietary Ca content is 0·8% or 1·2% at a Ca to P ratio of 10:1, the serum Ca concentration is within the normal range even in the absence of vitamin D, whereas the serum P concentration is grossly depressed. The provision of the vitamin raises serum Ca only when dietary P is grossly deficient and raises serum P at all levels but is without a significant effect on the amount of mineral deposited (Figs. 2, 4). The increase in the percentage of ash in these circumstances is due to the reduction in the amount of organic matter and water in the bone. This trend is also apparent at the two lower levels of mineral intake at a Ca to P ratio of 1:1. All these animals were rachitic. Underwood, Fisch & Hodge (1951) considered that the rise in serum P that followed administration of vitamin D to P-deficient animals was due to mobilization from the soft tissues, and that the vitamin accelerated the mineralization of bone. Our results (Harrand & Hartles, 1959) shown in Fig. 4 indicate no significant increase in bone mineral as a result of adding vitamin D to the diet. We cannot subscribe to the view that, in the rachitic state in the rat caused by a high Ca to P ratio in the diet, vitamin D

causes a shunt of P from the soft tissues to bone. It would appear from our results that there is no increased deposition of mineral attributable to vitamin D, and that the improvement, both histologically and chemically, is one of organization and utilization of mineral. The increase in percentage of ash and A:R ratio is due mainly to a reduction in the non-mineral portion of the bone.

Incisor tooth formation, mineral intake and vitamin D. Fig. 5 shows diagrammatically the results for the dry weights and weights of ash in the incisor teeth in the present study (Ca to P, 1:1) and in two earlier studies where the Ca to P ratios were 1:10 and 10:1 (Harrand & Hartles, 1968, 1969). It is apparent that from the quantitative point

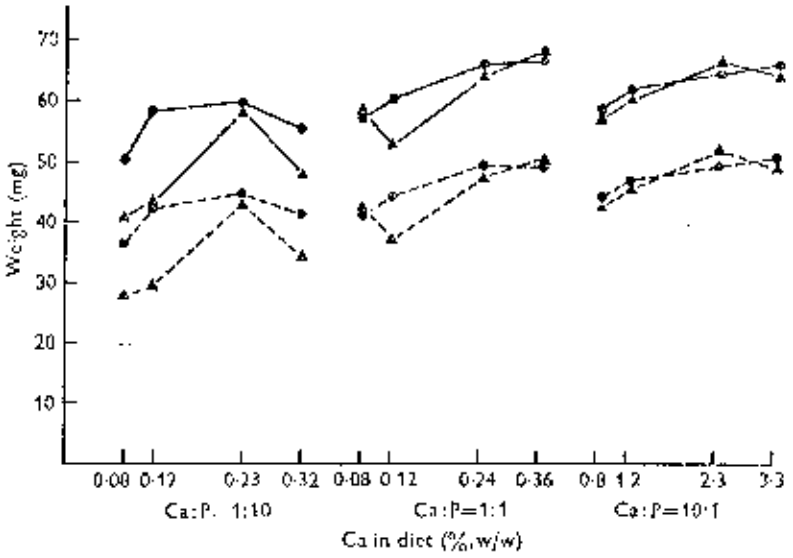

Fig. 5. Effect of vitamin D on the dry weight and weight of ash of the incisor tooth of the rat at different levels of mineral intake and with a calcium to phosphorus ratio of 1:10 (left), 1:1 (centre) and 10:1 (right). ▲——▲, dry weight without vitamin D; ●——●, dry weight with vitamin D; ▲---▲, weight of ash without vitamin D; ●---●, weight of ash with vitamin D.

of view the incisor tooth is most sensitive to lack of vitamin D when the diet is deficient in Ca at a Ca to P ratio of 1:10. The vitamin has least effect on the quantity of tooth formed when the Ca to P ratio is 10:1. It has been suggested that a simple deficiency of vitamin D results in a reduction of the amount of dentine formed (Jenkins, 1966), and, by implication, that a possible function of the vitamin may be the control of the amount of dentine matrix formed. The results of these experiments show that in the rat vitamin D had no effect on the mass of tooth formed when the Ca and P content of the diet was 0·24 or 0·36% (Fig. 5). When the diet was low in Ca (0·08, 0·12%) at a Ca to P ratio of 1:10, vitamin D greatly increased the mass of the tooth formed, but not when the Ca to P ratio was 10:1 (Fig. 5).

Thus vitamin D increases tooth mass only when the supply of Ca is deficient or when the Ca to P ratio is 1:10.

When the Ca to P ratio is 10:1 (Harrand & Hartles, 1969) provision of vitamin D

does not affect the quantity of tooth formed but it has a marked qualitative effect in the microscopic appearance of the dentine. It would appear, therefore, that in these circumstances one function of the vitamin is to prepare the matrix for a more orderly deposition of the available mineral.

Variation in dietary mineral content has a less dramatic quantitative effect on the tooth than on the bone, except when vitamin D is absent from the diet and the Ca to P ratio is 1:10. It is in these circumstances that the reactions of the tooth and bone show certain similarities (Figs. 4, 5).

Qualitatively, however, the teeth are severely affected by deficiencies of both vitamin D and mineral.

Comparative effect of mineral intake and vitamin D on the forming incisor tooth and bone

There is evidence to show that developing teeth and bone react differently to dietary changes in Ca, P and vitamin D (Gaunt & Irving, 1940; Gaunt, Griffith & Irving, 1942; Irving, 1957; McRoberts & Hill, 1962; Ferguson & Hartles, 1964, 1966).

The formed tooth, unlike bone, is extremely resistant to metabolic change and does not undergo remodelling. The only physiological resorption that occurs in the erupted teeth is in the deciduous dentition prior to natural exfoliation. The forming tooth, on the other hand, is responsive to metabolic influences, in some respects (e.g. fluorosis) more so than bone (Sognnaes, 1961).

The results of the present work and of earlier studies (Harrand & Hartles, 1968, 1969) can be summarized as follows.

(1) Lack of dietary mineral at Ca to P ratios of 1:10, 1:1 and 10:1 reduced the mass of mineral deposited in bone to a greater degree than in teeth.

(2) Variation of dietary mineral content had its least effect on tooth mass and weight of ash at a Ca to P ratio of 10:1. Bone mass and weight of ash, on the other hand, were least affected at a Ca to P ratio of 1:10.

(3) The mass of the forming tooth was more seriously affected by a poor dietary supply of Ca than by a lack of P. The converse was true for bone.

(4) The consumption of diets high in P (3·2%) with normal Ca (0·32%) reduced the mass and amount of mineral deposited in both bones and teeth compared with diets containing 2·3% P and 0·25% Ca. However, the provision of vitamin D mitigated this effect in the teeth and reversed it in bone. Thus an excessive intake of P has a more deleterious effect on teeth than on bone. An excessive consumption of Ca at a dietary Ca:P ratio of 10:1 had no such effect on either teeth or bones.

(5) In relation to the amount of mineral deposited in both bones and teeth, the effect of vitamin D varied with the Ca to P ratio in the diet. At a ratio of 1:10 the effect of the vitamin was highly significant, at 1:1 it was of less significance, and at 10:1 it was not significant.

(6) Notwithstanding the lack of quantitative effect of vitamin D at a Ca to P ratio of 10:1, there was a marked qualitative improvement in both bones and teeth. There is therefore a strong possibility that the vitamin exerts an effect on the organization of a calcifying tissue quite distinct from any function it may have in raising serum Ca.

It is postulated, therefore, that the calcaemic and mineralizing actions of vitamin D, though related, are independent effects.

Finally, it must be stated that the blocking by weight was effective in reducing residual variancy as the blocks removed a significant amount of the variation.

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