ALUMINUM COMPOUNDS VOL II #43
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SUMMARY

Aluminum Compounds-Volume 2

#43

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GRAS MONOGRAPH SERIES

ALUMINUM COMPOUNDS

(COPIES OF ARTICLES CITED
IN MONOGRAPH SUMMARY)

prepared for
THE FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION
AND WELFARE

JUNE 8, 1973

prepared by

Tracor Jitco, Inc.

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10. THE EFFECT OF ALUMINIUM HYDROXIDE ON CALCIUM, PHOSPHORUS AND ALUMINIUM BALANCES AND THE PLASMA PARATHYROID HORMONE IN PATIENTS WITH CHRONIC RENAL FAILURE

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Aluminium hydroxide has long been used in the treatment of the hyperphosphataemia of chronic renal failure. It is generally accepted that the compound is not absorbed from the gastrointestinal tract and acts by binding phosphate in the gut. The present study was undertaken to establish what effect this agent has on the calcium, phosphorus and aluminium balances and on the serum parathyroid hormone of patients with advanced renal failure. To investigate this, 75–150 ml of aluminium hydroxide (equivalent to 1·5–3·4 g aluminium) was administered daily to eight patients with chronic renal failure (creatinine clearance 4·9–22 ml/min). Calcium and phosphorus balances were measured using chromium sesquioxide as a continuous faecal marker. The effect of aluminium hydroxide on the calcium and phosphorus balances was variable, and in particular the phosphorus balance bore no relationship to the fall in plasma phosphorus. All patients were found to absorb and retain aluminium. Changes in serum aluminium, measured by atomic absorption spectrophotometry, however, were small. Plasma phosphorus fell in all patients, the mean fall being 2 mg/100 ml. The effect on plasma calcium was either nil or resulted in a small increase of up to 0·9 mEq/l. Similarly the effect on circulating immunoassayable parathyroid hormone was variable. If the plasma calcium remained unchanged the parathyroid hormone did not alter. Where a rise occurred in the plasma calcium there was a proportionate fall in parathyroid hormone. This would suggest the parathyroid gland was responding to a change in plasma calcium rather than plasma phosphorus. To explain the fall in plasma phosphorus following the administration of aluminium hydroxide it is suggested that aluminium combines with phosphate after absorption and is deposited in bone.

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HYPERALUMINÆMIA FROM ALUMINIUM RESINS IN RENAL FAILURE

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Summary The effect of ingestion of aluminium-cycle resins and aluminium hydroxide on serum-aluminium levels, determined by neutron activation, colorimetry, and atomic absorption spectrophotometry, has been studied in patients with advanced renal failure. Raised serum-aluminium levels were found in 6 out of 20 patients taking 45 g. of aluminium resin or more a day; in 2 out of 6 patients taking 3–6 g. of aluminium hydroxide a day; and in 3 out of 6 patients on dialysis who were not taking oral aluminium salts but were fortuitously exposed to dialysate with a relatively high aluminium content. Aluminium resins and salts should be avoided in renal failure until more is known about the possible toxic effects of hyperaluminæmia, and more rigid control of aluminium concentration in dialysis fluids is necessary.

Introduction

AFTER the introduction of calcium-cycle ion-exchange resins in the treatment of hyperkalæmia complicating renal failure,[1,2] two reports of calcæmia during the administration of calcium resins[3,4] prompted the suggestion that aluminium-cycle resins were safer, and these were recommended for use in the treatment of hyperkalæmic renal failure. Unfortunately, no data were produced showing aluminium resins were themselves free of the danger of causing elevation in serum-aluminium levels in patients with renal failure.[6] In this paper we show the effect on serum-aluminium levels of giving aluminium-cycle resins and aluminium hydroxide therapeutically in chronic renal failure to control hyperkalæmia and hyperphosphatæmia respectively. Because of the difficulty in accurately determining aluminium levels in the plasma and urine, three different methods were employed—neutron activation, colorimetry, and atomic absorption spectrophotometry. Plasma-aluminium levels were found to be raised in 1 person out of every 3 taking aluminium-cycle resins or aluminium hydroxide, and also in patients being dialysed.

Methods

The subjects were as follows: 5 male doctors, aged 23–29, in Manchester Royal Infirmary; 3 male doctors, aged 32–39, and 2 nurses, aged 25–26 in Central Negev Hospital Beer Sheva; 6 patients with chronic renal failure on regular dialysis at Manchester Royal Infirmary (glomerular filtration-rate [G.F.R.] < 1 ml. per minute); 16 patients with chronic renal failure not on regular dialysis (G.F.R. < 2 ml. per minute) at Manchester Royal Infirmary; and 7 patients with chronic renal failure at Central Negev Hospital, Beer Sheva. Aluminium levels were estimated in the serum before and after administration of aluminium-cycle 'Zeocarb 225' (made by the method of Chugh et al.[5]) in a dosage of 45 to 60 g. daily or of dry aluminium hydroxide 6 g. daily,

TABLE I—SERUM-ALUMINIUM LEVELS IN CHRONIC RENAL FAILURE: NON-DIALYSED PATIENTS

Case no.	Age (yr.)	Sex	G.F.R. (ml. per min.)	Al resin given		Aluminium hydroxide given		Serum-aluminium levels (mg. per l.)					
								Before Al therapy			After Al therapy		
				g. per day	No. of days	g. per day	No. of days	Act	Col	AA	Act	Col	AA
1	22	M	<3·0	0	0	0	..	0·77	0·77	1·7	..	..	..
2	35	M	<3·0	0	0	0	..	0·82	2·0	1·7	..	..	..
3	37	M	<3·0	..	..	6	>10	..	..	..	0·59	1·3	..
4	18	F	5·2	0	0	0	..	14·0	14·0	..	..	..	..
5	43	M	2·0	70	7	..	..	..	..	..	55·8	53·0	..
6	57	F	2·4	0	7	6	30	..	..	..	18·1	20·0	..
7	20	F	3·2	0	0	6	7	..	..	..	0·85	6·3	..
8	35	M	4·2	0	0	6	>10	..	..	..	1·12	4·0	..
9	21	F	<2·0	45	7	..	..	10·0	..	..	110	..	..
10	19	F	4·2	45	7	0	..	..	..	..	1·7	..	..
11	35	M	1·5	45	7	0	..	..	..	..	1·3	..	..
12	40	M	<2·0	45	7	0	..	..	..	..	..	5·0	..
13	42	M	<1·0	45	7	0	..	..	..	..	..	..	..
14	47	M	2·5	45	7	0	..	..	..	..	1·9	..	..
15	19	F	3·2	45	7	0	..	..	..	..	8·1	..	..
16	19	F	3·0	45	7	0	..	..	..	0·25	..	..	0·45
17	25	M	1·0	45	7	0	..	..	..	0·5	..	..	0·4
18	42	M	0·6	45	7	0	..	..	..	..	..	..	0·3
19	45	F	0·9	45	7	0	..	..	..	..	..	..	0·55
20	49	M	2·3	45	7	0	..	..	0·2	..	..	..	0·5
21	42	M	2·2	45	7	0	..	..	0·2	·2	..	..	0·3
22	38	F	3·0	45	14	0	..	..	..	0·2	..	..	1·0
23	45	M	<3·0	45	5	0	..	..	..	..	0·58	3·5	..
24	42	M	2·6	0	0	6	>10	..	..	..	0·36	1·3	..
25	40	M	1·9	0	0	3	7	..	..	..	19·2	21·0	..
26	45	M	2·8	0	0	0	0	1·23	2·3	..	..	..	..
27	60	F	1·8	45	10	0	0	..	..	0·5	..	..	1·4
28	37	F	4·5	45	10	0	..	..	..	0·4	..	..	1·0
29	26	F	1·2	45	10	0	..	..	..	0·4	..	..	0·45
30	36	F	3·0	45	14	0	..	..	..	0·4	..	..	0·5

Act = activation analysis. Col = colorimetric.

TABLE II—SERUM-ALUMINIUM LEVELS IN CHRONIC RENAL FAILURE: DIALYSED PATIENTS

Case no.	Age (yr.)	Sex	Type of dialysis*	Serum-aluminium levels (mg./l.)				Aluminium levels (mg./l.) in dialysis fluid	
				Before dialysis		After dialysis			
				Act	Col	Act	Col	Act	Col
31	40	M	P.D.	0·35	3·3	21·7	35·0	12·6	22
32	18	M	H.D.	0·22	1·5	0·64	2·5	0·79	2·6
33	17	M	H.D.	0·43	0·5	0·96	2·8	..	..
34	36	M	H.D.	0·74	1·5	1·00	2·5	61·7	50·0
35	46	F	P.D.	42·3	42	43·1	44	..	..
36	21	F	H.D.	21·5	24	30·7	29	38·9	36·0

* P.D. = peritoneal dialysis. H.D. = hæmodialysis

in persons who received drugs not known to contain aluminium.

G.F.R. was measured by urea or creatinine clearance, using urine collected over a period of 24 hours. Urea or creatinine in serum in urine was measured by 'AutoAnalyzer' method N 38.

Aluminium levels were measured by neutron activation and colometric methods carried out by G. R. G. at the universities joint reactor at Risley; in Israel estimation of serum and urine aluminium was by atomic absorption spectrophotometry by R. L. of the Israel Nuclear Research Unit.

Details of neutron-activation, colorimetric, and atomic absorption techniques of estimating aluminium can be obtained from G. M. B.

Results

The results are shown in tables I–IV.

Neutron activation and colorimetric methods agreed well with each other: $r = 0{\cdot}97769$, $p = < 0{\cdot}001$, $y = 0{\cdot}8954x + 2{\cdot}82297$ where x represents neutron activation results and y represents colorimetric results on the same specimens. It was not possible to make a comparison between atomic absorption methods and the other methods.

TABLE III—SERUM-ALUMINIUM LEVELS IN HEALTHY PERSONS

Subject	Age (yr.)	Sex	Serum-aluminium level (mg./l.) by:	
			Neutron activation	Atomic absorption
A	38	M	1·4	0·3
B	30	M	1·4	..
C	37	M	1·1	..
D	38	M	1·8	..
E	31	M	1·6	..
F	35	M	..	0·2
G	31	M	..	0·2
H	25	F	..	0·3
I	26	F	..	0·2
Mean			1·46	0·24

TABLE IV—RENAL CLEARANCE OF ALUMINIUM IN CHRONIC RENAL FAILURE

	Renal clearances (ml./min.)			$\frac{C_{Al}}{C_{urea}} \times 100$ (ml./min.)	$\frac{C_{Al}}{C_{creat}} \times 100$
	C_{Al}	C_{urea}	C_{creat}		
[illegible]	0·11	0·9	1·1	13·0	10·0
[illegible]	0·36	3·7	..	10·8	..
37	0·24	3·3	5·2	7·3	4·3
40	0·17	3·7	7·3	4·6	2·3
[illegible]	0·14	2·1	4·3	6·7	3·3
[illegible]	0·22	4·0	6·2	5·6	3·6
43	0·42	1·9	4·8	22·0	8·8

5 normal persons had serum-aluminium levels below 2 mg. per litre by neutron activation; and by atomic absorption 5 normal persons were found to have levels of 0·3 mg. per litre or less. This agrees well with previously reported results.[7] In 6 out of 20 patients (30%) given aluminium resins in a dosage of 45 g. or more for at least 5 days, serum-aluminium levels were increased up to 110 mg. per litre, by neutron activation measurements. Increases were also found by the atomic absorption technique. Hyperaluminæmia was found in 2 patients out of 6 (33%) taking aluminium hydroxide for the control of hyperphosphatæmia in renal failure. In 6 dialysed patients the dialysis fluid was found to have high initial aluminium concentration in 3 out of 4 samples tested (75%), levels being 12·6–61·7 mg. of aluminium per litre; and in 3 of the patients the serum-aluminium level was also raised (21·7–43·1 mg. per litre). In one patient with chronic renal failure, neither on dialysis nor receiving any drugs known to contain aluminium, the serum-aluminium level was raised at 14 mg. per litre.

Table IV shows the renal clearance of aluminium in 7 patients with chronic renal failure, determined by atomic absorption techniques. The overall tubular reabsorption of aluminium was:

$$\text{Mean } \frac{C_{Al}}{C_{urea}} \times 100 = 10\%;\ \text{range } 4{\cdot}6\text{–}22\%.$$

$$\text{Mean } \frac{C_{Al}}{C_{creat}} \times 100 = 5{\cdot}4\%,\ \text{range } 2{\cdot}3\text{–}10\%.$$

Discussion

The use of cation-exchange resins in the treatment of hyperkalæmia has been established since the pioneer work Evans et al.[8] using sodium-cycle resins. Because of the dangers of sodium retention resulting from the use of sodium-cycle resins in acute and chronic renal failure, we introduced calcium-cycle resins.[2] Calcium was chosen because it was known to be absorbed with difficulty from the gut in renal failure, and in treating over 100 patients in chronic renal failure (G.F.R. < 3 ml. per minute) we did not observe hypercalcæmia despite regular monitoring of serum-calcium levels. Nevertheless, aluminium-cycle resins were introduced by Chugh et al.[5] to overcome the occasional problem of hypercalcæmia resulting from the use of calcium-cycle resins, but their observation did not include any data on hyperaluminæmia in renal failure. We have found aluminium determination to be virtually impossible in the routine hospital laboratory, and have been obliged to use the facilities of two research institutions to measure aluminium concentration in serum. By each of three methods we have found that after either aluminium hydroxide or aluminium resin, serum-aluminium levels may be grossly elevated. We have also noted increased figures in some dialysed patients who were regularly consuming aluminium hydroxide. The hyperaluminæmia is apparently the result of ingestion of aluminium salts or aluminium-cycle resins or the addition of aluminium to the body from dialysis fluid. The toxic effects of hyperaluminæmia are unknown in man, and much of the published work on animals is related to the effects of concomitant phosphate depletion caused by the administration of

aluminium hydroxide.[7,9] Nevertheless, it is unwise to recommend the use of aluminium resin which can cause hyperaluminæmia in 30% of patients with advanced chronic renal failure when it is very difficult to measure aluminium levels accurately, and virtually impossible to obtain results of aluminium levels from routine biochemical laboratories. Serum-calcium is easy to measure accurately, and, in the rare event of hypercalcæmia occurring from calcium cycle resins, the serum-calcium level can be reduced by lowering the dose of drug given. Calcium resin is therefore recommended as the resin of choice in advanced renal failure where hyperkalæmia is a life-threatening factor.

In man hyperaluminæmia after large doses of aluminium salts in renal failure is probably accounted for by considerable absorption of aluminium ions from the intestine, and impairment of the normal efficient renal mechanism of excretion of aluminium. The lack of published data on aluminium metabolism in renal failure is partly explained by the difficulty in measuring aluminium concentration. Neutron activation, which is the most accurate method available, requires an atomic reactor. Colorimetric methods are not entirely specific. Atomic absorption spectrophotometry is moderately specific but the range of aluminium concentrations in human plasma is at the extreme lower range of sensitivity for this instrument. There is good correlation between the neutron activation and colorimetric methods, but, because atomic absorption tests were carried out on a different series of patients in Israel, there was no opportunity to compare the methods with atomic absorption.

Overall tubular reabsorption of aluminium is demonstrated by our data; the aluminium clearance varies from 2·3% to 22% of the G.F.R. according to whether urea or creatinine clearance was used as an index of G.F.R.

The amount of absorption from the intestine is unknown, but at normal plasma levels of aluminium not more than 1 mg. can be excreted per day in renal failure when the G.F.R. is 3 ml. per minute. At plasma levels of 50 mg. per litre, aluminium excretion may approach 250 mg. per day. Studies of aluminium metabolism are hampered by the lack of a suitable cheap radioactive isotope of aluminium. In addition the practice of cooking in aluminium utensils and wrapping foods in aluminium foils may result in gross changes in aluminium content of food before ingestion. Further studies are in progress to elucidate these points.

We acknowledge the help of Dr. M. Ronen, of the isotopes department, Central Negev Hospital, and our colleagues in Manchester and Beer Sheva who helped with the collections of urine and blood. We thank Mr. M. Soroka and Dr. H. Doron, of Merkaz Kupat Holim, for encouragement and support.

Requests for reprints should be addressed to G.M.B.

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ALUMINIUM TOXICITY IN RATS

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Summary Aluminium intoxication has been demonstrated in the uræmic and non-uræmic rat after modest doses of oral and parenteral aluminium salts. The clinical syndrome is associated with periorbital bleeding, lethargy, anorexia, and death. Plasma-levels of aluminium were greatly raised, as were tissue levels in liver, heart, striated muscle, brain, and bone. Histological changes were found in the cornea. Liver oxygen consumption was reduced by giving the animals aluminium salts before death or by adding aluminium in vitro to normal liver homogenates. It is recommended that aluminium salts should be withdrawn from use in patients with renal failure and their use restricted in normal persons pending clarification of the issue.

Introduction

THE question—is aluminium toxic?—has never been answered satisfactorily.[1-8] We have found that normal rats and rats in renal failure develop fatal intoxication from modest doses of aluminium salts; plasma-aluminium levels and tissue concentrations were increased, and the addition of aluminium to liver homogenates in vitro depressed oxygen uptake.

Materials and Methods

Littermate white adult male rats of Weizmann Institute strain weighing 200 g. were divided into control groups and test groups. The following test procedures were done (table I).

5/6 Nephrectomy

A total nephrectomy was carried out on one side and a 2/3 nephrectomy was done on the other side under chloral anæsthesia. The animals were allowed to recover for 1–2 weeks from the operation and were then divided into control and test groups. Both groups were given standard rat pellets as food.

Aluminium Salts

Test groups were given 1% or 2% aluminium chloride ($AlCl_3$) or sulphate ($Al_2(SO_4)_3$) as drinking-water or oral aluminium hydroxide ($Al(OH)_3$) 3 ml. daily (equivalent to 150 mg. of elemental aluminium per kg. per day) by gavage. Further test groups were given subcutaneous or intraperitoneal $Al(OH)_3$ in a dose of 3 ml. per day (equivalent to 90 mg. per kg. body-weight per day). All experiments were controlled with animals which had undergone 5/6 nephrectomy but received tap-water in place of aluminium salts to drink. In the experiments with intraperitoneal or subcutaneous injections, sham injections were not given to controls.

TABLE I—DESIGN OF IN-VIVO TOXICITY, INCLUDING ROUTE OF ADMINISTRATION AND MORTALITY

Aluminium salt	Route	Dose (mg. Al/kg./day)	No. of rats	No. with peri-orbital bleeding	No. of deaths
5/6 nephrectomised					
2% $Al_2(SO_4)_3$	Drinking-water	375	10	10	10
1% $AlCl_3$	Drinking-water	180	5	3	5
1% $Al_2(SO_4)_3$	Drinking-water	300	5	4	5
$Al(OH)_3$	Gavage	150	5	0	1
$Al(OH)_3$	s.c.	150	5	0	0
$Al(OH)_3$	i.p.	150	10	10	10
Control					
Water	Drinking-water	Nil	10	0	0
2% $Al_2(SO_4)_3$	Drinking-water	350	5	3	0
1% $Al_2(SO_4)_3$	Drinking-water	200	5	0	0
1% $AlCl_3$	Drinking-water	250	5	0	0
$Al(OH)_3$	Gavage	150	5	0	0
$Al(OH)_3$	s.c.	150	8	0	0
$Al(OH)_3$	i.p.	150	5	5	5

Normal Rats

Groups of non-operated (non-nephrectomised) rats were given 1% $AlCl_3$ and $Al_2(SO_4)_3$ as drinking-water, and $Al(OH)_3$ by gavage and intraperitoneal and subcutaneous routes. Control rats were given distilled water in place of aluminium salts.

Oxygen Consumption in Vitro

This was determined in liver homogenates in a Warburg apparatus, using liver from non-nephrectomised animals given oral or intraperitoneal $Al(OH)_3$ and normal rats drinking water as controls. Total protein was measured by the Lowrey technique and results were expressed as µl. oxygen consumed per minute per mg. protein. The test was repeated using normal homogenate of healthy rat liver to which $Al_2(SO_4)_3$ was added in various concentrations up to 40 mg. per litre and oxygen consumption compared with controls. Buffer used in all instances was Krebs-Ringer-phosphate buffer pH 7·4.

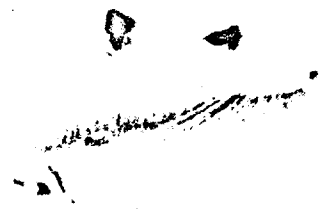

Fig. 1—Periorbital bleeding 6 days after one injection of intraperitoneal $Al(OH)_3$ in a non-operated rat.

Tissue Levels

Aluminium in tissues was extracted after drying in 'Pyrex' tubes at 100°C for 4–7 days and dissolving the residue in concentrated hydrochloric acid for 1 week. Aluminium was measured on a Unicam SP90 atomic-absorption spectrophotometer. (Heating at 600°C in a porcelain crucible gave spurious increases in aluminium because aluminium was leached out of ceramic crucibles, which contain 2% aluminium oxide.)

Results

Clinical Picture

5/6 nephrectomised controls recovered rapidly from the operation, and none died within 3 weeks of operation, whereas all animals receiving 1% of $AlCl_3$ or $Al_2(SO_4)_3$ died within 8 days and all receiving 2% $AlCl_3$ or $Al_2(SO_4)_3$ died within 3 days. The clinical picture was lethargy, failure to thrive, bleeding around the eyes (fig. 1), and reduced spontaneous movement. The test rats ate and drank very little in the last 2 days of life. Rats on 2% aluminium salts drank 11 (range 5–15) ml. water per day over 4 days, but intake on the first 2 days was probably around 20 ml. per day. They did not die of uræmia, the heart-blood urea being 320 mg. per 100 ml. at death. Rats on 1% aluminium salts drank 18 (range 16–21) ml. per day during 8–9 days' survival. Rats given aluminium intraperitoneally or subcutaneously and the control rats on pure water all drank 26–30 ml. per day. To exclude the possibility that the deaths were due in some measure to acidosis or hypophosphatæmia caused by oral aluminium salts, or to dehydration caused by voluntary limitation of fluid intake, other groups of animals were given 30 mg. of elemental aluminium as aluminium hydroxide in a single injection intraperitoneally or subcutaneously. Similar results with a high mortality within 5 days were seen in nephrectomised and control animals given intraperitoneal injections of $Al(OH)_3$. Subcutaneous injections were less toxic, resulting in no mortality, but periorbital bleeding occurred in nephrectomised animals. In non-operated animals 2% $Al_2(SO_4)_3$ caused periorbital bleeding in 3 out of 5 rats, but no mortality.

Histological Findings

Animals which died with periorbital bleeding had

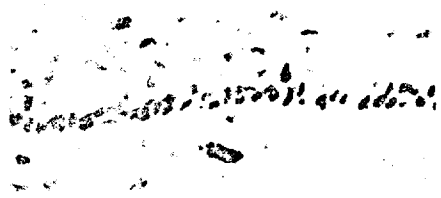

Fig. 2—Control cornea showing mitotic figures in stratum basale of epithelium and wide epithelial surface. (×250.)

Fig. 3—Cornea of animal given intraperitoneal Al(OH)$_3$ 5 days previously.

Note lack of mitoses in stratum basale and narrowed epithelial layer. (×250.)

conjunctival epithelial ulceration in the forniceal conjunctiva, and the bulbar conjunctiva was reduced in thickness compared with controls, with a striking lack of dense mitotic nuclei in the stratum basale of the corneal epithelium (figs. 2 and 3). The lungs showed a histological appearance of patchy pneumonia with desquamation of bronchial epithelium. The liver was normal apart from perihepatic aluminium granulomas and fibrinous peritonitis in animals given intraperitoneal aluminium hydroxide. The heart, spleen, pancreas, and skeletal muscle seemed normal. Histological examination of bone is not yet complete.

Biochemical Findings (tables II–IV)

The highest *tissue concentrations* in controls were found in bone (table II). In rats given intraperitoneal Al(OH)$_3$ the tissue concentration was highest in the liver (table III), but the concentrations for muscle, brain, bone, and heart were also several hundredfold higher than for the controls. 5/6 nephrectomised animals given oral $Al_2(SO_4)_3$ consumed an average of 18 mg. elemental aluminium; the highest levels are found in bone, but the levels in liver, heart, and muscle are also considerably increased in rats surviving to the 8th day (table IV). The serum-inorganic-phosphorus level in animals dying after parenteral Al(OH)$_3$ was 10·5 mg. per 100 ml. (mean of five rats). Aluminium concentrations were very high in brain, but were raised in all tissues (table IV).

Serum-levels of aluminium were high in all groups of test animals (table V), the highest figures (39·4 mg. per litre) being found in nephrectomised animals on oral 2% $Al_2(SO_4)_3$.

Oxygen consumption fell by 25% in aluminium-treated rats (table VI). In-vitro addition of aluminium to healthy rat liver led to an immediate depression of oxygen consumption, but this fell short of statistical significance.

TABLE II—ALUMINIUM CONTENT OF NORMAL RAT TISSUES: MEAN OF 5 RATS, POOLED TISSUES

Tissue	Aluminium (mg./kg.)	
	Wet weight	Dry weight
Bone	2·93	5·53
Muscle	0·56	1·93
Liver	0·12	0·39
Heart	0·34	1·23
Whole body	0·28	0·85

TABLE III—TISSUE LEVELS OF ALUMINIUM AFTER INTRAPERITONEAL Al(OH)$_3$: 5 RATS, MEAN OR MEAN AND RANGE

Tissue	Aluminium (mg./kg.) in:			
	Nephrectomised rats		Non-nephrectomised rats	
	Wet weight	Dry weight	Wet weight	Dry weight
Liver	1470	6280	2673 (1700–3600)	9966 (3000–17,800)
Muscle	116	220	389·3 (73–315)	1689 (307–3400)
Femur	36·5	76·5	113·3 (56·5–155)	18·27 (231–266)
Heart	600	4550	153·2 (18·1–395)	806·7 (180–1970)
Brain	15·6	93·0	36·6 (16·5–46)	161 (101–243)

TABLE IV—EFFECT OF ORAL 1% $Al_2(SO_4)_3$ ON TISSUE LEVELS OF ALUMINIUM (GROUPS OF 5 RATS)

Tissue	Aluminium (mg./kg., dry weight)	
	5/6 nephrectomised rats	Non-nephrectomised rats
Bone	17·7	5·2
Muscle	33·4	2·3
Liver	7·6	1·5
Heart	5·1	1·9
Total body	10·7	2·3

All animals died within 8 days of starting aluminium-salt drinking

TABLE V—EFFECT OF SUBCUTANEOUS ADMINISTRATION OF Al(OH)$_3$ ON TISSUE ALUMINIUM LEVELS: MEAN AND RANGE

Tissue	No. of rats	Aluminium (mg./kg. dry weight)
Bone	4	28·7 (5·3–49·2)
Muscle	2	13·9 (8–19·8)
Liver	2	25·2 (13·3–37·0)
Heart	4	48·3 (19·2–73·5)
Brain	4	339·5 (620–61)

TABLE VI—SERUM-ALUMINIUM LEVELS: 5 RATS IN EACH GROUP

	Serum-aluminium (mg./L.)
Controls	0·24
Oral 1% $Al_2(SO_4)_3$	
Non-nephrectomised	1·46
5/6 nephrectomised	2·25
Oral 2% $Al_2(SO_4)_3$	
Non-nephrectomised	2·94
5/6 nephrectomised	39·4
Subcutaneous Al(OH)$_3$	
Non-nephrectomised	0·49
5/6 nephrectomised	3·04
Al(OH)$_3$ by gavage	0·99

Discussion

Aluminium toxicity has been the subject of sporadic investigations in the past twenty years.[3,9] Aluminium has been administered principally in the form of oral $Al(OH)_3$ in the rat, in which a phosphorus-depletion syndrome is induced with depletion of red-cell A.T.P.[3,9] This should not be primarily considered as a manifestation of aluminium toxicity, but rather as a result of phosphate depletion resulting from phosphate binding in the bowel. A similar syndrome has been reported in man caused by mixed $Al(OH)_3$ and magnesium oxide intake.[7] In normal man absorbed aluminium is readily excreted by the intact kidney.[8] It is likely, therefore, that oral administration of aluminium salts will result in toxic manifestations in patients with renal failure, for raised plasma-aluminium levels have been found after aluminium salt or aluminium resin intake.[8] It is in these patients that a situation analogous to the aluminium toxicity of the 5/6 nephrectomised rat may occur if aluminium intake is increased by aluminium resins or other absorbed aluminium salts. The lack of observations on the toxic effects of aluminium (other than phosphate depletion) is possibly the result of giving poorly absorbed aluminium salts orally to rats with normal renal function. The combination of aluminium salts orally or parenterally to uræmic rats is presumably responsible for the high morbidity and mortality rates in our experiments. The minimum fatal dose in the rats given oral sulphate or chloride was about 1100 mg. elemental aluminium per kg. body-weight; in rats given intraperitoneal salts the dose chosen (150 mg. per kg. per day) was fatal in all rats.

In our experiments aluminium toxicity was most striking in uræmic rats, and manifested in a clinical syndrome of lethargy, periorbital bleeding, anorexia, and death. Plasma levels (normal value 0·2 mg. per litre) were raised to between 0·5 and 40 mg. per litre, depending on the route of administration of the aluminium salt. These levels correspond with those found in patients in renal failure taking aluminium resins or $Al(OH)_3$ therapeutically.[8] Although aluminium intoxication could be induced most readily in uræmic rats, toxicity could also be induced in normal rats by oral or intraperitoneal aluminium salts. The oral dose given to uræmic rats of 18 mg. elemental aluminium per day (90 mg. per kg. body-weight) is two or three times the dose given therapeutically to patients in renal failure, and about equal to the amount of aluminium theoretically available from aluminium resin if resin is taken in a dose of 45–60 g. per day. Since, at least for the uræmic rat, aluminium seems to be toxic in a dose close to or identical with the therapeutic dose used in uræmic man, we recommend that, pending further work, aluminium salts should not be prescribed for patients with renal functional impairment, and that they should be withdrawn from the market. A wide range of aluminium salts is used—they should not be regarded as inert.

The massive deposition of aluminium in various organs—notably, brain, liver, heart, and muscle—indicate that aluminium may have some responsibility in the production of several little understood uræmic syndromes, especially where bone is concerned. We have found aluminium levels, in samples of bone powder supplied by Dr. M. Kaye from uræmic men not known to have taken aluminium salts, to be twice those in non-uræmic men (unpublished), a result which accords well with that of Parsons et al.,[4] who also found a large amount of aluminium in uræmic bones. The implication of aluminium salts in the ætiology of the metatarsal type of osteoporosis seen in Newcastle is suggestive, in view of the high Al content of bone in Newcastle. We have suggested[5] that aluminium may damage bone formation, and this accords with the evidence of Bachra and van Harskamp[6] that 1 mg. of aluminium can initiate hydroxyapatite precipitation.

TABLE VII—IN-VITRO OXYGEN UPTAKE IN LIVER HOMOGENATES OF RATS

Group*	Preparation of liver homogenate	No. of rats	Mean (and range) oxygen uptake (µl./min. mg. protein)
1	i.p. $Al(OH)_3$	5	1·98† (1·56–2·36)
	Control	5	2·67 (2·01–3·71)
2	In-vitro addition of $Al_2(SO_4)_3$	5	1·72‡ (1·22–2·04)
	Control	5	1·79 (1·32–2·07)

* *Group 1.*—Treated with $Al(OH)_3$ intraperitoneally, and control group. *Group 2.*—All livers from normal (untreated) rats. 40 µg. per ml. aluminium as $Al_2(SO_4)_3$ added to homogenate at start of incubation, nothing added to controls.

† Significantly lower than control ($t = 2·78$, $P < 0·05$).

‡ Not significantly lower than control ($t = 2·42$, $0·1 > P > 0·05$).

The action of aluminium salts in vitro in depressing liver respiration is indicative of a direct toxic action on the liver cell. Further work is being done to determine the intracellular level and mechanism of aluminium poisoning. There is a reduction in liver-protein concentration which may be due to direct interference with protein synthesis, being found in non-uræmic rats drinking $Al(OH)_3$.

The previous work on aluminium toxicity has to a certain extent been befogged by the use of oral routes of administration of aluminium salts and rats with normal renal function. This allows phosphate depletion to develop and is responsible for the incorrect assumption that aluminium toxicity is really phosphate depletion.[7] That this is not so is demonstrated by slightly raised levels of phosphate in the uræmic rat with aluminium poisoning, in which phosphate depletion can be confidently excluded.

The reduction in mitoses in the stratum basale of the corneal epithelium is presumably a response to severe aluminium intoxication. Periorbital bleeding is a very regular finding in the non-uræmic rat given intraperitoneal aluminium hydroxide, and may well be due to damage to the forniceal conjunctiva.

Although it is difficult to extrapolate from the rat to man it would seem advisable to suspend the widespread use of aluminium salts in man pending further studies of this problem.

We thank Dr. H. Doron and Mr. M. Soroka, of Mercaz Kupat Holim, Tel-Aviv, for making this work possible.

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#77

Diminished blood ATP levels induced in the chick by dietary aluminum hydroxide gel[1]

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BISHOP, C., C. WILLIAMS AND S. RODBARD. *Diminished blood ATP levels induced in the chick by dietary aluminum hydroxide gel.* J. Appl. Physiol. 14(2): 259–260. 1959.—When newly hatched chicks are placed on a diet containing a reactive aluminum hydroxide gel, the fatal syndrome of progressive leg and wing weakness which they develop is accompanied by a reduction in the quantity of adenosine triphosphate (ATP) in the blood. The intramuscular injection of an emulsion of inorganic phosphate tends to prevent this change. It is suggested that these chicks fed alumina gel are deficient in inorganic phosphate and that their reduced ability to maintain blood ATP levels is indicative of reduced phosphorylating ability elsewhere in the body.

When newly hatched chicks are placed on a diet containing a reactive aluminum hydroxide gel (Amphogel, obtained through the courtesy of Dr. Elmer H. Funk, Jr., Wyeth Labs, Philadelphia, Pa.), a syndrome of progressive leg and wing weakness develops, leading to death within the first month (1). The aluminum hydroxide apparently interferes with enteric phosphate absorption, leading to phosphate deficiency. Evidence for this lies in the following observations: *a*) the serum inorganic phosphate level falls markedly in aluminum hydroxide-fed chicks and *b*) intramuscular injection of an emulsion of inorganic phosphate prolongs survival time (2).

Since muscular activity requires a supply of high energy phosphate compounds, it seemed reasonable to assume that in phosphate deficiency, the amounts of these high energy phosphate compounds might be reduced. Furthermore, this reduction might be observable not only in muscle tissues, but throughout the body. Since erythrocytes contain relatively large amounts of adenosine triphosphate (ATP), it was decided to examine these cells for changes in ATP levels after aluminum hydroxide feeding in chicks.

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[2]The University of Buffalo Chronic Disease Research Institute supported in part by the New York State Department of Health.

METHODS

The first series of experiments was performed on three groups of newly hatched DeKalb chicks. The control group (*I*) was placed on a diet of regular mash. *Groups II* and *III* were fed the same mash to which 25 mg of aluminum hydroxide gel was added per gram of food. The *group III* chicks also received intramuscular injections twice daily of a fat emulsion of Na_2HPO_4 containing approximately 10% phosphorus. The initial injection of the emulsion was 0.1 ml. This was increased 0.025 ml daily to 0.2 ml per injection. Within approximately 2½ weeks, chicks receiving aluminum hydroxide gel and no phosphate (*group II*) developed the characteristic muscular weakness of the syndrome. The weakness was also present in some chicks receiving injections of phosphate emulsion. Blood was then collected from each group by heart puncture, using heparinized syringes. Individual blood samples were pooled in tubes chilled in ice. Blood taken from 6 to 10 chicks was used for each pool. Five milliliters of blood from each of three pooled samples (*groups I, II, III*) were expressed into 5 ml of ice cold 5% trichloracetic acid (TCA). TCA was removed by extraction with ether and the aqueous layer stored frozen.

The frozen extract was carefully thawed and poured onto a 1 x 20 cm column of Dowex 1-x 10 formate resin. After washing with water, the nucleotides in the extract were successively eluted by stepwise increases in the concentrations of formic acid and ammonium formate passing through the column. The eluates were collected in 5-ml fractions and each fraction was read at 260 mμ on the Beckman DU spectrophotometer. Results were plotted and the amounts of the nucleotides present were determined by integration of the areas under the various peaks.

A second series of experiments provided results identical to the first series, except that the groups were larger, so that two pooled samples of blood could be obtained under each condition. The chicks contributing to each pool were chosen according to a predetermined random pattern. It should be noted also that in the first series of experiments, muscle weakness and death occurred within

J. Appl. Physiol. 14(2) 259-260 (1959)

TABLE I

(1) ATP (μmoles)		(2) DNA-P (μmoles)	(3) 100 ATP/DNA-P		(4) % of Control
Series 1					
Control	3.19	91	3.50		100
Al	1.78	85	2.09		60
Al + PO_4	2.34	88	2.66		76
Series 2				Av.	
Control	5.05	105.6	4.78	4.86	100
	5.09	102.8	4.95		
Al	3.33	93.5	3.56	3.44	71
	2.98	89.6	3.32		
Al + PO_4	3.89	90.5	4.30	4.16	86
	3.79	94.4	4.01		

Values expressed in terms of 5.0 ml of whole blood.

about 2½ weeks, whereas in the second series, these changes occurred in about 1½ weeks.

RESULTS

The ATP levels in the various experimental groups are shown in table 1. Because the amount of ATP is related to the amount of erythrocytic material present, it seemed desirable to use deoxyribonucleic acid phosphorus (DNA-P) as a reference. The amount of ATP, as referred to DNA-P, is shown in *column 3*. In *column 4* the ATP levels in the two experimental situations are expressed as percentage of the control ATP levels.

The ATP levels of the blood were markedly depressed in both series of experiments in which aluminum hydroxide gel was administered. The results also show that these effects were partially counteracted by intramuscular injections of phosphate.

It has been previously reported from this laboratory (2) that the serum inorganic phosphate level falls rapidly in chicks fed on aluminum hydroxide gel. The present studies demonstrate that the blood does not maintain a normal ATP level under these circumstances. These results suggest the possibility that the muscle weakness in this syndrome may be accompanied by lowered ATP levels in muscle. Since the erythrocytes are unable to maintain their ATP levels under these experimental conditions, a general failure of phosphorylation, due to a decreased availability of inorganic phosphate, is suggested. When more inorganic phosphate is supplied, this difficulty is overcome, at least partially.

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Osteomalacia with Pseudofractures Caused by the Ingestion of Aluminum Hydroxide

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Osteomalacia in the adult is usually attributed to defect in renal tubular reabsorption, or to the failure to respond to vitamin D, or to the loss of excessive amounts of calcium in the feces.[1] The case reported presents severe osteomalacia with pseudofractures in a patient who ingested large amounts of aluminum hydroxide.

Report of Case

During the last five years, a 50-year-old housewife gradually developed an increase in diffuse back and leg pain. She noted much discomfort in her feet from time to time, with difficulty in "taking-off." Several physicians had seen her and various shoe changes had been suggested and tried. None, however, provided any relief of the symptoms. An internist felt that there was some osteoporosis and administered estrogen therapy. She herself stopped this hormone treatment because of a lump in her breast. She was seen occasionally by one of us during the past two years. When first seen, a fatigue fracture was present in the left second metatarsal. Dicalcium phosphate and vitamin D were prescribed and taken periodically by the patient. Some relief of the pain was noted from this medication, but she spontaneously stopped taking it as soon as she felt better. Prior to hospital admission, more pain developed and she had difficulty in walking. Roentgenograms revealed spontaneous fractures of the pelvis and femoral neck (Fig. 1 and 2). She had had a spastic colon for many years. This had been associated with cramps and diarrhea, but no mucous, blood, or fat was present in the stools. She

From the Research Laboratory of the Piedmont Hospital.

JAMA 174: 1327-1330 (1960)

voluntarily began taking an aluminum hydroxide preparation (Malcotabs) about six years ago. She never told anyone how much she took but careful questioning revealed that she had ingested between one-half and one bottle a day. This amounts to 66 1-Gm. tablets.

The family history was noncontributory and a review of systems added no further information. The physical examination revealed a well-developed and well-nourished woman. Her blood pressure was 120/80 mm. Hg bilaterally; her pulse was 80 beats per minute and regular. Her respirations were 16 per minute, and the temperature was normal. The eyes were negative except for some increase in the light reflex of the arterioles on funduscopic examination. The general physical examination was normal for her age, except that there was definite bone tenderness to pressure over the tibia. She had pain on moving her left hip. She could walk only with the help of crutches. The peripheral pulses were intact, and the reflexes were normal.

The laboratory findings at the time of admission were as follows: hematocrit determination, 32; hemoglobin level, 8.3 Gm.%; white blood cell count was 5,000 per cu. mm., with 43% segmented cells, 4% eosinophils, 48% lymphs, and 5% monocytes. Urine specific gravity was 1.005; reaction, 5.5; sugar, albumin, and acetone tests were negative. Urine concentration was 1.025; Bence-Jones protein, negative; and microscopic examination, negative. The nonprotein nitrogen content was 34 mg.%, and fasting blood sugar was 102 mg.%. The serum calcium level was 10.4 mg.% and the serum inorganic phosphorous level, 2.9 mg.% fasting in the morning and 2.0 mg.% in the afternoon while the patient was taking the aluminum hydroxide tablets. Serum chloride level was 103 mEq. per liter; sodium level, 140; potassium level, 4; and carbon dioxide combing power, 23 mEq. per liter. Total serum protein level was 7.2; albumin, 4.5 and globulin, 2.7. Phenolsulfonphthalein excretion was 47% in 15 minutes, with total excretion, 62.2%. Gastric analysis revealed no free hydrochloric acid and total acidity was 8 units after a test meal.

While in the hospital, a diet containing 200 mg. of calcium was instituted for four days. During this time, her medication was continued as before. It was found that the serum phosphorus level decreased to approximately 2 mg.% each afternoon. Total 24-hour urine phosphorus excretion was 1.7 mg. in 1,390 cc. Urine calcium excretion totaled 164 mg. in 24 hours.

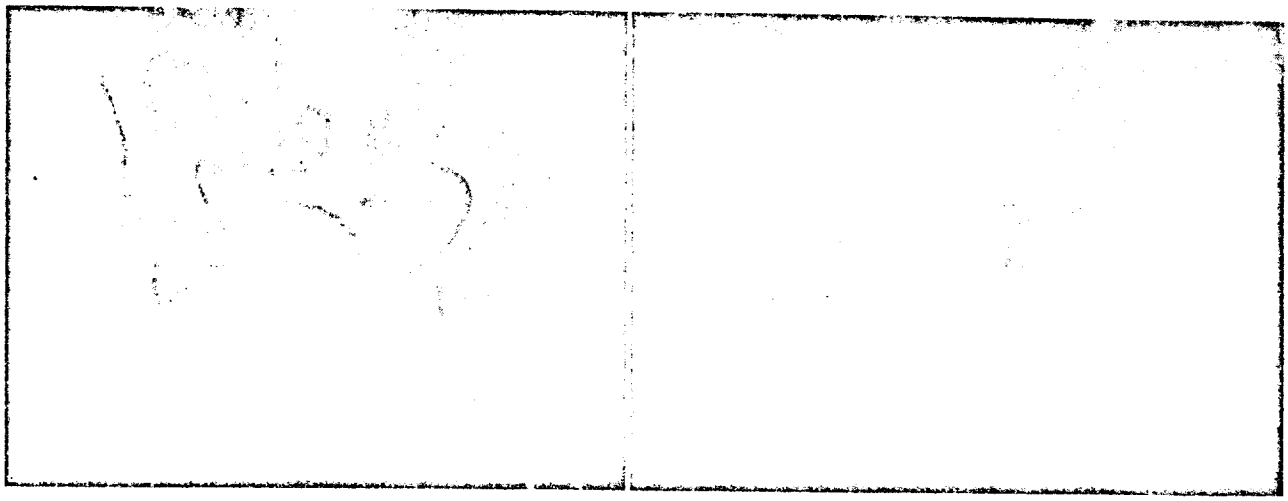

Fig. 1.—*Left,* roentgenogram of pelvis shows fractures in both superior pubic rami, left femoral neck, and left ischium. *Right,* close-up view to show fracture line in pubic ramus.

The medication of aluminum hydroxide was stopped, and calcium gluconate and 50,000 units of vitamin D were given each day. One month later she began to get up from her chair without help, and the bone pain was subsiding. At this time, the reticulocyte count was 2.3%; serum inorganic phosphorus level, 4.7 mg.%; calcium level, 9.3 mg.%; and alkaline phosphatase level, 9 King-Armstrong units. Two months later the hemoglobin level was 9.2 Gm.%; reticulocyte count, 1%; calcium level, 10.2 and phosphorus level, 4.4 mg.%; and alkaline phosphatase level, 9.9 King-Armstrong units. Total 24-hour urine phosphorus excretion was 420 mg. in 1,500 cc. Bone pain at this time had completely disappeared. Her fractures had healed

Progressive Laboratory Findings in Patient with Osteomalacia with Pseudofractures Caused by Ingestion of Aluminum Hydroxide

Date	Serum Ca, Mg. %*	Serum P, Mg. %	Alkaline Phosphatase, King-Armstrong Units	24-hr. Urine Phosphorus, Mg.	Bone Pain
2- 4-58	10.4	2.90	7.5	1.7	Severe, limited walking
3-20-58	9.3	4.75	9.6	420.0	Some pain, walking better
5-19-58	10.2	4.40	9.6	...	Pain gone, walking well

* Total serum protein level was normal in all determinations.

(Fig. 2 and 4). She was walking well but said that she still missed her "stomach medicine."

Comment

Soon after the introduction of aluminum hydroxide in the treatment of peptic ulcers, it was found that this compound bound inorganic phosphate in the gastrointestinal tract and resulted in increased phosphate excretion in the feces [2] as well as decreased the phosphate level in the blood during the time that aluminum hydroxide was administered. Along with the increased phosphate excretion in the feces and decreased serum phosphate level there was a reduction in excretion of phosphate in the urine and, although the total output of phosphorus was not changed, a marked alteration occurred in the normal physiological transfer of phosphate ions in the body. Experimental animals have shown mineral reduction when aluminum salts were ingested for several months.[3]

The clinical report presents a clinical complication which might be expected from the protracted use of large doses of aluminum hydroxide. Continual reduction of phosphate ions in the extracellular fluid is evidenced in this patient by low serum phosphate concentrations, particularly during the time of aluminum hydroxide ingestion. The low available phosphate in the extracellular fluid would lead to a physiological state incompatible with deposition of calcium in bone. To quote Albright and Reifenstein, "after all, calcium phosphate cannot be deposited in bone if there is no phosphate!" [1] As was evidenced in earlier work, the phosphate concentration in the body fluids will fluctuate with aluminum hydroxide ingestion, as was shown in the serum phosphate changes in this patient. This was well demonstrated by Freeman and Freeman [4] in their studies of phosphate retention in children; they found that lowering of the level of phosphate in the extracellular fluid and serum was a function of the time and amount of aluminum hydroxide ingested. As soon as the aluminum hydroxide had bound the phosphorus and passed on through the gastrointestinal tract, the serum phosphorus level would tend to rise unless further aluminum hydroxide was administered. This is precisely what happened to this patient, who had taken large doses of aluminum hydroxide. The fasting serum inorganic phosphorus level was considerably higher than the level noted during medication.

Aluminum hydroxide has been used therapeutically in an attempt to reduce the formation of renal stones by diminishing the phosphate excretion in the urine and thus the tendency to form calcium phosphate stones.[5] It has also been used to treat the phosphate retention of renal failure.[4] It is surprising that, with all this evidence, there are no reports on the effects of this type of medication on calcification of bone. Many patients take large amounts of aluminum hydroxide and frequently do so on a semi-self-medication basis although under physician supervision. In our patient, the dosage of aluminum hydroxide taken was large. It is quite clear that the aluminum hydroxide reduced the extracellular fluid phosphorus content to the point where bone calcification could not take place, with the eventual demineralization of bone and pseudofractures (Milkman's syndrome) which went undiagnosed for a long period of time. The clinical study indicated that there was no renal abnormality, no steatorrhea, and no vitamin D resistance. Thus, the accepted causes for osteomalacia in an adult were not present. Furthermore, by merely stopping the intake of aluminum hydroxide and giving minimal doses of calcium and vitamin D, the patient's response was dramatic, as noted in Figures 1 and 2.

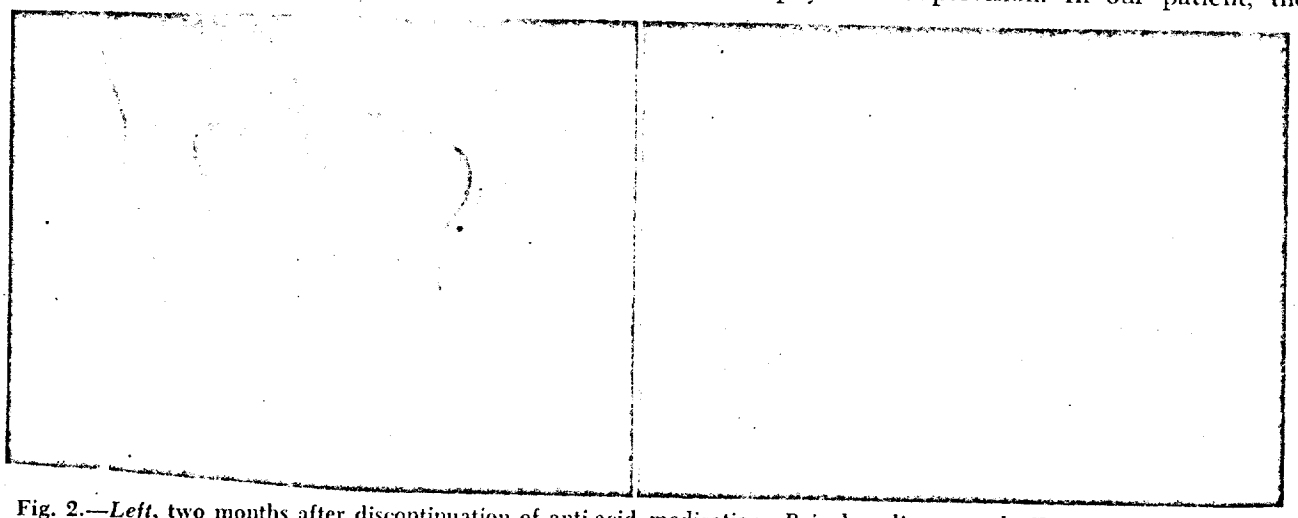

Fig. 2.—*Left,* two months after discontinuation of anti-acid medication. Pain has disappeared. Fractures are noted to be healed. *Right,* close-up view of superior pubic ramus, showing that fracture has now healed.

Although this is probably a rather rare occurrence, the question is raised concerning the frequency in which bone aching and demineralization

of unknown etiology may be related to hypophosphatemia as an added factor to some underlying bone disease. For instance, aluminum hydroxide ingestion in the osteoporotic patient might intensify the bone problem. Certainly a more careful search for similar patients who have metabolic bone disease resulting from aluminum hydroxide ingestion may be invaluable. Physicians should be alerted to the fact that aluminum hydroxide ingestion, unless carefully supervised with some knowledge of the phosphate changes in the patient, may not be as innocuous as suggested in brochures advocating the use of the medicament.

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Aluminum in the Environment of Man

A Review of Its Hygienic Status

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With the Assistance of Lilli B. Lange and Ellinor Poeck

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Foreword

One of the most fascinating aspects of general physiology, and one of the abiding interests of physiological investigators, the world over, has been the role fulfilled in the living organism, for better or worse, by the mineral elements which, occurring in varying forms and concentrations in the earth's crust, find their way by various means and in varying concentrations into the tissues and tissue fluids of plants and animals. Time and a multitude of observations, some of which have been fortuitous, have served to demonstrate that both beneficent and noxious effects are associated with the presence of one or another of the many mineral elements in the environment and substance of some specific plant or animal. In certain instances the physiological mechanisms involved have been indicated. Rarely, however, have such mechanisms been elucidated fully, and it is fair to say that herein reside some of the most elusive and intriguing mysteries of the processes of life.

An important principle emerges from th many and often unrelated observations, i that favorable (or innocuous) environment conditions, with respect to a specific miner element which gains access to the tissues o a plant or animal, may be differentiated fro unfavorable, on the basis of the ranges o concentration below or above a thresho level found in the environment or within th organism itself. Variations in such conce trations, which coincide with health or di ease in the living organism, occur often nature in consequence of the variability o the richness of the element in the soil water of the earth's areas, while artifici factors have been imposed by man's ceas less effort to obtain and make use of t minerals of the earth, thereby altering t natural environment and reducing or i creasing the ease and extent of access of t element to plants or animals in the are involved.

This brief epitome includes within scope the considerations, hypotheses, a experimental efforts put forth in the fiel

of horticulture, animal husbandry, and plant and animal physiology with respect to the mineral metabolism. Further, it encompasses the search of the alchemist and medicine man and, in more modern times, that of the biochemist, pharmacologist, veterinarian, and physician for mineral agents for the relief or cure of the diseases of plants, domestic animals, and men. More pertinently, for present purposes, it extends to the attempts of the pharmacologist, toxicologist, and industrial hygienist to explore the combined effects, upon the health of industrial workers and the general public, of natural and artificial variations in the content of mineral elements and their compounds in human food and beverages and in the air breathed by men in the modern world of industry and technology.

In this Laboratory, any unhampered opportunity to widen the scope of our knowledge of the metabolism, as well as other aspects of the behavior, of metallic compounds in the animal organism is welcomed with enthusiasm. Accordingly, when representatives of industries engaged in marketing finished aluminum products communicated to us their desire to establish the truth or falsity of certain persistent allegations and inferences that human health is being jeopardized by the use of their products, due notice was provided thereby that such an opportunity had presented itself. It was not that this was a new or unfamiliar problem. Rather, the possibility existed that certain items of new and useful information might be found in as yet unearthed or insufficiently explored experimental reports on the subject. More to our taste was the likelihood that certain additional investigations might be justified by the gaps in the available information, or by previously unrecognized suggestions of harmful effects which would provide experimental leads. In any event, the first step in the process had to be a thorough and critical search of the literature, in the expectation that this would provide a proper basis for advice to those who had sought our counsel, and for a decision on our own part as to the scope and design of further investigations, should the latter be indicated.

The publication which follows is the product of the review and interpretation of the observations and reports of many investigators. Some 1500 books, reports, and articles by authors and investigators in more than 20 countries have been assembled and, for the most part, abstracted in some detail. Of these, about 500 have been cited in documentation of facts and statements in the text. An unstinted effort has been put forth to sift out the pertinent and reliable information. While it cannot be said that every significant observation recorded by every capable investigator in every corner of the world has been taken into account, it seems unlikely that any very significant body of fact has been missed or ignored. The result must stand for itself in the opinion of those who may exercise capable and critical judgment.

I. History of the Development and Uses of Aluminum and of the Principal Aluminum Minerals

Isolation, Production, and Uses of Aluminum.—In December, 1856, in the weekly publication *Household Words*, which he was "conducting," Charles Dickens [112] wrote an article on a newly found metal—aluminum. He traced its development, beginning with Lavoisier's assertion that alumina, among other fixed alkalies and earths, is nothing else than the oxide of a metal, through Sir Humphrey Davy's failure to isolate the metal and Wöhler's success in reducing it in 1827. Although Dickens included H. C. Oersted's attempts among the failures, the part played by this Danish chemist cannot be overlooked, for he was really the first to obtain a small lump of aluminum—or, as he called it, "metal of clay"—in 1825 by heating potassium amalgam with aluminum chloride and distilling the mercury from the resulting aluminum amalgam. This achievement then prompted Wöhler to react metallic potassium with aluminum chloride, and the particles obtained, as large as "big pinheads," made it possible for him to work out the

first data on the physical and chemical properties of the metal aluminum. His determination of the specific gravity was remarkably close to the accepted value, 2.7 (about one-third of that of iron), although he was unable to get the particles to coalesce because of their accompanying oxide film.

About 30 more years were to elapse before aluminum became available in commercial, but still small, quantities through the discovery in 1854 by Henri Sainte-Claire Deville that, by using sodium, the sodium chloride obtained from the reduction of aluminum chloride reacted with an excess of aluminum chloride to give a fusible double chloride of sodium and aluminum; and that this salt in turn acted as a flux which allowed the aluminum globules to coalesce. Further developments, encouraged by a grant from the French Academy and Napoleon III, who was anxious to use the new light metal for armor and armaments, were responsible for the first public showing of aluminum—in the form of a bar—at the Paris Exposition in 1855. At this time it was so costly (about 1000 francs per pound) that Napoleon III used an aluminum dinner service at important state functions. It is reported[185] that the first objects made of it were a rattle for Napoleon III's son and a medal honoring Wöhler, struck by Deville. By 1856, at the time Dickens was writing, improvements in the process and reductions in the cost of sodium had brought the price down to 300 francs ($27.00) per kilogram, so that this familiar author could envisage all sorts of applications and thought its properties of ease of workability and resistance to corrosion so valuable as to say: "Henceforward, respectable babies will be born with aluminium spoons in their mouths." Until that year Deville had made about 60 lb. altogether,[126] and throughout the following 30 years only about 100,000 lb. had been produced[62]; for, while other processes were introduced in that period, none was sufficiently economical to pave the way for the production of a large volume.

The year 1886 is an important one in the history of aluminum. On July 9 of that year, Charles Martin Hall, a recent graduate from Oberlin College, applied for a patent based on the discovery that aluminum oxide could be dissolved in molten cryolite and that the dissolved oxide could then be reduced electrolytically to produce metallic aluminum. In the same year, working entirely independently, Paul L. T. Héroult of France succeeded in isolating the metal by the same process. Neither knew of the other's discovery until Héroult applied for a patent in the United States. Priority was given in the latter to Hall, and in France to Héroult, and the process came to bear both names. It may be of interest to note here that the coincidence in the lives of the two men goes even farther, for both were born in the same year, 1863, and both died in 1914. Hall was able to interest Captain Alfred E. Hunt and George H. Clapp, of the Pittsburgh Testing Laboratory, in his patent. They raised the necessary funds to organize a company, the Pittsburgh Reduction Company (later named Aluminum Company of America), around a small pilot plant, and the first aluminum was produced by this process in November 1888.[71]

In 1888 K. J. Bayer, of Austria, perfected his method for treating bauxite in order to obtain the purified alumina needed for the reduction of the metal. Both the Hall-Héroult and the Bayer processes have undergone many refinements, but they are still used for almost all of the production of primary aluminum in the world. The impact of these processes has been enormous. The estimated output of aluminum in the United States in 1887 was 9 short tons, and its cost per pound, $7.00; in 1900, a total of 3575 tons were produced, and the price had dropped to 23 cents per pound.[62] By 1910 primary production in this country annually was 18,000, and world production, 41,000 short tons; it rose by 1940 to 206,300 and 902,800 and by 1954 to 1,461,000 and 3,400,000 (estimated) short tons, respectively.[359]

In the Hall process, the alumina is reduced in furnaces known as "pots." These are insulated, carbon-lined steel boxes con-

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taining a mixture of molten cryolite and aluminum fluoride in which the alumina is dissolved. Electric current, introduced through carbon electrodes suspended in the mass, dissociates the alumina into molten metal; which collects on the bottom of the pot and is then drawn off at intervals and cast into pigs or conveyed to holding furnaces for further treatment.[257,360]

Dickens foresaw accurately that aluminum would be "applied to the fabrication of vessels and instruments of all kinds in which resistance to the action of the air and to the chemical agents is indispensable . . . for the decoration of interiors . . . for traveling purposes, for which its lightness is no small merit." The author's imagination and faith in "human wit," as he expressed it, are all the more remarkable because until the Hall-Héroult-Bayer processes made extensive production possible the metal remained a curiosity and a luxury. Like so many other developments, its greatest impetus in production has taken place in times of war. The applications have been growing rapidly, and today the availability of aluminum and its alloys for civilian use, without war-time restrictions, has been responsible for many commerical developments. These are led by building products, followed by transportation equipment, electrical and industrial equipment, and consumer goods.[25]

Probably the first architectural application of the metal dates back to 1884, when a small cast aluminum pyramid, weighing 100 oz. and costing $225.00 for the metal and its manufacture, was mounted on the tip of the Washington Monument.[74] In 1897 aluminum sheets and rivets were used to cover the dome of the Church of San Gioacchino in Rome.[485] The many other applications in the building industry, both for structural and finishing purposes,[51,52] were climaxed in 1954 when three crews each composed of five men completely enclosed the 26-story Tishman building in the City of New York in six and a half working days.[25]

In the transportation field, the metal finds increasing uses in air, land, and sea craft.[30] It may be of interest that more aluminum had gone into the construction of the S. S. *United States* than into any other single structure until that time (maiden voyage in 1952) on land or sea.[235]

The uses in other industries are too many to be detailed. The metal finds application in the atomic energy program and in all types of chemical industries, as well as the pharmaceutical industry.[8,51,52,162,163,313,422,460,461]

Among the durable consumer goods, aluminum is used for a list of items ranging from artists' materials and equipment to window shades and accessories.[460] Discussions of these many applications may be found in the sources cited above. Suffice it to say that today aluminum boasts some 4000 terminal uses and tops all other nonferrous metals in the volume of its production.[359]

Aluminum Minerals and Early History.—Aluminum does not occur on the earth's surface in the metallic state. However, in combination with oxygen, fluorine, silicon, and other elements, it is distributed widely in rocks, clays, and soils. Assuming a thickness of 10 miles, F. W. Clarke and H. S. Washington had estimated the rock composition of the earth's crust to be as follows: igneous rocks, 95%; shales, 4%; sandstones, 0.75%; limestones 0.25%. Although about 1000 different minerals are known, the number of those composing the great majority of igneous rocks (over 99%) is very small; only about 12 of these are really important, among which are the silicates, metasilicates, and orthosilicates of aluminum.[478] Alumina is the second most abundant constituent of the earth's crust next to silica (15.34% and 59.12%, respectively), and the element itself is listed third (7.51%) in the order of abundance in the lithosphere, hydrosphere, and atmosphere after oxygen (49.52%) and silicon (25.75%), iron ranking fourth (4.70%).[94]

In the tables of minerals given in Dana's Textbook of Mineralogy, the statement is made that "aluminum is of such common occurrence among silicate minerals that it is impracticable to list all of these minerals

that contain it." Among them are such gems as the ruby, topaz, turquoise, and garnet,[305] while those of industrial importance include bauxite, cryolite, alum, corundum, and the kaolin minerals.

In spite of the great abundance of aluminum, bauxite still remains the most important ore for the production of the purified alumina needed as the basic material in the manufacture of the metal. Second in importance in this respect is cryolite, which serves as the flux.

The physical characteristics of bauxite are extremely variable. Mineralogically it occurs primarily as the trihydrate (gibbsite) in the western hemisphere and as a monohydrate (boehmite or diaspore) in other parts of the world. Most major deposits are concentrated in a zone extending about 20 degrees on each side of the equator, but they extend to other areas as well. They are found in the Caribbean (British Guiana, Surinam, Jamaica, Hispaniola), Brazil, North and Central Africa, South Asia, in the Mediterranean region of Southern Europe, and to some extent in the United States.[150]

Most of the bauxite consumed in the United States (89%) is used in the production of aluminum; some of it (6%) is used for the manufacture of abrasives, and the greater part of the remainder (3%) for the production of aluminum sulfate (alum).[257]

The only commercial source of natural cryolite, which originally was the chief constituent of the alumina reduction bath, is in Greenland. These deposits are near exhaustion, but methods of producing this compound artificially have been developed, so that synthetic cryolite is now used almost entirely for the production of aluminum.[359]

The third aluminum-bearing mineral of importance, which must be included in the present discussion, although it has nothing to do with the metal, is "alum." In present common usage, "alum" refers to hydrated aluminum sulfate. However, in strict chemical terminology, alum pertains to a group of crystalline double salts of which the most common are ammonium-aluminum sulfate and potassium-aluminum sulfate. Apparently these double salts occur as minerals in nature,[12] although aluminum sulfate containing some iron sulfate may be the naturally occurring mineral. The information on that point is not clear.[305] Although the metal itself had so long defied isolation (disregarding the legend dating back to Tiberius' time that a common worker had succeeded in obtaining it, only to be beheaded for his pains, for fear that a metal derived from clay would replace silver[111]), alum has been used for about 4000 years and has been in great demand as a mordant for dyes, as a substance for the treatment of hides, and, in Roman times in individual households, as an agent for the purification of water. In the Middle Ages it even assumed political importance, for the Papacy established a monopoly on the sale of alum which it derived from a large deposit of trachite in Italy, thus shutting off the Turkish markets to the western countries.[12] Among the uses of alum (or, as referred to by the ancient name, *alumen*) enumerated by Pliny[3] is that of the liquid form, which, in combination with honey, healed ulcers. As a solid, it is said to be "less astringent than the others, and is less useful as a check upon bad humors of the body." The best type, obtained from the Island of Melos, was "injected for dysentery and employed as a gargle."

Derivation of the Term Aluminum.—The term alum, as applied to the above-described substance, was derived from the Latin *alumen*, meaning substance with an astringent taste. In 1684, it was discovered that alum is obtained by the action of sulfuric acid on clay, and later opinion confirmed that the base of alum is a true, distinct earth. This gave rise to the German designation of *Tonerde* (earth of clay) for what came later to be known as "alumina" in England, upon argument that, since alum is a *sel alumineux*, the proper name for the base is "alumine." Much later, Sir Humphrey Davy stated that had he been fortunate enough to isolate the metal, he would have given it the name "alumium," which he

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later changed to "aluminum," to name it after the oxide. Later writers have adopted "aluminium," which is still used in most countries.[305] The spelling "aluminum" was adopted in 1926 by the American Chemical Society "in conformity with almost universal popular usage in the United States."[11]

II. Chemistry. Aluminum, Its Physical and Chemical Characteristics, and Its Analysis

Physical Properties of Metallic Aluminum.—Aluminum has an atomic number of 13 and an atomic weight of 26.97. It has a density of 2.7 gm. per ml. and melts at 660.2 C. It is a silvery white metal which is very ductile and malleable in the pure form. The pure metal is very soft and as such finds many uses where a high degree of malleability is required. High strength is imparted to the metal by appropriate alloying additions and subsequent working or heat treatment. Copper, silicon, zinc, manganese, and magnesium are the most common alloying elements. These are added singly or in combination to achieve the desired properties for a particular application. Small quantities of other elements are also used for special purposes.

Aluminum of commercial purity contains small amounts of other elements, particularly iron and silicon. These metals add strength to the product so that commercially pure metal (1100 grade) is one of the most important alloys of commerce.

Aluminum of the highest purity is extremely resistant to corrosion in the atmosphere, or by most chemicals. The strong, structural alloys are appreciably less resistant, but judicious alloying and thermal treatment give a high degree of resistance to corrosion along with adequate strength for a very large number of applications in corrosive environments. The great strength and the resistance to corrosion of structural alloys are achieved by a special type of application of a thin layer of pure aluminum on a base alloy of great strength.

Bright aluminum has the highest reactivity of any common metal in the visible and ultraviolet regions and is exceeded only slightly by polished gold and silver in the infrared. The high reflecting properties are only slightly impaired by the application or development of a thin, protective, film of oxide.

Aluminum is an excellent thermal and electrical conductor.

For the practical applications of these physical and chemical characteristics of aluminum, see Chapter I on Uses.

Analysis of Aluminum in the Metallurgic Aluminum Industries.—The earliest significant work on the analysis of aluminum is described in "De l'Aluminium."[110] Development of the Hall-Héroult process stimulated further work in the United States on the chemical analysis of aluminum. Chemists in France[317,318] and Switzerland[361,362] also provided important contributions to the analytical methods.

In recent decades procedures have been standardized throughout the industry.[90] These methods with various modifications and additions have been adopted and issued by the American Society for Testing Materials,[15] Aluminum Research Institute,[9] and others. Prior to World War II, spectrochemical methods of analysis had been perfected.[91] By the end of the war, spectrographic methods had largely superseded chemical methods for production control.

Since that time, photographic spectroscopy has been largely replaced by photoelectric spectrochemical analysis in the industry.[92] In the laboratory of one company approximately 90% of the 10,000,000 to 12,000,000 analytical determinations reported annually are carried out with photoelectric spectrochemical equipment of the Quantometer type. The elaborate instrumentation required for rapid photoelectric spectrochemical analysis is justified only when large-scale operations are involved. Such equipment is not likely to be available in laboratories in which only occasional aluminum analyses are required or the work load is relatively small. Under these circumstances, conventional chemical methods are necessarily employed.

Of all the common elements, aluminum is probably the most difficult to determine accurately, because of the interference of other elements which normally accompany it and because of the incomplete separation of other metals prior to its determination. Gravimetric methods, following separation by precipitation with ammonia, 8-hydroxyquinoline, phosphate, and various organic acids in alkaline medium (precipitation with ammonia being the most common), have been proposed. These methods are used for minerals, metals, chemical products, and other materials in which aluminum is a major constituent.

If aluminum is accompanied by titanium, zirconium, or other metals which precipitate with it, an indirect determination is ordinarily employed, based on correcting the total weight of precipitate after an individual determination of each element separated with aluminum. Methods for the direct determination of aluminum in the presence of other elements have been proposed but have not been adopted generally.

Intermediate quantities of aluminum down to about 1 mg. are commonly precipitated with 8-hydroxyquinoline, which gives a precipitate suitable for oven-drying or ignition to the oxide. The precipitate can also be dissolved and titrated with a standard bromate solution. Acidimetric titration following precipitation of potassium cryolite is also used commonly.[48]

Trace amounts of aluminum in concentrations too low to be precipitated are determined colorimetrically, and various reagents have been proposed for this purpose, including aluminon, hematoxylin, alizarin sulfonic acid, and eriochrome cyanine. These reagents are typical of a large number of lake-forming dyes, some of which are used in biological staining tests. Interference by other metals which form lakes under existing conditions is common in tests of this kind, and separations are usually required. Aluminon is the most commonly used of these colorimetric reagents, although they are all similar in sensitivity and in their reactions with other metals. A fluorescence test using Pontachrome blue black R is also highly sensitive and can be used for quantitative purposes.

For establishing the presence (or absence) of aluminum in virtually any type of material, the spectrograph offers the most sensitive, reliable, and general means available. Lines at 3082, 3093, 3944, and 3962 A. are among the most useful at low concentrations of aluminum. The spectrograph is also useful for rapid quantitative determinations, particularly at low concentrations, if methods appropriate to the material under examination are applied.

Chemistry of Aluminum Compounds in Relation to the Functions of the Body.—The physical and chemical properties of aluminum and its compounds help to explain to some extent their actions and reactions in the body. Aluminum is capable of combining with both acids and bases, thus forming aluminum salts and aluminates. The oxidation number of Al in its compounds is always +3. Because of this high charge, and the rather small size of the Al^{+++} ion, negative groups such as Cl^- and OH^- ions are attracted strongly to it. On this account, aluminum chloride and aluminum hydroxide do not ionize readily, but behave as covalent compounds. The chloride, for example, is like the chloride of phosphorus or of carbon, in that it dissolves readily in organic solvents and is readily vaporized (properties which chlorides of typical metals such as sodium and calcium do not possess). The solubilities of these various aluminum compounds are noted in Table 1.[49]

The following is a brief discussion of some compounds of aluminum which man is likely to take into his body from his environment.

Aluminum Chloride: In the presence of water aluminum chloride forms a hydrate $AlCl_3.6H_2O$ (m.w. 241.43) or $[Al(H_2O)_6]Cl_3$, in which the Cl has its usual ionic character. The chloride also reacts with water to form aluminum oxychlorides, and, with the splitting off of HCl, the resultant solution is acid. As such, it possesses astringent properties and is used

as an antiseptic and deodorant. Aluminum chloride to which a solution of sodium hydroxide at 25 C is being added progressively, begins to form a precipitate [$Al(OH)_3$] at about pH 3; this precipitate ceases to form when the mixture reaches about pH 7, and it redissolves as further sodium hydroxide is added. It is uncertain whether metaaluminates (K or $NaAlO_2$) may be present in the resolution of the precipitate above pH 7 in such a system.[305] Double salts of aluminum chloride with metal chlorides, and with various organic compounds (for example, ketones, esters, nitro compounds, amines), are known.

Hydrated Aluminas (Aluminum Hydrogels): These are precipitates of the formula $Al_2O_3 \cdot nH_2O$, obtained by treating solutions of aluminum salts with OH ions. Alumina gel is formed by adding ammonia or alkali to a solution of a salt such as aluminum chloride. Hydrated alumina not only occurs in crystalline forms as definite hydroxides but may be obtained in a noncrystalline or amorphous state, as exemplified by the colloidal alumina hydrogel, which is sparingly soluble in water, and the so-called soluble hydrate in the form of an alumina hydrosol. Aluminum hydrogels, whether freshly prepared or dried, adsorb acids, bases, and salts from solution. It is impossible to precipitate hydrated alumina from solutions containing other salts without the occurrence of adsorption phenomena.

Alumina hydrogel, freshly precipitated and washed in the cold, is readily soluble in acids, but with aging for some days it becomes sparingly soluble (associated with change from an amorphous to a crystalline state).

Aluminum Hydroxide: Aluminum hydroxide [$Al(OH)_3$, m.w. 77.99] is insoluble in water between pH 4 and 8 to 10.5. When freshly precipitated, it dissolves readily in cold hydrochloric acid, in sulfuric acid, and in solutions of fixed alkali, but its solubility is impaired by drying. Aluminum hydroxide is formed as a white gelatinous precipitate when Al^{+++} ions occur in a solution of a metal hydroxide, sulfide or carbonate:

$$Al^{+++} + 3(OH)^- \rightarrow Al(OH)_3$$
$$S^{--} + HOH \rightleftharpoons HS^- + OH^-$$
$$CO_3^{--} + HOH \rightleftharpoons HCO_3^- + OH^-$$

The OH^- in aluminum hydroxide is so closely bound to the Al^{+++} that the repulsion between the H and the Al ions liberates H^+ in water, thus in fact producing a slightly acid solution:

$$Al^{+++} + 3OH^- \overset{1}{\rightleftharpoons} Al(OH)_3 \overset{2}{\rightleftharpoons} AlO_2^- + H^+ + HOH$$

If aluminum hydroxide is precipitated from a solution which contains suspended or dissolved matter, it adsorbs some of these substances and carries them down in the precipitate. According to a patent,[305] the hydrolysis of a solution of basic aluminum salts is retarded in the presence of 0.1% phosphoric acid or soluble phosphate.

Aluminum hydroxide in water is assumed to ionize in two ways:

$$Al(OH)_3 \begin{cases} \rightleftharpoons Al^{+++} + 3OH^- \\ \rightleftharpoons H^+ + H_2AlO_3^- \rightleftharpoons H^+ + H_2O + AlO_2^- \end{cases}$$

The process by which aluminum hydroxide dissolves in alkalies is that of the removal of hydroxyl ions and the formation of complex anions $Al(OH)_3 + OH^- = Al(OH)_4^-$ rather than of neutralizing bases by means of H^+ ions. Stated in another way, it neutralizes not by its acidity but by its affinity for hydroxyl ions. The hydrolysis of aluminates is then affected not by the scarcity of H^+ ions from aluminic acid but by the incompleteness of the complex ion formation. The first product of hydrolysis is alumina hydrosol (this occurs fairly rapidly, although no precipitation occurs in 96 hours), which then changes to gel form and then to crystalline aluminum hydroxide. The degree of hydrolysis of potassium aluminate is said to be 14 times that of aluminum sulfate in aqueous solution.[305]

It has been noted that freshly precipitated $Al(OH)_3$ suspended in water does not dissolve during the passage of SO_2 gas, but the gas is absorbed by the solid. On the other hand, $Al(OH)_3$ is partially soluble in an aqueous solution of carbonic acid and is reprecipitated on warming or exposure to air.

Aluminum Phosphate: The Al^{+++} ion in solution may be expected to form a number of phosphates: the orthophosphate $AlPO_4$; primary and secondary orthophosphates $Al(H_2PO_4)_3$ and $Al_2(HPO_4)_3$, as well as some mixed combinations; pyrophosphate $Al_4(P_2O_7)_3$; the acid pyrophosphate $Al_2H_6(P_2O_7)_3$, and the metaphosphate $Al(PO_3)_3$. Aluminum phosphate is said to be insoluble, but there is some evidence that phosphoric acid goes into solution slowly (8% to 24% PO_4 in 34 days) and that, while a greater proportion of the solid passes into solution with increasing proportions of water to solid, the concentration of the aluminum phosphate in the solution diminishes.

In aqueous solution aluminum phosphate is relatively insoluble. Between pH 3.9 to 5.5 and 8.6 it dissolves readily in a solution of alum or other aluminum salts (for example, a solution containing 2 mols of aluminum sulfate dissolves 1 mol of aluminum phosphate).

Calcium hydroxide increases the solubility of aluminum phosphate. Calcium hydroxide or chloride precipitates all of the phosphoric acid in solution as calcium phosphate, while the alumina remains in solution.[395] Organic hydroxy acids tend to prevent the precipitation of aluminum phosphate. Hydrogen sulfide precipitates aluminum phosphate from an alkaline solution, while some of the phosphoric acid remains in solution. The amount of phosphoric acid passing into solution from an aluminum phosphate precipitate is less as the proportion of alkali salt (potassium chloride or potassium sulfate and sodium nitrate) in the solution increases. The presence of CO_2 in the solution has no effect upon the solubility of aluminum phosphate even in the presence of lime or magnesium. Similarly, calcium carbonate has no effect upon its solubility.

Aluminum Sulfate: Aluminum sulfate [$Al_2(SO_4)_3 \cdot 18H_2O$, m.w. 666.40] is used in the preparation of some of the alums. It is soluble in water and is converted by strong and weak alkalies to $Al(OH)_3$. Following its use as a coagulant in the treatment of water supplies, some of the aluminum goes into solution if the pH of the water is lower than 5.7 or higher than 7.3.[203]

A solution containing $Na_2CO_3:Al(SO_4)_3$ does not precipitate until a molar ratio of 3:5 is reached. When the ratio is 7.5:5, half of the aluminum is precipitated as hydroxide, and when the ratio is 12:5 all of the aluminum is precipitated as hydroxide.

Sodium thiosulfate reacts with aluminum sulfate in cold solution:

$$Al_2(SO_4)_3 + 3Na_2S_2O_3 = 3Na_2SO_4 + Al_2(S_2O_3)_3$$

the latter of which decomposes:

$$Al_2(S_2O_3)_3 + 3H_2O = 3H_2S + Al_2(SO_4)_3$$

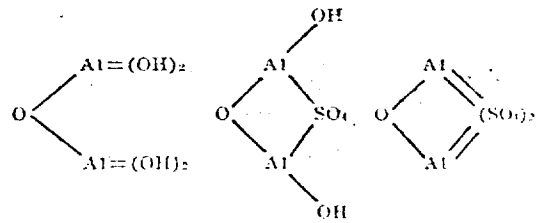
Acid Aluminum Sulfates: Three have been reported:

$(HSO_4)_2 = Al_2 = (SO_4)_2$

$(HSO_4) \equiv Al_2 = SO_4$

$(HSO_4) \equiv Al_2 = (HSO_4)_2$

Basic Aluminum Sulfates: $Al(OH)_3$ in a solution of aluminum sulfate at different temperatures is considered to give rise to $Al_2(OH)_4$ and two hydrated basic salts:



Alums: Aluminum salts have a marked tendency to form double salts, as illustrated by the sulfate, which forms a series of salts of the type $R'_2SO_4 \cdot Al_2(SO_4)_3 \cdot 24H_2O$ or $R'Al(SO_4)_2 \cdot 12H_2O$, where R′ represents a univalent radical. Allowing for the relatively low stability of the trivalent forms of some of the elements, as a rule, the greater the molecular weight of the univalent radical the greater the stability of the alum and conversely, the greater the molecular weight of the trivalent metal the less the stability of the alum. Thus, sodium forms alums only with the lighter trivalent elements aluminum, vanadium, and chromium, whereas the only stable potassium alums are those of aluminum and chromium. Ammonium and potassium alums have an indescribable

taste, for they are sour and yet sweet. Dilute solutions of alum in water behave like solutions of the mixed sulfates; only in concentrated solutions are there signs of the formation of complexes. The liberated aluminum sulfate in a dilute aqueous solution of alum should be hydrolyzed more or less by water as in the case of aqueous solution of aluminum sulfate alone, excepting as it may be modified, perhaps, by some protective action of the alkali sulfate.

Basic Alums: When an alkali is added gradually to an aqueous solution of alum, the resultant precipitate redissolves on stirring, until a certain proportion of alkali has been added, any further addition producing a permanent precipitate (called "neutral alum" in commerce).

Summary.—The physical and chemical characteristics of aluminum may be expressed in relation to the particular anion with which it is associated in a compound. Aluminum chloride forms oxychlorides, gives off hydrochloric acid, and acts as an astringent. In the presence of OH^- ions it may precipitate as the hydroxide. In addition, the chloride forms salts with several organic compounds. The hydrated aluminas ($Al_2O_3 \cdot nH_2O$) adsorb acids, bases, and salts from solution. Aluminum hydroxide is insoluble between pH 4 and 8 to 10.5. When precipitated, it adsorbs suspended and dissolved substances from suspensions. Both the hydroxide and hydrated aluminas are gelatinous in nature. The Al^{+++} ion in solution may be expected to form a number of phosphates. Aluminum phosphate is relatively insoluble between pH 3.9 to 5.5 and 8.6. This solubility is modified by the presence of other substances in the solution; for example, aluminum salts and calcium hydroxide increase the solubility of aluminum phosphate. Organic hydroxy acids tend to prevent, and hydrogen sulfide, to promote, the precipitation of aluminum phosphate, while neither carbon dioxide nor calcium carbonate exerts any effect.

The Al^{+++} ion from aluminum sulfate is free in an aqueous solution to some extent, when the pH is lower than 5.7 or higher than 7.3. All salts of aluminum in solution have a marked tendency to form double salts, called alums. These are recognized only in concentrated solution, since in dilute solution the various components of these alums behave like individual substances in a mixture.

Under appropriate conditions, aluminum may be expected to form complex compounds with proteins (as do most of the other common metals). The reactions and solubilities of aluminum compounds may be expected to be modified by the range of pH from about 2 to 7.4 in the presence of enzymes, catalysts, and protective substances which exist in the digestive tract and in the diet.

Some of the physicochemical characteristics of aluminum and its compounds display themselves when these are taken into the animal organism. However, this is observed only when the quantities of these substances ingested are far in excess of those likely to be taken or prescribed. The effects of the combination of aluminum with phosphate may be noted when large amounts of aluminum compounds are administered experimentally by mouth (see Chapter VI. Aluminum in the Digestive Tract). The ingestion of unusually large quantities of alumina and aluminum hydroxide gels has led occasionally to alimentary obstruction and to the dehydration of intestinal contents owing to the gelatinous and astringent properties (see Chapter VII. The Uses of Aluminum in Medicine). The characteristic of dilute sulfate alum solutions to behave like solutions of mixed sulfates manifests itself in a laxative effect, due to the SO_4^{--} ion, when the latter is present in the digestive tract in medicinal quantities (for example, this effect has been observed with the quantity of sulfate associated chemically with about 225 mg. and more of aluminum in sodium aluminum sulfate).

Analysis of Aluminum in Body Tissues, Body Fluids, and Other Biological Materials.—Aluminum has been determined in biological matter by a variety of methods, including gravimetric measurement follow-

ing precipitation as the phosphate[389] or hydroxide,[380] by the use of colorimetric or fluorescent properties of lakes produced by combining aluminum with certain organic reagents,[31,42,81,86,99,100,173,216,333,371,380,439,481,488] and spectrographically.[41,86,303,420]

The gravimetric method is suitable for the analysis of more than 50γ of aluminum or for large samples of biological material, whereas the colorimetric methods are not completely satisfactory for traces of aluminum because of various interferences by many metals and ions. The organic reagents that have been employed to produce lakes with aluminum include alizarin red S,[31,86] 8-hydroxyquinoline,[42,173] hematoxylin,[216] eriochromecyanine,[439] and aluminon (ammonium aurintricarboxylate),[81,99,100,333,371] but no one of these reagents has such a marked superiority as to be preferred above the others. Certain colorimetric methods are very sensitive, but the wide distribution of aluminum makes it difficult to obtain reagents free from it or other interfering ions, and therefore the results of quantitative determinations of traces of aluminum by chemical procedures are always open to question.

The fluorimetric method employing morin[488] or Pontachrome blue black R[481] is very sensitive (0.5γ Al/50 ml.), provided traces of iron, copper, and cobalt are completely removed (mercury cathode method) and the concentrations of titanium and vanadium are below limited values. The difficulties generally associated with the lakes produced in the colorimetric procedures are also encountered in the fluorescent procedure.

The most specific and sensitive analytic methods for traces of aluminum make use of the spectrographic technique.[41,86,420] Manipulations and chemical treatment are generally at a minimum in this method, and therefore the possibility of contamination is greatly reduced. Graphite electrodes can generally be obtained free from aluminum, and the method gives reliable results when it is used with an understanding of the precautions that must be taken to avoid contamination of the sample. Concentrations as low as 0.05 ppm have been determined reliably by its application to the analysis of biological material.[86]

A polarographic method has been based on the measurement of the polarographic step produced by the reduction of Pontachrome violet S. W. (the sodium salt of 5-sulfo-2-hydroxy-α-benzene-azo-2-naphthol).[491] This reagent in an acetate buffer produces a polarographic wave at −0.3 volt versus the calomel electrode, but in the presence of aluminum the step is reduced in height and a second step, proportional in length to the concentration of aluminum, appears at −0.5 volt. As with the other colored lakes used in the chemical method, many metals and ions interfere, and so reagents must be free of these ions as well as of aluminum. The results of analyses can be reproduced well when applied to known concentrations of aluminum; however, this method has not been applied to the analysis of biological materials.

III. Occurrence of Aluminum in the Normal Environment

Aluminum is the most widely distributed metallic element in the earth's crust (see Chapter I). It is concentrated there almost entirely in the lithosphere, the highest content being found in the uppermost parts.[357]

Aluminum in Soil.—Soils are constituted of varying proportions of organic and mineral materials, ranging from virtually 100% of mineral matter in selected sandy soils to more than 95% of organic matter in certain peat soils. Most upland soils contain at least 95% of mineral materials by weight. The bulk of the inorganic material is composed of oxygen, silicon, aluminum, and iron in proportions comparable to those which obtain in the earth's crust (see page 363).[293]

Aluminum occurs in undecomposed rock fragments, in secondary aluminosilicate clays, as solid or hydrosol hydrated oxides, as phosphates, and in ionic form. The origin of aluminum in soils may be attributed to decomposition initiated by mechanical subdivision of rock fragments and to the action of colloidal clays and organic matter where-

by cations are removed. Carbon dioxide and organic acids have generally been regarded as important agents in the process.[221]

Aluminum in combination is the least soluble of the major elements of the soil. Its solubility is affected by the presence of other ions, among which the hydrogen ion is of greatest importance,[294] the phosphate ion ranking next. Only the very acid soils can dissolve significant quantities of aluminum hydroxide (presumably at and below pH 5). However, the solubility at a given pH is not the only factor, for in the presence of complex forms of organic matter in the soil much aluminum is probably held in solution at hydrogen ion concentrations that would completely precipitate pure aluminum hydroxide in the laboratory.[293]

Knowledge as to the form in which aluminum ions exist in soil is not precise. It is believed that Al^{+++} ions appear only at very low pH values and that normally aluminosilicate and aluminohydroxyl ions, $Al(OH)^{++}$, are the chief ionic forms of aluminum in solution in the soil.[302] More recent investigations of a certain podsol soil showed 90% of the soluble aluminum to be present as oxyaluminum ions, $Al_2O_3H^+$, the remainder being referred to as colloidal alumina and Al^{+++}.[148,221]

The concentrations of aluminum as they may occur in various soils of the United States are given in Table 2.

In lateritic soils, which represent the end-product of the soil-leaching processes, aluminum is one of the main constituents. Concentrations of aluminum as high as 37.1% (200,000 ppm expressed as Al) have been reported.[266,293]

Aluminum in the Atmosphere.—Analyses of aluminum are not always included in surveys of atmospheric pollution, because its presence in air-borne particulate matter may, to a certain extent, be taken for granted as a consequence of its occurrence in soil. Some recently reported data are given in Table 3, in order to give some general comparison of the concentrations of aluminum with certain other metallic constituents in the atmosphere of various cities. These data are not representative of the average concentrations of aluminum in the atmosphere generally inhaled by man, because of a number of factors. The amount of dust present in the air may vary greatly (*a*) depending upon meteorological conditions as well as the extent and nature of vehicular traffic; (*b*) with the location of the sampling site, whether it is on a paved street in a city or in a rural area; (*c*) with the type of samples, whether they represent settled dust, in which case the large and therefore irrespirable particles are collected, or whether they represent only that portion of the suspended matter which has physiologic significance.

In areas where coal is burned, the concentration of aluminum in the air may be high. In a recent investigation,[205] analyses of the ash of coal disclosed the presence of concentrations of alumina ranging from a minimum of 5.3% (Al 28,042 ppm) to a maximum of 52.3% (Al 276,719 ppm). Among the other constituents, only silica exceeded alumina throughout. Mention is made of the fact that, in the analysis of the coal and its ash, some of the aluminum may be volatilized in the ashing process.

Aluminum in Vegetation.—In 1845 Will and Fresenius cast some doubt on the presence of aluminum in the ash of all plants and pointed to the probability of the external contamination of the plants with dust, in respect to the high values reported by earlier investigators.[499] It is now established that aluminum occurs in the ash of all plants, ranging from mere traces to considerable quantities.[44,273,502] The concentrations of aluminum found in most plants, including the edible varieties, are generally very low. The latter are discussed in Chapter V, and the concentrations reported by the various investigators are listed in Table 6. On the basis of the most reliable data available, the mean content of aluminum is estimated to be about 0.02% (200 ppm in the dry matter) in herbaceous vegetation, the mean value being 0.002% in the woody part.[221,376] Certain species, however, accumulate much higher concentrations of aluminum.[222] Plants are

considered to be "accumulators" when the concentration of aluminum ions equals or surpasses 1000 ppm in their dried leaves, in contrast to a more usual content of less than 300 ppm.[80] The most remarkable cases are found in the Proteaceous tree, Orites excelsa R. Br.; in various Theaceae (the tea family); in most Melastomaceae (woody greenhouse plants growing naturally in Australia and tropical Asia); in the Euphorbiaceous genera, Baccaurea and Aporosa (cactus-like plants found chiefly in South Africa); in hickory, Hicoria ovata; in the whole family Diapensiaceae (small evergreens), and in Symplocos (among them plants having medicinal properties).[221] Very recently massive deposits of aluminum succinate were found in the heartwood of a log of Cardwellis sublimus F. Muell. (Proteaceae), commonly known as northern silky oak, in Queensland, Australia. The alumina content of the dried material was 40.9% (Al 216,402 ppm). No other metals were identified, nor were there organic acids other than succinic acids.[483] A survey in Australia and New Guinea has revealed aluminum deposits in the bark and leaves, as well as in the timber, of about 80 species (dicotyledons and Filicales). These accumulators were confined to leached acid soils in areas of comparatively high rainfall. The redeposition of aluminum in litter from these plants may be an important factor.[482] This agrees with an earlier observation that hydrophytes and hydrophiles are noted for their high aluminum content.[125] In a comprehensive survey made at the Kew Botanical Gardens concentrations of aluminum as high as 29,300 ppm in dry material (using the "aluminon" test) were found in cryptogams. This work was stimulated by the recognition that the use of certain plants as mordants for dyes was based upon the concentrations of aluminum in their tissues. In addition to these properties, the following indicate an accumulator species: the presence of a characteristic yellowish-green color of the leaves, the presence of pH-sensitive anthocyanin pigments which respond to concentrations of aluminum by the change in the color of the flower, as in the hydrangea, and the presence of bright blue fruit.

As to distribution within the plant, it is generally concluded that, especially in species growing in very acid soil, there is a gradient in the aluminum content, the concentration falling off from the root to the leaf.[221] More commonly, however, the concentration of aluminum, as of other ash constituents, is often greater in the leaf than in the stem.[265,411] The higher accumulation of aluminum in green leaves as compared with the interior, or blanched, leaves has been associated with the chlorophyll content.[44] The form in which the aluminum is combined in the plant has been thought to be (*a*) an arabate in the case of the lacquer tree[500]; (*b*) a succinate in the center of a silky oak tree, as mentioned above[416,482,483]; (*c*) a malate in the sap of the latter[416]; (*d*) an acetate in the sap of the birch[468]; (*e*) a phosphate in the case of unspecified plant ash[40]; (*f*) an oxalate in the case of tea leaves, which together with calcium forms a mineral similar to vevelite,[341] and (*g*) in the case of plants such as the hydrangea, an aluminum complex of delphinidine diglycoside.[79] While, generally, in earlier analytical data, the presence of aluminum was expressed in terms of the oxide, phosphate, or sulfate, more recent and current results are given as the ion concentration.

The soil is composed of many chemical compounds, a number of which are essential for the growth of plants. Most of them, however, even those belonging to the latter group, are injurious to plants when their concentrations within the soil exceed a certain range.[293] The role of aluminum as an essential element for the growth of plants is not completely known. Some workers have considered it to be necessary for the best development of plants, such as corn, for the setting of seed,[280,419] and it has been regarded as important for the uptake of water by plants, although the latter has not been substantiated.[221] It has been said to be beneficial not only to the roots but also to the tops of citrus seedlings[192] and to be capable of protecting citrus cuttings from

damage induced by excess of copper.[278] Tea bushes have been reported to develop better with adequate quantities of available aluminum.[82,341] In recent analyses, concentrations of aluminum in tea leaves from Ceylon, Tanganyika, and Kenya ranged from less than 100 ppm to more than 17,000 ppm (dry basis), depending upon the age of the leaf. The greater part of the storage takes place during the seven months following maturity. In the flush (the commercial part of the plant), the aluminum content of the leaf is about 50 ppm, and of the mature leaf, as much as 1500 ppm. A well-plucked flush most commonly contains between 150 and 250 ppm. Some plants, such as Vaccinium, Rhododendron and several species of Ficus (the fig family), and blueberries, as well as ferns and club mosses, to mention only a few, require aluminum for normal growth.[137,501] However, no deficiency diseases due to lack of it are known.

A great deal of work has been done on the growth of plants when aluminum is in solution in soil in relatively high concentrations. This matter was reviewed thoroughly by Hutchinson in 1943.[229] It now appears that acid soils are unsuitable for some plants because of their acidity but that, in other cases, an increasing concentration of hydrolyzate-oxidate elements may exert an inhibitory influence upon their growth.

There is much variation among plants with respect to the concentrations of aluminum, in the soil or other media, required to induce harmful effects. There is also a great difference between solution cultures and pot cultures, containing equivalent concentrations of soluble aluminum, as media for the demonstration of the effects of aluminum upon the growth and development of plants. The form in which aluminum is present in the soil solution is also of great importance.[220]

The treatment of soil with either phosphate or lime may modify the activity of aluminum by fixing the aluminum as the relatively insoluble aluminum phosphate, particularly in the roots, on the one hand, or by increasing the pH, and thereby decreasing the solubility of the aluminum, on the other.[221]

Aluminum in Waters.—In comparison with the abundance of aluminum in the earth's crust, the concentrations found in most waters are negligible. The explanation for this is that the aluminum compounds that occur in water are soon precipitated from solution or adsorbed on sediments, so that only traces or relatively small quantities remain in solution. For this reason, the aluminum content of streams and other water supplies is seldom determined, unless the streams are known to be polluted with acid wastes or to have received acid drainage suspected of carrying considerable quantities of aluminum from mines.[284] This is shown clearly in data relating to the Raccoon Creek basin in Ohio, where the raw water is frequently acid. When the pH of the water exceeded 5.5, the highest concentration of aluminum found was 0.5 ppm, whereas lower levels of pH were accompanied by concentrations as high as 29 ppm.[260]

Some waters, particularly those in volcanic districts, may contain extremely high concentrations of aluminum. The clear, yellow, very acid water of a hot lake on White Island in the Bay of Plenty, New Zealand, was found to have 0.3546% of aluminum sulfate (Al about 560 ppm), and a thermal spring at Paramo de Ruiz, New Granada, contained alumina in the concentration of 1660 ppm (Al 878.31 ppm). Salt deposited on the shores of Lake Djouvan-Tubé, Akmolink Province, Russia, was said to contain 13.27% of aluminum sulfate (Al about 20,940 ppm).[305]

In a recent publication, analysis of the dry mud of a thermal spring in Messina showed, among other constituents, 14.22% of alumina (Al 75,240 ppm); the water-soluble components found included 0.0017% of alumina.[259] Langworthy and Austen[262] had compiled reports of nearly 500 determinations of aluminum in natural waters in different parts of the world, including the so-called alum springs. Some of these have been included in Tables 4 and 5.

The accuracy of the earlier analyses is, of course, open to question. However, the data give an indication of relative values.

Ocean Waters: Sea waters contain relatively low concentrations of aluminum. Monthly determinations (colorimetric method using oxine) for over a year at different depths in the northeast Pacific Ocean yielded a range of concentrations averaging 0.54 ppm.[195] In recent investigations (involving the use of the new fluorimetric reagent, Pontachrome blue black R) samples of open ocean water, collected on a voyage from Baytown, Texas, to Halifax, Nova Scotia, contained quantities of aluminum ranging from 0 to 0.010 ppm and averaging 0.0025 ppm.[414] Analyses of the water of the Sea of Japan yielded from 0.315 to 0.360 ppm (±5%, as determined colorimetrically)[225]; 0.288 ppm was found in the sea water at Suma near Kobe city, and 0.009 ppm was found at Shirahama, Kii Peninsula (on the Pacific Ocean side of Japan) by the use of a polarographic method.[224] The suspended matter in sea water from the English Channel is reported to have contained (in the ignited residue) from 1% to 20% of alumina.[29]

Public Water Supplies: Few data are available on the levels of aluminum in public water supplies, because it is presumed that aluminum would not be present in significant concentrations following proper treatment of the water.[260] Since whatever is known on the levels of aluminum in these waters is of direct interest in relation to the amounts that may be ingested by man, the available data are included in Table 7.

Summary.—Aluminum constitutes about 8% of the earth's crust and is the most abundant metallic element, ranking third among all elements after oxygen and silicon.

Although aluminum does not exist naturally in the metallic state, it is present in most minerals. It is found in all types of soil, varying from low to extremely high concentrations. As a consequence, aluminum is contained in all air-borne dusts.

Except for the so-called "accumulator" plants, most plants absorb aluminum from the soil only to a limited degree. Furthermore, aside from the "accumulators," and a few other exceptions, aluminum does not seem to be required for growth or for any other vital function, nor are there known any deficiency diseases due to the lack of this element. However, all vegetation contains some aluminum, although in most plants the concentrations are of a relatively low order of magnitude.

In natural waters, aluminum compounds which may occur normally in solution are precipitated rapidly together with suspended matter. For this reason, relatively low concentrations are generally present in rivers, lakes, oceans, and most ground waters. There exist, however, acid springs, and surface waters that carry acid drainage from mines, which may contain relatively high levels of aluminum in solution. According to some early analytical data, the so-called alum springs show levels up to 7500 ppm, in contrast to less than 1 ppm in the greater majority of natural waters.

IV. Aluminum and Food

Among the many predictions made by Dickens in 1856, long before the production of aluminum had reached the point that would permit the average housewife to own a single article made of the new metal, was that of its use for cooking utensils. While Dickens' prediction of the almost exclusive use of aluminum in the home has not come true, the growing popularity of utensils made of this metal has been evident from the time they came into general use, around 1890.[115] The many advantages of aluminum for this use are well recognized. The chief of these lie in its high and even conductivity of heat, attractive appearance, light weight combined with strength and durability, ease of cleaning, and resistance to corrosion.

Food in contact with any metal or any surface will be affected by it to varying degrees, and, conversely, the food itself may affect the metal or surface. As will be shown later, the amounts of aluminum dissolved by food are minute, and its effect on the food itself, in contrast to certain other metals, is

negligible, since most of its salts are colorless and (in the concentrations in which they may occur) tasteless, and do not affect the nutritive qualities. These same properties have been responsible for the growing use of aluminum *foil* in the home, especially in the preparation of meats for the table.

Beyond its uses in the home, the metal has found increasing application in the food processing, pharmaceutical, and cosmetic industries. In fact, in these industries processing and handling equipment made of aluminum is used extensively. The advantages, in addition to those mentioned earlier, are that it is nonadsorptive and splinter-proof and that it can be cleaned with steam. Its high thermal conductivity also results in economies when containers or conveyors must be moved in and out of heated or refrigerated areas.[8,30,67,271,313]

Suitability and Uses of Aluminum in Food Processing.—There can be no better assurance for a person using any product in his own home than the knowledge that it has been tested, not only under ordinary but also under a variety of extreme conditions, and that it has been found suitable under these conditions. In the home, the housewife, when selecting and using a utensil, is concerned as to its advantages or disadvantages in relation to a small group, principally her family. In the food and beverage processing industries, the concern of the manufacturers extends to all purchasers of their products.

Modern man has departed far from a former way of life in which he produced and stored or preserved most of the food he consumed. The growing trend toward urbanization and dependence on processed foods has resulted in extensive search within the food industries to find the best and most economical materials for the preservation of foods in a manner that will retain their taste and nutritive qualities and meet the standards of government agencies. The growth of this industry is clearly evident from the rise in the consumption of canned foods alone in the United States from 1,500,000,000 cans, or about 20 cans per person in 1900, to 23,000,000,000, or about 145 cans per person in 1955, more than half of that increase having occurred since 1935.[33] Among the per capita figures for 1953 of food products consumed in the United States, as compiled by the Department of Agriculture,[459] about 875 lb. represented foods that had been subjected to some sort of processing in flour mills, dairies, or other food processing plants. This does not include fresh meats, fish, fruits, and vegetables, all of which are handled extensively before they reach the consumer.

From the time that the "art" of canning was discovered in 1809 by Nicholas Appert, a Parisian confectioner who won the prize offered by the French Directory for a new, successful method of preserving foods to supply French armies,[10] the metal industries also have sought to develop alloys and protective coatings that would resist attack by the complicated mixtures of chemical compounds, of either more or less neutral or acid reaction, which occur in the food we eat.

The cooking utensils used in the home differ greatly in their construction from the vats and kettles used in the processing industries. The first are relatively simple structures, shaped of one piece (or two, including the lid), whereas the latter may be complicated by valves, pipes, and other apparatus, the construction of which requires the combined technical knowledge and skill of those concerned with the processing of the food as well as of those engaged in fabricating the metal, so that not only the proper alloys will be used but also the possibilities of galvanic and chemical corrosion may be avoided. The prevention of corrosion is very important in industry, where the durability of equipment, as well as its tendency to transfer metals into the substance being processed, must be considered.[8,402] From the time aluminum became available in commercial quantities, investigations seeking to determine its reactions with substances with which it might come in contact began to be made. Guided by its many technical investigations, which continue to improve the quality of the metal and its alloys,

as well as experience in the field, the aluminum industry learned that not all aluminum alloys are satisfactory for use in food processing industries and that aluminum is not suitable for all types of equipment or under all conditions of service. Alloys that contain controlled amounts of manganese or silicon and magnesium give good service generally, as does commercially pure aluminum. Alloys of higher strength, clad with either pure aluminum or with an alloy highly resistant to corrosion, may also be used where superior mechanical properties are required.[8,274]

The concentrations of aluminum which may be transferred into foods from metallic surfaces will be discussed later. For the most part, the tests, particularly those employed early, have been carried out by immersing strips of the metal in the various substances (foods and beverages, or the acids that may be contained in them) at various temperatures and for various periods of time. Alkali and salt solutions, which are known to attack all metals, have been used extensively. These tests yield no valid information as to the actual amounts of aluminum that may be ingested with foods cooked in actual utensils, but they have been valuable in contributing knowledge as to the suitability of the metal for various processes, in spite of the fact that the quality of the metal used during the earlier years was relatively poor.

The extensive literature up to 1946 on corrosion, the reactions of the metal with foods, beverages, and other chemicals, the effects of cleaning agents, and the development of protective coatings, has been reviewed thoroughly by J. M. Bryan for the Food Investigation Board of the Department of Scientific and Industrial Research of Great Britain.[67]

It became evident very early in the history of the use of aluminum that the resistance of this metal to corrosion is due to the formation of a protective film on its surface. This film was found to be oxide or hydroxide.[32,66] Under certain conditions this film is broken down, whereupon further action of the attacking solution may cause corrosion.[66] The term "corrosion" means the chemical or the electrochemical reaction of a metal in the environment in which it is placed. In the case of aluminum, the products formed in the pH range from 4 to 9 are again mainly aluminum oxides or their hydrates.

Among factors that may have given rise to concern, the mechanism of the stain that sometimes develops on aluminum utensils has been the subject of considerable study and controversy.[102,116,151,442] The stain has no significance in terms of the contamination of the article being cooked, stored, or conveyed,[102] and it can be removed by acidulating water with vinegar or acetic acid and boiling it in the utensil.[67]

Suitability for Conveying or Storage of Water: As might be expected, many investigations have been published on the effects of water on aluminum. Probably the earliest of these were begun in 1891[350] at the request of the Prussian war office, which was considering the use of aluminum for soldiers' water bottles and cooking utensils. No change in the taste of the water kept in these containers for long periods of time was observed after hundreds of tests, even though white spots formed on the walls of the vessel and the water became somewhat turbid. Distilled water had no effect even at high temperatures.[116,300,319,353] For this reason aluminum is used extensively in distillation plants for condensing coils, pipe lines, and storage tanks.[8,88,89] The quality of the metal in the earlier tests can only be surmised. In certain tests made later,[356] the purest grade available at that time (1935) was used: 99.26% (0.53% iron, 0.03% copper, 0.16% silicon). The present metal, on the other hand, reaches a purity of 99.99%.[7]

The action of urban and industrial waters on aluminum and its alloys is generally small. Soft waters are more corrosive than hard, because protective coatings are more readily formed on the metal with the latter.[300] However, the nature of the water

and its dissolved mineral constituents may affect corrosion: Nearly neutral tap waters, low in chlorides and dissolved metal salts, exert little effect generally, while contamination by heavy metal salts, particularly iron and copper at a pH below 6.0 or above 8.0, may greatly accelerate the rate of corrosion[8,55,277,354] and cause localized pitting. The effects of dissolved oxygen and carbon dioxide have also been investigated.[63,67,89,312,419] Recent developments in protective coatings have greatly increased the resistance of aluminum to corrosion by weak alkalies and chloride ions that may occur in water.[163] When potable water is boiled in utensils, the protective film that forms prevents further corrosion under the same conditions.[156,300,449,498,499] Aluminum and its alloys are not attacked by ice; hence it is used extensively in refrigeration and in ice plants.[60]

Suitability in Respect to Fruits and Fruit Juices: In general, cold fruit juices have but little effect on aluminum which, therefore, may be used in contact with practically all types of fruit products.[8,274] Hot and especially boiling citrus juices, however, do attack it severely.[50,321,351] Sugar added to fruit juices inhibits corrosion,[38,65,351] and the successful use of aluminum boiling pans for the manufacture of jams and jellies is attributable mainly to this fact. On the whole, corrosion by fruit and berry juices is relatively slow; some fruits, such as cherry and blackberry, which seem to form a protective film on the metal, exert a negligible effect.[65] The extent to which the juices react with aluminum depends also on their dilution, the more dilute juices having a more pronounced effect.[366] The differences in the rate of attack by individual juices cannot be attributed to differences in pH or to their total acidity or to their content of pectin and soluble solids.[65] The presence of sodium chloride increases corrosion.[105,404] Certain fruit colors are reduced by long standing in aluminum vessels in the absence of air, but the color may sometimes be restored by exposure to air.[158] For practical purposes, in home processing, there are few problems. Generally, fruit juices are not boiled alone; when sugar or starch is added, as will usually be the case, the protective effect will be exerted.

Suitability in Respect to the Manufacture of Sugars: Although not much information is available on the use of the metal in the processing of sugar, extensive tests made in Canada have led to the recommendation that boilers and evaporators for processing maple sap for syrup and sugar be made of aluminum.[54]

Suitability for the Processing of Vegetables: The corrosion of aluminum by vegetables is negligible, unless the vegetables contain free acids or have been rendered alkaline with sodium bicarbonate.[38,300] The addition of salt may also increase the action if the foods are allowed to remain in the vessel for long periods of time.[17,18,103] Even so, one investigator[118] found that sauerkraut had less action on aluminum than on certain other metals commonly used for utensils. The extent of the attack on aluminum by tomato juice compared well with that on Monel metal or nickel when the temperatures did not exceed 200 F.; in boiling tomato catsup and juice, aluminum was somewhat less resistant than the other two but more resistant than a number of other metals.[263] Of the metals tested, smaller amounts of aluminum than of most of the other metals dissolved into the product at the temperatures associated with cooking or pasteurizing.[118]

Suitability for the Storage of Vinegar and Pickling Processes: While aluminum vessels are suitable for the storage of vinegar and unfermented apple juice, they cannot be recommended for the manufacture of pickles and sauces, since the presence of salt may intensify the action of the acid.[67] This applies to other metals as well,[208] and cookbooks commonly recommend that no metal containers be used for home pickling.[372]

Suitability for Fermentation Processes: Aluminum is immune to attack by alcohol, provided the latter contains a small amount of water and is free from impurities, such

as tannin, traces of acids or alkalies, and heavy metals.[8,67] The metal must not be in electrical contact with copper. Anodic treatment of the metal (to provide a protective film of oxide) is recommended, and fermentation vats so treated have proved satisfactory for the manufacture of alcohol from molasses, there being no detrimental effect on the color of the product or on the stability of the yeast.[240,449] Aluminum and its alloys have been used only to a limited extent in the wine industry. Chemically oxidized "Aluman" was recently found to be the most suitable alloy for transporting and storing wines[172]; however, aluminum containers have not been recommended, thus far, for storing wines.

The use of aluminum in breweries began about 1908, with the construction of fermentation vessels.[163] Subsequent tests and applications have shown it to be particularly well suited for many other items, such as wort coolers, brew kettles, storage tanks, and beer barrels.[8] Aluminum pipes for carrying beer have many advantages over tin or tinned copper pipes, and they have been admitted in the German regulations as harmless for this purpose for more than 20 years.[373] This metal produces no turbidity or haze, has no effect on the taste or odor of beer, and does not bring about any deterioration in the vitality of yeasts.[77]

Suitability for the Processing of Meats and Fish: Numerous investigations have shown that aluminum is practically free from attack by meats[260,300,103] and that it does not bring about the objectionable dark discoloration imparted by certain other metals. Since the fat in meat acts as a protective agent, probably by forming a thin coating on the surface of the metal, the presence of salt, which otherwise attacks aluminum, has no effect.[405] In the meat packing industry, aluminum and its alloys are used for steam-jacketed pans, ham cookers, and other items of equipment.

The same practices obtain in the packing of fish and other seafood products. One of the new uses of aluminum applies to fishing boats, where its high thermal conductivity, which expedites the freezing of the catches, and its ease in cleaning are responsible for greater profits to fishermen, since they can thus stay at sea longer.[271]

Suitability for the Manufacture of Gelatin: Several metals were tested in 1924 to find a suitable material for the manufacture of gelatin. Aluminum bronze and pure aluminum were found to be best.[215] They are used extensively for the construction of cookers, vacuum evaporators, pans, vats, cooling coils, and other equipment.[8]

Suitability for the Processing and Storing of Fats and Oils: The resistance of aluminum to attack by fatty acids has resulted in the wide application of the metal in the processing and storing of fats and edible oils.[8] Unlike certain other metals, it does not catalyze the oxidation of fats, which promotes rancidity in lard and vegetable oils,[121,160,368] nor does it cause discoloration. The lack of attack by fats and fatty acids was observed early in the history of aluminum, and the use of the metal for the construction of equipment and for containers for the transport of fatty foods and preserved meats was recommended as early as 1895.[116]

Suitability for Processing Dairy Products: Aluminum has had wide use in the dairy industry. The results of many tests fully justify the conclusion that this metal is highly resistant to the action of fresh milk in the cold[148,208,219,256] and at pasteurizing or boiling temperatures[190,397]; also, free access of air does not promote corrosion.[171] No effect on the color, odor, and flavor of milk has been observed.[175,176,190,256,479] Strongly soured skim milk, semisolid sour buttermilk, and sour rennet whey attack aluminum,[316] especially at high temperatures.[117] Moderately sour milk products, such as sour cream and buttermilk, induce either no corrosion or only slight corrosion within the period of five days.[219,256,479] On the whole, the attack on aluminum by acid milk products is less intense than would be expected, considering their lactic acid

content. This is explained by the protective influence of the nonacid constituents.[219] As early as 1919, the suitability of aluminum for the production, storage, transportation, and processing of milk products was believed to be unlimited.[467] In Great Britain, Switzerland, and Denmark aluminum dairy farm equipment has been used for over 20 years. This has included pasteurizing equipment, coolers, churns, vats, storage tanks, milk containers, piping, strainers, and stirrers, as well as the tanks of trucks used for the transport of milk. Aluminum milking machines have been in service in Great Britain since 1928, and aluminum milk bottles, recommended in 1921,[120] are used on the continent.[332] Equipment made of this metal is also used in the preparation of evaporated and powdered milk and ice cream, for processing and storing cottage cheese, and in butter-making equipment, handling in some cases a butter containing up to 8% of salt.[67] Forms suitable for pressing round and brick cheese have been known to give good service without deformation for over two years.[417] In India, aluminum containers were found to be the best for the storage of ghee.[343]

Suitability for Processing Tea and Coffee: Except for early tests,[288,350,377] very little information is available on the effects of tea, coffee, and cocoa in contact with aluminum. Bleyer[48] found that hot tea and coffee (made with Munich water), after the initial attack, had no greater action on aluminum than on other metals in common use. In fact, after one hour of contact, the rate of corrosion dropped below that of iron and tin. In one test, tea, coffee, or cocoa, when boiled for half an hour, or allowed to stand for four days at room temperature in contact with Duralumin, caused no more effect on the alloy than did ordinary tap water.[144] Tea was said to be darkened sometimes when made with water boiled in aluminum kettles, which suggested a colloidal change in the tannin to one investigator,[131] while another,[97] observing that the effect was especially pronounced when water was boiled in new kettles, believed it to be due to particles of iron left on the surface of the vessel by tools used in its shaping. The effect was no longer observed after the kettles had been used for some time.

Miscellaneous Uses: The world's first all-metal flour mill, in which aluminum was used extensively, was installed in 1953 at Duffield, Yorkshire.[162,332] In France also, a similar application resulted in the replacement of wooden gravity conveyors by tubular ducts of an aluminum-magnesium alloy.[163] Investigations carried out by the U. S. Department of Agriculture on the use of sheet aluminum alloys in grain bins and the effect on them of various liquid fumigants, led to the conclusion that most of the alloys tested could be used in properly sealed and calked bins for the storage of grain.[128]

Suitable Detergents for Aluminum Equipment.—The importance of using suitable detergents for cleaning equipment employed in processing food and beverages has been stressed frequently and is reemphasized by Bryan.[67] Cleansers of strongly alkaline nature should not be used. The addition of sodium silicate or some other suitable inhibitor to mild alkalies will render them harmless and efficient. Among abrasives, copper wool should not be used. In the dairy, a sterilizing solution is required to destroy residual bacteria. A dilute solution of chloramine-T has been found to be satisfactory.[67] Recent corrosion tests have demonstrated that cleansing solutions which, in general, when used continuously for dairy equipment, have corroded aluminum, have also corroded tinned steel, while commercial alkaline compounds that contained sufficient silicates and did not contain sodium hydroxide were noncorrosive to both metals. Among five commercial chlorine preparations, in which the available chlorine reached the strength of 100 ppm, only one of the sodium hypochlorite type, stabilized with trisodium phosphate, corroded both aluminum and tinned steel.[479]

Aluminum Containers.—Aluminum cans have been in commercial use in Norway for fish products since about 1933.[285] This use arose out of the shortage of tinplate after

World War I, in contrast with the plentiful supply of aluminum in that country. In subsequent years this use has been extended to other products in Denmark, France, Portugal, Italy, Switzerland, Sweden, and Japan.[37,219,252,320] Tests extending over a three-year period led the German State Health Department to the conclusion that plain uncoated aluminum containers could be used without reservation for meats, sausage, fats, fish-in-oil, milk, peas, and asparagus, but that the inside of the containers required protection when acid products were to be packaged.[165] Similar recommendations were made recently in Denmark and Italy.[58,218] In Germany, beer is sold in aluminum cans produced by impact extrusion.[332]

The higher cost of aluminum, in comparison with some other materials suitable for this purpose, lower mechanical strength, and certain difficulties in fabrication are no doubt responsible for the fact that the former is not used more widely for vacuum packing.[67,351] On the other hand, aluminum, either alone or with an organic coating, is used for coating the inside of cans of steel plate.[33,97] Aluminum is also used for bulk containers for sugar, flour, and other products and for the caps and seals of containers for liquors, food products, and medicines.[8]

Aluminum Foil.—The use of aluminum foil for wrapping meats was recommended as early as 1895.[119] The gauge of the foil then available was not stated, and it may well be doubted that the thinness of the modern foil was achieved in those days.

The foil, as it is now known, was produced experimentally in 1905, but it was unavailable commercially until shortly before World War I. By 1939, aluminum foil, combined with paper, was being used as a wrapping on cartons of dried fruit and as a direct wrapping for cream cheese and butter.[214,358] Strategic allocation of metals during World War II prevented its use for packaging foods, with the exception of a few items for the armed forces.

In more recent years, during which the foil has found increasing application, the aluminum industry has adopted the thickness of 0.006 in. as the point of differentiation between sheet and foil. Below this gauge it is considered foil; at 0.006 in. and above, sheet. Foil, rolled as thin as 0.00017 in., is used in the electrical industry. For flexible packaging material, the finest foil normally used is 0.00035 in. thick; at this gauge it is almost always bonded to paper. The ordinary household foil is 0.0007 in. thick. The metal used today is 99.35% pure, at the minimum, the remainder consisting principally of silicon and iron. Foil may be coated with a vinyl resin, laminated with cellophane, or mounted on parchments, tissue paper, or fibers, depending upon what coating or combination will offer satisfactory protection to the product as well as to the foil itself. Or, as in the case of butter, oleomargarine, and certain cheeses, parchment-lined foil is used to prevent the product from sticking to the wrapper. The lamination with other packaging materials, such as papers and, particularly, plastics, which seal any unavoidable breaks in the foil, has resulted in an extremely low transmission rate of water vapor. It can also be heat-sealed readily by the application of thin coatings of heat-reactive lacquers and by being combined with polyethylene or Pliofilm. Today the foil is used for an increasing variety of food products for purposes both of cleanliness and of preservation of nutritive qualities. It is used also for numerous medicinal products.[8,67,166,415] More recently, containers of foil of heavy gauge, made still more rigid by fluting, have come into use for soft cheeses, baked goods, and, more extensively, frozen food.[26,56,142,329] In an appraisal of containers for frozen foods, aluminum was among those best suited for foods packed under vacuum, for fowls or irregular cuts of meats, for precooked products requiring heating for serving, and for rigid, tight containers which may be used repeatedly.[409] The impermeability of the foil-wrapped products to moisture aids in the prevention of "freezer burn" (spoilage of frozen foods caused by evaporation of ice crystals), while its ability to conduct, as well as to reflect, heat has proved to be valuable in the frozen

food industry.[436] The latter property is very important for products such as ice cream stored in self-service display cabinets. Recent tests showed that ice cream stored in cartons overwrapped with foil was protected against deterioration through softening, shrinking, and coarsening of texture.[340] Among other desirable attributes of the foil-wrapped carton is that of its resistance to the penetration of greases and oils, volatile substances that may impart foreign tastes and odors to foods, and other contaminants such as dust, molds, bacteria, and insects. As an effective barrier against light, including ultraviolet rays, such a container inhibits rancidity in butter, bacon, cheese, candy, potato chips, cookies, and other foods containing fats.[97]

In a recent appraisal of the safety of food-packaging materials by the U. S. Food and Drug Administration, aluminum foils, along with other aluminum containers, are said to be ideal.[268]

Addition of Aluminum Compounds to Foods.—The Use of Alum: Most of the work on aluminum, as it relates to health, has been done not because of concern about the wide use of aluminum cooking utensils but because of a controversy which arose out of the sale of baking powders containing "alum." However, even earlier, its effects came into question because of the addition of alum to flour to disguise certain inferior qualities, among them to arrest fermentation and to renovate flour damaged by damp storage.[415] Just when this fraudulent practice came into being has not been determined. It was forbidden in England by the Bread Acts of 1822 and 1836.[319]

On the other hand, the addition of alum to cucumber pickles and to maraschino cherries to preserve their firmness was never questioned. Smith, who reviewed the relationship of aluminum to health in 1928,[415] attributes this attitude to the fact that, in contrast to the fraudulent use in flour, this application served a useful purpose. It began in domestic pickling and is still continued in the home and in commercial establishments. In a recent report on the preservation of frozen strawberries, the addition of a solution of alum and sugar is recommended to make the berries hard, give them luster, and protect vitamin C from decomposition in storage.[4] Also, the green color of pickles is fixed best by heating them in a 0.2% aluminum sulfate solution during the presouring process.[437]

Baking Powder Containing Aluminum: The practice of combining baking soda and cream of tartar in exact proportions as a baking powder, to replace the separate use of these ingredients by the housewife, began in the middle of the 19th century. In the 1870's alum came into use as an economical substitute for cream of tartar, and this was later replaced by sodium aluminum sulfate. Competing manufacturers took advantage of the situation that had developed because of the unwarranted implications based on the fraudulent use of alum in flour and circulated statements which attributed harmful properties to the so-called "alum baking powders." Extensive litigation, beginning about 1879, both in England and in the United States, as reviewed in great detail by Smith, arose as a result of this. Actually, there are two types of these powders. Correctly speaking, they should not be designated as "alum." Sodium aluminum sulfate (S. A. S.), which is a comparatively nonhygroscopic substance* and in the presence of moisture serves as an acid to liberate carbon dioxide from sodium bicarbonate, is used in both types. In the "straight" powders, S. A. S., added to the extent of 30.5%, is the only acid ingredient; in the "combination" type, calcium acid phosphate is also present. The residue of a "straight" powder, after reaction, has been calculated to be composed of about 10.1% of aluminum hydroxide, 35.6% of sodium sulfate, and 37.8% of starch (used as a filler). The proportions in the "com-

* In the U. S. Department of Agriculture Bulletin No. 103,[458] the term "alum," which is used to distinguish aluminum-containing baking powders, applies to the "calcined sodic aluminum sulphate commonly used in alum baking powders and not to the ordinary crystallized alum."

bination" type are regarded as considerably less certain. Through the use of 1 rounded teaspoonful, or 7.5 gm., of the "straight" powder to leaven 1 qt. of flour, a finished biscuit (weighing 545 gm.) was said to contain a residue of 0.139 gm. $Al(OH)_3$ per 100 gm., or 0.048 gm. expressed as aluminum; where the quantities in 2 teaspoonfuls (rounded) of the "combination" powder (15 gm.) were used per quart of flour, the residue per 100 gm. of biscuit was set approximately at 0.147 gm. of $Al(OH)_3$, or 0.051 gm. of aluminum (assuming that 1 lb. of this powder would leave a residue of 0.56 oz. of Al_2O_3).[415] Such calculations, however, cannot yield valid results. On the basis of the above figures, the concentrations of aluminum would be 480 and 510 ppm, respectively, in a biscuit. However, since modern cookbooks[159] recommend allowing the quantity in 2 teaspoonfuls (when level, about 14 gm.) of baking powder for each cup of flour (¼ qt.), when no eggs are used, the corresponding concentrations of aluminum would then be approximately 3584 ppm and 1904 ppm, respectively, or, if the suggestions for "double action" baking powders are followed strictly, about half of these proportions. More realistic data are provided by accurate analyses of the finished product.

In 1908 President Theodore Roosevelt appointed a Referee Board of Scientific Experts, with Ira Remsen, Professor of Chemistry and President of Johns Hopkins University, as Chairman, to study aluminum compounds in relation to the health of man. A summary of the results of the investigations of the Board and their unanimous conclusions was published in 1914 in a U. S. Department of Agriculture Bulletin, which ended with the statement that baking powders containing aluminum are no more harmful than are any other baking powders but that it is wise to be moderate in the use of foods that are leavened with baking powder. This qualification was made on the basis of Dr. Alonzo E. Taylor's findings that any baking powder provides a saline cathartic residue in the alimentary tract. In the case of the cream of tartar type it is a tartrate; in that of phosphate powders it is sodium phosphate, and in the case of the "alum" type it is sodium sulfate. The aluminum itself contained in the latter is harmless.[458]

Then, in 1923, the Federal Trade Commission charged the Royal Baking Powder Company, makers of a tartrate powder, with unfair practice in claiming that "alum"-containing powders of competitors were harmful. After lengthy hearings of testimony by a large number of expert witnesses for both sides,[462] the case was dismissed by the Commission in 1926 on the understanding that the attacks of the Royal Baking Powder Company on the product of its competitors had been discontinued.

In spite of the findings of safety in the use of baking powders containing aluminum, their sale is still not permitted in England and certain other European countries. S. A. S. baking powder was permitted in Germany in 1945 as an emergency measure,[229] and attempts to have it approved permanently are being made there.[145]

Use of Aluminum Compounds in Brewing and in the Manufacture of Sugar.—Aluminum compounds are used also in brewing to remove organic colloids.[874] In Germany, the recently reported Protex process represents the addition of Deglutan, a mixture of aluminum silicates of the bentonite type, which acts as a protein stabilizer because of its adsorptive action. Up to 200 gm. of Deglutan is added per liter of beer 8 to 10 days before it is drawn off.[302] In Japan, aluminum chloride or sulfate (or both) is added to the brewing water at the rate of 60 mg. of aluminum chloride or more per liter, to give a superior quality to the beverage known as sake.[111]

Another use for aluminum compounds in connection with foods is found in the manufacture of sugar. Bauxite is now being introduced as an adsorbent, and sodium aluminate has been used as an auxiliary coagulant for the clarification of juices. In this capacity it serves to remove some of the

aluminum present in cane juices. This element is always present in sugar cane in high concentrations together with iron; in fact, the sesquioxides are generally determined together and reported as $Al_2O_3+Fe_2O_3$. In screened mixed juices, Al_2O_3 varies between 20 and 100 mg. per liter. Most of it is precipitated in the usual clarification with lime. In clarified juices the concentration is 5 to 25 mg. per liter, about one-third of the iron content. The carbonation process reduces it further, and subsequent liming to pH 8 removes the cations almost completely.[213]

Use of Aluminum Compounds in the Treatment of Water.—Methods of water purification generally characterized as coagulation have been practiced since ancient times. The fundamental factors involved in the process became known only after 1884, when Isaiah Smith Hyatt patented the combined use of iron perchloride (or any other suitable agent) as a coagulant with his system of rapid filtration in the treatment of a turbid water. The first careful investigation of alum as a coagulant was made in 1885. Throughout subsequent developments in this field, aluminum sulfate has been, and still is, the most widely used agent. Other aluminum compounds so used are sodium aluminate and ammonium alum. Coagulation finds its greatest usefulness in the removal of turbidity, organic color, and bacteria from unsoftened surface waters. In many water works, where other treatment such as chlorination requires the addition of activated carbon for the removal of tastes and odors, coagulation may be an important aid in these operations while serving its own specific functions.[16] The extent of the removal of bacteria by coagulation and sedimentation has been found to average about 80%, varying between 50% and 99%.[167]

Aluminum sulfate is added to the water at the rate of 5 to 40 ppm or even to 70 ppm.[438] The reactions which occur during coagulation are complex, involving not only the direct combination of the coagulant with impurities but also the formation of complex hydrous oxides. The presence of various ions in the water is responsible for further reactions. Whatever these may be, the successful use of alum in the treatment of water depends upon its change to an insoluble form and its subsequent precipitation.

Along with many other methods that have been introduced to guard the public health, the addition of alum to water supplies met with opposition. However, it is well established that modern purification practice results in the presence of less, rather than more, aluminum in the treated water than in raw water.[16] This is well illustrated by data provided by the Supervisor of Water Purification of the Cincinnati Water Works, which now serves more than 600,000 people.[132] For the period of September, 1954, through August, 1955, the amounts of alum added varied from 0.3 to 1.5 grains (19.5 to 97.5 mg.) per gallon of raw water (5.13 to 25.65 ppm). Alum was not added every day of each month, but only when it was required. The concentration of soluble aluminum in the finished water ranged up to 0.30 ppm and averaged 0.04 ppm over the year. On the other hand, the total aluminum content of a sample of raw water in the Ohio River was found to approximate 21 ppm.

It is significant that aluminum is not included among the chemical substances for which the U. S. Public Health Service has established maximum allowable concentrations,[466] no hygienic problems having arisen in this relationship. The recommendation was made in 1925 that when waters are treated with chemicals any excess of the chemicals be avoided, and in respect to aluminum no more than a trace should result in the effluent from the purification plant.[465] The determination of aluminum is included among the chemical analyses of water supplies by the U. S. Geological Survey only when the waters are acid.[260] Data on the aluminum content of a number of potable waters are listed in Table 7.

Effects of Aluminum and Aluminum Compounds on the Nutritional and Other

Qualities of Food.—Extensive investigations in the field of nutrition have been concerned with the attenuating or destructive influence of various types of processes for the preservation and preparation of foods upon the vitamins. It is not surprising, therefore, that the effects of various metals, including aluminum, have been subjected to scrutiny. Before discussing what has been found in respect to this metal, it may be well to consider certain conditions (without regard to the utensils in which foods may be cooked or processed) which affect the stability of the better known vitamins.

The effects of hydrogen ion concentration, atmospheric oxygen, light, and heat on the various vitamins, as well as losses encountered in ordinary cooking, have been summarized recently in "Standard Values in Nutrition and Metabolism."[5] Among the more common vitamins, ascorbic acid (or vitamin C, the chief sources of which are fruits and vegetables) is the most labile, its stability being greatest under acid (less than pH 7) conditions (except in the presence of oxygen and certain metallic ions, principally iron and copper),[206] while it is decomposed by atmospheric oxygen, light, and heat. Vitamin A is affected by atmospheric oxygen and light, riboflavin by light, and thiamine by oxygen and heat. Niacin is the most stable to these influences. Ascorbic acid and the B-complex vitamins, among them thiamine, riboflavin, and niacin, are soluble in water. It is evident, therefore, that in the course of the simplest preparation of food, and even in some cases of its storage, certain of these substances, particularly ascorbic acid, may undergo partial destruction because of exposure to air and light, while heat and the discarding of water in which the food is cooked may cause complete loss. The tabulated data in the publication mentioned earlier[5] indicate that the losses in ordinary cooking may range as follows: vitamin A, from 10% to 30%; ascorbic acid, from 20% to 80%; niacin, from 0% to 72%; riboflavin, from 0% to 48%, and thiamine, from 25% to 45%.

The length of time and the manner of cooking (whether the food being cooked is covered or uncovered, steamed, or prepared in a pressure cooker), the amount of water used, peeling, and, especially, shredding or mashing play a decided role. A few years ago, the retention of the nutrients in 20 foods typical of those a homemaker might choose was determined in the laboratories of the U. S. Department of Agriculture[211] after preparing and cooking these foods by more or less usual household procedures. As might be expected from its properties, ascorbic acid was the most sensitive of all the nutrients studied. In general, the greater the volume of water employed in the cooking, the longer the time of cooking, or the smaller the article to be cooked, the less ascorbic acid was retained. This may be illustrated by the following examples: When fresh lima beans were cooked in water sufficient to cover them, only 23% of the vitamin was retained; when they were cooked in half as much water, they retained 38%. Wax beans cooked for two hours in water sufficient to cover them retained 35%, while 54% was retained when they were cooked for only 20 minutes in the same quantity of water. Only 18% of the ascorbic acid content of raw cabbage remained after it had been cut into strips 1 in. wide and boiled for one hour. Sweet potatoes baked whole retained 89% of their original content of this vitamin, but when cut in half before baking, only 31%. The other nutrients included in this investigation were more stable, especially those resistant to heat. The experiments were not designed to determine any effects introduced by specific utensils, but aluminum pans were used for baking, aluminum skillets for braising and frying, and cast aluminum Dutch ovens for pot roasting, while enameled pans were used for boiling foods on the top of the stove. Although there is no basis for comparisons, it may be concluded from the results shown in Table A that home cooking in aluminum results in only such losses in nutrients as may be anticipated from the

TABLE A

	Percentages Retained		
	Nicotinic acid	Riboflavin	Thiamine
Beef pot roast braised in aluminum for 222 and 244 min., with and without water	89*; 115	107; 114	--
Pork chops and liver braised or fried in aluminum for 11.5 to 30 min.	57 to 105	91 to 116	65 to 102
Biscuits, rolls, and bread baked in aluminum	76 to 118	129 to 189	65 to 93
Chicken steamed or boiled in enamel for 194 and 240 min., with water to cover	32	55; 72	--

*Adapted from Hewston et al. 211.

characteristics of the product, the properties of the vitamins, and the manner of cooking.

The effects of canning and other commercial processing on the nutrients of foods have also been studied extensively and reviewed only recently.[95] It is obvious that in the course of commercial canning some losses in nutrients will occur, not only through exposure to heat but also in preparatory procedures such as cleaning, washing, cutting, blanching or scalding; in filling containers, and in brining, exhausting air from products before closure, and closing. Retention of nutrients, especially those which are water-soluble, and ascorbic acid in particular, may vary according to the practices in various canneries. For instance, retention of 94% of ascorbic acid during the canning of tomato juice was achieved in one cannery, while in another only 32% was retained.[95] Retention of vitamins in various foods depends also upon the size, maturity, grade, and characteristics of the raw products.[175] Two recent compilations of the composition of foods, including raw, processed (canned, frozen, and dehydrated), and prepared, provide a fairly broad portrayal of what may be expected with respect to the various nutrients under various conditions.[207,450] These compilations did not concern themselves with the materials of which processing equipment or containers were made, but they do furnish a background of information on other general factors which arise out of the ordinary preservation and preparation of food. As such they are pertinent here.

The effects of the use of aluminum containers in the processing of milk have been investigated extensively. The first observations were reported by investigators at the Mellon Institute.[395] In these tests, the antiscorbutic action (a function of vitamin C) of milk, boiled in various types of vessels, was tested. The milk, fed to different groups of guinea pigs, was heated as rapidly as possible to the boiling point, then allowed to continue boiling lightly for five minutes in an aluminum saucepan or in a glass beaker. Unheated milk was also fed in parallel experiments. There was no detectable difference in the condition of the animals fed upon the two lots of heated milk. The loss of vitamin C in both lots amounted to about 20% and was referable to the boiling itself. Similar results were obtained by feeding animals milk pasteurized aerobically in aluminum.[386,394]

In later tests, the ascorbic acid content of foods was determined chemically. In one of these, milk brought to the boiling point in aluminum pans lost less of its vitamin C than did milk boiled in glass vessels. This was attributed to the shorter period of time required to bring the milk to the boiling point in aluminum. When the time of heating to the boiling point was made uniform, artificially, slightly less destruction of the vitamin occurred in the glass containers. On the other hand, when the same intensity of heat was used for cooking potatoes, kohlrabi, cauliflower, and apricots, in a variety of utensils (aluminum, newly and poorly tinned copper, enameled ware, glass, glazed earthenware, and a double boiler and steam table of unspecified material), the loss of ascorbic acid was least in the aluminum ware.[152,153]

The stability of vitamin C was also examined by immersing strips of different metals, among them plate and cast aluminum, in separate portions of aqueous solutions of ascorbic acid[258] as well as by

immersing aluminum, its alloys, and other metals and their alloys in milk.[365] From all points of view, aluminum and chrome-, vanadium-, and chrome-nickel steels were considered to be the best of the metallic materials tested.

Two investigations reported in the Russian literature deal with the influence of different alloys of aluminum on aqueous ascorbic acid solutions, either alone or following their addition to foods. According to the findings, the higher the copper content of an alloy the greater was the loss of the vitamin content.[440] Strips of sheet aluminum (Mark A-3) and of secondary Duralumin (Mark AVD-1-1) caused complete destruction of the vitamin in aqueous solutions boiled for 40 minutes and then stored at 75 C for 2 hours. However, when ascorbic acid was added to potato, cabbage, onion, tomato, spinach, meat, or starch in amounts sufficient to equalize the concentrations of the vitamin therein with those in the control solutions in water, the destruction of the vitamin by the means indicated above was limited to 30% to 50%. The same strips of aluminum had very little influence on the ascorbic acid content of milk after two minutes of boiling and two hours of standing at room temperature.[441] The difference in the behavior of the strips of aluminum in the aqueous solutions and in the foods is not clear and is contrary to other findings.[258]

Recently the Department of Dairying of the Ontario Agricultural College[175,176] examined the quality of cream and milk stored in new cans made of aluminum, stainless steel, and tinned steel and in slightly rusty, and very rusty, tinned steel cans. A 2-gallon glass bottle, wrapped in asphalt-coated paper to prevent the undesirable effect of light, was used as control. The conditions were such as would obtain in actual practice, and even overemphasized, since the cans were only one-quarter full, and the ratio of the area of contact of cream and metal to the volume of cream was thus in excess of that of a full can by about 40%. Samples removed from all the containers on the fifth day of storage at 60 to 70 F showed no differences in the content of vitamin A or carotene of cream or butter from the various cans, nor any differences in the riboflavin or ascorbic acid content of the stored milk. The mean values for the acidity of the cream from the new cans were the same; those of the cream in slightly and very rusty cans were somewhat higher because of the presence of iron. Flavor scoring showed close ratings for the products in the new cans and in the glass bottle. No significant differences in the quality of butter from creams stored in these last-mentioned containers nor in the quality of the milk held in them for 16 to 120 hours at 38 to 65 F could be observed. Milk and cream held in these cans, and butter made of this cream, showed only very slight differences when they were subjected to the thiobarbituric acid test (which indicates the degree of oxidation of milk products) and this test was correlated with the flavor score.

In another test, storage of whole or centrifuged milk in aluminum containers was accompanied by little greater loss of ascorbic acid than that associated with storage of the milk in dark glass bottles.[348]

There seems to be little published information on the preservation of vitamins in food products stored in metal containers. One report indicates that the carotene content of some vegetables was affected neither by the material of the can nor by the length of storage, while the losses in the ascorbic acid content of green kale were greatest in black plate containers, less in aluminum, and least in tin plate.[385] This paper could not be seen in the original, and so the conditions could not be verified. The factors that affect nutrients in the course of canning have been discussed previously. While ascorbic acid is sensitive to the presence of iron, the variations in its destruction, as indicated above, should not be attributable to the metal alone. Further investigations are needed to give more complete information. Duralumin and aluminum strips, immersed in Squalus suckleyi liver oil and

heated under vacuum, caused less decomposition of vitamin A than did most of the 12 other metals tested. At atmospheric pressure the action of the metals was the same.[335]

The persistence of catalase and reductase in milk stored in vessels made of aluminum of 98% and 95% purity, tinned copper, and tinned iron at 20, 15, and 6 C for periods up to 120 hours showed only minor variations. The influence of pure aluminum on the pH of milk was the same as that of tinned copper and somewhat more favorable than that of aluminum of 95% purity and of tinned iron.[75]

In respect to the effects on other food catalysts in the laboratory, a 0.05% aqueous solution of aluminum chloride and one of 0.1 N aluminum nitrate inhibited only weakly, the coagulation of milk with rennet in comparison with certain other metal salts which caused marked effects.[477]

In its use as a packaging material, aluminum foil contributes to the preservation of the nutritive qualities and to the appearance of foods. This is particularly true of fats. Fats or fat-bearing foods exposed to light, air, heat, moisture, and certain metals soon become rancid, and through this change they suffer a destruction in vitamins A and E.[130] In early tests, aluminum as well as dark or green wrappers afforded effective protection against light, which was shown to be the principal cause of rancidity in oil-bearing substances.[96] Since this foil has come into wider use, tests and experience have shown that butter wrapped in parchment-lined aluminum foils keeps much longer without developing surface taint, rancidity, and loss of moisture than that wrapped in parchment alone.[211,322,391]

Recent investigations, in which the riboflavin and moisture content of "brown and serve" rolls packaged in different materials were measured after exposure to light, further support these observations. Aluminum foil proved to be superior to the other materials tested in preventing loss of riboflavin and in preserving natural flavor.[47] Candies also, when wrapped in aluminum foil or stored in boxes overwrapped with foil, were found to have undergone less deterioration in appearance ("sugar and fat bloom") and in flavor after storage for as long as three months at temperatures of 0 to 70 F[206] than did those in other wrappings. In the freezing of foods, particularly, proper containers or wrappings are important, for the reason that inadequate packaging is one of the principal causes of loss of water vapor. Desiccation affects appearance, weight, vitamins, and flavor.[149] Because of physical properties that promote close wrapping and impermeability to water vapor, aluminum foil aids in the retention of these properties.[409]

From time to time articles appear in reputable journals that arouse public fear in relation to foods and their processing or preparation, without giving the details of experimental observations upon which the pronouncements are based and without documentation. Experimental observations and common experience have shown that one of the most desirable properties of aluminum is that foods are not discolored through contact with it. Yet one author[433] states that some hours (2 to 10) after food (potatoes, beets, milk) had been prepared in aluminum its color had darkened, its taste had been altered, and its pH had been changed toward the alkaline side. The conditions under which the food was kept were not described; that is, whether under refrigeration or otherwise; moreover, it is well known that the taste of food, particularly of vegetables and milk, is changed even when they are stored after cooking, in the refrigerator, in vessels other than those made of metals.

Aside from the findings of the Referee Board[458] that "when aluminum compounds are mixed or packed with a food, the quality or strength of said food has not been found to be thereby reduced, lowered, or injuriously affected," not much information appears to have been published on the effects on the nutritive qualities of breads and cakes from the use of baking powders containing sodium aluminum sulfate. In a recent study on factors affecting the

persistence of B vitamins in corn bread,[339] no significant changes were found in the content of thiamine, riboflavin, and niacin when Calumet (containing sodium aluminum sulfate), Royal (tartrate), or Rumford (phosphate) baking powders were used (4.5 gm. of baking powder to 250 gm. of meal).

Sanitary Aspects.—So far as can be determined, there have been but few thorough investigations of the sanitary qualities of aluminum utensils. For the most part, this matter is not of first importance, since in the process of cooking, bacteria of significance to health are destroyed.

A laboratory investigation was reported recently[367] on the persistence of bacteria on various types of dinner plates, including one of stainless steel and one of aluminum, both before and after washing with a detergent in a commercial washer. The results appeared to be unfavorable to aluminum. However, without knowledge of the quality of the aluminum used, or of the type of detergent, it is impossible to judge the results in the light of actual practice.

Of more significance is the comparison of aluminum with tinned steel as materials for milk cans under conditions simulating those of actual use. The reports of experiments of this type have been favorable to aluminum.[75,311] A recent investigation deserves special mention.[179] Some 1400 cans of all types were examined over a period of 12 months, with use of various washing solutions. Except for a very short time when the cans were new (about one month in use), 12% to 40% more of the Danish type (seamless) of aluminum cans could be placed in the first and most satisfactory classification in relation to their production of bacterial colonies (0 to 40,000) per can. The same trend of a more complete destruction of strongly acid and caseolytic types of colonies in aluminum cans was evident. In a creamery, where cream remains in the can for three to seven days during hot summer months, 85% of the aluminum cans were in the first classification, as compared with 47% of tinned cans. The aluminum cans remained 3 to 7 degrees C hotter than the tinned cans for three to five minutes after their removal from the washers. The more complete destruction of bacteria was attributed to this longer retention of heat. The authors advocate further observations on seamless tinned cans, but they believe that once the tin wears off satisfactory cleaning is hard to accomplish.

Summary.—The suitability of aluminum for the processing and storage of foods has been studied extensively since the metal became available in adequate quantities in 1890. The various advantages of aluminum, that is, the high and uniform conductivity of heat over its entire surface, the ability to withstand steam-cleaning, and the relatively low rate of attack by food products have resulted in its wide application not only in the home but in the food industry as well.

A number of investigations have shown that the processing and packaging of foods in aluminum does not contribute to the destruction of nutrients over and above that caused by the handling of the food in the home or in the food industry; neither does it bring about discoloration or change in the flavor of the product. In comparison with other materials designed for the same use, the sanitary qualities of aluminum are equal or superior.

Compounds of aluminum are used widely as baking powders, in the preservation of products such as pickles, cherries, and other fruits, and in the manufacture of sugar and beer. Aluminum sulfate (alum) is used most extensively in the processes of purification of drinking water supplies.

V. Aluminum in Human Food

In his book, "The Chemistry of Food and Nutrition," Henry C. Sherman[410] confines his discussion on the significance of aluminum in man's diet to the following statement: "Our bodies have evolved in, and we live in, an environment so rich in aluminum and silicon compounds that it seems improbable that we should be injured by the

small amounts of our usual incidental intake; or that we should need more than we regularly get incidentally."

The "incidental intake" of aluminum and certain other "trace elements" is illustrated, in at least one of its quantitative aspects, by recent observations of Kehoe et al.[241] on the concentrations of the trace metals manganese, lead, tin, copper, silver, and aluminum in the tissues of a number of American adults, all but one of them women whose life histories were such as to exclude the likelihood of their having sustained any occupational or other unusual exposure to these metals. The levels of concentration of aluminum found in the lungs of these adults were higher than those of the other metals investigated and also higher than those of the same element in other tissues of the body. (The forms in which the metallic components of man's environment reach him are variable, no doubt, and largely unknown. In the case of aluminum, that portion which derives from soil dispersed in the atmosphere is made up, in all probability, of di- or trisilicates, which are essentially insoluble in water. To what extent, if any, these dissolve slowly in the medium of the lung and are transported to other tissues is unknown. Moreover, the nature of the compounds which are formed during and after the absorption of aluminum [and other metals] into the body from the lung or alimentary tract is a theoretical rather than a factual consideration. In the preparation of tissues or other biological materials for analysis, the organic components are destroyed completely by chemical or thermal means, to provide an artificial aqueous solution of the metal or metals being determined. The results of the analyses are then expressed, as a general rule, in terms of the metallic ion. Data on the concentrations of aluminum in biological materials generally are to be interpreted in the light of these facts.)

Since aluminum is the most widely distributed metallic component of the earth's surface, it is to be expected that it should occur in significant concentrations in inhaled dust. In fact, one of the major problems in the analysis of aluminum in tissues and in foods has been the avoidance of the contamination of the atmosphere and equipment of the laboratory with particulate material in the outside atmosphere and with incidental dust carried into the laboratory on the shoes and clothing of the personnel.[241] The fate and significance of the inhaled aluminum compounds does not belong in this discussion of the significance of its presence in the diet, except that its omnipresence poses the constant likelihood of the contact of foodstuffs with the dusts present in the atmosphere even in the most meticulously kept household. Moreover, aluminum in the dust inhaled under ordinary or unusual circumstances may be trapped, in part, in the upper respiratory tract and subsequently swallowed with the nasopharyngeal secretions. From the very fact that aluminum is so widely distributed in the universe, its presence in all plant and animal tissues—thus in man's daily diet—appears to be inevitable.

A number of reports have been published on the natural occurrence of aluminum in foodstuffs, and it is necessary to consider the quantities from this source before drawing conclusions as to the amounts that may be added by cooking foods in aluminum utensils, by processing, by canning, or by using aluminum-containing baking powders. The data reported vary widely, not only because of the variability of the analytical methods used, especially in early investigations, but also because of the variety of the individual foods. The concentrations in foods from animal products are consistently low. Greater variations occur in fruit and vegetable products. Although certain plants appear to accumulate aluminum, the known edible varieties (with the exception of tea plants) seem not to do so. On the whole, the outer leaves and coverings yield the greater concentrations—largely, in all probability, because of external deposition.

The methods of analysis have been discussed earlier. It is evident that the spectrographic technique has provided the

more consistent results, and that the concentrations of aluminum in natural foods are much lower than was earlier supposed. Indeed, the first use of this method gave rise to a controversy as to whether aluminum occurred regularly in plant and animal tissues. In 1928[398] attention was called to the probability of contact with aluminum contained in the air in the laboratory and with that in reagents used during chemical analysis. Then, by means of a spectrograph, the following substances were examined: wheat germ, yeast, navy beans, lima beans, potatoes, carrots, and cottonseed meal, as well as hen's eggs and several tissues from laboratory animals. All except the skin, the intestinal walls, and the lungs of the rat showed the absence of aluminum lines. Hence it was concluded that aluminum is not a constituent of either plant or animal matter, or, if it is present, then only in quantities no greater than 0.5 ppm. This work was immediately challenged by others,[238] who analyzed, also spectrographically, hen's eggs, potato, carrot, and other foods, including meat, and found the element to be present in amounts sufficient to be detected with certainty. Several other reports were published subsequently, demonstrating aluminum by this method in marine products,[331] in hen's eggs,[41] and in human and bovine milk,[111] although quantitative data were not recorded until more recently.

Concentrations of Aluminum in Raw Foods and Potable Waters.—The concentrations of aluminum in the various foodstuffs as they are reported in the literature are compiled in Table 6. The foods have been arranged according to classes—that is, cereals and breads, dairy products, meats, vegetables, fruits, desserts, condiments, and miscellaneous products, as representatives of each group found in the daily diet. The data are tabulated according to author, where numerous products were included in the investigation, and by grouping together the methods of analysis used, to enable easy comparison of results. It may be pointed out that a large number of the foods listed are not in the fresh, unaltered state but have been subjected to some sort of processing, such as milling in the case of flour or drying and treatment with preservatives in the case of raisins, and so forth. It must be borne in mind that any sort of processing, even in containers other than aluminum, may increase the aluminum content, by reason of handling and exposure to air-borne dust particles, or decrease it through procedures of cleansing or removal. All of the values have been recalculated to parts of aluminum per million parts of food (which is equivalent to milligram per kilogram in the case of solids and milligram per liter in the case of liquids which are essentially aqueous or of comparable density). No attempt has been made to recalculate the values so as to express them uniformly on either a fresh (wet) or dry basis. Where the values for fresh products were included in the reports, these were chosen. When the information as to the state of the substance was not clear, it has been taken to apply to the common form of the article. The conversion of the values could hardly be exact, in any case, unless the moisture content were specified. The concentrations of aluminum found in foods cooked in vessels made of this metal, under conditions such as would occur in usual practice, are also included in the Table, so that the significance of these values may be apparent.

The analyses made by Cholak in 1940[55] cover a wider variety of foods and beverages than those reported elsewhere. The experiments which provided these results were not designed to compare the concentrations of aluminum in foods cooked in aluminum vessels with those cooked in other types of utensils; consequently no direct correlation in this respect is possible. However, the results obtained in the case of foods that had been subjected to contact with aluminum raise some interesting issues which will be discussed later. But for a few data on water boiled in aluminum utensils under conditions comparable to those of normal practice, the

concentrations of aluminum found in drinking water supplies are listed separately in Table 7. On the whole, these are very low; yet in some instances (Arkansas) they attain appreciable proportions. The aluminum contents of other ground and mineral waters, as determined mainly in early investigations, are listed in Table 5.

It is now well established that aluminum is present in all foods and beverages, the levels, for the most part, being of a low order of magnitude.

Concentrations of Aluminum in Foods Cooked in Aluminum Utensils or Stored in Aluminum Containers.—Very shortly after the Hall-Héroult process had made the general use of aluminum possible, information appeared in the literature on the safety of the use of this metal for the preparation of foods in the home or among military personnel, together with evidence concerning the amounts that may be so introduced into the food. The first of these, begun in 1891 by Plagge and Lebbin,[350] were mentioned earlier, in connection with the effects of water on aluminum. During the course of their investigations the authors cooked the noon meal for two men in the laboratory, over a period of a year and a half, in vessels made of this metal and demonstrated that the typical items of the daily diet so processed contained but a few milligrams of aluminum.[398] No effects on the well-being of the persons who consumed the food were observed.

In the investigations that followed, the amounts of the metal which may be added to foods by aluminum vessels were estimated by measuring losses in the weight of strips immersed in a variety of beverages or solutions of acids that occur commonly in foods. The liquids, with the strips suspended in them, were allowed to stand at room temperature or at elevated temperature for various periods of time. This method was used (and is still in use) for most of the corrosion tests discussed earlier. Some tests were also carried out by measuring the loss in weight of the vessel in which the foods had been cooked. As might be expected, there were wide variations with time and temperature and with the acidity or alkalinity of the product.

The first of these observations were made in 1892.[288] Strips of aluminum, after standing in wines and various food acids of 1% to 5% concentrations for six days, lost from 1.08 to 4.77 mg. per 100 sq. cm. Hot coffee caused a 0.50 mg. loss in weight, but tea and beer had no effect. An estimate was made that a field flask of 1-liter capacity, having a surface of about 600 sq. cm. and a weight of 200 gm., attacked twice as vigorously by its contents as it would be by wine, on the basis of the experiments above (2.84 and 3.27 mg. per 100 sq. cm.), would give up no more than 5 mg. of aluminum within 24 hours. Similar experiments were performed in France under more rigorous conditions by keeping the strips immersed in the foods for several months.[34] Again, these tests were done in the interests of the use of aluminum field equipment for military personnel. Apparently 1500 utensils were so used in the expedition to Madagascar and were found to have given satisfactory service.[189]

The formation of white and dark spots on aluminum strips immersed in filtered river and well water was attributed in an early report[116] to impurities such as iron, carbon, and silicon. Even when this type of corrosion was considerable, only comparatively small quantities of alumina or, possibly, iron oxides were dissolved and they were not considered of significance from the point of view of health. By cutting sheet metal sections out of army field flasks and utensils and placing them in boiling tap water for at least three hours daily over a period of three years, one investigator,[156] in 1897, satisfied himself that a cooking vessel with a surface of 732 sq. cm. would lose 0.105 gm. in weight, corresponding to 0.09 mg. per day, after three years of use. In another experiment, strips of aluminum, boiled in milk for half an hour, developed white spots but sustained no loss in weight.[147]

While these and many other experiments of this type have given valuable information as to the suitability of the metal for use in domestic and other cooking, they fail to reveal the actual quantities of aluminum that may be contributed to the food under practical conditions of cooking or other use in the home. The metal available in the early years contained many impurities, and, although some investigators have stated that they used aluminum of a purity as high as 99%, the analytical methods of the period were far from exact, and these statements cannot be accepted. Moreover, the earlier metal had many discontinuities, causing uneven wear. The modern metal reaches a degree of purity of 99.99%, and present milling operations provide a homogeneous surface. It must also be borne in mind that tests in which strips of metal are immersed for various lengths of time in acid, neutral, or alkaline solutions, in order to determine the effects of individual components of foods upon the metal, are indicative rather than definitive. As will be shown later, the mass of food cooked in a utensil reacts entirely differently on the outer surface of contact than does a strip of metal immersed in a solution.

In 1913, investigators in the Lancet Laboratory,[17] as well as others in Great Britain,[18] undertook to find out what happens under conditions simulating everyday use. While their observations were mainly qualitative, they found virtually no evidence of a solvent action on the vessel when water, salt solution, and acetic and tartaric acid solutions, with and without salt, were boiled in an ordinary aluminum saucepan for several hours, unless the solutions remained in the pan for 24 hours. Sodium carbonate solution produced a distinct darkening of the vessel, and alumina was formed. Bacon fried in an aluminum pan was not discolored, and no alumina was introduced into the residual fat. Other foods, as beefsteak and tomatoes, together with butter, salt and pepper, seasoned soups, onions, carrots, brussels sprouts, and apples, when cooked were found to contain only small quantities of aluminum. It was concluded that aluminum was not more susceptible than iron to the action of water and foods during the processes of cooking and that "any suspicion that it may communicate poisonous qualities to food in the process of cooking may safely be dismissed." [17]

These and later experiments [19,260,300] are of particular interest because they were the first to show that when foods are cooked in aluminum vessels their aluminum content may differ greatly from the amounts that might be calculated from immersion tests using food acids or other solutions. The complex physicochemical system represented by most foods is much less corrosive, generally, than are solutions of seemingly comparable concentrations of the acids or salts found in foods. This was proved some years later [161] by suspending pieces of chemically pure metal foil, 4×5.5 sq. cm. in area, within glass jars containing 150 ml. of the corroding medium and allowing them to remain therein for about three weeks at 18 C. In the experiments with aluminum, the suspension of the strip in a 3% solution of sodium chloride caused 0.0028 gm. loss in weight, or 100% mean relative corrosion, whereas when 0.1% of gelatin was added to the salt solution only 0.0012 gm. was lost, equivalent to 44.6% mean relative corrosion. Later investigations further corroborate these results: When an egg was boiled in water in an aluminum vessel a dark stain was produced which was absent when an egg was scrambled.[142]

Unfounded reports alleging disastrous effects from the preparation of food in aluminum utensils led to further investigations by European authorities concerned with the public health. In 1928 a Swiss investigator employed used and cleaned aluminum vessels of varied capacity in his experiment.[143] Among his findings the most important are that sodium chloride (table salt) solution dissolves much less aluminum than was earlier supposed, that fats (butter) heated in a pan for 20 minutes to about 200 C absorb but negligible quantities (0.012 mg. Al in 20 gm. of butter), and that the cook-

ing of apples in a vessel in which water was previously boiled, without cleaning, dissolved somewhat more aluminum than did apples cooked in a cleaned pan. Milk, meat stock, and even sauerkraut, however, took up only minute amounts of aluminum, indicating that the protective layer of the hydroxide on the walls of the vessel was not destroyed. He also considered whether the aluminum combines with the food in an organic form, but he concluded that it does not do so. While he expressed some reservation on the use of the metal with acid foods, he saw no danger in its use for cooking most foods.

Other significant observations were reported in 1929 from Germany.[300] Berlin tap water (total hardness 21.84°) and soft Rhineland water (total hardness 5.74°) were boiled repeatedly in aluminum vessels over different periods of time up to 60 minutes. Only traces of aluminum were dissolved by bringing the hard or the soft water to a boil. When the boiling was continued for five minutes, the aluminum content of the two waters increased to 8 and 5 ppm, respectively. However, when the same pots were used repeatedly the concentrations of aluminum decreased, so that with the third use only 4 ppm was found in the boiled water. This decrease in solvent action on aluminum by water or foods with repeated use was demonstrated in the earliest investigations[156,350] and confirmed later.[109, 498] An important fact mentioned in the report cited earlier[300] is that in the home the water is brought to the boiling point and used immediately, so that the higher concentrations associated with prolonged boiling (even though the highest value was only 17 ppm) are of no consequence. When food products are added to the water, the modifying influence gives entirely different results. Thus, the only valid results are those obtained by using finished food for analysis. After determining the content of aluminum in the raw foods, the same investigator prepared them in aluminum vessels. The concentrations of aluminum obtained in the finished meals were small (6.4 mg. and lower). Only cabbage (650 gm.) boiled with vinegar, and apples (1 kg.) cooked without sugar, gave higher values, 35.0 mg. (but 8.5 mg. when the experiment was repeated) and 24.3 mg. (in the ash), respectively.

J. H. Burn,[68] of the Pharmaceutical Society of Great Britain, who appraised the hygienic status of aluminum for the British Non-Ferrous Metals Research Association in 1932, calculated, on the basis of these meals, that a person would ingest 13.16 mg. of alumina (6.92 mg. of Al) daily. This estimate of the average daily intake of aluminum in a hypothetical diet is open to some question, but it is lower than estimates made by others (see Table 8). The relative percentages of increase in the alumina content of 75 different foods after cooking were also determined.[405] Most of them ranged below 0.009%, and only eight exceeded 0.025%. The highest was 0.082%.

The publications of the well-known German pharmacologist, K. B. Lehmann, which also appeared in 1929, contributed further information on the subject.[269] He analyzed the aluminum content of a number of products as they were obtained on the market and that of various materials—water, aqueous solutions of salt and acetic acid, and a number of foods—heated in aluminum pans. Of particular interest is the following experiment: Into a new aluminum saucepan of 3-liter capacity, the author introduced 750 gm. of meat, 950 gm. of white cabbage, 900 gm. of potatoes, and 30 gm. of salt, to which 2000 ml. of water was added and the whole cooked for three hours. Then the entire contents were emptied into an enamel dish, without cleaning the saucepan thoroughly. Two days later the contents of the enamel dish were divided into five portions of 500 gm. each and one of 440 gm. The aluminum pan was freed thoroughly of all adhering food with a rubber spatula and about 100 gm. of water, and this material was analyzed separately. The results were as follows:

In the five 500 gm. portions	17.2 mg. Al
In the 440 gm. portion	3.1 mg. Al
In the remnants in pot	3.1 mg. Al
Total	23.4 mg. Al

On the basis of his analyses of raw products he expected to find

From 900 gm. potatoes	8.2 mg. Al
From 750 gm. meat	11.2 mg. Al
From 950 gm. white cabbage ...	5.7 mg. Al
Total	25.1 mg. Al

The fact that this cooked food contained no more aluminum than did the raw substance is explained by the large mass of food in relation to the surface area of the pan, the almost neutral reaction, the colloidal nature, and the high phosphate content of the food.

In another publication, two years later,[270] Lehmann reported the results of an experiment based on the use of two aluminum vessels of different quality: one, a pot of good quality used only two or three times, but somewhat darkened; the other, a new cheap pot, and very shiny. Into these he put 253 gm. and 261.5 gm., respectively, of tomatoes, to each of which 5 ml. of 5% acetic acid and 5 gm. of salt were added. After 18 hours, he found 0.65 mg. of aluminum in the one, and 1.07 mg. in the other. Two hundred fifty grams of fresh tomatoes (control) yielded 0.62 mg. of aluminum. Thus, the one batch of tomatoes took up virtually no aluminum; the other, less than 0.5 mg.

The concentrations of aluminum in foods cooked in utensils of this metal, then allowed to stand in them for various lengths of time, were also found to be of relatively low order of magnitude (from 2.6 to 19.74 ppm), even in somewhat acidic foods.[103,319,409] Only a combination of acid fruit, vinegar, and salt yielded a notable quantity of aluminum (1153 ppm) after 20 days of storage.[103] In view of the apparent fact that in these experiments the foods were left in open containers at room temperature, the conditions would scarcely apply to general practice, and for this reason the results are not tabulated.

Of interest in this connection is a report [129] that the cavalry of the British Army had used an aluminum water bottle in which was placed sodium bisulfate, giving the water a pH value of 3 and making it sterile. Depending upon the period of contact, this water contained concentrations of aluminum up to 17 ppm. The men who used these bottles were said to have had no more gastric or other disturbances than those who had used the enameled iron bottle.

Investigations of greater significance are those in which foods have been cooked in duplicate lots—one in a glass vessel, the other in aluminum—as was done by Beal and his co-workers at the Mellon Institute.[35] These were the more valuable because the periods of cooking corresponded closely to those in actual practice. The data are given in Table 6 for comparison with those obtained under other conditions. They need not be commented on here in detail. Those derived from foods processed in glass are considered to represent essentially control values in relation to cooking. The findings indicate that the amounts of aluminum taken up by neutral foods in clean utensils are insignificant; that acid foods, or those to which baking soda has been added, dissolve small amounts of aluminum; that dark (stained) utensils yield more aluminum to foods than do bright ones, but probably only in proportion to the quantity of the stain, and that sugar acts as a retardant of aluminum uptake. The largest amount found was that in apple butter, cooked for six and one-half hours.

Only a few additional experiments of this type have been reported.[109,191,261,319] In one of them [109] it is pointed out that certain raw products contain much higher concentrations of aluminum than are found in the cooked lots. It is somewhat difficult to make exact comparisons of these data, since the aluminum in the cooked products is expressed in terms of the weight of the samples, while that in the raw products is based upon their dry weight. For instance, dry spinach (raw) was found to contain 242.5 to 604.9 ppm, while there were 15.2 and 39.5 ppm in two batches cooked in glass and 17.8 and 49.6

ppm in those cooked in aluminum. Without exact knowledge of the amount of water used, and the subsequent moisture content, it is impossible to establish any relevant relationship. In the case of spinach and other leafy vegetables, as well as root vegetables to which soil and dust may adhere, the degree of washing and the inclusion or exclusion of outer leaves and coverings may make a gross difference, and this variable may introduce apparent variations in the distribution of the element in different parts of the plant. Thus, when separate results have been obtained on pared potatoes and peelings, much higher values have been found in the latter. According to recent analyses,[191] pared potato contained aluminum in concentrations ranging from 1.8 to 2.2 ppm, while the peelings contained 238 to 308 ppm on the fresh basis (or 9.2 to 11 ppm and 1450 to 1880 ppm, respectively, in dry material). In another experiment[169] there were "traces" and 146.7 ppm in the respective dry substances. These differences seem unduly large and may have been due to insufficient scrubbing of the surface. It appears, however, that there is some selective distribution of aluminum within this root, whereby the skin is favored.

Not much information is available on the concentrations of aluminum taken up by raw milk products under conditions that occur on the farm, in the creamery, or in the pasteurizing plant. According to one analysis, the aluminum content of milk six hours after milking, 0.07 ppm, was increased to 0.7 ppm after its pasteurization in laboratory apparatus made of this metal.[395] Although no separate determinations of the aluminum content of cream stored in cans made of aluminum, stainless steel, new tinned steel, rusty tinned steel, and glass are reported in recent observations,[175] no apparent differences in the content were found among the lots analyzed after periods of storage ranging up to four days. The concentration found was 7.75±5.35 ppm (average of 62 samples). This is attributed to the fact that the milk was carried from the dairy barn to the laboratory in aluminum and tinned cans. The use of a glass container for the latter purpose would have given better information in respect to the possible uptake of aluminum during storage in cans made of this metal; yet it is significant that the cream kept in such cans for several days differed insignificantly in its aluminum content from that kept in cans made of other metals or of glass. Of interest also are the concentrations of metals found in the butter from these lots of cream, which were neutralized and churned on the fifth day. Just as the iron content was found to be about half that of the cream, the aluminum concentrations averaged only 1.52 ppm. The latter are said to have been variable (many samples showing only traces), but there was no definite trend in relation to the different types of cans. This agrees with the values found in another experiment of this type.[479] Even at high acidity (0.83%), aluminum was found in the low concentration of 2.4 ppm in milk stored in aluminum cans for 48 hours at room temperature; acid cream so stored for 72 hours contained less than 1.2 ppm. The values at the start of the test were 0.84 and less than 0.1 ppm, respectively.

It was mentioned earlier that the addition of sugar to acid foods materially decreases the amount of aluminum dissolved thereby from utensils made of this metal. Two experiments[351,352] showed a relationship between the acidity of fruit juice and the amounts of aluminum taken up by it, and they also demonstrated that the addition of sugar reduced the uptake of aluminum in proportion to the quantity of sugar added. Under the conditions of the experiment (bringing to a boil and simmering in the covered pan for 30 minutes) the average amount of aluminum dissolved in wrought utensils by unsweetened juices was 23.9 ppm and in cast utensils 39.1 ppm; the corresponding values obtained with sweetened juices were 13.3 and 29.6 ppm, respectively.[352]

Comment.—The analytical findings of Cholak[85] raise some interesting considerations. The range of the concentrations of aluminum in this large series of foods is

generally low. It must be borne in mind that these results are expressed in terms of the weight of the product as such, rather than on the basis of dry weight.

In examining the data obtained by the analyses of foods that have been prepared in aluminum, the composition and quantity of a representative meal may be considered. Whether roast beef prepared in glass is chosen or ground beef fried in an aluminum pan, about the same quantities of aluminum are involved, the concentrations in the former being 1.15 ppm as against 1.20 ppm in the latter. If instead of mashed potatoes cooked in aluminum (1.8 ppm) a baked potato (10 ppm), cooked without contact with an aluminum utensil, is selected, almost six times as much aluminum will be ingested because of the choice. Raw cabbage would give 1.3 ppm, or green canned lima beans 2.05 ppm, whereas canned green beans or fresh brussels sprouts boiled in an aluminum pan contributed 2.8 and 1.65 ppm, respectively. Within this class of vegetables, special attention may be called to asparagus. Canned asparagus heated in an aluminum pan might contain 3.2 ppm, while the fresh vegetable boiled in the same type of vessel might yield as much as 25 parts per million. This high value cannot be attributed entirely to the pH of this product, which is about 5.4 to 5.8.[78] The more logical explanation is that the canned product had given up surface dust (soil) to the liquor in which it was processed, whereas the fresh tips, even under careful laboratory manipulation, may well have retained particles of soil within the "blossoms" and scales. If the meal had started with soup, canned turtle soup, having had no known contact with an aluminum vessel, would have contributed 1.80 ppm, whereas canned cream of tomato soup heated in an aluminum pan might have yielded 1.65 ppm. Among the "fruit," only rhubarb prepared in aluminum (apparently without sugar) gives a relatively high value—15 ppm. Cakes show consistently higher concentrations, although only in the case of waffles is it specified that double-action baking powder (containing aluminum) was used, as well as an aluminum griddle; the concentration found therein was 260 ppm.

Apparently recurrent propaganda against the use of aluminum utensils in Europe was responsible for a recent publication[199] in which the author emphasizes the fact that some foods prepared in utensils made of this metal may have lower concentrations of aluminum than do natural products. Yet in his conclusions he expresses doubt as to whether acid foods should be so prepared, warns against the use of baking soda in the cooking of green vegetables, advises against the use of alkaline cleansers, and urges people not to use a new aluminum pan without prior boiling out with water.

Such precautions can hardly be carried out with uniformity in homes generally, and there is no reasonable justification for their promulgation. The mean quantities of aluminum represented in the daily diet of adults, as reported by Kehoe and his associates, were obtained by the analyses of duplicate samples of the food eaten daily by two healthy American adults without reference to culinary equipment and habits of the homes involved. Yet they were only 17.31 mg.±S. D. 27.67 and 36.43 mg.±S. D. 61.97. These, and a number of estimates or calculations arrived at by other investigators, are shown in Table 8.

In a discussion on the safety of aluminum vessels, published in 1950,[450] it is pointed out that much larger amounts than would ordinarily come into question from the preparation of foods in aluminum can safely be tolerated, but the suggestion is advanced that maximum allowable concentrations be fixed as follows: 20 ppm for fresh meat, poultry, and seafood and 100 ppm for the same foods processed or canned; 50 ppm for processed eggs, oils and fats, and alcoholic and nonalcoholic beverages; 250 ppm (dry basis) for processed foods and vegetables, and 200 ppm (dry basis) for cereals, cheese, and confectionery products. There is no mention of the method whereby these determinations should be made, although in one discussion on the subject of tolerable limits for various metals the gravimetric and

volumetric procedures, depending on the quantities involved, are favored in the case of aluminum.[108] The adoption of fixed limits for the concentration of this metal, which occurs in natural products to such a variable degree and is further augmented to such a variable extent by the contact of many vegetable products with ordinary soil, seems somewhat unrealistic. For example, it is very unlikely that the aluminum content of meats would be of any significance under any but extraordinary circumstances. Indeed, the analytical data available (see Table 6) point to the occurrence of but small fractions of the proposed limit values, even in commercially processed foods,[85] which may well have been exposed to handling equipment made of aluminum. It may be inferred that any such concern for the application of regulatory standards arises out of the increasing use of aluminum for food containers, principally for vacuum-packed foods. Two relatively recent articles deal with the types of aluminum best adapted for use as containers.[58,218] For cannery products in the pH range of 6.2 to 7.2, for most fish in oil, and for sweetened condensed milk ordinary aluminum serves well; for products of pH 5.5, tuna and certain sardines in oil, and unsweetened condensed milk anodized aluminum is recommended; for smoked herrings and kipper snacks an alloy with 1.25% of manganese may be used; for vegetables varnished cans (with the addition of a small amount of calcium chloride to relieve corrosion by foods high in oxalic acid) are suitable, and for stews and smoked ham products as well as for fruits anodized and varnished cans are advised.

At the present time there are but few analytical data on products stored in aluminum containers. Lunde,[256] who determined the concentrations in fish foods canned in aluminum, considered them to be within levels comparable to those of raw products. Actually, the values found are comparatively high, probably because of the use of the gravimetric method of analysis. Unfortunately, the products were not analyzed before being stored. Proper specifications or recommendations for the type of aluminum to be used in canning various foods would diminish the likelihood of an excessive uptake of the metal by the food.

No data are available concerning the possible introduction of aluminum into food from foil used as packaging material or as a wrapping for meats and poultry in the oven or for raw vegetables and leftover foods in the home refrigerator. In view of the appearance and the durability of foil used for such purposes, however, it is unlikely that the quantities involved are significant from either the chemical or the physiological viewpoint. Direct contact of the foil with liquid materials of high acid and salt content may, obviously, result in attack upon the metal. In the United States, the Food and Drug Administration guards closely the acceptability of the interior coatings for food containers and packaging materials,[268] giving consumers further assurance that negligible quantities of the basic metal will be introduced into food and that no hazardous contamination by coatings or any other lining materials will result.

In its general aspects, a statement made in 1932 with respect to the hygienic problem of aluminum in food still stands: "Fortunately the widespread interest which the possible danger of aluminum in food has aroused has led to investigations by workers of the highest standing whose considered opinion, apart from their experimental results, should be a sufficient guide for others."[68] The opinion that aluminum cooking vessels offer no danger to health has been expressed by numerous authorities and official agencies,[20,21,142,194,304,311,319,350,363,364,419,453,493] as well as by leading pharmacologists.[186,269,418]

The American Medical Association has expressed itself on the safety of the use of aluminum for the preparation of food,[22,23] and in 1951 its Council on Foods and Nutrition published its opinion that "the use of aluminum cooking utensils is in no way injurious to health."[13] The Medical and Scientific Director of the American Cancer Society has recently added his assurance that

there is no evidence to suggest that the use of aluminum cooking utensils can cause cancer.[71] Finally, in a recent editorial in the *Quarterly Bulletin of the Association of Food and Drug Officials of the United States*[125] assurance is given that on the basis of pharmacologic data "aluminum compounds are harmless when ingested even when the administration is continued over long periods, and the casual introduction of aluminum to foods prepared in aluminum cooking utensils loses practical significance."

Summary.—Aluminum is present in man's natural diet, ranging from extremely low concentrations, particularly in foods derived from animals, to relatively high values in certain plant products. Some of the early analytical results must be regarded as unduly high, not only because of the inadequacies of the analytical methods employed but also because of sources of contamination in the reagents and equipment and in the samples themselves.

The amounts of aluminum that may be added to foods by utensils made of aluminum in the process of cooking have been studied by numerous responsible investigators. It has been found that the amounts extracted during contact with neutral foods are negligible. Acid foods exert some degree of solvent action upon aluminum, but this effect is greatly diminished by the presence of sugar. Foods that are alkaline or are made alkaline by the use of soda in cooking also attack aluminum. The complex physicochemical system that is represented in the composition and structure of foods generally serves to inhibit the chemical attack of the acids, alkalies, and salts contained in the food upon metallic containers, including those made of aluminum.

From all reported data it is evident that, except for a few isolated instances, the concentrations of aluminum in foods after being cooked in aluminum fall well within the ranges of concentrations found in raw products. Moreover, those that go beyond this range fall short of physiologic significance.

In viewing the reported concentrations of aluminum in food products, it must be remembered that they refer to material that had been cleaned and handled carefully in the laboratory. It is on the basis of these values that most of the estimates of the daily human intake of aluminum have been made. Only two sets of values listed in Table 8 are derived from analyses of food comparable in composition and quantity to that freely chosen and consumed by ordinary human adults, the samples analyzed being exact duplicates of meals prepared and eaten in an ordinary modern American household. Even so, except for the highest estimate,[227] which was based on earlier analyses, all are in fairly close agreement. This fact indicates that the factors which influence the content of aluminum in human food, while numerous and variable, tend, by their commonness and variety, to yield a fairly uniform composite result. Despite such appearances, however, the results reported by Kehoe,[212, 214] as well as the data obtained on the differences in the aluminum content of certain processed foods, as compared with raw foods,[87] suggest the likelihood that larger variations in the actual intake would occur in a larger group of persons of more varied dietary habits. That is, persons who depend mainly upon canned or frozen foods may be expected to ingest relatively small quantities of aluminum, even if all the food is subsequently cooked in aluminum pans (since the product will have been subjected to careful washing as well as preliminary processing) than do those who eat mainly fresh fruits and vegetables. Finally, persons who bring fresh fruits and vegetables from their gardens into their kitchens and subject them to merely cursory washing, safe in the knowledge that only "mother earth" had touched them, are likely to ingest rather large amounts of aluminum.

VI. Aluminum in the Body

Under ordinary circumstances, aluminum and its compounds enter the body by various routes, each of which, under certain conditions, provides some portion of the total which is absorbed into the tissues of the body. The digestive tract is probably the

principal route, because of the quantities of aluminum taken in regularly with food and beverages. Additional quantities of aluminum, inhaled mainly as soil dispersed in the atmosphere, are trapped or otherwise deposited in the upper respiratory tract and in the nasopharynx, and much of this may be swallowed. Still further quantities gain entrance to the alimentary tract from time to time because of the use of certain salts of aluminum in the medical treatment of digestive disorders. Finely divided aluminum compounds in the general atmosphere and in that of certain industrial establishments are inhaled and, dependent on the size of the particles, some proportion of that thus inhaled is retained within the finer passages and alveoli of the lung. Some of that which is so retained within the lungs, as well as some portion of that which comes in contact with the respiratory tract, is probably absorbed. Although the alimentary and respiratory tracts constitute the primary avenues for the absorption of aluminum into the body, small quantities may gain entrance through the subcutaneous or intramuscular injection of therapeutic agents containing aluminum. Minute quantities of aluminum are absorbed through the skin, in all likelihood, when aluminum compounds are applied externally in the treatment of burns and other cutaneous disorders, but the actual extent of such absorption is probably negligible, in the quantitative sense.

The sources and fate of aluminum compounds which find their way into the alimentary and respiratory tracts (and into the subcutaneous tissues by injection) may be sketched briefly, the former factually, in the main, the latter largely by inferences derived from the known behavior of other metallic compounds.

A. Aluminum Taken into the Mouth.—Quantities Commonly Ingested: Aluminum is ingested from two principal sources: (1) that which occurs naturally in foodstuffs, culinary water, drinking water, and other beverages (see Tables 6 and 7); (2) that which is added during the handling and processing of foods and beverages and in their preparation for consumption.

Many of the experiments undertaken to learn the sources and extent of the ingestion of aluminum with the diet were reviewed and summarized up to 1933.[211] These observations were made, as a rule, before analytical methods had reached their present specificity and accuracy. Apparently, as more specific and precise methods have been developed and employed, the amounts of aluminum found in the ordinary diet have decreased. Taking the available data as they are, however, it would appear that the quantities of aluminum in the diet of the ordinary adult, including amounts derived from aluminum utensils, range from less than 10 mg. to about 100 mg. of aluminum per day. The sources of this aluminum in the diet have been investigated.[119,194,269,307,455,496,503] For more detailed information concerning the aluminum content of foods and beverages consult Chapter V of this report.

Unusual Quantities Administered by Mouth Experimentally: Experiments designed to investigate the immediate toxicity of aluminum for experimental animals through the oral administration of various of its compounds have had to contend with two troublesome obstacles—the relative insolubility of many of these compounds, on the one hand, and the complicating effects of the acid radical of certain of the soluble salts, on the other. Briefly to summarize the results, the lethal dosages of the acetate and the chloride for experimental animals appear to lie between 5 and 15 gm. per kg. in the case of the former and between 1 and 3 gm. per kg. in that of the latter.[199] Anhydrous sodium alum also has a low order of toxicity, the lethal oral dose for the rabbit being approximately 2 gm. per kg.[245] A mixture of insoluble aluminum phosphates (35% normal phosphate and 65% pyrophosphate) can be administered by stomach tube to rabbits up to the limit of feasibility, without inducing harmful effects.[245] Since the aluminum content of the acetate is much greater than that of the chloride, it is ap-

parent that the toxicity of these two compounds does not depend entirely upon their aluminum content, the toxic effect being due in some indeterminate but considerable degree to the acid radical. The matter is of academic, rather than of practical, interest, in view of the low toxicity of these compounds. It is of some importance, however, to recognize that injurious effects can be induced by the sudden introduction of very large doses of soluble aluminum compounds into the alimentary tract, since this fact, demonstrable experimentally, is in such striking contrast with general medical and human experience with aluminum compounds. Of greater significance is the fact that no delayed or chronic form of aluminum poisoning has been observed or described in connection with the many and extensive investigations of the effects of feeding aluminum compounds to animals and man.

As suggested above, numerous investigations have been concerned with the human absorption of quantities of aluminum well above the usual dietary range.[14,164,242,275,36?,383,398,453,458,497] Laboratory animals used to extend this type of information include rats,[154,217,245,286,290,301,303,327,447] guinea pigs,[393] mice,[286,492] rabbits,[46,245,292] pigs,[289,291] and dogs.[136,326,361,452,457,497] In the commonest type of animal experiments a compound of aluminum was administered orally at regular intervals or added to the food in known quantities over a definite period of time, during and after which the aluminum content of the blood and urine, and often that of the tissues of killed animals, was determined at intervals. An alternative procedure, applicable to human subjects, has been that of measuring the total intake of aluminum by mouth and the total elimination of aluminum in the urine and feces. A less elaborate, but unfortunately faulty, form of experimental procedure has consisted of determining the output of aluminum in the urine and feces during a period of oral administration, disregarding the influence of the quantities which occur in the diet during such a period. Under these experimental conditions, a slight increase, or an apparent increase, in the concentration or output of aluminum in the urine has been interpreted erroneously, as evidence of the absorption of some of the administered aluminum.

A common finding of the first type of experiment has been the lack of any significant increase in the concentration of aluminum in the blood or urine, following the administration of large quantities of aluminum compounds.[127] This is consistent with the results of balance experiments on human subjects, in which apparently all of the aluminum taken in by mouth was found in the feces. The facts have not been established fully under varied experimental conditions such as the use of a representative variety of specific compounds of aluminum, but the available evidence demonstrates that a number of aluminum compounds are very poorly absorbed, if at all, following their administration orally to experimental animals and men. Some representative results on man are presented in some detail in the following section. In view of the occurrence of aluminum in animal and human tissues, albeit in minute concentrations, it is evident that some aluminum is absorbed from some source, and it seems highly probable that some of this enters the tissues by way of the alimentary tract.

No aluminum (within the limits of analytical sensitivity) was found in the blood of four individuals who had ingested the large quantity of 1000 mg. of aluminum (for not stated) per day for "several days.[458] A healthy human subject gave no evidence of having absorbed a compound of aluminum fed in his diet at levels of about 300 mg. per day for nine weeks and about 145 mg. per day for another period, of eight weeks.[242] The concentration of aluminum in the blood and urine of this person underwent no increase during the periods of elevated intake. Moreover, between 81% and 91% (95% confidence limits) of the 300 mg. and 88% and 110% of the 145 mg. of the aluminum taken in daily was evacuated from the digestive tract.

Other experiments have been reported involving 19 persons, in which after the ingestion of from 100 to 970 mg. of aluminum per day (as alum baking powder residue, $AlCl_3$ or $Al(OH)_3$), over periods of 70 to 118 days, between 95% and 102% of the total quantities ingested was found in the feces.[415,458]

In another experiment, 89% of a single oral dose of about 540 mg. of aluminum [as $Al(OH)_3$] was eliminated in the feces in two days, and 98% of a second similar dose was so eliminated in the subsequent four days.[497] One investigator has offered dubious evidence of the absorption of aluminum by a man who had taken meals rich in aluminum. The reported elevation of the concentration of aluminum in the blood cannot be accepted as significant.[458]

Other investigators found no significant differences between the levels of concentration of aluminum in the blood of a group of normal adults and those of a corresponding group undergoing oral therapy with large amounts of aluminum as aluminum hydroxide or aluminum phosphate.[127,164]

Further evidence of the slight or negligible extent of the alimentary absorption of aluminum is found in experiments on laboratory animals. In one experiment, four generations of rats were fed on diets containing 8 mg. of aluminum (as potassium aluminum sulfate) per kilogram of body weight. In no instance did the tissues from the rats of these four generations contain significantly higher concentrations of aluminum than did the corresponding tissues from control rats.[327] Another group of growing rats was fed on a diet which contained aluminum in the concentration of 800 ppm (as decomposed baking powder containing calcium phosphate and sodium aluminum sulfate) over a period of 12 to 16 weeks.[290] Analyses of tissues from the control and aluminum-fed rats yielded no significant differences in the aluminum content of the corresponding organs of the two groups. The aluminum fed to the rats was recovered completely (within the limits of experimental error) in the feces collected over a period of 7 to 12 days.[290] In a third experiment, after growing rats had been fed for three months on a diet containing aluminum (as $AlCl_3.6H_2O$) in the concentration of 600 ppm the levels of concentration of aluminum in their tissues were the same as those in the tissues of controls.[447] Similar results have been reported by other investigators, even with a diet containing 1.7% of aluminum (as aluminum phosphate).[286,291,303]

To each of a group of dogs, one of the following—3.2 to 6.4 gm. of potassium-alum, 4.5 gm. of aluminum sulfate, or a quantity of aluminum acetate equivalent to 0.125 to 0.25 gm. of aluminum oxide—was fed daily for several weeks, and the aluminum was recovered completely in the feces. No retention of aluminum in the tissues of dogs could be demonstrated; nor was there any elevation of the concentration of aluminum in their urine during or following the feeding of 0.3 gm. of $AlCl_3.6H_2O$ to them daily for 8 to 13 months, or of comparable daily dosages of $Al(OH)_3$ for 10 to 15 months.[497] No absorption occurred following the injection of aluminum into experimentally prepared loops in different parts of the intestines of dogs.[326] Experiments are cited[364] in which dogs were fed 529 mg. of aluminum (as hydroxide) daily for one year, during which time all of the ingested aluminum was eliminated in their feces and no absorption of aluminum was demonstrated. One group of investigators has suggested that dogs absorbed small amounts when the diet contained elevated amounts of aluminum. However, the limited evidence in support of the suggestion is unacceptable in the light of the wide variation of the analytical results and the overwhelming weight of evidence to the contrary. The blood of the dogs did not show any significant increase in concentration.[152,157]

The preponderance of experimental reports on man and other animals are in agreement that various compounds of aluminum, when taken into the body by mouth in even large quantities and over long periods of time, are insignificantly absorbed.

Some of the data which appear in the earlier literature imply that alimentary absorption has occurred, but these are highly questionable and have not been confirmed.[305,453] (For data pertaining to intravenous injection see the section on Distribution in this chapter.) The analytical methods used in early experiments were inadequate to determine the minute amounts of aluminum present in body fluids and tissues. They tended to err on the high side.[35,239,421]

B. Aluminum in the Alimentary Tract.—There is evidence to indicate that aluminum, as well as certain other metals (e. g., calcium and magnesium) taken in with the diet, reacts with some of the phosphate present in the alimentary tract. The following is a simplified example.

$$[Na_2HPO_4 + NaH_2PO_4] + 2AlCl_3 = 2AlPO_4 + 3NaCl + 3HCl$$

This binds a portion of the phosphate in the diet so that it passes unabsorbed through the bowel. Aluminum compounds, even in solution, do not ionize readily to release the Al^{+++} ion, and so it is likely that only a portion of any aluminum present in the digestive tract is free to combine with phosphorus.[325] When the aluminum in the diet amounts to more than 200 mg. per day (a situation that would occur only rarely from dietary variations but may well occur occasionally under experimental or therapeutic conditions), a measurable portion of the phosphates in the diet may be bound by the aluminum in the digestive tract and passed out with the feces. It has been shown, however, that quantities of aluminum roughly 25 to 50 times those commonly present in the diet, administered daily (as much as 1118 mg. of aluminum [as $Al(OH)_3$] and 970 mg. of aluminum [as $AlCl_3$]), are insufficient to so combine with the alimentary phosphates as to deprive the body of its necessary supply of phosphorus.

In one experiment, eight human subjects ingested from 65 to 260 mg. of aluminum daily for 130 days without showing any change in the urinary phosphate or in the phosphorus to nitrogen ratios.[83] There was a measurable but physiologically insignificant decrease in the phosphorus in the urine in six subjects that had taken in from 223.6 to 1118 mg. of aluminum daily over a period of 70 days. Six additional persons, fed for shorter periods of time on a diet containing about 225 mg. of aluminum per day, showed a slight decrease in phosphorus in their urine.[248]

In another experiment, the phosphorus content of the urine and feces of six persons was followed and found to be unchanged when the daily diet contained 100 or 149 mg. of aluminum (as sodium aluminum sulfate). However, at daily levels of 209 mg. and 298 mg. of aluminum there was 10% to 25% deflection of phosphorus from the urine to the feces. The experiment was continued, using sodium aluminum sulfate, aluminum hydroxide or aluminum chloride in daily dosages above 227 mg. (of Al) over periods of 6 to 40 days in each instance. The sulfate and the hydroxide induced a decrease of the phosphorus in the urine and a proportional increase in the feces to the extent of about 20%. The chloride induced smaller changes of the same type. Despite this change in the route of some of the dietary phosphorus, it was found that the total elimination of phosphorus was no more than that taken in. In order to check whether only the route of elimination of phosphorus was changed or whether phosphorus normally required in tissues and fluids was being drawn upon, two human subjects were fed on low-phosphorus-containing diets. They were given 540 and 660 mg. of aluminum [as $Al(OH)_3$] per day, respectively. In both instances the urinary phosphorus decreased and the fecal phosphorus increased, but the total elimination of phosphorus by both routes was unchanged as compared with that of a control period.[432] When aluminum was ingested as aluminum phosphate gel in quantities up to 650 mg. per day, the feces gave evidence of the additional phosphate but the urinary excretion of phosphorus continued at its previous level.[248] The smallest quantities of aluminum (greater than 250 mg. of aluminum per day) which led to a decrease in the absorption of phosphorus

from the alimentary tract were roughly 5 to 10 times greater than those which occur commonly in the daily diet.

Other experiments, wherein human subjects were given excessive quantities of aluminum hydroxide by mouth (240 ml. per day as compared with the usual dosage of less than 100 ml.), have shown the change in the route of elimination of inorganic phosphate from the urine to the feces.[110,248] Organic phosphorus may also combine with aluminum in the digestive tract.[350]

Observations on laboratory animals have yielded results in conformity with those obtained through the use of human subjects and have brought to light other aspects of the affinity of large amounts of aluminum for phosphates. Young domestic pigs given 0.8 to 1.4 gm. of aluminum [as $NaAl(SO_4)_2$] in the diet daily for 6 to 11 days (or about 10,000 ppm) did not show a significant reduction in their urinary phosphorus.[258] Similar results were obtained with equivalent amounts of aluminum as aluminum phosphate. A diet containing large quantities of aluminum [5% of $Al_2(SO_4)_3.18H_2O$, or Al about 4045 ppm] caused a decrease of the inorganic phosphorus in the blood serum of rats along with a resultant lower mineral ash content in a long bone (femur)[254] and a diversion of phosphorus from the urine to the feces.[267]

The experimental introduction of aluminum into the diet in concentrations (about 1400 ppm) sufficient or more than sufficient to combine with the total phosphorus has been found to lower, markedly, the inorganic phosphorus level in the blood and tissues of guinea pigs,[98,393] of rabbits (their urine contained practically no phosphorus after nine days),[98] and of baby chicks (they developed severe "rickets").[107] Puppies were shown to have suffered a loss of phosphorus from the body while being fed on a low-phosphorus-containing diet and given large quantities of aluminum hydroxide (100 or more mg. of aluminum per kilogram of body weight daily).[267]

Certain experiments have provided evidence that even very large quantities of aluminum in the human diet fail to interfere with the assimilation of such food components as protein, carbohydrates, and fats.[83,188,415,458] Corresponding results have been obtained in experiments with animals (dogs[39,391] and rats[255]).

Investigations concerned with the possible effects of aluminum upon digestive enzymes and upon various functions of the digestive tract have been carried out on man,[2,59,127,296,325,358,381,415] dogs,[1,93,226,254,383,384] and rats,[301,408] as well as in vitro.[39,253,296,301,385,381,387,415,443] The more significant findings in these investigations are related to elevated quantities of compounds of aluminum [$Al(OH)_3$ and $AlPO_4$ gels, also basic aluminum carbonate (approximately $Al_2O_3.CO_2$)] which have been used extensively in the treatment of certain disorders of the digestive tract (essentially local therapy involving little or no absorption of aluminum)[127,464] (see Chapter VII, Uses of Aluminum in Medicine). They are noted briefly as follows:

After the administration of medicinal amounts (starting with approximately 10 to 100 times the quantities of aluminum found in the daily diet), the protein-digesting enzyme of the stomach, pepsin, was inactivated (but not destroyed) in varying degrees, depending upon the concentration of aluminum.[253,254,296,387,408] Stomach acidity was decreased without upsetting the acid-base balance in the body.[2,14,127,408] Various aluminum compounds, insoluble in water, were found to be soluble in the stomach and, to a less extent, in the intestines.[174,296,325,358,384] There appeared to be no relationship between gastric acidity and the solubility of aluminum.[325] The volume of gastric secretions was decreased[2,59,254] or unchanged,[1,226] and the mucin secretion was increased.[127]

Pancreatic juice was found to be unaltered by aluminum hydroxide.[59] Constipation, sometimes associated with the use of massive doses of aluminum gels, is considered to be due to their slightly astringent property. Some laxative effect is associated with the ingestion of aluminum salts which contain

sufficient quantities of the SO_4^{--} ion, the latter ion being responsible for the observed effect.[283]

C. Injection of Aluminum into the Body.

The question of the absorption of aluminum after its injection into various tissues or body cavities has not been investigated systematically. Ample evidence has accumulated, however, to permit the conclusion that when aluminum is injected, whether under the skin, into muscle tissue, or into the peritoneal cavity, it finds its way from the site of the injection into various tissues and organs. Data obtained by the analysis of the tissues of a variety of laboratory animals have demonstrated the presence of significant quantities of aluminum in certain organs. Aluminum was retained particularly in the ventricular muscle of the heart following the injection of aluminum hydroxide into the heart's blood of guinea pigs. The aluminum content of the livers and spleens of these animals remained elevated for at least a year after the injections. No evidence of injury to the tissues was found at any time.[179] Intraperitoneal injections of potassium aluminum sulfate into rats resulted in increased concentrations of aluminum in their livers and blood.[327] Acute and even fatal poisoning may be induced by the injection of very large doses of salts of aluminum subcutaneously [the lethal dose of $Al_2(SO_4)_3$ for rabbits approximates 100 mg. per kg.] or intraperitoneally (lethal dosages by this route of administration are apparently somewhat lower than those given subcutaneously).[45] Solutions of aluminum compounds can be injected intravenously only in very dilute forms (1% or less), since at higher concentrations precipitation occurs in the blood stream, with the formation of emboli. In consequence of this, fine dispersions of insoluble aluminum compounds have been employed, for the most part, in intravenous injections. Reference to experiments of this type will be made under Distribution Under Artificial Conditions.

D. Topical Application.—The extent of the absorption of aluminum compounds through the unbroken skin of experimental animals and men has not been investigated in a satisfactory manner. Only a few recorded observations bear on this subject, and they do not relate to the intact skin.

The use of aluminum foil as a dressing on burns of the human skin has given no evidence of any systemic toxic effect.[64] No aluminum accumulated in the scavenger cells (reticuloendothelial cells) of patients whose burns had been treated with powdered metallic aluminum.[138]

E. Inhaled Aluminum.—There appears to be no experimental or clinical evidence that aluminum is absorbed within the lungs even under the conditions associated with the prolonged inhalation of aluminum and its compounds in the industrial atmosphere. That some absorption occurs slowly seems likely, but the quantities of aluminum found in human tissues other than the lungs and regional lymph glands have been insufficient to demonstrate the occurrence of such absorption under ordinary circumstances,[244] and comparably negative results have been obtained in the tissues of rabbits following the direct intratracheal injection of aluminum.[272]

Most of the reports on the inhalation of aluminum have been concerned with workmen in industries involved in processing aluminum or its alloys who are exposed regularly to mixed dusts containing various relatively insoluble compounds of aluminum. Such reports have been devoted principally to descriptions of clinical findings or the lack of them or to roentgenographic or microscopic examinations of the respiratory tissues and, particularly, of the lungs following the inhalation of dust laden air known to contain aluminum.[116,223,230,231,232,425,434] Many investigators (particularly in reports published before 1938) have called attention to certain changes in the lungs of exposed workmen. Minor changes, unassociated with disability, may be grouped together, for present purposes, in the category of tissue responses to "inert" particles of dust, or foreign bodies, rather than as evidence of significant disease processes.[223,251,428]

It was not until 1938, in fact, that aluminum dusts were indicted as causative agents in the production of pulmonary irritation and fibrosis. The earliest findings of abnormalities in the lungs of workers with aluminum compounds were published by Doese and Goralewski.[115,182,185] The former reported the case of a spray painter who used aluminum paints for six months and whose lungs demonstrated marked anatomic changes. The latter described several cases of roentgenographic abnormalities in the lungs of workmen in a stamping mill, who had been exposed to dust containing high concentrations of both aluminum and copper. In all instances the atmosphere was known to have been excessively dusty, and physical and chemical factors other than the presence of aluminum were assumed to have exerted some (if not the major) effect.[159,299]

Descriptions of tissue abnormalities have been provided in detail by several other investigators.[236,237,247,250] In no instance was it possible to determine the precise nature or the amounts of the materials to which the workmen had been exposed, and the authors differed in their descriptions of the tissues and their interpretations of the findings. Some investigators have attempted to attribute the development of isolated cases to the specific size of the particles involved or to various unspecified changes in coatings on the aluminum particles. Jaeger and Jaeger[225] have postulated that uncoated particles of aluminum dust are ionized in the tissue fluids of the lungs and transformed into compounds that injure the cells. This hypothesis was not confirmed by the direct intratracheal injection of pure alumina into the lungs of rabbits, wherein only a massive "scavenger cell" reaction (such as can be obtained with any inert dust) was demonstrated.[272]

With the advent of the inhalation of aluminum[199] for the prophylaxis and treatment of silicosis, the question of the development of "aluminosis" of the lung received greater and more specific attention. For a period of more than 10 years, hundreds of thousands of such treatments were administered to individuals with varying degrees of silicosis and to other persons with silicosis whose occupational exposure to harmful concentrations of silica dust was being continued. Although the administration of aluminum (also aluminum oxide and aluminum hydrate) was not continuous, relatively high concentrations of particles of small size were employed in such a fashion as to ensure delivery and deposition of the particles into the bronchioles and alveoli. In no instance has there been a report of adverse tissue reactions among the persons who had taken daily treatments over periods of many years. Although it has been demonstrated that aluminum may stimulate the spread of tuberculosis in experimentally infected animals, no evidence has been obtained to suggest that the inhalation of aluminum dusts by human beings increases the incidence of, or susceptibility to, new tuberculous infection. Therefore, whether or not the specific entity "aluminum lung" as described by Mödder and Schmitt[315] is indeed a consequence of the inhalation of aluminum alone has not been confirmed and is open to serious doubt, since other environmental factors have not been taken into account.

In 1947, Shaver and Riddell[406,407] first reported a series of cases, on this continent, of "Lung Changes Associated with the Manufacture of Alumina Abrasives." These authors reported a lung disease, similar to that described by Goralewski and others,[159,181,185,196,316,396,471] in 344 workers who were exposed to high concentrations of alumina and silica, both in a very fine state of division. Other unidentified materials were also known to exist in the working environment. Of additional significance was the fact that in these operations the rate of production and the temperature at which the process was being carried out had been increased greatly just prior to the time that chest complaints were reported by the men.

It would seem, from the foregoing paragraphs, that under certain conditions of exposure in industry where aluminum or certain of its compounds is present in the

atmosphere, a deleterious effect can be exerted upon the pulmonary epithelium. Whether or not the presence of aluminum is an important etiologic factor in the production of the disease is uncertain. The information available indicates that aluminum, aluminum oxide, and aluminum hydrate do not injure the pulmonary system.

Summary.—Extensive though somewhat scattered evidence from human experience, supported by the results of experiments on laboratory animals, indicates that aluminum and its compounds are absorbed only slowly and slightly, if at all, from the respiratory membranes. Apparently, the quantities of aluminum absorbed from the respiratory and alimentary tracts combined, under ordinary environmental conditions or in those of the aluminum industries, are little more than traces (measurable only by highly sensitive analytical methods). That some slight absorption occurs from one or the other or perhaps from both of these sources is proved by the presence of aluminum in extremely small but measurable amounts in the tissues, organs, blood, and urine of man and other animals. There being no evidence to indicate that aluminum is retained within the body or any of its specialized tissues to any considerable extent (cf. Section F below), it may be assumed from the general level of the urinary excretion of ordinary individuals that a quantity of aluminum, perhaps as much as 100γ of aluminum, may be absorbed from all sources daily. It is likely that some portion of that which occurs in the feces is also the result of a true excretion from the tissues into the alimentary tract. No estimate of this quantity can be made from available evidence, but it may be taken as reasonably certain, in view of the low and relatively uniform rate of the urinary excretion of aluminum, that the extent of the absorption of aluminum is conditioned much more by the physical and chemical characteristics of the animal organism (as a medium for absorption) than it is by the amounts of aluminum available for absorption within the respiratory or alimentary tracts.

F. Distribution of Aluminum in the Animal Organism.—Distribution Under Ordinary Environmental Conditions: The complete pattern of the normal or usual distribution of aluminum in the tissues of the mammalian organism, including that of man, has not been established satisfactorily. Among the reasons behind this situation, which include the difficulties associated with the determination of minute quantities with a high degree of precision, lies the fact that the stimulus, which might have been provided by dire results from human experience with this metal and its compounds, has been lacking. With minor and only apparent exceptions, which have been or will be discussed elsewhere in this text, there have been no episodes of acute or chronic poisoning to arouse the interest of toxicologists and physicians and no cases of industrial poisoning to create concern among industrial hygienists. As to the analytical problem presented by the "trace" metals generally—difficult enough, in any case, because of the large quantities of organic material which must be eliminated by harsh, destructive procedures before any analytical procedure can be applied—the minuteness of the quantities of aluminum available within human or animal tissues, in sharp contrast with the great abundance of the element in the natural environment, has been baffling. The effective barriers to the absorption of aluminum at the portals of entry have been commented on previously, but the scantiness of such absorption is worthy of further note here because of the troublesome problem which it has created with reference to the determination of the physiological pattern of the aluminum content of the various organs and tissues. When metallic compounds are absorbed readily, or even with moderate ease, into the body, the manner in which they distribute themselves within the tissues can often be observed to advantage by the introduction of significant quantities for absorption through natural avenues. Traces in the tissues can often be converted thereby into significant quantities, with reasonable assurance that physiological relationships have

been preserved, and with considerable augmentation of analytical precision. Such a procedure has been of little avail in the case of aluminum compounds, and, moreover, the injection of these compounds into the blood stream or beneath the skin cannot be counted on to provide the same type of distribution in the tissues as that which would result from slow absorption from the alimentary or respiratory tract. Indeed, one may assume that the intravenous injection of a soluble salt of aluminum will result in a dispersion of solid particles within the blood stream and that most of these particles will find their way fairly promptly into the reticuloendothelial system. That such an initial distribution is an artifact which bears no necessary or likely relationship to that built up slowly by the always low concentrations which must be assumed to be gaining entrance to the blood stream and to the tissues from one or both of the natural avenues of absorption is apparent, and certain of the experimental data (cf. below) tend to show that such is the case.

The foregoing considerations point up the fact that the only acceptable information concerning the normal pattern of the distribution of aluminum in the human body is to be found in the fairly meager results of analyses of the organs and tissues of persons whose exposure to aluminum has been of the normal (in the sense of the usual and incidental, as well as the natural) type. Various items of such available data are given in Table 9. The levels of concentration in the various tissues, not all of which are represented, range from 0.004 to 0.5 mg. per 100 gm. The data are much too meager to be representative, and consequently they must be regarded as merely scattered observations in a field yet to be explored. Moreover, they cannot be accepted as entirely reliable, if for no other reason than that the development of spectrographic techniques (which do not lack specificity but must be viewed critically on the score of the limitation in their precision) and the elimination of contaminating reagents and interfering substances have yielded results significantly lower than those provided by earlier and less specific methods of analysis. Some evidence has been submitted in support of the presumption that the aluminum content of the animal organism increases with advancing age (man,[156] dog[451]), and it has also been estimated that the adult human body may contain between 50 and 150 mg. of aluminum.[210] Opinion on both of these matters must be tentative. It may be said with assurance that human tissues, in so far as they have been investigated, contain minute but measurable quantities of aluminum. As evidence of the source of such aluminum in the common environment of animal life, without special reference to artificial factors that exert their influence upon man, are the data from many sources on the aluminum content of the tissues of the rat,[101,286,290,303,327,378,423,447,448] the cat,[271] the dog,[135,157,326,452,471,497] the domestic pig,[289,308,421] the sheep,[308] the rabbit,[292] the horse,[279,308,421] the cow,[271,305,330,421] the guinea pig,[170,303] the mouse,[289] the chicken,[119] and marine animals.[179,308] In these animals, as in man, aluminum was found to be a regularly occurring but small component of various tissues and organs.

Distribution Under Artificial Conditions: Aside from the results obtained by the analysis of the tissues of animals to which compounds of aluminum have been administered by injections of one or more types, there are but few data in illustration of the distribution of unusual quantities of aluminum in the body. It would seem that some opportunity might have been afforded for the analysis of the tissues of deceased workmen in fairly close temporal relationship to prolonged employment in industries in which respiratory exposure to aluminum had occurred. This seems not to have been the case, and, on the basis of experiments in which animals have been subjected to the inhalation of aluminum compounds over comparatively brief periods of time, such analyses of human tissues might be expected to yield essentially negative results, with the exception of the lungs and the adjacent lymph nodes. In view of the factor of time,

however, as well as that afforded by the extensive surface of the human lungs, opinion on this matter should be reserved. The same reservation may well apply to the results of experiments in which human subjects have ingested large quantities of aluminum over considerable but not greatly prolonged periods of time. Obviously, such experiments, as cited previously, have succeeded in demonstrating that practically all of the ingested aluminum has traversed the alimentary tract without absorption, but the intrinsic errors of sampling and analysis are such as to defy conclusions with respect to the absorption or failure of absorption of small quantities. That the quantities must indeed be small is shown by the analysis of the tissues of guinea pigs fed for long periods on diets containing aluminum in concentrations ranging between 25 and 1167 ppm, in the form of the chloride or lactate.[393] Some slight increase in the aluminum content of certain organs was found in association with the higher dietary levels (700 ppm and above) after prolonged feeding. (The analytical findings of these experiments are not altogether reassuring as to their complete reliability, because of the variability of the data on the brain and spinal cord in particular. Since other investigators[245] have found only low concentrations in the brain of rabbits even after intravenous injections of aluminum, the contamination of these tissues with aluminum from extraneous sources suggests itself as a distinct possibility.) The data of these experiments are consistent with other evidence of the slightness of the absorption of aluminum in the alimentary tract, but they contribute little to the portrayal of the distribution of aluminum in animal tissues.

Some analyses have been carried out on tissues of the dog after the injection of aluminum compounds. Although these are not concerned with the usual routes of entry of aluminum into the body, they demonstrate certain changes in the distribution of aluminum in the tissues of the dog following the introduction of relatively large quantities into the blood stream. Each of four dogs was given an intravenous injection of 1 mg. of aluminum per kilogram of body weight on each of 10 successive days.[326] The dogs were killed 78 to 150 days after the last injection, and the concentrations of aluminum in their tissues were compared with those in the corresponding tissues of five control dogs. The data show elevated concentrations in the liver (the values remained at 6 to 16 times the control level over the 150-day period), the kidney (about 2½ times the control concentrations at 78 days, gradually falling to 1½ times the control values at 150 days), and the spleen (increasing with time to 22 times the control values at 150 days). No other tissue (including brain, lung, gallbladder, bile, pancreas, stomach, intestine, muscle, or gonad) showed any significant change in its concentration of aluminum, as measured by the rosolic acid (aurin) technique of analysis. In another experiment a dog was given 5 mg. of aluminum (as potassium alum) intravenously on each of 2 days, followed by injections of the same dose into the abdominal cavity on each of the subsequent 12 days.[326] The dog was killed eight days after the last injection. Another dog was given 1 intravenous and 13 intraperitoneal injections of 5 mg. of aluminum (as potassium alum) on consecutive days and was killed 34 days after the last injection. Thus, each animal was given a total dose of 70 mg. The concentrations (expressed as milligrams of aluminum per 100 gm. of tissues) found in the tissues of the two dogs are compared with those found in control dogs as shown in Table B.

The elevation of the concentrations of aluminum in the liver, heart, and kidney is evident and hardly unexpected, while the

TABLE B

	Range, Normal Dogs	Experimental Dogs, 70 Mg. Al (as Alum)	
		8 Days After Last Injection	34 Days After Last Injection
Blood	--	0.18	0.12
Liver	0.08 to 0.34	2.25	0.87
Heart	0.02 to 0.17	2.10	0.66
Kidney	0.03 to 0.14	1.50	0.33
Spleen	0.03 to 0.14	--	--
Bile	0.33 to 0.38	0.32	0.41
Intestine	--	1.87	--
Abdominal muscle	--	4.38	1.15
Abdominal skin	--	5.01	0.99

values for the intestine and skin are open to question on the score of surface contamination, but the results recorded for the abdominal musculature are due in all probability to local absorption and are hardly characteristic of the musculature generally. The persistence of relatively high concentrations for 34 days is not as remarkable as the extent of the decrease. One investigator has reported that aluminum appeared in significant concentrations in the bile, lymph, and urine of dogs shortly after the intravenous injection of 1 or 2 or 10 mg. of aluminum (as aluminum chloride) or 1 mg. of aluminum (as aluminum sulfate), per kilogram of body weight.[157] The significance of the findings is rendered dubious by the apparent assumption on the part of this investigator that these body fluids were free of aluminum prior to the injection.

G. Excretion of Aluminum.—Little work has been done to clarify the pathways and mechanisms of the excretion of aluminum. A few data are available from observations made on man, the rat, and the dog which provide tentative conclusions regarding the main routes by which aluminum is eliminated from the body.

Excretion in Urine: The available evidence indicates that large doses of aluminum taken by mouth do not increase the excretion of aluminum in the urine. One investigator ingested about 540 mg. of aluminum (as hydroxide) on two occasions and could recover none from his urine after this large dose. No increase could be detected in the aluminum content of the urine of a man who had ingested between 50 to 100 mg. of aluminum hydroxide per day for 70 days.[497] In contrast with such results are those reported in connection with balance experiments on three preschool age boys, in which 5% to 29% (sic) of the total amount (ranging from 0.24 to 0.69 mg. of aluminum per day) in the daily diet was found to be excreted in the urine.[398] The validity of these results is open to serious question for a number of reasons, but especially because of their own extreme variability. No significant increase was found in the urinary aluminum of 10 human subjects who ate four meals of (among other items) bread made with aluminum-containing baking powder (dose of aluminum not stated).[453] In one experiment "large amounts" of aluminum (dose and compound not stated) were introduced into the stomachs of human subjects, with no resultant increase in urinary aluminum over control levels.[361] One normal young adult was fed 145 mg. of aluminum per day for eight weeks and 300 mg. of aluminum per day (in bread) for nine weeks. These diets were alternated with others low in aluminum. No significant change was found in the rate of the urinary excretion of aluminum during the entire experiment.[212] Another investigator reported that aluminum was not found in the urine of six subjects who had ingested aluminum in amounts increasing from 100 to 298 mg. per day, as potassium or sodium alum, over a period of seven to eight weeks.[432]

Rats given 2 mg. of aluminum per day (as a salt in their drinking water) for three months eliminated 70% in the feces and 30% in the urine.[151] On the other hand, almost no aluminum was excreted in the urine of rats that had been fed aluminum as the phosphate.[290] The data of both of these experiments[151,290] are probably meaningless, since insensitive methods (gravimetric) were applied to comparatively small samples. It would also be of interest to know the means by which the urine of these rats was obtained without some contact with the feces.

After dogs had been fed 3.2 to 6.4 gm. of potassium alum (equivalent to about 450 mg. of aluminum), 4.5 gm. of aluminum sulfate, or 0.125 to 0.25 gm. of aluminum oxide (as aluminum acetate solution) per day for several weeks no aluminum was found in their urine. A corresponding series of five dogs, to which aluminum hydroxide in suspension was given daily for 10 to 15 months in amounts totaling for the period from 100.53 to 178 gm. of aluminum per dog, and two other dogs, to which 53 mg. of aluminum (as $AlCl_3$) was administered daily, developed no significant elevation in

the concentration of aluminum in their urine. The same investigator fed about 33.5 mg. of aluminum (as $AlCl_3.6H_2O$) or about 103.7 mg. of aluminum [as $Al(OH)_3$] daily to dogs over periods of 10 to 15 months. The rate of elimination of aluminum in the urine of these dogs was unchanged.[497] A gravimetric method of analysis was used in these experiments, but since the samples were comparatively large the results may be considered to be reasonably reliable. In another experiment[364] dogs were fed 529 mg. of aluminum (as hydroxide) daily for 12 months. Their urine contained traces of aluminum of the same order as those in the urine of control dogs. Six dogs were fed 230 mg. of aluminum (in baking powder) and two dogs were fed 1550 mg. of aluminum (as $AlPO_4$) daily for 90 days. The urinary concentrations were 0.01 and 0.02 mg. per 100 gm., respectively, for the aluminum-fed dogs, but no figure for comparison was given for the control dog.[326] The evidence is consistent that there is no significant increase in the output of aluminum in the urine of the dog after the ingestion of very large doses of various aluminum compounds. In the only article found[157] concerning excretion after the injection of aluminum into dogs, it was reported that the aluminum content of the urine increased sharply soon after the intravenous injection of aluminum chloride in doses between 1 and 10 mg. per kg. of body weight. The inadequacy of the analytical methods employed in these experiments has been commented on previously. Other results suggest that aluminum may be excreted by the kidney if it is available in sufficient quantities. Thus, the absence of a significant increase of aluminum in the urine of dogs to which large quantities of aluminum have been given is probably the result of poor alimentary absorption of aluminum.

Excretion in Feces: In view of the evidence that aluminum is very poorly absorbed after being swallowed, it is to be expected that most or all of that taken in by mouth should be eliminated in the feces. This has been found to be true of man as well as of all species of animals investigated. It may reasonably be assumed that some quantity of aluminum occurs in the alimentary tract and in the feces as a true excretion. The somewhat sparse evidence of the presence of aluminum in the bile is sufficient to strengthen such an assumption. The order of magnitude of the rate of such excretion, in itself or by comparison with that in the urine, is entirely speculative. The available facts are reviewed in the following paragraphs.

In an experiment on an adult male human subject, in which the analyses were made spectrographically, it was found that "ingested aluminum traverses the alimentary tract without absorption and is evacuated in the feces."[242] The dosage of aluminum averaged about 300 mg. per day over the period of nine weeks—a much larger daily dose than is ingested with a normal diet. Other persons have been fed sodium or potassium alum in doses furnishing from 100 to 300 mg. of aluminum per day over a period of 70 days, during which they passed 97% to 101.7% of the ingested aluminum in the feces.[115] When the dose was increased to 970 mg. of aluminum per day over 118 days, the fecal output came to be equivalent to 99% of the total intake. One investigator has reported data on the fecal and urinary output of aluminum by three children, but the range of values was so wide as to make the information virtually meaningless (for example, 31% to 171% of ingested aluminum was excreted in the feces).[398] The results of another experiment were also variable[244] (for example, the mean fecal output per day was 41.9 mg., standard deviation 46.8 mg.), but within these wide limits the quantities found in the feces were equivalent to those taken in with the normal daily diet.

An experiment involving the use of the aluminum phosphate precipitation method of analysis showed that "practically all" of the oral dose of 0.8 to 1.4 gm. of aluminum [as $NaAl(SO_4)_2$ or $AlPO_4$] was evacuated in the feces of domestic pigs.[289] The fecal elimination of aluminum by rats among

to about 73% to 112% of the total intake of 0.39 to 0.59 mg. of aluminum over the period of 7 to 12 days.[290] The wide variability of these results is probably an expression of the use of the relatively insensitive gravimetric method for the determination of aluminum in small samples. Between 84% and 95% of a larger total dose of 274 to 575 mg. of aluminum taken in over a period of 7 to 12 days was found in the feces.[290] In one report of an experiment in which rats were fed elevated quantities of aluminum (about 600 ppm in the diet) it was stated that "all dietary aluminum is excreted in the feces," though quantitative data to support this statement were not given.[293] Two unrelated investigations have provided results that agree in demonstrating that the total amount of aluminum fed to dogs, even in very high dosage (up to 104 and 530 mg. of aluminum [as hydroxide], daily, for 12 months, and about 33 mg. of aluminum [as chloride], daily, for 8 to 13 months) was recovered in the feces.[361,497]

H. Aluminum in Bile, Lymph, Milk, Saliva, and Sweat.—As might be expected from the evidence presented and discussed previously, efforts made to find elevated amounts of aluminum in bile, lymph, or other secretions, following the oral administration of aluminum, have been unsuccessful.[326,152,197,475] One investigator claimed that his data support the idea that aluminum is excreted in the bile of dogs after the oral administration of aluminum.[452] However, the differences found in the concentration of aluminum in the bile before and after the feeding of aluminum were not significant, and his argument for biliary excretion is not supported. No data are available which deal with biliary excretion in man after the oral administration of aluminum.

Data from experiments in which animals were injected with aluminum compounds indicate that after injection into a vein or into the abdominal cavity aluminum may appear in the bile. There is disagreement on this point. One investigator,[156] although stating that a dose of aluminum injected into a vein "appeared promptly in the bile," reported an increase of only 50% above the normal biliary aluminum concentrations at the peak after injection (from a control level of 0.28 mg. per 100 gm. to a peak of 0.41 mg. per 100 gm.), at the 78th hour after the injection. The apparent increase is probably within the experimental error of the colorimetric method as applied to these samples, and this response can hardly be regarded as prompt. Little change in the biliary concentrations of aluminum was found after the intravenous and intraperitoneal injection of a total dose of 70 mg. of aluminum (as potassium alum).[326] Aluminum is said to have appeared in the bile of dogs shortly after the injection of doses ranging up to 10 mg. of aluminum (as chloride) per kilogram of body weight.[157] Taking such discrepant data into account, it is still uncertain whether or not a significant increase occurs in the biliary excretion of aluminum in the dog following its injection. Data on other species were not found, though it is stated[327] that the large amounts of aluminum in the livers of rats following injections of 4 mg. of aluminum (as potassium alum) into the abdominal cavity "suggests its excretion in the bile." More suggestive is the occurrence of aluminum in the bile under ordinary circumstances, a fact that argues fairly conclusively for some excretion of aluminum in this form.

There is no evidence for the appearance of aluminum in lymph after oral feeding. Aluminum is said to have appeared in the lymph of dogs soon after intravenous injection.[475]

An increase in the concentration of aluminum in milk from cows to which aluminum was fed is supported by extremely few data. Cows were given 1.25 to 1.5 oz. (35.4 to 42.5 gm.) of alum by mouth, and traces of aluminum were found in their milk nine hours later. When 0.375 and 0.25 oz. (10.6 and 7 gm.) doses were fed to a goat, the milk gave dubious evidence of absorption.[275] In a small series of analyses aluminum was found to be a constant constituent of cow's milk in concentrations of about 1 ppm or

less. The milk from three cows, to which approximately 114 mg. of aluminum [as $AlK(SO_4)_2.12H_2O$] was fed daily for two periods of two months, contained 0.39 to 1.42 ppm (average 0.81 ppm), in comparison with 0.15 to 0.97 ppm (average 0.46 ppm) in the milk of three controls.[27] Two to three and one-half hours after 1.6 gm. of alum was administered orally to two women, the concentration of aluminum in their milk rose from nil and 0.02 ppm to between 0.05 and 0.15 ppm, respectively.[275] That aluminum is a normal constituent of human and bovine milk was shown spectroscopically by another investigator.[593]

VII. Uses of Aluminum in Medicine

Concomitant with the evidence of the exceptionally poor absorption of aluminum within the respiratory and digestive tracts (see Chapter VI, Aluminum in the Body), several widely employed medicinal uses have developed for certain preparations containing aluminum.

Aluminum hydroxide gel is used extensively and with apparent success (initially reported in 1929) in aqueous media in the handling of peptic ulcers and symptomatic gastric hyperacidity.[11,121,146,276,336,491,495] Its commonly used dosage is 4 to 8 ml. [4% to 6% $Al(OH)_3$] taken in water or milk every two to four hours or administered by a continual drip of diluted gel through a tube directly into the stomach [equivalent to as much as 6 gm. $Al(OH)_3$, or 2.075 gm. of aluminum, per day]. This is often continued over periods of months or even of years without eliciting ill effects. Aluminum phosphate gel, in a 4% aqueous solution, when given in about twice the above dosage, has the same action as the hydroxide gel, without interfering with the absorption of phosphate from the digestive tract—an effect which may be significant under unusual circumstances.[155] The only undesirable effect associated at times with the continued use of massive doses of the gel is that of the promotion of constipation. This effect may be controlled by the concurrent use of a suitable intestinal lubricant.

Aluminum gels in large doses by mouth have been prescribed to assist in the prevention of kidney stones (composed of phosphates), on the theory that excessive quantities of phosphate in the diet may be diverted from the urine into the feces.[121,298,412,413]

Experimental evidence has been adduced that aluminum sulfate or aluminum chloride may counteract the toxic effects of sodium fluoride when the latter and one of the former pair of substances are taken simultaneously into the digestive tract of animals.[297,469] It has been demonstrated by balance experiments that the Al^{+++} ion binds the F^- ion in the digestive tract to form a tightly joined compound which is less readily absorbed (cattle, sheep[212]). The dose of aluminum sulfate required to prevent poisoning in cattle and sheep is said to be between 10 and 100 times the quantity of fluoride taken into the body daily.[212,435]

Dry particulate forms of aluminum hydroxide, alumina, and metallic aluminum have been administered by inhalation to workmen in certain industries for the prevention and, in some instances, for the therapy of silicosis.[72,73,170,195,200,338,347] Its value in the prevention of this disease in man has not been established,[61] although the evidence obtained in experiments with animals has been favorable.[106,123,170,216] The most common prophylactic or therapeutic procedure in the United States has been that of subjecting workmen, for a period of about 10 minutes daily, to the inhalation of finely divided metallic aluminum delivered through a tube from a small grinder (a ball mill which produces about 3.2 mg. of dust per liter in an air stream flowing at the rate of 10 liters per minute). Another procedure is that of introducing the finely divided metal into the air of the quarters in which workmen change their clothing, this being so regulated as to provide about 0.0353 mg. of powdered aluminum per liter. Approximately 73 1/3 million individual treatments are known to have been administered from 1943 through 1956 (including about

64 4/5 million in change houses in Canada, Mexico, and Western Australia and about 8½ million involving dust from ball mills in the United States). In the 13 years of experience with this technique, no injurious effect from the inhalation of the aluminum dust has been observed.[291] Another report made after nine years of daily use of the method (representing approximately 1,250,000 mass preventive treatments and over 40,000 individual treatments) cites no instance of damage to the lungs of workmen from the inhalation of the aluminum.[370] Other clinical reports have provided similar results.[61,73,206]

Certain uses have developed for aluminum compounds as astringent preparations in the treatment of dermatitis, wounds, and burns. Burns have been treated with aluminum foil[64] and powdered metallic aluminum,[138] as well as with dilute solutions of neutral aluminum acetate (5% Burow's solution) and basic aluminum acetate (about 8%). Colloidal aluminum hydroxide (as a powder) has been used to treat sore and fissured nipples during lactation.[282] There are reports of the use of metallic aluminum as an aid in the healing wounds.[36,76,101]

One-half per cent solution of aluminum acetate may assist in the mechanical removal of surface bacteria from the skin, through its softening effect, without bactericidal action.[349] Aluminum sulfate (10% aqueous solution) hardens the skin to such an extent that surface bacteria may be imprisoned but not killed; such organisms may be released on subsequent softening of the skin.[369]

Potassium or ammonium aluminum sulfate has been used as a 2% solution for the control of excessive sweating. Aluminum chloride likewise has found use as a deodorant and antiperspirant in local sweating of the skin of the feet and axillae, when applied as a 25% solution once or twice per week.[418] A variety of ointments, pastes, and solutions of aluminum compounds have provided the basis for an extensive antiperspirant industry.

Aluminum compounds have been tested for their germicidal properties. Aluminum chloride in slightly alkaline aqueous solution exhibits some bactericidal activity at low dilutions (0.09 to 9 parts per billion).[337] Varying concentrations of aluminum chloride have an effect different from that of other salts (potassium chloride, magnesium chloride, aluminum iodide, aluminum bromide, and ferric chloride) on the number of surviving bacteria, through its capacity to induce agglutination.[294] A strip of metallic aluminum exerted no bactericidal action when immersed in a culture of bacteria.[439] The germicidal action of aluminum foil is small.[193] Aluminum was one among several elements that exerted some inhibiting effect on the growth of Escherichia coli[209] and Proteus vulgaris,[105] both common bacteria in the intestinal tract of man.

Aluminum compounds have been used occasionally in the treatment of infectious diseases. A preparation of aluminum has been used in the treatment of gonorrhea.[356] Aluminum hydroxide and colloidal kaolin have been used in vaginal hygiene.[382] A purulent infection was treated with aluminum sulfate.[427] A nasal spray containing 3% or 4% of sodium alum was effective in increasing the resistance of monkeys to poliomyelitis virus instilled into the nasal passages.[28,379] This action was believed to be due to a decrease in the permeability of the mucous membranes of the nose rather than to any antiviral action. A 4% solution of sodium alum can be sprayed repeatedly into human nostrils without any apparent harmful effects.[379]

In 1889[375] an attempt was made to purify diphtheria toxin by precipitation with aluminum chloride. In 1926[177] the protective properties (antigenicity) of diphtheria toxoid were found to be improved by the addition of potassium alum. The increase in antigenic properties is thought to be due to the delayed release of the antigen from the alum precipitate under the skin. Although different batches of toxoid vary in the concentration of alum required for precipitation, 1% or 2% of alum is usually satis-

factory. The potassium alum content of each immunizing dose is limited to less than 20 mg. Potassium alum as a precipitant is now used regularly in the production of diphtheria toxoid, tetanus toxoid, and pertussis (prophylactic) vaccine for whooping cough.

Aluminum hydroxide has been used to control digestive disturbances elicited occasionally by the oral administration of certain antibiotics. The evidence indicates that aluminum hydroxide adsorbs and, in some instances, lowers markedly the absorption of chlortetracycline (Aureomycin) taken by mouth at the same time.[115,342,399,476] On the other hand, penicillin, adsorbed on aluminum hydroxide, has been used successfully in the postoperative control of oral infection.[358]

Among several other trace elements, aluminum has been found occasionally in minute concentrations in human cancers.[334] No significance has been attributed to such findings, beyond the fact that they provide evidence of some continuing absorption and distribution of aluminum into the tissues of the body. A few compounds of aluminum have been employed in attempts to prevent cancer or to inhibit its growth. The intraperitoneal injection of 3.37 mg. of aluminum (as anhydrous aluminum chloride) in 1 ml. of vehicle into rats with ascites sarcomas caused a temporary retardation of the growth of the tumors.[295] On the other hand, the injection of several complex organic compounds containing aluminum[195] failed to inhibit the growth of tumorous tissue.

Concluding Comments

The discovery of aluminum, the production of the metal in large quantities, and its use as an extensive and expanding part of industrial and household materials and equipment are essentially modern developments. Despite this fact, human experience with the metal and with its chemical compounds and preparations has been widespread and intimate. Only in industrial experience has there been any basis for the opinion that the aluminum-containing earths or the metal itself may possess properties that may be inherently dangerous to man. Even here, difficulty has been encountered rarely and only under conditions of unusual urgency for production (as in war time), and these sporadic difficulties, in the form of "Shaver's disease" and the "aluminum lung," have been such as to be highly questionable, with respect to the role played by aluminum in the mixture of raw materials and industrial products that find their way, via dusty atmosphere, into the human respiratory apparatus. Moreover, these industrial hazards have been amenable to control by appropriate measures of industrial hygiene.

It is prudent to emphasize the fact that the quantities of aluminum which gain entrance into the "internal medium" of the body, under industrial conditions and, indeed, under any other than those devised artificially in the laboratory, are small. This factor, without doubt, is highly important for the safety of workmen in certain of the industries in which aluminum is produced and used. In this connection, a certain note of caution may well be sounded. Modern technologic research, in its ceaseless quest for new materials and for new forms of familiar materials, has brought forth a wide variety of organic compounds of the metals for specialized uses. In some of these, the usual properties of the metals, including their biological effects, have been so masked or modified as to be virtually unrecognizable, but in some instances the organic radical has provided a vehicle for the ready carriage of the metallic compound into the tissues of the complex animal organism and actually into the very cells of such tissues. Under such circumstances, an entirely new and artificial set of physiological reactions are produced within the organism, the consequences of which may be serious. The organic compounds of aluminum, their uses, and their properties, have not been dealt with in this review, for the reason that they present a totally different type of situation from those with which the review is concerned. By the same token, it should be understood by those

who may be involved in any manner with the preparation, handling, and use of organic compounds of aluminum that very little that has been said about the characteristics of the inorganic compounds of aluminum may be applied in the anticipation of the physiological behavior of its organic compounds, especially of those which are volatile at ordinary temperatures or at those which occur under the conditions of their preparation and use. Gases and vapors of any kind, unless they are decomposed or acted upon otherwise within the respiratory tract and lungs, are absorbed into the body fluids and tissues in accordance with the laws that govern the diffusion and movement of gases. Likewise, liquid compounds, especially those soluble or miscible in fats, often penetrate the intact skin with comparative ease. Since, under these circumstances, the normal barriers of the body do not protect the tissues against the intrusion of these foreign substances, the outcome is unpredictable and assumptions are fraught with risk.

Aside from the foregoing reservations which rise out of the complexities of modern industry and the purposefully venturesome character of modern technologic and industrial research, little need be said to summarize or emphasize the results of this review. There are voids in our information concerning the effects of aluminum in a variety of forms and quantities if and when such forms and quantities should gain entrance to the tissues and fluids of the body. These voids exist in part, as suggested previously, because the apparently innocuous properties of aluminum and its ordinary compounds, as encountered in human experience, have acted to retard rather than to stimulate intensive inquiry. Questions have been raised, as indicated, and these have been answered to the satisfaction of mature and reliable investigators. The resultant investigations have provided the great bulk of the information presented in the foregoing chapters. There is no reason for concern, on the part of the public or of the producer and distributor of aluminum products, about hazards to human health derived from well-established and extensive, current uses of such products. Nor need there be concern over the more extended uses which would seem to be in the offing.

While missing details of the physiological and pharmacodynamic behavior of aluminum will pique the curiosity of investigators until our knowledge has been more fully rounded out, and while more representative and strictly quantitative data on a number of facets of the problem would be desirable, the essential facts as to the more important practical aspects of human risk in relation to aluminum compounds are available. These facts, while not amenable to expression with rigid quantitative precision, may be stated, with confidence in their validity, as follows:

A. The quantities of aluminum which are required within the animal (human) organism to induce harmful effects, as disclosed by laboratory experiments in which such compounds have been introduced into the tissues, fluids, and cavities of the bodies of experimental animals by artificial means, are large (e. g., of the order of 100 mg. per kg. injected subcutaneously), as compared with those found in the tissues of the body under the severest conditions of ordinary or occupational exposure to aluminum and its compounds or as compared with the quantities of certain other metals (and their compounds) which are known to be dangerous.

B. Despite the abundance of aluminum compounds in the earth's crust and in the natural environment, and despite the artificial increase in the quantities of aluminum in the environment of modern man, the absorption of aluminum into the bodies of animals and men occurs to only a slight extent. The rate of absorption via the normal avenues of entry into the body is so slight as to escape quantitative measurement in any other manner than that of observing the end-result of prolonged exposure—namely, by determination of the small quantities that occur in the tissues of the body.

C. The quantities of aluminum and its inorganic compounds which are absorbed

TABLE 1.—*Solubility of Various Aluminum Compounds in Water**

Compound	Temperature, C	Solvent	Solute
		Gm./100 Gm. Saturated Solution	
$AlCl_3 \cdot 6H_2O$ in water	25		31.6 $AlCl_3$ [as $AlCl_3 \cdot 6H_2O$]
	40		32.1 $AlCl_3$ [as $AlCl_3 \cdot 6H_2O$]
	100		32.9 $AlCl_3$ [as $AlCl_3 \cdot 6H_2O$]
$AlCl_3 \cdot 6H_2O$ in aq. sol. HCl	25	0.00 HCl	34.081 $AlCl_3$ [as $AlCl_3 \cdot 6H_2O$]
	25	4.15 HCl	29.750 $AlCl_3$ [as $AlCl_3 \cdot 6H_2O$]
	25	5.09 HCl	27.980 $AlCl_3$ [as $AlCl_3 \cdot 6H_2O$]
$Al(NO_3)_3 \cdot 9H_2O$ in water	25		39.5–43.5 $Al(NO_3)_3$ [as $Al(NO_3)_3 \cdot 9H_2O$]
	40		43.0–47.0 $Al(NO_3)_3$ [as $Al(NO_3)_3 \cdot 9H_2O$]
	90	Transition point	60.5 $Al(NO_3)_3$ [as $Al(NO_3)_3 \cdot 9H_2O$ + $Al(NO_3)_3 \cdot 8H_2O$]
	100		61.5 $Al(NO_3)_3$ [as $Al(NO_3)_3 \cdot 8H_2O$]
$Al_2(NO_3)_6 \cdot 18H_2O$ in aq. sol. nitric acid	25	0 HNO_3	39.0 $Al_2(NO_3)_6$ [as $Al_2(NO_3)_6 \cdot 18H_2O$]
	25	10 HNO_3	30.5 $Al_2(NO_3)_6$ [as $Al_2(NO_3)_6 \cdot 18H_2O$]
	20	0 HNO_3	40.0 $Al_2(NO_3)_6$ [as $Al_2(NO_3)_6 \cdot 18H_2O$]
	20	10 HNO_3	31.0 $Al_2(NO_3)_6$ [as $Al_2(NO_3)_6 \cdot 18H_2O$]
	40	0 HNO_3	44.0 $Al_2(NO_3)_6$ [as $Al_2(NO_3)_6 \cdot 18H_2O$]
	40	10 HNO_3	36.0 $Al_2(NO_3)_6$ [as $Al_2(NO_3)_6 \cdot 18H_2O$]
$Al(OH)_3$ in aq. NaOH	30	3.95 Na_2O	0.70 Al_2O_3 [as $Al_2(OH)_6$]
	30	9.68 Na_2O	1.82 Al_2O_3 [as $Al_2(OH)_6$]
$Al_2(SO_4)_3 \cdot 18H_2O$ in water	20		26.7 $Al_2(SO_4)_3$ [as $Al_2(SO_4)_3 \cdot 18H_2O$]
	40		31.4 $Al_2(SO_4)_3$ [as $Al_2(SO_4)_3 \cdot 18H_2O$]
	100		47.1 $Al_2(SO_4)_3$ [as $Al_2(SO_4)_3 \cdot 18H_2O$]
$Al_2(SO_4)_3 \cdot 18H_2O$ in aq. sol. H_2SO_4	25	0.00 H_2SO_4	27.82 $Al_2(SO_4)_3$ [as $Al_2(SO_4)_3 \cdot 18H_2O$]
	25	5.13 H_2SO_4	29.21 $Al_2(SO_4)_3$ [as $Al_2(SO_4)_3 \cdot 18H_2O$]
$AlK(SO_4)_2 \cdot 12H_2O$	25		6.62 $AlK(SO_4)_2$ [as $AlK(SO_4)_2 \cdot 12H_2O$]
		Gm./100 Gm./H_2O	
$Al(OH)_3$ moist, fresh ppt'd in aq. sol. $Al_2(SO_4)_3$	20	2.37 $Al_2(SO_4)_3$	0.15 $Al(OH)_3$ [as $Al_2O_3 \cdot SO_3 \cdot 9H_2O$]
	20	5.00 $Al_2(SO_4)_3$	0.30 $Al(OH)_3$ [as $Al_2O_3 \cdot SO_3 \cdot 9H_2O$]
	20	7.00 $Al_2(SO_4)_3$	0.65 $Al(OH)_3$ [as $Al_2O_3 \cdot SO_3 \cdot 9H_2O$]
	40	5.22 $Al_2(SO_4)_3$	1.33 $Al(OH)_3$ [as $Al_2O_3 \cdot SO_3 \cdot 9H_2O$]
		Normality	Gm. Dissol./100 Cc. Sat. Sol.
$Al(OH)_3$ fresh ppt'd suspended in aq. NH_4OH	20	0.050 NH_4OH	0.007 $Al(OH)_3$
	20	0.100 NH_4OH	0.018 $Al(OH)_3$
	20	0.125 NH_4OH	0.025 $Al(OH)_3$
	20	0.200 NH_4OH	0.038 $Al(OH)_3$
		Normality	Gm./Liter
$Al(OH)_3$ in aq. NaOH sol. (acetic acid soluble "tonerde")	20–23	0.49 NaOH	9.27 Al_2O_3
		0.99 NaOH	13.90 Al_2O_3
		Gm./100 Gm.	
		Saturated Solution	Water
$AlNa(SO_4)_2$	25	39.1 $Na_2Al_2(SO_4)_4$	43.1 $Na_2Al_2(SO_4)_4$
$AlNa(SO_4)_2 \cdot 24H_2O$	25	56.9 $Na_2Al_2(SO_4)_4 \cdot 24H_2O$	131.8 $Na_2Al_2(SO_4)_4 \cdot 24H_2O$
$AlNa(SO_4)_2$	38	30.21 $AlNa(SO_4)_2$	133.7 $AlNa(SO_4)_2 \cdot 12H_2O$
			Mg./Liter
$Al(OH)_3$ in water	18		0.94–1.04 Al_2O_3 [as $Al(OH)_3$]
	29		0.98 Al_2O_3 [as $Al(OH)_3$]

* Adapted from Seidell.[61]

into the body are independent, for all practical purposes, of the quantities in the environment—specifically, in the food and in the atmosphere. From the theoretical viewpoint, no doubt, some influence is exerted by the actual mass of the compounds present in the alimentary and respiratory tracts, but the influence is so slight as to be of little significance. This means, practically, that a significant increase in human exposure to the same type of situations and compounds that are now prevalent will result in no significant increase in aluminum in the body. This also means, of course, that new developments may create new situations which will call for investigation. There is no such problem of public health at present, however, and none appears upon the horizon.

University of Cincinnati, The Kettering Laboratory (19).

TABLE 2.--*Occurrence of Aluminum in Some Soils of the United States*

State	Soil	Parts per Million
Alabama	Top	46,037
	Sub	67,725
Connecticut	Top	5,334–14,233
	Sub	8,730–36,614
District of Columbia	Top	32,963–43,669
	Sub	19,648
Florida	Top	11–135,079
	Sub	85–26,349
Georgia	Top	1,296–246,984
	Sub	5,767–228,730
Maine	Top	65,767–68,889
	Sub	72,910–77,407
Maryland	Top	20,370–146,190
	Sub	9,894–154,815
Massachusetts	Top	11,055–58,677
	Sub	54,603–58,995
Missouri	Top	51,154
	Sub	71,121
New Hampshire	Top	36,899–98,624
	Sub	37,302–70,053
New Jersey	Top	159–42,116
	Sub	2,635–97,196
New York	Top	645–55,592
	Sub	741–83,915
North Carolina	Top	10,952–90,329
	Sub	16,667–145,026
Pennsylvania	Top	19,550–60,294
	Sub	29,312–78,307
Rhode Island	Top	14,524–29,099
South Carolina	Top	294–108,485
	Sub	3,261–140,401
Virginia	Top	7,249–152,045
	Sub	13,328–265,449
West Virginia	Top	22,751–42,963
	Sub	28,413–74,973
Wisconsin	Top	18,148
	Sub	50,212

TABLE 3.--*Concentrations of Aluminum and Certain Other Metallic Constituents of the Atmosphere of Several Communities*

Community or Area	Aluminum, γ/Cu.M.	Iron, γ/Cu.M.	Manganese, γ/Cu.M.	Vanadium, γ Cu.M.
Baltimore	4.0 (av.)	15.1 (av.)	0.3 (av.)	0.2 (av.)
Cincinnati				
All areas (range)	0.1–25*	0.1–110.0 †	0.0–2.0 ‡	<0.2–0.4§
Rural	1.9 (av.)	5.7 (av.)	0.2 (av.)	<0.2
Business and industrial area				
Over-all average	5.3	12.7	0.4	
Cold months	7.5	17.5	0.36	
Cool months	4.1	10.7	0.4	
Warm months	4.7	9.2	0.6	
Residential and semi-res. areas	4.0	7.7	0.4	
Detroit area, Mich. City	3.2 (av.)	3.0 (av.)	0.2 (av.)	0.002 (av.)
Detroit area, Mich. City ¶	0–27.8	0–17.4	Trace–2.66	0–0.042
St. Clair-Marine City (nonindustrial)	0–6.0	0–1.4	0.003–0.10	0–0.03
Donora, Pa.	6.0 (av.)	14.0 (av.)	0.6 (av.)	
Kanawha River Valley				
Belle	4.4; 12.5	1.76; 7.2	0.18; 0.7	0–<0.02
Charleston	1.3–10.0	0.19–4.4 (av.)	0.13–0.3 (av.)	0–<0.02 (av.)
Charleston, north	1.97; 10.8	0.99; 2.6	0.10; 0.2	0–<0.02
Charleston, south	0.55–9.5	0.03–15.0	0.02–0.5	0–<0.03
Cladyan	3.50–7.0	1.05; 6.0	0.21	0–<0.02
Kanawha City	3.1; 10.0	1.17; 5.0	0.16; 0.3	Trace
Nitro	2.89–7.8	1.40–4.0	0.21; 0.3	Trace–<0.01
Saint Albans	2.6; 3.6	0.60; 4.0	0.12; 0.2	0–<0.01
Los Angeles	2.5–6.0	2.5–6.0	Trace–0.24	
Windsor, Ontario	0.71–6.04	0.59–8.77	0.064–0.91	0–0.28
Windsor, Ontario	3.0 (av.)	2.8 (av.)	0.3 (av.)	0.019 (av.)

* 723 samples.
† 719 samples.
‡ 719 samples.
§ 141 samples.
‖ 1,035 samples, semiquantitative analysis.
¶ 31 sampling stations
# 25 sampling stations.
** 82 samples, semiquantitative analysis.

TABLE 4.—*Occurrence of Aluminum in Surface Waters*

	Parts per Million
Rivers and Creeks	
United States	
Allegheny [illegible]	4.2
Casselman [illegible]	0.6; 9.7
Conemaugh [illegible]	2.4
Delaware R. Bed Creeks in Pennsylvania [illegible]	15.0–77.0
Detroit [illegible]	5.5
Humboldt [illegible]	0.7
James [illegible]	0.04
Kiskiminetas [illegible]	0.6–21.0
Lackawanna [illegible]	1.3–23.0
Lehigh [illegible]	1.2
Lehigh R. Basin Creeks in Pennsylvania [illegible]	11.0–16.0
Little Conemaugh [illegible]	4.5; 29.0
Little Schuylkill [illegible]	0.2–47.0
Little Schuylkill West Branch [illegible]	11.0
Mississippi [illegible]	16.6; 15.9
Monongahela [illegible]	0.2–9.4
Neponset [illegible]	0.33
Ohio [illegible]	0.0–3.2; 21.1
Ohio R. Basin Creeks in Ohio [illegible]	0–29.0
in Pennsylvania [illegible]	0.4–67.0
Passaic [illegible]	7.1
Rio Grande [illegible]	1.1
Schuylkill [illegible]	0.4–26.0
Schuylkill, West Branch [illegible]	2.4–13.0
Susquehanna, West Branch [illegible]	0.4–31.0
Susquehanna R. Basin Creeks in Pennsylvania [illegible]	0.4–45.0
United States (Cont.)	
Susquehanna R., West Branch Bed, Creeks [illegible]	0.8–12.0
Youghiogheny [illegible]	0.0–3.5
Australia, New South Wales	
Bekabula, Clifden Run [illegible]	Trace
Targagar [illegible]	Trace
Canada	
Ottawa [illegible]	Trace
St. Lawrence [illegible]	Trace
England	
Thames [illegible]	Trace
France	
Doubs [illegible]	0.041
Loire [illegible]	0.057
Seine [illegible]	0.026
Germany	
Rhine [illegible]	0.01; 0.73
South America	
Parana [illegible]	1.6
Rio de la Plata [illegible]	3.2
Rio Vinagre [illegible]	211.6
Switzerland	
Rhone [illegible]	0.021
Lakes	
United States	
Borax, Calif. [illegible]	1.5
Michigan [illegible]	Trace
Mono, Calif. [illegible]	242.2
Owens, Calif. [illegible]	12.2
Superior [illegible]	0.5
Yellowstone [illegible]	2.1
Austria-Hungary	
Gmunden [illegible]	0.5
Canada	
Goodenough, B. C. [illegible]	182.6
Japan	
A number of lakes [illegible] (bottom layers of waters)	2.0–8.0
Scotland	
Loch Katrine [illegible]	0.51

TABLE 5.—*Occurrence of Aluminum in Ground Waters, Mineral Springs, and Wells*

	Parts per Million
United States	
Arkansas	
Columbia Co. [illegible]	0.2–3.4
Hot Springs [illegible]	4.1
Iowa	
Acid Springs [illegible]	1,790.2
Kansas	
Burr Oak [illegible]	62.2
Kentucky	
Alum Springs [illegible]	109.0
Michigan	
Mt. Clemens Mineral Well [illegible]	267.5
St. Clair [illegible]	7,509.5
Missouri	
Blue Lick [illegible]	92.8
Montana	
Hunter's Hot Springs [illegible]	1.2
Nevada	
Hot Springs [illegible]	1.0
New Hampshire	
Londonderry Lithia [illegible]	13.7
New York	
Geneva Lithia [illegible]	23.7
Oak-Orchard Acid [illegible]	34.4
Saratoga Springs [illegible] (4 springs)	1.2–11.1
Texas	
Overall [illegible]	221.3
Sour Lake Mineral Springs [illegible]	123.3
Virginia	
Alum Spring, Rockbridge Co. [illegible]	6,568.2; 398.9
Alum-water, Lee Co. [illegible]	471.4
Buffalo Lithia [illegible]	20.5
Jordan Alum Springs [illegible]	219.6
Shenandoah Alum Springs [illegible]	123.3
Wisconsin	
Bethesda Springs [illegible]	1.1
Wyoming	
Old Faithful Geyser, Yellowstone Park [illegible]	0.9
Austria	
Elizabethenquelle, Baden [illegible]	0.4
Canada	
Tuscarora Sour, Brantford [illegible]	347.7
Czechoslovakia	
Karlsbad Sprudel [illegible]	11.8
England	
Old Alum Well, Harrowgate [illegible]	647.1
Orchard Alum [illegible]	18.7
Fiji Islands	
Kantava [illegible]	48.1
France	
Celestine, Vichy [illegible]	6.9
Grande Grille, Vichy [illegible]	95.2
Germany	
Bad Ingelfingen [illegible]	Trace
Italy	
Civillina [illegible]	238.1
Luxembourg	
Mondorf-Etat [illegible]	3.2
New Zealand	
Acid water, Lake White Island [illegible]	4,028.0
Kopuowhara Makia [illegible]	18.7
Switzerland	
Hebenen Spring [illegible]	0.06; 0.05
Urien Spring [illegible]	0.02
Luzius Spring [illegible]	0.05

Table [illegible] Aluminum Content of Foods and Beverages

	Aluminum Content, PPM*										
	Spectrographic		Colorimetric				Gravimetric			Other Methods, Various Authors, 1935–1955	Methods?, Early Analyses
	Cholak,[illegible] 1940	Various Authors, 1931–1955	Various Authors, 1926–1955	Beal,[illegible] 1932	Underhill,[illegible] 1929	Winter & Bird,[illegible] 1929	Various Authors, 1928–1953	Bertrand & Levy,[illegible] 1931	Berg,[illegible] 1929		
Cereals and cereal products											
Barley						11.3*		6.0			8.07[illegible]
Buckwheat											19.25[illegible]
Corn											
Flint						2.1*					
Pop						4.0*					
Corn flakes	7.0					2.8*					
Cornmeal	7.4		9.4–11.2[illegible]								17–6.0[illegible]
Corn oil cake											8170.0[illegible]
Flour (not spec'd; see also rye, wheat flour)											0.009–[illegible]
Grits											[illegible]
Hominy											[illegible]
Oatmeal						15.1*			29.2		1750.0[illegible]
Cooked in glass 150 min.				1.43; 1.51							
Cooked in Al 150 min.				*8.28; 9.13*							
Oats, entire grain								4.4		15.0*†	1133.3[illegible]
Potato starch									1.2		
Rice, unpolished	5.6										
Rice											
Half-polished									10.1		
Polished	5.1						151.0*[illegible]	1.2			
Not spec'd						4.7*	17.7*[illegible]			0.45‡	5.6–7.4[illegible]
Cooked in glass 45 min.							31.7109				
Cooked in Al 45 min.							*30.2–32.2*[illegible]				
Rice starch									1.6		
Rye, entire grain							16.3–18.5*[illegible]	4.8			2730.0[illegible]
Rye bran							396.8*[illegible]				
Rye flour			19.0–19.5*[illegible]								
Soya bean, extracted meal									251.8		
Wheat											
Entire grain	0.95–9.0		6.65(av.)[illegible]			4.5*	16.2*[illegible] 4.5*[illegible]	3.45			0.49–14.7[illegible]
Finely ground								11.1			
Coarsely ground								4.5			
Wheat bran											
Coarse								10.8			1.10[illegible]
Fine								8.9			
Not spec'd						6.0*				4.5‡	
Wheat bran flakes	15.0										
Wheat flakes	12.0										
Wheat flour	1.25–5.10		8.8–13.0*[illegible]		11.6–23.0	2.0*	38.7*[illegible] (bazaar sample)	0.83–1.7			450.0[illegible]
Wheat germ			15.0[illegible]					126.0			
Wheat middlings						3.3*					0.75[illegible]
Wheat puffs	4.5										
Wheat starch									0.5		
Wheaties	5.0										
Breads											
Not spec'd							301.6–1883.0*[illegible]				4.87–21.33[illegible]

TABLE 6.—*Aluminum Content of Foods and Beverages*—Continued

	Aluminum Content, PPM*										
	Spectrographic			Colorimetric			Gravimetric			Other Methods, Various Authors, 1935–1955	Methods?, Early Analyses
	Cholak,[44] 1940	Various Authors, 1931–1955	Various Authors, 1926–1955	Beal,[88] 1932	Underhill,[485] 1929	Winter & Bird,[487] 1929	Various Authors, 1928–1953	Bertrand & Levy,[44] 1931	Berg,[44] 1929		
Knaeckebrod			21.16[436]								
Kommis rye											
Pumpernickel			7.86[436]						1.2		
Rye											
Rycerisp (Swedish)									1.2		
Simmons Health									107.6		
White	0.40		10.32[436]						74.7		
Whole wheat			61.52[436]				0.53*[300]		1.2–6.0		
Schinterbrot									17.1		
Zwieback									2.1		
Macaroni									30.2		
Cooked in glass 45 min.							8.5*[109]				
Cooked in Al 45 min.							1.5[109]				
Cooking water (glass)							*2.8–6.6*[109]				
Cooking water (Al)							0.2[109]				
Dairy products							*2.1*[109]				
(for butter, see Animal and Vegetable Fats)											
Buttermilk, dried											
Cottage cheese	0.15					21.2–28.0*					
Cream, unpasteurized, stored in cans, *incl. Al*, up to 4 days		7.75; *7.75*[174]									
Cream, acid											
Stored in Sn cans		<0.1[479]									
Stored in Al 24–72 hr.		*<0.1–1.2*[479]									
Milk, cow's, acid		0.81[479]									
Stored in Sn cans up to 48 hr.		0.31–0.21[479]									
Stored in Al cans 24–48 hr.		*1.4; 2.4*[479]									
Milk, cow's, dried skim											
Milk, cow's						4.0*					
Fresh, unpasteurized			0.018–0.027[217]								
			0.4[167]		13.9–17.1						
Cooked in Al 0.5 min.			*0.6*[241]								
Fresh, unpasteurized			0.46§								
Cooked in Al 5 min.			0.5[396]								
Pasteurized	0.07		*0.6*[396]								
Cooked in Al 30 min.			0.6[396]								
(stirred with Al spoon)			*1.0*[396]								
Fresh, 6 hr. after milking			0.07[396]								
Pasteurized in Al 30 min.			*0.67*[396]								
Not spec'd			1.5; 2.1[167]								
Boiled and simmered in Al 20 min.							0.53[300]		0.5	1.9‡‡	
Cooked in Al 2 min.							0.18[191]				
Powdered (whole)							*0.16–0.88*[141]				
Milk, goat's			3.0–1.0[396,391]				*0.18*[191]				
Meat, poultry, and seafood products											
Bacon									0.5		
Fried in glass 5 min.				0.25							
Fried in Al 5 min.				*0.68*							
Fried in Al 2 min.	1.85										
Salted, lean											
Beef					5.0		5.0; 8.4–17.0[illegible]		5.3 2.7		

Meat, Poultry, and Seafood Products (Cont.)
Beef

Meat, Poultry and [illegible]									
[illegible]									
Beef									
Dried						13.9			
[illegible], *fried in Al*	[illegible]								
Beef [illegible]			7.6-14.8*[illegible]						
Beef [illegible]	1.15								
Beef pot roast with gravy									
Cooked in glass 120 min.				0.64					
Cooked in Al 120 min.				*0.37*					
Beef shank, medium fat									
Beef short ribs								2.5	2.3-7.1[illegible]
Beef tongue			1.2 ¶					0.5	
Ham									
Boiled in glass 120 min.				0.78					
Boiled in Al 120 min.				*2.10*					
Fried in Al	*0.75*								
Smoked									
Ham sausage	1.70							4.8	
Liver									
Beef			4.0 ¶						
Beef (baby), *fried in Al*	*0.90*								1.7[illegible]
Calf			1.2[illegible]		17.3				
Lamb			2.0 ¶						
Pig			3.0 ¶		15.4-20.7				
Mutton					4.3				
Pork, fresh					4.4				
Cooked in Al	*0.80*								
Pork, salt, cooked 2 hr. with 3.5% salt in *Al*							*4.4*[illegible]		
Veal brisket (fat-free)			0.7*[illegible]						
Gravy, *cooked in Al*	*0.21*								
Chicken, creamed									
Cooked in glass 2 hr.				0.95; 0.98					
Cooked in Al				*2.18; 2.43*					
Egg									
Whole		0.05-0.5[illegible]; 0.2 (est'd)[illegible]			0.17			0.5	5.5[illegible]
White			0.99[illegible]						
			0.2; 0.4[illegible]						0.2-0.9[illegible]
Yolk			0.32-1.6[illegible]						
			2.2-11.2[illegible]						3.8-5.8[illegible]
Membrane			11.9-29.1[illegible]						
Dried						12.0*			
Fried in Al	*1.75*								
Fish, not spec'd., *cooked in Al*	*2.50*								
Fish balls, *canned in Al 18 mo.*							*20.0-21.2*[illegible]		
Carp, boned									
Cod						3.1		16.4	
Crab			8.0 ¶						
Crab, *canned in Al 15 mo.*							*9.8-18.0*[illegible]		
Herring						8.8			
Fresh, *canned in Al 7 mo.*							*19.3-64.0*[illegible]		
In oil, *canned in Al up to 25 mo.*							*18.5-74.0*[illegible]		
Kipper, *canned in Al 6 mo.*							*21.3*[illegible]		
Lobster			13.2 ¶						
Pike, boned								15.4	
Rockfish			3.0 ¶						
Sardines									
Bristling, in oil, *canned in Al up to 38 mo.*							*31.2-85.9*[illegible]		
Sild, in oil, *canned in Al up to 23 mo.*							*4.9-104.0*[illegible]		
Sole, boned									
Tench								0.10	
Tuna, canned	0.35							8.0	
White fish						3.0			

TABLE 6.—*Aluminum Content of Foods and Beverages*—Continued

	Aluminum Content, PPM[a]										
	Spectrographic			Colorimetric			Gravimetric			Other Methods, Various Authors, 1935-1955	Methods?, Early Analyses
	Cholak,[b] 1946	Various Authors, 1931-1955	Various Authors, 1926-1955	Beal,[c] 1932	Underhill,[d] 1929	Winter & Bird,[e] 1929	Various Authors, 1928-1953	Bertrand & Levy,[f] 1931	Berg,[g] 1929		
Animal and vegetable fats and oils											
Butter	0.70		4.2[h]						1.6		
Made from creams stored in metal cans, *incl. Al*		Trace-1.52[i] Trace-1.52[i]									
Lard									1.2		
Olive oil									0.3		
Palm oil									1.2		
Vegetables											
Artichoke											
Chinese								13.5			
Jerusalem								5.9			
Asparagus						3.5		7.1	0.1		
Canned, *heated in Al*	3.2										
Fresh, *cooked in Al*	25.0										
Beans											
Green					3.6-9.1	5.0		0.8	2.7-4.2		
Cooked in glass 90 min.				0.91							
Cooked in Al 90 min.				2.28							
Cooked in Al ? min.	3.2										
Canned, *cooked in Al*	2.8										
Beans											
Flageolet variety								1.1			
Soissons var.								2.8			
Early prol.; pea; sulfur						9.0; 10.5[e] 7.0[e]					
Lima, green, canned	2.05										
Yellow string						9.1					
Baked, canned, *heated in Al*	1.15										
Kidney, light and dark red						7.0; 11.5[e]					13.21[j]
Navy											
Cooked in glass 3 hr.				1.44							
Cooked in Al				3.00							
Beets						1.9-3.7		1.1		28.0[e]†	149.1-198.5[j]
Cooked in glass 2.5 hr.				0.58							
Cooked in Al 2.5 hr.				0.74							
Peeled					3.1-6.1	0.8					
Beet tops						23.5				72.0[e]†	132.4-1639.4[j]
Brussels sprouts							28.8-31.0[k]	2.5	35.5	8.6[l]	
Cooked in Al	1.65										
Cooked in Al 45 min.							3.9[k]				
Cooked in glass 45 min.							3.8[k]				
Cooking water, in glass							0.3[k]				
Cooking water, *in Al*							0.5[k]				
Cabbage			8.5[m]			2.3	8.1[k]		20.1		
Outer leaves			19.6-22.0[n]					22.7			
Interior leaves			0.45[n]					0.7			
Stem									0.2		
Cooked	1.3										
Cooked in Al with 0.02% soda +0.25% salt			9.5[m]								
Cabbage, curly									1.2	6.6[l]	
Cabbage, Savoy											

Cabbage, Savoy												
Cooked in glass												
Cooked in Al 1 hr.												
Cooking water, in glass												
Cooking water, *in Al*												
Cabbage, red												
Cooked in glass 90 min.												
Cooked in Al 90 min.												
Cabbage, white												
Creamed in glass 45 min.												
Creamed in Al 45 min.												
Creamed, with soda, in glass 45 min.												
Creamed, with soda, *in Al* 45 min.												
Carrots												
Dried												
Peeled												
Cauliflower												
Creamed in glass, 20 min.												
Creamed *in Al*, 20 min.												
Celery												
Blanched												
Creamed in glass, 20 min.												
Creamed *in Al*, 20 min.												
Chicory												
Corn												
Cream style, canned												
Cream style, canned, *heated in Al*												
Whole kernel, canned												
Cucumber												
Dandelions												
Wild, green												
Cultivated, blanched												
Endive, blanched												
Escarole												
Kale												
Kohlrabi												
Leek, entire plant												
Lentils												
Lettuce												
Green leaves												
Interior leaves												
Root leaves												
Whole												
Head, very young												
Roots												
Lamb's												
Roman												
Mushrooms												
Onion												
Peeled												
Creamed in glass 30 min.												
Creamed in Al 30 min.												
Onion, green												
Parsnips												
Dried												
Peas												
Shelled												
Cooked, not spec'd												

TABLE 6.—*Aluminum Content of Foods and Beverages*—Continued

	Aluminum Content, PPM*										
	Spectrographic			Colorimetric			Gravimetric			Other Methods, Various Authors, 1925-1955	Methods?, Early Analyses
	Cholak,[illegible] 1940	Various Authors, 1931-1955	Various Authors, 1926-1955	Beal,[illegible] 1932	Underhill,[illegible] 1929	Winter & Bird,[illegible] 1929	Various Authors, 1928-1953	Bertrand & Levy,[illegible] 1931	Berg,[illegible] 1929		
Canned, *heated in Al*	*5.0*		13.7[illegible]			5.8*	31.0*‡‡				
Yellow						8.8*					
White											
Potato						2.2	Trace*[illegible]	15.9-78.7			290.0[illegible]
Whole		0-0.05[illegible]	3.3[illegible]								1260.0[illegible]
Whole, dried											
Whole, baked	10.0			0.25; 0.26			0.0[illegible]				
Whole, cooked in glass 30 min.											
20 min.				*0.25; 0.27*			*0.6*[illegible]				
Cooked in Al 30 min.							*0.0*[illegible]				
20 min.							0.6[illegible]				
60 min.							*1.2*[illegible]				
Cooking water, in glass							8.7*[illegible];9.4‡‡		0.5		
Cooking water, *in Al*			1.8-2.2[illegible]		3.2-22.0	0.8					
Peeled			2.0[illegible]								
Peeled, cooked in glass 30 min.				0.55							
Peeled, *cooked in Al* 30 min.			*6.0*[illegible]	*1.10*							
Peeled, *cooked in Al* with 0.5% salt, 30 min.							146.7*[illegible]				
Peel			238.0-308.0[illegible]				25.1[illegible]				
Peel, cooked in glass											
Mushed, *cooked in Al*	*1.80*					4.8		8.0-23.3			
Radish	2.10							10.4			
Salsify							78.0*[illegible]				
Sauerkraut							2.1[illegible]				
Cooked in glass 3 hr.							*5.1*[illegible]				
Cooked in Al 3 hr.				0.83			*4.4*[illegible]				
Cooked in glass 45 min.				*16.4*				6.7	7.4		
Cooked in Al 45 min.								2.9*		7.5*†	
Sorrell								6.8	4.8	102.0*†	
Soya bean							212.5-601.9*[illegible]				
Spinach							15.2-39.5[illegible]				
Cooked in glass							*17.8-49.6*[illegible]				
Cooked in Al							3.7-5.1[illegible]				
Cooking water, glass							*7.2-8.3*[illegible]				
Cooking water, Al									643.4		
Canned, *heated in Al*	*2.5*						1.61*[illegible]	1.1		2.5‡‡	
Dry powder											
Tomato	0.30			0.12							
Cooked in glass 20 min.				0.14							
Cooked in Al, bright pan, 20 min.				*4.20-4.30*							
Cooked in Al, dark pan, 20 min.				*15.6*			3.1[illegible]				
Juice, fresh											
Juice, canned	1.50						0[illegible]				
Purée, cooked in glass 1 hr.							*4.6-6.6*[illegible]				
Purée, *cooked in Al* 1 hr.							3.1[illegible]				
Yellow, juice, canned								20.8-22.0	2.1	32.0*†	
Turnips								3.9-1.5			
Turnip tops								17.1	0.5		
Vegetable gelatin salad *made in Al*	*1.75*										910.0[illegible]
Watercress											3[illegible]
Yams											
Dried											

[illegible]										
[illegible]										
Cooked in glass 2.5 hr.			0.16							
Cooked in Al 2.5 hr.			*0.39*							
Julienne soup										
Cooked in glass 3 hr.						0[109]				
Cooked in Al 3 hr.						*0.4–2.75*[109]				
Mutton broth with veg.										
Cooked in enamel 3 hr.									1.13‡	
Cooked in Al 3 hr.									*7.0*‡	
Stew with meat, peas, potatoes						15.4[illegible]				
Tomato paste, Italian		15.0–45.9[illegible]								
Tomato soup										
Cooked in glass						1.0[109]				
Cooked in Al						*2.2–4.0*[109]				
Tomato soup, cream of, canned, *heated in Al*	*1.05*									
Tomato soup (peeled tom. and stock)										
Before cooking		1.0[illegible]								
Cooked in Al 30 min.		*10.0*[illegible]								
Turtle soup, canned	1.80									
Fruits and berries										
Apple	<0.05			0.15–0.8	1.1	2.86*[illegible]	1.5	0.5	2.8[illegible]	
Pared		1.1[illegible]			0.68					12.7[illegible]
Peel		5.7[illegible]								
Stewed in glass 10 min.			0.28							
Stewed in Al 10 min.			*1.40*							
Stewed in Al 20 min.						*1.6–3.5*[illegible]				
With sugar and water		2.0[illegible]								
Cooked in Al 30 min.		*14.0*[illegible]				*2.9–4.2*[illegible]				
Apple Cider								15.4		
Apple juice										
Canned						2.2[illegible]				
Fresh								0.2		
Apricot							8.0	0.4		
Cooked in glass 40 min.			21.6							
Cooked in Al 40 min.			*78.8*							
Apricot juice, canned, sweetened						1.8[illegible]				
Banana	<0.05						0.3		6.0*‡5.2[illegible]	330.0[illegible]
Blueberries								2.1		26.45[illegible]
Cantaloupe, edible portion				4.0–11.4						
Cherries										27.9–69.6[illegible]
Pitted				31.8–38.0			9.3	0.4		
Stewed	3.7									
Cherry juice, fresh						5.3[illegible]				
Cranberries								0.2; 0.3		
Cranberry sauce										
Cooked in glass 10 min.			0.52–0.54							
Cooked in Al 10 min.										
Bright pan			*7.9*							
Dark pan			*28.0*							
With sugar										
Cooked in glass 10 min.			0.62–0.75							
Cooked in Al 10 min.										
Bright pan			*3.12; 3.20*							
Dark pan			*3.40; 3.50*							
Currants										
Black								1.2		
Red							1.6	0.5		
White							3.8	0.5		
Dates		4.16[illegible]			10.7 (pulp)					
Dried								0.6		
Figs					18.4					
Gooseberries								0.2		
Grapes (see also Raisins)							1.8			
Spiced, canned	0.05									

TABLE 6.—*Aluminum Content of Foods and Beverages*—Continued

	Aluminum Content, PPM*										
	Spectrographic			Colorimetric			Gravimetric			Other Methods, Various Authors, 1935-1955	Methods?, Early Analyses
	Chohak,** 1940	Various Authors, 1931-1955	Various Authors, 1926-1955	Beal,** 1932	Underhill,*** 1929	Winter & Bird,*** 1929	Various Authors, 1925-1953	Bertrand & Levy,** 1931	Berg,** 1929		
Grape juice											
Canned											
Fresh			0.41; 0.45***				3.1***				
Grapefruit									1.3		0.37-25.97***
Grapefruit juice						0.9					
Canned											
Canned, sweetened							4.4***				
Lemon							4.0***				
Lowanberry juice, canned, sweetened						1.5					
Orange							4.4***				
Rind											
Section								1.0			
Whole					0.9	1.6		0.6		0.5 ##	
Mandarin									80.6		
Rind											
Section								0.8			
Orange juice								0.8			
Fresh	0.11										
Canned							1.3***				
Canned, sweetened							4.3***				
Peaches							4.0***				
Peeled and stoned											
Canned	0.35				8.3-9.3			5.0	0.4		
Peach juice, canned, sweetened											
Pear juice, canned, sweetened							2.0***				
Pineapple juice, canned							2.0***				
Sweetened											
Unsweetened							4.0***				
Plum							3.0***				
		46.0-62.0***							0.4		0.063***
Plum juice, canned, sweetened											
Pomegranate							2.7***				
Meat									0.1		
Kernels									0.2		
Prunes, pulp						15.6					
Cooked in glass 10 min.				4.6							
Cooked in Al 40 min.				*7.1*							
Prune juice, canned, sweetened											
Raisins							6.2***				
Raspberries, canned	2.2					16.9-20.0			26.5		
Raspberry juice											
Rhubarb									6.9		
Stewed in Al	*15.0*					1.9	270.3***	1.6			
Stewed in glass 5 min.				0.95							
Stewed in Al 5 min.											
Bright pan				*13.56*							
Dark pan				*41.8*							
Stewed in glass 15 min.							13.3***				
Stewed in Al 15 min.							*40.1-43.7***				
Stewed in glass with sugar 15 min.							5.6***				
Stewed in Al with sugar							*62.6***				
Rhubarb juice, fresh							23.5***				
Strawberries	0.65					3.1		11.5	0.5		
Watermelon, edible portion					0.28						
Puddings, cake, other desserts											
[illegible]	[illegible]										
[illegible]	[illegible]										
Puddings, cake, other desserts—Cont.											
Coffee cake	76.0										
[illegible]											

[illegible]												
Stewed in Al [illegible]												
Light p[illegible]												
Dark pan								13.5				
Stewed in glass 15 min.								*40.5-48.7*[illegible]				
Stewed in Al 15 min.								5.0[illegible]				
Stewed in glass with sugar 15 min.								*62.6*[illegible]				
Stewed in Al with sugar								23.5[illegible]				
Rhubarb juice, fresh							3.1		11.5	6.5		
Strawberries	0.65					0.28						
Watermelon, edible portion												
Puddings, cake, other desserts												
Brownies, chocolate, baked in glass	23.0											
Cake, fudge, baked in tin	120.0											
Puddings, Cake, Other Desserts (Cont.)												
Coffee cake	50.0											
Cookies, chocolate, sandwich	6.3											
Waffles made with S.A.S. baking powder, *baked in Al* iron	*260.0*											
Blackberry pie (canned berries), glass and *Al* pans used	*1.5* 1.5											
Butterscotch pie, baked in glass	2.1											
Lemon pie, baked in glass	1.5											
Lemon pie filling												
Cooked in glass 2 min.					0.31							
Cooked in Al 2 min.					*2.24*							
Pineapple cream pie, canned fruit, baked in tin	1.3											
Raspberry cobbler (canned berries), glass *and Al* used	*10.0* 10.0											
Ice cream, butter-pecan	0.35											
Chocolate pudding, *cooked in Al*	*2.90*											
Beverages												
Coffee												
Drip												
In glass 5 min. cooking + 5 min. drip					0.097							
In Al, same method					*0.58*							
Percolator												
In glass 15 min.					0.25							
In Al 15 min.					*1.05*							
In Al 7 min.	*0.20*											
Tea, iced, *made in Al*	*0.36*											
Coffee bean		0.30.0[illegible]							41.2			11709.0[illegible]
Washed											0‡	
Unwashed											72.0†	
Tea leaves				150.0-250.0*[illegible]						465.0*		
Pepsi Cola	0.13											
Beer								10.21[illegible]			0.02-0.11†	
Wine												
Red				0.8[illegible]							5.0-13.0*	
White				0.64[illegible]								
Dessert				0.96[illegible]								
Blueberry										0.5		
Wines, fermented and unfermented												1.47-20.1[illegible]
Water												
Berlin, tap												
Brought to boiling *in Al*								*Trace*[illegible]				
Boiled in Al 5 min.								*4.0-7.9*[illegible]				
Cincinnati, Ohio	0.02											
London, tap				0.3[illegible]				0.17[illegible]				
Boiled in Al, 0.5 min.				*2.0*[illegible]				*22.02*[illegible]				
Pittsburgh, tap				0.6[illegible]								
Boiled in Al 1 min.				*0.8*[illegible]								
Rhineland, tap												
Brought to boiling *in Al*								*Trace*[illegible]				
Boiled 5 min. *in Al*								*4.1-4.7*[illegible]				
Sendai, Japan, tap												
Boiled *in Al, pressed*												
1 min.				*0.2; 0.2***								
5 min.				*0.6; 0.9*								
Boiled *in Al, cast*												
1 min.				*2.2***								
5 min.				*2.8*								
Condiments, preserves, and miscellaneous products												
Cardamon seeds and husks												339.2[illegible]
Chevril									31.0			
Chile sauce	1.1											
Cinnamon bark												149.0[illegible]
Cocoa bean (defatted)				10.8-60.3[illegible]								

TABLE 6.—*Aluminum Content of Foods and Beverages*—Continued

	Aluminum Content, PPM*										
	Spectrographic		Colorimetric				Gravimetric			Other Methods, Various Authors, 1935–1955	Methods?, Early Analyses
	Cholak,[illegible] 1940	Various Authors, 1931–1955	Various Authors, 1926–1955	Beal,[illegible] 1932	Underhill,[illegible] 1929	Winter & Bird,[illegible] 1920	Various Authors, 1928–1953	Bertrand & Levy,[illegible] 1931	Berg,[illegible] 1929		
Cocoa powder			37.4[illegible]				16.0*[illegible]				
Defatted								13.9			
Not spec'd										160.0*†	
Garlic											
Mint								67.6			
Leaf						67.0*					
In flower											
Mustard seed	2.2										
Mustard, prepared											
Olives		0.7*[illegible]									
Pickled		0.1*[illegible]									
Brine			42.8–41.8[illegible]								65.88[illegible]
Parsley											
Peppers											
Pickles, sweet	90.0										
Salad dressing											
Mayonnaise type	0.38–2.0										
Sandwich spreads and 1000 island dressing	1.72–2.4										
French	1.22–8.5							8.6			
Saxifrage, leaves								12.8			
Sedge, leaves									0.1		
Soya root											
Vinegar, cider											
Homemade	0.1						0.3–5.3[illegible]				
Not spec'd											
Apple butter	3.5										
Cooked in glass 6.5 hr.				5.28							
Cooked in Al 6.5 hr.				*118.0*					3.7	3.2§§	21.31[illegible]
Honey											
Maple sap	0.4–0.52										
Late run	0.01										
Maple syrup, *stored in Al can*	*0.75*										
Orange marmalade											
Cooked in glass 1.5 hr.				0.30							
Cooked in Al 1.5 hr.				*5.06*				18.7*			
Sorgo, entire grain										55.0††	
Sugar	0.60								33.4		
"Trace Element Salt"									25.4		
Almonds								1.0	1.2		11.38[illegible]
Brazil nuts									1.6		
Chestnuts								2.9*	0.5		
Hazelnuts						7.6*					
Peanuts											
Peanut butter	3.2					4.2*					
Pecans						5.0*		2.0*	18.0		
Walnuts			4.61[illegible]								
Malt grist (light)			30.87*[illegible]					4.8			147.1–387.5[illegible]
Malted barley								8.4	0.1		256.9–581.9[illegible]
Hops									159.0		
"Promonta" nerve tonic									97.0		
"Feovin" tonic											

* Values marked with an asterisk are on the basis of the dry product; others, on the basis of the fresh products.
† Meunier,[illegible] opacimetric method. ‡ Monier-Williams,[illegible] volumetric method. § Archibald,[illegible] photometric method. ¶ Meunier,[illegible] electrophotometric method.
§ Tuile,[illegible] fluorimetric method. ** Goto,[illegible] photometric method. †† Reference 21, method not given. §§ Lehmann,[illegible] volumetric method.

TABLE 7.—*Occurrence of Aluminum in Drinking Water Supplies*

	Parts per Million
United States	
Alabama	
Birmingham[x,xx]	0.13
Arkansas[x,xx]	
153 communities	0.0–1.8
12 communities	2.1–3.8
7 communities	6.1–21.0
4 communities	32.0–42.0
California	
San Francisco[x,xx]	0.7
Colorado	
Denver[x,xx]	1.0
Connecticut	
Stafford[x,xx]	1.0*
District of Columbia	
Washington[x,xx]	0.4
Florida	
Jacksonville[x,xx]	0.4
Georgia	
Albany[x,xx]	0.01
Atlanta[x,x,xx]	0.01; 0.2
Hogansville[x,xx]	0.04
Illinois	
Arlington Heights[x,xx]	0.005
Chicago[x,x,xx]	0.01; 0.6
Geneseo[x,xx]	77.35*
Indiana	
Indianapolis[x,xx]	0.4
Kansas	
Atchison Parker Springs[x,xx]	1.69*
Wichita[x,xx]	0.01
Kentucky	
Louisville[x,xx]	0.09
Louisiana	
New Orleans[x,xx]	0.1
Maryland	
Baltimore[x,x,xx]	0.01, 0.035; 0.2
Massachusetts	
Boston[x,x,xx]	0.01; 0.1
Fall River[x,xx]	0.005
Michigan	
Ann Arbor[x,xx]	0.011
Birmingham[x,xx]	0.01
Detroit[x,xx]	0.25
E. Lansing[x,xx]	0.01
Pontiac[x,xx]	0.01
Minnesota	
Minneapolis	0.2; 0.8
Missouri	
Kansas City[x,xx]	0.09–0.27
St. Louis[x,xx]	0.2
Weston[x,xx]	0.005
Montana	
Helena[x,xx]	0.4
New Jersey	
Montclair[x,xx]	0.012
United States	
New Mexico	
Santa Fe[x,xx]	0.2
New York	
Merrick, L. I.[x,xx]	42.3*
New York City[x,x,xx]	0.015; 0.2
Rochester[x,xx]	0.05
North Dakota	
Bismarck[x,xx]	1.0
Ohio	
Cincinnati[x,x,x,x,xx]	0.01–0.10
Cleveland[x,x,xx]	0.07–0.19
Columbus[x,xx]	0.015
Dayton[x,xx]	0.05
Kent[x,xx]	0.07
Lima[x,xx]	0.015
Oakwood[x,xx]	0.02
Springfield[x,xx]	0.015
Toledo[x,xx]	0.03
Oklahoma	
Bristow[x,xx]	0.013
Sapulpa[x,xx]	0.013
Tulsa[x,xx]	0.013
Pennsylvania	
Philadelphia[x,x]	0.3
Pittsburgh[x,x,xx]	0.4–0.6
Rhode Island	
Providence[x,xx]	0.015
Tennessee	
Nashville[x,x]	0.4
Texas	
Houston[x,x]	1.0
Utah	
Salt Lake City[x,x]	0.7
Virginia	
Richmond[x,x]	0.3
Wisconsin	
Milwaukee[x,xx]	0.10
White Rock[x,xx]	5.96*
Canada	
Ontario[x,x]	
Aurora	0.25
Brantford	20.11
Dresden	0.35
Ottawa	4.92
Toronto	0.53
England	
London[x,x,xx]	0.17–0.3
Germany	
Appolinarius Spring[x,xx]	10.86*
Driburg[x,xx]	0.07*
Würzburg[x,xx]	0.12
Panama	
Emperador[x,xx]	5.8; 10.5S*

* Early analysis.

TABLE 8.—*Estimates of Daily Intake of Aluminum by Man*

Date	Author	Daily Aluminum Intake, Mg.	
		From Natural Diet; No Known Contact with Al	From Natural Diet and Contact with Al Utensils and/or Use of Al Baking Powder
1914	U. S. Dept. of Agriculture (based on Ref. Bd. findings; Al incorporated as baking powder)[x,xx]		25–75 in usual diet; 130–250 extreme conditions } baking powder only
1926	Jacobs (estimate based on early high values in raw products; calculated Al content in meals over a period of 7 days)[x,xx]	472 (av.)	
1929	Lehmann (estimate based on own experiments)[x,xx]	20–25	40–60; 100 maximum } including acid foods cooked in aluminum
1931	Fellenberg (estimate based on own experiments)[x,xx]	5–10	5–10 + 5–10 from natural sources + from aluminum utensils
1931	Engelmer (estimate based on literature)[x,xx]		50
1932	Born (estimate based on Massarsch's values)[x,xx]		7
1932	Beal (estimate based on own experiments)[x,xx]	7 (av.)	12 (if all foods are cooked in Al utensils)
1935	Datta (estimate based on own experiments)[x,xx]		<50 (under conditions found in India)
*1940	Kehoe et al. (analysis of total intake of food and beverages of 1 male adult for 25 days)[x,xx]		26.33 ± S.D. 61.97 mean values
1942	Calvery (estimate based on literature)[x,x]		10–50 ppm
*1943	Kehoe et al. (analysis of mean intake of food and beverages of 1 male adult (other than above) for 15 wk.)[x,xx]		17.50 ± S.D. 27.07 mean values; <9.0–>100 daily
1947	Hadorn (estimate based on own experiments)[x,xx]	1.5–10; 6 in 1,000 gm. food	1.5–10 + 0.1–8 from natural sources (maximum includes 2 meals with acid foods) (in 250 gm. baked goods) baking powder only
1955	(estimate from Smith[x,x]) Editorial (estimate based on 1.5 kg. daily food consumption; review of literature)[x,xx]		115; 8–10 + natural content; 135 maximum (if all foods were cooked with soda)

* See summary, Chapter V.

TABLE 9.—*Compilation of Data on the Occurrence of Aluminum in the Tissues of Man*[*]
(mg. Al/100 gm. fresh tissue except where indicated)

Tissue	Meth. of Analysis			
	Spectrographic	Boric Acid	Alizarin	Not Given
Whole blood (ppm)	0.13±0.01[***] Nil-0.3 gm. 0.21-0.94,[***] mode 0.54 0.1-0.5[***]	<2.0[***]	Trace-2.10,[***] av. 0.6	
Blood partition (ppm)	Plasma 0.24±0.02[***] Cells 0.03±0.03[***]	Plasma 0.15-0.5[***]		
Liver	0.160[***] 3.0[***]	0.03-0.14,[***] av. 0.08	0.17-1.17[***]	
Kidneys	0.042[***] +(no figures given)[***]	0.02-0.51,[***] av. 0.10	0.13-0.87[***]	
Gallbladder bile		0.01-0.17,[***] av. 0.07		
Stomach	0.073[***]			
Small intestine	0.037[***]			
Lungs	5.94[***] +(no figures given)[***]			
Spleen	0.130[***]	0.03-0.13[***] av. 0.07		
Brain	0.004[***] Trace (ashed)[***] 10 cerebral and cerebellar cortex (ashed)[***]	0.07-0.15,[***] av. 0.25		
Heart	0.056[***] +(no figures given)[***]	0.11-0.29,[***] av. 0.21		
Muscle	0.015[***]			
Pancreas	±[***]			
Testicles	±[***]			
Long bone	0.500[***]			
Rib bone	0.210[***]			
Skin	Dry tissue 0.0000053–0.0000345[***]			
Hair	Dry tissue 0.0000055–0.0000214[***]			
Nails	Dry tissue 0.0000345–0.0000831[***]			
Whole organism				50-150 mg. est. total[***]

* Milligrams of Al per 100 gm. fresh tissue, except where indicated otherwise.
† There is no good explanation for these tissues containing so little aluminum.

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The Formation of Concretions of Aluminum Salts of Fatty Acids After the Use of Aluminum Hydroxide

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SINCE a careful search through the literature concerning the use of aluminum hydroxide for peptic ulcer failed to disclose any description of the formation of aluminum soap concretions, it was thought that the following observations would be of interest.

Aluminum hydroxide was used first by Crohn in this country in the treatment of peptic ulcer. He found no deleterious side effects. After the ingestion of 168 cc. of a heavy aluminum hydroxide suspension, Myers found less than 0.12 mgm.% in the blood stream. Quigley, Einsel and Meschan noted that large amounts of this substance when fed to animals over long periods had little if any undesirable side action.

Monaghan, however, reported that in persistent pylorospasm, large doses of aluminum hydroxide delayed the stomach emptying in many patients. Havens described one case of complete intestinal obstruction following the use of the antacid in the treatment of severe ulcer hemorrhage. He found at autopsy that a large mass of blod clot and aluminum hydroxide had completely obstructed the bowel.

Fauley and others have pointed out that aluminum chloride may be formed in the stomach and unless enough alkali is present in the intestine to convert the salt to the hydroxide, gastric and intestinal irritation may result. These apparent objections are few and aluminum hydroxide has enjoyed wide clinical use with remarkably satisfactory results. Bockus recommends, however, that in gastric stasis the rate of evacuation of this antacid from the stomach should be checked frequently.

The patient in whom the unusual aluminum concretions were found was a 36 year old male with a 15 year history of chronic duodenal ulcer. Following a severe hemorrhage in March 1945 the patient made an uneventful recovery on a bland diet with atropine and aluminum hydroxide medication. Three months after this hemorrhage and while still taking the antacid and maximum doses of tincture of belladonna he suffered another acute episode of severe pain culminating in hemorrhage. His stools were found to contain small formed white masses. These masses became more apparent after the melena had cleared up. Their passages were accompanied by intermittent periods of constipation and diarrhea, cramps and pain suggesting intestinal irritation. The patient recalled a similar sequence of events with approximately the same interval between hemorrhages in 1941. At that time the white concretions were not analyzed.

This condition cleared up after five days. During this time, however, he passed about 40 small masses which were egg shaped and varied from 5 to 25 mm. in length. Their surface was convoluted bearing a hollow viscus impression. The color was white with yellow greenish lines in the crevices of the convolsions. On cutting, the cut surface was a pure white. Gross and microscopic examination showed no evidence of any organized structure. Their consistency was firm but somewhat rubbery. On drying the concretions they would shrink to two-thirds their former size but without any appreciable change in appearance.

The concretions burned readily with a yellow smokey flame leaving a considerable amount of ash.

They were only sparingly soluble in boiling chloroform, 10% aqueous sodium hydroxide or 10% hydrochloric acid. The chloroform extract gave a faint Liebermann Burchard test for cholesterol and the NaOH extract gave a very faint biuret test. An acrolein test was positive for glycerol. A small amount of material could be dissolved from the concretion with boiling alcohol. On evaporation of the alcohol, a greasy residue remained. This fatty residue gave negative tests for amines and phosphates.

On saponification with alcoholic KOH small whitish flakes of undissolved material remained. The flakes, as well as the ash which remained after burning a concretion, gave the usual qualitative tests for aluminum. On dilution with water and acidification of the alcoholic KOH solution remaining from the saponification, oily droplets of fatty acids separated from the solution.

These qualitative tests indicate that the concretions consisted of aluminum salts of fatty acids together with a small amount of neutral fat. There apparently was some water emulsified within the concretion judging from the diminution in size upon drying.

The site of origin of the aluminum soaps is open to speculation. Their size and surface markings indicate that the concretions were compressed in some hollow viscus of small bore. Their uniform texture and lack of admixture with other substances may be attributed to the relative absence of other substances in the gastro-intestinal tract of the pa-

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tient during the first days following the hemorrhage

The condition apparently cleared spontaneously when the patient was given increasing amounts of food.

SUMMARY

Following a hemorrhage of duodenal ulcer origin in a patient using aluminum hydroxide as an antacid, small concretions were found in the stool. The passage of these concretions through the intestinal tract was accompanied by severe intermittent cramps and pain.

Chemical analysis of these concretions showed them to be composed of fatty acid salts of aluminum with a small amount of neutral fat and traces of cholesterol and protein.

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FOOD CHEMICALS CODEX

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ALUMINUM AMMONIUM SULFATE

Ammonium Alum

$AlNH_4(SO_4)_2 . 12H_2O$ Mol. wt. 453.32

DESCRIPTION

Large, colorless crystals, white granules, or a powder. It is odorless and has a sweetish, strongly astringent taste. One gram dissolves in 7 ml. of water at 25° and in about 0.3 ml. of boiling water. It is insoluble in alcohol, and is freely, but slowly soluble in glycerin. Its solutions are acid to litmus. A 1 in 20 solution gives positive tests for *Aluminum* and for *Ammonium*, page 926, and for *Sulfate*, page 928.

SPECIFICATIONS

Assay. Not less than 99.5 percent of $AlNH_4(SO_4)_2 . 12H_2O$.

Limits of Impurities

Alkalies and alkaline earths. Passes test.

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Fluoride. Not more than 30 parts per million (0.003 percent).

Heavy metals (as Pb). Not more than 20 parts per million (0.002 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Selenium. Not more than 30 parts per million (0.003 percent).

TESTS

Assay. Weigh accurately about 1 gram, dissolve it in 50 ml. of water, add 50.0 ml. of 0.05 *M* disodium ethylenediaminetetraacetate, and boil gently for 5 minutes. Cool, and add in the order given and with continuous stirring 20 ml. of pH 4.5 buffer solution (77.1 grams of ammonium acetate and 57 ml. of glacial acetic acid in 1000 ml. of solution), 50 ml. of alcohol, and 2 ml. of dithizone T.S. Titrate with 0.05 *M* zinc sulfate to a bright rose-pink color, and perform a blank determination (see page 2). Each ml. of 0.05 *M* disodium ethylenediaminetetraacetate is equivalent to 22.67 mg. of $AlNH_4(SO_4)_2 . 12H_2O$.

Alkalies and alkaline earths. Completely precipitate the aluminum from a boiling solution of 1 gram of the sample in 100 ml. of water by the addition of enough ammonia T.S. to render the solution distinctly alkaline to methyl red T.S., and filter. Evaporate the filtrate to dryness, and ignite. The weight of the residue does not exceed 5 mg.

Arsenic. A solution of 1 gram in 35 ml. of water meets the requirements of the *Arsenic Test*, page 865.

Fluoride

Lime Suspension. Carefully slake about 56 grams of low-fluorine calcium oxide (about 2 parts per million F) with 250 ml. of water, and add 250 ml. of 60 percent perchloric acid slowly and with stirring. Add a

few glass beads, and boil to copious fumes of perchloric acid, then cool, add 200 ml. of water, and boil again. Repeat the dilution and boiling once more, cool, dilute considerably, and filter through a fritted glass filter, if precipitated silicon dioxide appears. Pour the clear solution, with stirring, into 1000 ml. of sodium hydroxide solution (1 in 10), allow the precipitate to settle, and siphon off the supernatant liquid. Remove the sodium salts from the precipitate by washing 5 times in large centrifuge bottles, shaking the mass thoroughly each time. Finally, shake the precipitate into a suspension and dilute to 2000 ml. Store in paraffin-lined bottles and shake well before use. (*Note:* 100 ml. of this suspension should give no appreciable fluoride blank when evaporated, distilled, and titrated as directed in the *Fluoride Limit Test*, page 917.)

Procedure. Assemble the distilling apparatus as described in the *Fluoride Limit Test*, page 917, and add to the distilling flask 1.67 grams of the sample, accurately weighed, and 25 ml. of dilute sulfuric acid (1 in 2). Distil until the temperature reaches 160°, then maintain at 160° to 165° by adding water from the funnel, collecting 300 ml. of distillate. Oxidize the distillate by the cautious addition of 2 or 3 ml. of fluorine-free 30 percent hydrogen peroxide (to remove sulfites), allow to stand for a few minutes, and evaporate in a platinum dish with an excess of *Lime Suspension.* Ignite briefly at 600°, then cool and wet the ash with about 10 ml. of water. Cover the dish with a watch glass, and cautiously introduce under cover just sufficient 60 percent perchloric acid to dissolve the ash. Add the contents of the dish through the dropping funnel of a freshly prepared distilling apparatus (the distilling flask should contain a few glass beads), using a total of 20 ml. of the perchloric acid for dissolving the ash and transferring the solution. Add 10 ml. of water and a few drops of silver perchlorate solution (1 in 2) through the dropping funnel, and continue as directed in the *Fluoride Limit Test*, page 917, beginning with "Distil until the temperature reaches 135°. . ."

Heavy metals. Dissolve 1 gram in 20 ml. of water, add a few drops of diluted hydrochloric acid T.S., and evaporate to dryness in a porcelain dish. Treat the residue with 20 ml. of water, and add 50 mg. of hydroxylamine hydrochloride. Heat on a steam bath for 10 minutes, cool, and dilute to 25 ml. with water. This solution meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) and 50 mg. of hydroxylamine hydrochloride in the control (*Solution A*).

Lead. A solution of 1 gram in 10 ml. of water meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Selenium. A solution of 2 grams in 40 ml. of dilute hydrochloric acid (1 in 2) meets the requirements of the *Selenium Limit Test*, page 953.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Buffer; neutralizing agent.

ALUMINUM POTASSIUM SULFATE

Potassium Alum

$AlK(SO_4)_2.12H_2O$ Mol. wt. 474.38

DESCRIPTION

Large, transparent crystals or crystalline fragments, or a white crystalline powder. It is odorless and has a sweetish, astringent taste. One gram dissolves in 7.5 ml. of water at 25° and in about 0.3 ml. of boiling water. It is insoluble in alcohol, but is freely soluble in glycerin. Its solutions are acid to litmus. A 1 in 20 solution gives positive tests for *Aluminum*, page 925, for *Potassium*, page 928, and for *Sulfate*, page 928.

SPECIFICATIONS

Assay. Not less than 99.5 percent of $AlK(SO_4)_2.12H_2O$.

Limits of Impurities

Ammonium salts. Passes test.

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Fluoride. Not more than 30 parts per million (0.003 percent).

Heavy metals (as Pb). Not more than 20 parts per million (0.002 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Selenium. Not more than 30 parts per million (0.003 percent).

TESTS

Assay. Weigh accurately about 1 gram, dissolve it in 50 ml. of water, add 50.0 ml. of 0.05 *M* disodium ethylenediaminetetraacetate, and boil gently for 5 minutes. Cool, and add in the order given and with continuous stirring 20 ml. of pH 4.5 buffer solution (77.1 grams of ammonium acetate and 57 ml. of glacial acetic acid in 1000 ml. of solution), 50 ml. of alcohol, and 2 ml. of dithizone T.S. Titrate with 0.05 *M* zinc sulfate to a bright rose-pink color, and perform a blank determination (see page 2). Each ml. of 0.05 *M* disodium ethylenediaminetetraacetate is equivalent to 23.72 mg. of $AlK(SO_4)_2.12H_2O$.

Ammonium salts. Heat 1 gram with 10 ml. of sodium hydroxide T.S. on a steam bath for 1 minute. The odor of ammonia is not perceptible.

Arsenic. A solution of 1 gram in 35 ml. of water meets the requirements of the *Arsenic Test*, page 865.

Heavy metals. Dissolve 1 gram in 20 ml. of water, add a few drops of diluted hydrochloric acid T.S., and evaporate to dryness in a porcelain dish. Treat the residue with 20 ml. of water, and add 50 mg. of hydroxylamine hydrochloride. Heat on a steam bath for 10 minutes, cool, and dilute to 25 ml. with water. This solution meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) and 50 mg. of hydroxylamine hydrochloride in the control (*Solution A*).

Fluoride. Determine as directed in the test for *Fluoride* under *Aluminum Ammonium Sulfate*, page 34.

Lead. A solution of 1 gram in 10 ml. of water meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Selenium. A solution of 2 grams in 40 ml. of dilute hydrochloric acid (1 in 2) meets the requirements of the *Selenium Limit Test*, page 953.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Buffer; neutralizing, firming agent.

ALUMINUM SODIUM SULFATE

Soda Alum; Sodium Alum

$AlNa(SO_4)_2$ Mol. wt. 242.09

DESCRIPTION

Aluminum sodium sulfate is anhydrous or may contain up to 12 molecules of water of hydration. It occurs as colorless crystals, or white granules or powder. It is odorless and has a saline, astringent taste. The anhydrous form is slowly soluble in water. The dodecahydrate is freely soluble in water, and it effloresces in air. Both forms are insoluble in alcohol. It responds to the flame test for *Sodium*, page 928, and gives positive tests for *Aluminum*, page 925, and for *Sulfate*, page 928.

SPECIFICATIONS

Assay. *Anhydrous form*, not less than 96.5 percent of $AlNa(SO_4)_2$ after drying; *dodecahydrate*, not less than 99.5 percent of $AlNa(SO_4)_2$ after drying.

Loss on drying. *Anhydrous form*, not more than 10 percent; *dodecahydrate*, not more than 47.2 percent.

Neutralizing value. *Anhydrous form*, between 103 and 107.

Limits of Impurities

Ammonium salts. Passes test.

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Fluoride. Not more than 30 parts per million (0.003 percent).

Heavy metals (as Pb). Not more than 20 parts per million (0.002 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Selenium. Not more than 30 parts per million (0.003 percent).

TESTS

Assay. Weigh accurately about 500 mg. of a sample previously dried as directed in the test for *Loss on drying*, moisten with 1 ml. of acetic

acid, and dissolve it in 50 ml. of water, warming gently on a steam bath until solution is complete. Cool, neutralize with ammonia T.S., add 50.0 ml. of 0.05 *M* disodium ethylenediaminetetraacetate, and boil gently for 5 minutes. Cool, and add in the order given and with continuous stirring 20 ml. of pH 4.5 buffer solution (77.1 grams of ammonium acetate and 57 ml. of glacial acetic acid in 1000 ml. of solution), 50 ml. of alcohol, and 2 ml. of dithizone T.S. Titrate with 0.05 *M* zinc sulfate to a bright rose-pink color, and perform a blank determination (see page 2). Each ml. of 0.05 *M* disodium ethylenediaminetetraacetate is equivalent to 12.10 mg. of $AlNa(SO_4)_2$.

Loss on drying, page 931. *Anhydrous form:* dry at 200° for 16 hours. *Dodecahydrate:* dry first at 50–55° for 1 hour, then at 200° for 16 hours.

Neutralizing value. Weigh accurately 500 mg. of the anhydrous form into a 200 ml. Erlenmeyer flask, add 30 ml. of water and 4 drops of phenolphthalein T.S., and boil until the sample dissolves. Add 13.0 ml. of 0.5 *N* sodium hydroxide, boil for a few seconds, and titrate with 0.5 *N* hydrochloric acid to the disappearance of the pink color, adding the acid dropwise and agitating vigorously after each addition. Calculate the neutralizing value, as parts of $NaHCO_3$ equivalent to 100 parts of the sample, by the formula $8.4V$, in which V is the volume, in ml., of 0.5 *N* sodium hydroxide consumed by the sample.

Ammonium salts. Heat 1 gram with 10 ml. of sodium hydroxide T.S. on a steam bath for 1 minute. The odor of ammonia is not perceptible.

Arsenic. A solution of 1 gram in 35 ml. of water meets the requirements of the *Arsenic Test*, page 865.

Fluoride. Determine as directed in the test for *Fluoride* under *Aluminum Ammonium Sulfate*, page 34.

Heavy metals. Dissolve 1 gram in 20 ml. of water, add a few drops of diluted hydrochloric acid T.S., and evaporate to dryness in a porcelain dish. Treat the residue with 20 ml. of water, and add 50 mg. of hydroxylamine hydrochloride. Heat on a steam bath for 10 minutes, cool, and dilute to 25 ml. with water. This solution meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) and 50 mg. of hydroxylamine hydrochloride in the control (*Solution A*).

Lead. A solution of 1 gram in 10 ml. of water meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Selenium. A solution of 2 grams in 40 ml. of dilute hydrochloric acid (1 in 2) meets the requirements of the *Selenium Limit Test*, page 953.

Packaging and storage. Store in tight containers.

Functional use in foods. Buffer; neutralizing, firming agent.

ALUMINUM SULFATE

$Al_2(SO_4)_3 . xH_2O$ Mol. wt. (anhydrous) 342.15

DESCRIPTION

Aluminum sulfate is anhydrous or contains 18 molecules of water of crystallization. Due to efflorescence, the hydrate may have a composition approximating the formula $Al_2(SO_4)_3 . 14H_2O$. It occurs as a white powder, as shining plates, or as crystalline fragments. It is odorless and has a sweet taste, becoming mildly astringent. One gram of the hydrate dissolves in about 2 ml. of water. The anhydrous product approaches the same solubility, but the rate of solution is so slow that it initially appears to be relatively insoluble. The pH of a 1 in 20 solution is 2.9 or above. A 1 in 10 solution gives positive tests for *Aluminum*, page 925, and for *Sulfate*, page 928.

SPECIFICATIONS

Assay. $Al_2(SO_4)_3$ (anhydrous), not less than 99.5 percent of $Al_2(SO_4)_3$, calculated on the ignited basis; $Al_2(SO_4)_3 . 18H_2O$ (hydrate), not less than 99.5 percent and not more than the equivalent of 114.0 percent of $Al_2(SO_4)_3 . 18H_2O$, corresponding to not more than approximately 101.7 percent of $Al_2(SO_4)_3 . 14H_2O$.

Limits of Impurities

Alkalies and alkaline earths. Passes test (about 0.4 percent).

Ammonium salts. Passes test.

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Heavy metals (as Pb). Not more than 40 parts per million (0.004 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Loss on ignition. $Al_2(SO_4)_3$ (anhydrous), not more than 5 percent. [*Note:* This specification does not apply to $Al_2(SO_4)_3 . 18H_2O$.]

Selenium. Not more than 30 parts per million (0.003 percent).

TESTS

Assay. Weigh accurately an amount of sample equivalent to about 4 grams of $Al_2(SO_4)_3$, transfer into a 250-ml. volumetric flask, dissolve in water, dilute to volume with water, and mix. Pipet 10 ml. of this solution into a 250-ml. beaker, add 25.0 ml. of 0.05 *M* disodium ethylenediaminetetraacetate, and boil gently for 5 minutes. Cool, and add in the order given and with continuous stirring 20 ml. of pH 4.5 buffer solution (77.1 grams of ammonium acetate and 57 ml. of glacial acetic acid in 1000 ml. of solution), 50 ml. of alcohol, and 2 ml. of dithizone T.S. Titrate with 0.05 *M* zinc sulfate until the color changes from green-violet to rose-pink, and perform a blank determination (see page 2), substituting 10 ml. of water for the sample.

Each ml. of 0.05 *M* disodium ethylenediaminetetraacetate is equivale to 8.554 mg. of $Al_2(SO_4)_3$ or to 16.67 mg. of $Al_2(SO_4)_3.18H_2O$.

Alkalies and alkaline earths. To a boiling solution of 2 grams 150 ml. of water add a few drops of methyl red T.S., and then a ammonia T.S. until the color of the solution just changes to a distin yellow. Add hot water to restore the original volume, and filter whi hot. Evaporate 75 ml. of the filtrate to dryness, and ignite to consta weight. Not more than 4 mg. of residue remains.

Ammonium salts. Heat 1 gram with 10 ml. of sodium hydroxi T.S. on a steam bath for 1 minute. The odor of ammonia is not perce tible.

Arsenic. A solution of 1 gram in 35 ml. of water meets the requir ments of the *Arsenic Test*, page 865.

Heavy metals. Dissolve 500 mg. in 20 ml. of water, add a fe drops of diluted hydrochloric acid T.S., and evaporate to dryness in porcelain dish. Treat the residue with 20 ml. of water, and add 50 m of hydroxylamine hydrochloride. Heat on a steam bath for 10 mi utes, cool, and dilute to 25 ml. with water. This solution meets t requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of le ion (Pb) and 50 mg. of hydroxylamine hydrochloride in the contr (*Solution A*).

Lead. A solution of 1 gram in 10 ml. of water meets the requir ments of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) the control.

Loss on ignition. Weigh accurately about 2 grams of $Al_2(SO_4$ (anhydrous), and ignite, preferably in a muffle furnace, at about 50 for 3 hours. [*Note:* This test does not apply to $Al_2(SO_4)_3.18H_2O$.]

Selenium. A solution of 2 grams in 40 ml. of dilute hydrochloric ac (1 in 2) meets the requirements of the *Selenium Limit Test*, page 953.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Firming agent.

ALUMINUM SULFATE SOLUTION

Alum Liquor

DESCRIPTION

Aluminum sulfate solution is usually available as a solution containin approximately 50 percent of $Al_2(SO_4)_3.18H_2O$. It is a clear to slightl turbid, colorless to slightly colored, liquid having a mildly astringe taste. It is acid to litmus, and a 1 in 20 solution [based on $Al_2(SO_4)_3.18H_2O$] has a pH between 2.5 and 3.5. Diluted 1 in 5, it gives positiv tests for *Aluminum*, page 925, and for *Sulfate*, page 928.

SPECIFICATIONS

Assay. Not less than 90.0 percent and not more than 110.0 percent, by weight, of the labeled amount of aluminum sulfate, expressed as $Al_2(SO_4)_3$, $Al_2(SO_4)_3 \cdot 18H_2O$, or Al_2O_3.

Limits of Impurities

Alkalies and alkaline earths. Passes test.

Ammonium salts. Passes test.

Arsenic (as As). Not more than 3 parts per million (0.0003 percent), calculated on the $Al_2(SO_4)_3 \cdot 18H_2O$ determined in the *Assay*.

Heavy metals (as Pb). Not more than 40 parts per million (0.004 percent), calculated on the $Al_2(SO_4)_3 \cdot 18H_2O$ determined in the *Assay*.

Lead. Not more than 10 parts per million (0.001 percent), calculated on the $Al_2(SO_4)_3 \cdot 18H_2O$ determined in the *Assay*.

Selenium. Not more than 30 parts per million (0.003 percent), calculated on the $Al_2(SO_4)_3 \cdot 18H_2O$ determined in the *Assay*.

TESTS

Assay. Transfer an accurately weighed amount of the solution, equivalent to about 7.5 grams of $Al_2(SO_4)_3 \cdot 18H_2O$, into a 250-ml. volumetric flask, dilute to volume with water, and mix. Pipet 10 ml. of this solution into a 250-ml. beaker, add 25.0 ml. of 0.05 *M* disodium ethylenediaminetetraacetate, and boil gently for 5 minutes. Cool, and add in the order given and with continuous stirring 20 ml. of pH 4.5 buffer solution (77.1 grams of ammonium acetate and 57 ml. of glacial acetic acid in 1000 ml. of solution), 50 ml. of alcohol, and 2 ml. of dithizone T.S. Titrate with 0.05 *M* zinc sulfate until the color changes from green-violet to rose-pink, and perform a blank determination (see page 2). Each ml. of 0.05 *M* disodium ethylenediaminetetraacetate is equivalent to 8.554 mg. of $Al_2(SO_4)_3$, 16.67 mg. of $Al_2(SO_4)_3 \cdot 18H_2O$, or 2.549 mg. of Al_2O_3.

Alkalies and alkaline earths. Add a few drops of methyl red T.S. to an accurately weighed amount of the solution equivalent to 2 grams of $Al_2(SO_4)_3 \cdot 18H_2O$, dilute to about 150 ml. with water, heated to boiling, and then add ammonia T.S. until the color of the solution just changes to a distinct yellow. Add hot water to restore the original volume, and filter while hot. Evaporate 75 ml. of the filtrate to dryness, and ignite to constant weight. Not more than 4 mg. of residue remains.

Ammonium salts. Heat an accurately weighed amount of the solution equivalent to 1 gram of $Al_2(SO_4)_3 \cdot 18H_2O$ with 10 ml. of sodium hydroxide T.S. on a steam bath for 1 minute. The odor of ammonia is not perceptible.

Arsenic. Dilute an accurately weighed amount of the solution equivalent to 1 gram of $Al_2(SO_4)_3 \cdot 18H_2O$ to 35 ml. with water. The resulting solution meets the requirements of the *Arsenic Test*, page 865.

Heavy metals. Dilute an accurately weighed amount of the solution equivalent to 500 mg. of $Al_2(SO_4)_3 \cdot 18H_2O$ to 25 ml. with water, add a few drops of diluted hydrochloric acid T.S., and evaporate to dryness in a porcelain dish. Treat the residue with 20 ml. of water and add 50 mg. of hydroxylamine hydrochloride. Heat on a steam bath for 10 minutes, cool, and dilute to 25 ml. with water. This solution meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Lead. Dilute an accurately weighed amount of the solution equivalent to 1 gram of $Al_2(SO_4)_3 \cdot 18H_2O$ to 10 ml. with water. The resulting solution meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Selenium. Dilute an accurately weighed amount of the solution equivalent to 2 grams of $Al_2(SO_4)_3 \cdot 18H_2O$ to 40 ml. with diluted hydrochloric acid T.S. The resulting solution meets the requirements of the *Selenium Limit Test*, page 953.

Packaging and storage. Store in tight containers.

Functional use in foods. Firming agent.

AMBRETTE SEED OIL

Ambrette Seed Liquid

DESCRIPTION

The volatile oil obtained by steam distillation from the partially dried and crushed seeds of the plant *Abelmoschus moschatus* Moench, syn. *Hibiscus Abelmoschus* L. (Fam. *Malvaceae*). It is refined by solvent extraction to remove fatty acids, or precipitation of the fatty acid salts. It is a clear yellow to amber liquid, having the strong musky odor of ambrettolide. It is soluble in most fixed oils and in mineral oil, often with cloudiness. It is relatively insoluble in glycerin and in propylene glycol.

SPECIFICATIONS

Acid value. Not more than 3.0.

Angular rotation. Between −2.5° and +3°.

Refractive index. Between 1.468 and 1.485 at 20°.

Saponification value. Between 140 and 200.

Specific gravity. Between 0.898 and 0.920.

TESTS

Acid value. Determine as directed in the general method, page 893.

Angular rotation. Determine in a 100-mm. tube as directed under *Optical Rotation*, page 939.

Loss on drying. Not more than 15 percent.

TESTS

Assay. Proceed as directed in the *Alginates Assay*, page 26. Each ml. of 0.25 *N* sodium hydroxide consumed in the assay is equivalent to 27.75 mg. of sodium alginate (Equiv. wt. 222.00).

Ash. Determine as directed under *Ash* in the monograph on *Alginic Acid*, page 26.

Arsenic. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 865.

Heavy metals. Prepare and test a 500-mg. sample as directed in *Method II* under the *Heavy Metals Test*, page 920, but use a platinum crucible for the ignition. Any color does not exceed that produced in a control (*Solution A*) containing 20 mcg. of lead ion (Pb).

Insoluble matter. Determine as directed under *Insoluble matter* in the monograph on *Alginic Acid*, page 26.

Lead. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Loss on drying, page 931. Dry at 105° for 4 hours.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Stabilizer; thickener; emulsifier.

SODIUM ALUMINUM PHOSPHATE, ACIDIC

$NaAl_3H_{14}(PO_4)_8 \cdot 4H_2O$ Mol. wt. 949.88
or or
$Na_3Al_2H_{15}(PO_4)_8$ Mol. wt. 897.82

DESCRIPTION

A white, odorless powder. It is insoluble in water, but is soluble in hydrochloric acid. A 1 in 10 solution in dilute hydrochloric acid (1 in 2) gives positive tests for *Aluminum*, page 925, and for *Phosphate*, page 928, and it responds to the flame test for *Sodium*, page 928.

SPECIFICATIONS

Assay. Not less than 95.0 percent of $NaAl_3H_{14}(PO_4)_8 \cdot 4H_2O$, or not less than 95.0 percent of $Na_3Al_2H_{15}(PO_4)_8$.

Loss on ignition. $NaAl_3H_{14}(PO_4)_8 \cdot 4H_2O$, between 19.5 and 21 percent; $Na_3Al_2H_{15}(PO_4)_8$, between 15 and 16 percent.

Neutralizing value. Not less than 100.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Fluoride. Not more than 25 parts per million (0.0025 percent).

Heavy metals (as Pb). Not more than 40 parts per million (0.004 percent).

Lead. Not more than 10 parts per million (0.001 percent)

TESTS

Assay. Transfer about 2.5 grams, accurately weighed, into a 250-ml. volumetric flask, add 15 ml. of hydrochloric acid and one glass bead, and boil gently for about 5 minutes. Cool, dilute to volume with water, and mix. Transfer 10.0 ml. of this solution to a 250-ml. beaker, add phenolphthalein T.S., and neutralize with ammonia T.S. Add dilute hydrochloric acid (1 in 2) until the precipitate just dissolves, then dilute to 100 ml. with water and heat to 70°–80°. Add 10 ml. of 8-hydroxyquinoline T.S. and sufficient ammonium acetate T.S. until a yellow precipitate forms, then add 30 ml. in excess. Digest at 70° for 30 minutes, filter through a previously dried and weighed Gooch crucible, and wash thoroughly with hot water. Dry at 105° for 2 hours, cool, and weigh. Each mg. of the precipitate so obtained corresponds to 0.689 mg. of $NaAl_3H_{14}(PO_4)_8 \cdot 4H_2O$, or to 0.977 mg. of $Na_3Al_2H_{15}(PO_4)_8$.

Loss on ignition. Ignite at 750° to 800° for 2 hours.

Neutralizing value. Transfer 840.1 mg., accurately weighed, into a 375-ml. casserole, and add 20 grams of sodium chloride, 5 ml. of sodium citrate solution (1 in 10), and 25 ml. of water. Stir vigorously at once for about 30 seconds, then add exactly 120 ml. of 0.1 *N* sodium hydroxide, bring the suspension to a boil in exactly 2 minutes, and boil for 5 minutes. While the solution is still boiling hot, add exactly 0.05 ml. of phenolphthalein T.S., and titrate the excess alkali with 0.2 *N* hydrochloric acid until the pink color has almost disappeared. Boil the solution for 1 minute, and titrate again with 0.2 *N* hydrochloric acid until the pink color just disappears. Calculate the neutralizing value, as parts of $NaHCO_3$ equivalent to 100 parts of sodium aluminum phosphate, by the formula $120 - 2V$, in which V is the total volume of 0.2 *N* hydrochloric acid consumed.

Arsenic. A solution of 1 gram in 10 ml. of dilute hydrochloric acid (1 in 2) meets the requirements of the *Arsenic Test*, page 865.

Fluoride. Weigh accurately 2.0 grams, and proceed as directed in the *Fluoride Limit Test*, page 917.

Heavy metals. Dissolve 500 mg. in 2.5 ml. of diluted hydrochloric acid T.S., and add water to make 25 ml. This solution meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Lead. A solution of 1 gram in 5 ml. of diluted hydrochloric acid T.S. meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Leavening agent.

SODIUM ALUMINUM PHOSPHATE, BASIC

Kasal

DESCRIPTION

A white, odorless powder comprised of an autogenous mixture of an alkaline sodium aluminum phosphate [approximately $Na_8Al_2(OH)_2(PO_4)_4$], with about 30 percent dibasic sodium phosphate. It is soluble in hydrochloric acid; the sodium phosphate moiety is soluble in water whereas the sodium aluminum phosphate moiety is only sparingly soluble in water. A 1 in 10 solution in dilute hydrochloric acid (1 in 2) gives a positive test for *Aluminum*, page 925, and for *Phosphate*, page 928, and it responds to the flame test for *Sodium*, page 928.

SPECIFICATIONS

Assay. Not less than 9.5 percent and not more than 12.5 percent of Al_2O_3, calculated on the ignited basis.

Loss on ignition. Not more than 9 percent.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Fluoride. Not more than 25 parts per million (0.0025 percent).

Heavy metals (as Pb). Not more than 40 parts per million (0.004 percent).

Lead. Not more than 10 parts per million (0.001 percent).

TESTS

Assay. Transfer about 2.5 grams, accurately weighed, into a 250-ml. volumetric flask, add 15 ml. of hydrochloric acid and one glass bead, and boil gently for about 5 minutes. Cool, dilute to volume with water, and mix. Transfer 10.0 ml. of this solution to a 250-ml. beaker, add phenolphthalein T.S., and neutralize with ammonia T.S. Add dilute hydrochloric acid (1 in 2) until the precipitate just dissolves, then dilute to 100 ml. with water and heat to 70°–80°. Add 10 ml. of 8-hydroxyquinoline T.S. and sufficient ammonium acetate T.S. until a yellow precipitate forms, then add 30 ml. in excess. Digest at 70° for 30 minutes, filter through a previously dried and weighed Gooch crucible, and wash thoroughly with hot water. Dry at 105° for 2 hours, cool, and weigh. Each mg. of the precipitate so obtained corresponds to 0.111 mg. of Al_2O_3.

Loss on ignition. Ignite at 750° to 800° for 2 hours.

Arsenic. A solution of 1 gram in 10 ml. of dilute hydrochloric acid (1 in 2) meets the requirements of the *Arsenic Test*, page 865.

Fluoride. Weigh accurately 2.0 grams, and proceed as directed in the *Fluoride Limit Test*, page 917.

Heavy metals. Dissolve 500 mg. in 2.5 ml. of diluted hydrochloric acid T.S., and add water to make 25 ml. This solution meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Lead. A solution of 1 gram in 5 ml. of diluted hydrochloric acid T.S. meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Emulsifier.

Am J Physiol. 111 (118–23) 1935

THE EFFECT OF FEEDING HIGH AMOUNTS OF SOLUBLE IRON AND ALUMINUM SALTS[1]

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Received for publication November 6, 1934

Studies which have been in progress in this laboratory for several years dealing with the availability of various forms of calcium for bone formation included a number of determinations on calcium aluminum silicates and calcium iron silicates. These salts were included in an attempt to find forms of calcium which were unavailable to the body. However, when they were fed at fairly high levels to chicks on rations containing adequate vitamin D, a more severe rickets than is usually obtained on a low calcium intake was observed. We immediately suspected that the deficiency was dependent upon a decreased phosphorus utilization due to the large amounts of aluminum and iron ingested.

Some work has been done on the effect of iron and aluminum on phosphorus assimilation. Cox, Dodds, Wigman and Murphy (1) using guinea pigs and rabbits found that rations containing soluble aluminum salts in excess of the total phosphorus in the diet produced a severe lowering of the blood phosphorus and bone ash. Ferric salts produced similar but less marked changes. They found that the addition of sodium acid phosphate would prevent these changes and concluded that the effects were due to a precipitation of ferric and aluminum phosphates in the tract. Brock and Diamond (2) working with rats found that the addition of large amounts of ferric or ferrous salts to a non-rachitogenic diet produced rickets. They also showed that additions of inorganic phosphate would prevent the rickets. Guyatt, Kay and Branion (3) obtained somewhat similar results with beryllium.

In this paper we wish to present the results obtained from iron and aluminum feeding in the case of chicks.

EXPERIMENTAL. Day-old White Leghorn chicks were used in all the work. They were housed in the usual way in warmed cages with wire screen bottoms. The basal ration used was the rachitic ration described by Hart, Kline and Keenan (4) supplemented with 1 per cent cod liver oil. This ration contains 0.91 per cent Ca, 0.51 per cent P and when supplied

[1] Published with the permission of the Director of the Wisconsin Agricultural

with adequate vitamin D allows rapid growth and normal bone formation. Detailed studies on blood calcium and phosphorus and bone ash in chicks on various modifications of this ration have been published by Elvehjem and Kline (5).

For bone analyses the left tibia was removed from each chick and stored in alcohol until a group of bones had collected. The bones were then crushed, extracted with hot alcohol for 72 hours, dried, and ashed in an electric furnace. The percentage of ash was calculated on the moisture- and fat-free basis. The blood calcium was determined on the serum by Clark and Collip's modification of the Kramer and Tisdall method and inorganic phosphorus by the Fiske and Subbarow method.

When chicks were placed on the basal ration in which the $CaCO_3$ was replaced by an equivalent amount of calcium as fused calcium silicate ($CaOAl_2O_3(SiO_2)_2$) the chicks grew fairly well but the bone ash at 5 weeks

TABLE 1

The effect of iron and aluminum salts on bone ash and blood phosphorus

GROUP NO.	SALTS IN RATION					BONE ASH	P PER 100 CC. SERUM	Ca PER 100 CC. SERUM
	$Fe_2(SO_4)_3$	Fe	$Al_2(SO_4)_3 \cdot 18H_2O$	Al	Na_2HPO_4			
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mgm.*	*mgm.*
1	3.22	0.90				23.41	4.6	12.2
2	2.42	0.67				25.47	4.4	12.2
3	1.61	0.45				27.68	2.7	12.7
4	3.22	0.90			5.77	44.51	6.8	10.4
5			5.37	0.44		31.74	3.5	10.6
6			4.03	0.33		24.61	2.4	12.3
7			2.69	0.22		25.98	2.3	14.0
8			5.37	0.44	5.77	44.72	6.2	10.5

of age was only 37 per cent. When the amount of silicate was doubled in an attempt to supply more available calcium the bone ash was only about 27 per cent at 5 weeks. The addition of the larger amount of the silicate had a very deleterious effect on bone formation. Additional results obtained with silicate are given in another paper (6).

In the next trial the calcium was supplied as calcium carbonate but sufficient ferric citrate or aluminum sulfate ($Al_2(SO_4)_3 \cdot 18H_2O$) was added to unite with the total phosphorus contained in the ration as $FePO_4$ or $AlPO_4$. In spite of the fact that the chicks received adequate calcium and vitamin D those fed the soluble aluminum salt exhibited severe symptoms of rickets as early as the tenth day and were all dead by the end of the third week. The bones contained an average of 31.74 per cent ash. The group on ferric citrate survived five weeks but had a bone ash of only 34.12

iron in the form of ferric citrate did not produce as severe a condition as the more soluble aluminum salt.

Since ferric citrate is somewhat insoluble and the iron less available to unite with the phosphates of the ration another experiment was conducted using ferric sulfate and aluminum sulfate. In this series three different levels of the salts were used. The highest level in both cases corresponded to equal amounts of iron or aluminum and phosphorus calculated on the basis of $FePO_4$ and $AlPO_4$. The intermediate level supplied 0.75 and the lowest level 0.5 of this amount. In addition one group on high aluminum and one on high iron received sufficient disodium acid phosphate to react with the added metals. The amount of each salt added is given in table 1. All groups received 1 per cent cod liver oil. Ten chicks were used in each

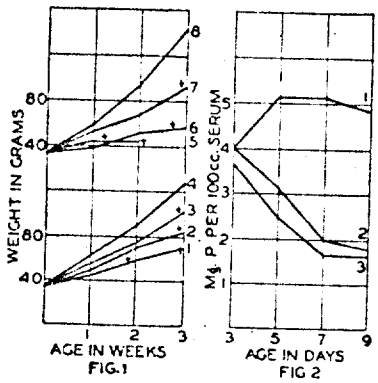

Fig. 1. Average growth records for chicks on iron and aluminum salts. 1, high iron; 2, intermediate iron; 3, low iron; 4, high iron plus phosphate; 5, high aluminum; 6, intermediate aluminum; 7, low aluminum; 8, high aluminum plus phosphate. Arrow indicates time at which majority of chicks exhibited severe rickets.

Fig. 2. Daily changes in blood phosphorus. 1, basal ration; 2, basal + 4.03 per cent $A_2(SO_4)_3 \cdot 18H_2O$; 3, basal + 2.42 per cent $Fe_2(SO_4)_3$.

group and the averages of the growth records for each group are given in figure 1. The arrow indicates the time at which the majority of the chicks exhibited rickets. It is readily seen that all chicks getting the iron or aluminum salt alone developed rickets between 1 and 3 weeks of age. The rickets developed in all cases at an earlier time than in chicks reared on a diet deficient in vitamin D alone. Rickets is very seldom observed in chicks on a standard rachitic ration before the fourth week.

Most of the chicks on the high aluminum were dead at 2 weeks of age while those on the high iron survived about 3 weeks. The chicks on the lower levels did not succumb but all showed signs of rickets. All remaining chicks were killed at 3 weeks of age for bone and blood analysis. The

very low ash content of the bones. The ash in the bones from the chicks on the highest level of aluminum was higher than the other groups, probably because these animals died very early. The values for the other groups were lower than those usually obtained for chicks of the same age deprived of vitamin D. The chicks receiving added phosphate gave bones with normal ash content. The inorganic phosphorus content of the blood of these chicks was also high while the animals receiving the iron or aluminum alone showed very low values. The group receiving the lowest level of iron and aluminum gave values slightly above 2 mgm. per 100 cc. of serum. The figures for the high levels ranged from 3.5 to 4.6 mgm. due undoubtedly to a dehydration of the blood during the period of very severe rickets. There was no drop in blood calcium. The values for calcium were increased somewhat when the blood phosphorus values were low.

TABLE 2

The effect of feeding iron and aluminum salts for 11 days on bone ash and blood phosphorus

GROUP NO.	SALTS IN RATION				BONE ASH	P PER 100 CC. SERUM
	$Fe_2(SO_4)_3$	Fe	$Al_2(SO_4)_3 \cdot 18H_2O$	Al		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*mgm.*
1	3.22	0.90			25.38	1.7
2	1.61	0.45			29.54	2.2
3			5.37	0.44	25.93	3.5
4			2.69	0.22	28.34	2.1
5					38.33	5.7

In order to study the possible effect of dehydration on the blood results and the rate at which the iron and aluminum affect the blood phosphorus another series of chicks was run for a shorter period of time. In this series only the highest and lowest levels of iron and aluminum were used and the animals were killed at 11 days of age. The results are given in table 2. Even at this early age the phosphorus values are very low as compared with the normal. Again the highest blood phosphorus values were found in the high aluminum group. Only two chicks in this group were able to stand when the blood sample was taken. They had consumed very little food the last two days. There was a spread of at least 10 per cent in the bone ash between the normal chicks and those fed iron and aluminum.

Three additional groups of chicks were started on the normal ration and on the intermediate levels of iron and aluminum. Five chicks from each group were killed for blood analyses at 3, 5, 7, and 9 days of age. The results are given in figure 2. The blood phosphorus at 3 days of age is 3.8 to 4.0 mgm. per 100 cc. serum in all groups. By the 6th day there is a

definite drop in the iron and aluminum groups and an increase in the normal group. Values as low as 1.5 to 1.7 are obtained by the ninth day in the groups without added phosphate.

Discussion. The results presented in this paper demonstrate conclusively that the addition of soluble iron and aluminum salts to a natural diet produces very severe rickets in chicks. Levels of iron and aluminum sufficient to unite with one-half the total phosphorus in the ration as $FePO_4$ or $AlPO_4$ will reduce the bone ash to 25 to 27 per cent at 3 weeks of age. Higher levels produce rickets in a shorter time. The rickets is undoubtedly due to a lowered blood phosphorus which may be observed within 5 days after the iron or aluminum is added and which may fall to levels of 1.5 mgm. per 100 cc. of serum.

From a practical point of view these observations may not be of great significance because iron and aluminum ingestion will never reach the levels used in the work in ordinary diets. However many workers are now recommending the use of very high doses of iron in the treatment of secondary anemia, dosages equivalent to the highest level used in these experiments. We have called attention to this fact in a recent paper (7) on the use of iron in the treatment of anemia in children. Precautions against high ingestion of iron should be made not only in the case of infants but also with adults because any change in the diet which will reduce the blood phosphorus to levels reported here surely will affect other biological processes in addition to bone formation.

The fact that one-half of the amount of iron and aluminum needed to combine with the total phosphorus in the diet will produce severe disturbances indicates that only a portion of the phosphorus in the ration is available. The possibility of variations in the availability of phosphorus in different rations was suggested in an earlier paper (4). Recently Bruce and Callow (8) point out that certain forms of phosphorus are less available than others. The use of diets high in iron or aluminum may be valuable for the determination of the availability of phosphorus compounds.

SUMMARY

1. Day-old chicks placed on a normal ration to which was added large amounts of soluble iron or aluminum salts (0.9 per cent Fe, 0.44 per cent Al) developed severe rickets in one to two weeks and were all dead within 3 weeks after being placed on the ration.

2. Levels of iron and aluminum salts equivalent to 0.5 and 0.75 of the amount necessary to unite with the total phosphorus in the ration as $FePO_4$ and $AlPO_4$ reduced the bone ash to about 25 per cent and the blood phosphorus to 2 to 4 mgm. per 100 cc. of serum at three weeks of age. Similar results were obtained after the chicks had been on the rations only 11 days.

Aluminium Hydroxide Granuloma

MAGDA ERDOHAZI, R. L. NEWMAN

British Medical Journal, 1971, 3, 621–623

It is well known that subcutaneous injection of triple vaccine may cause a granuloma, but it is usual for such nodules to subside without treatment after some months. In each of the two cases reported below a postimmunization granuloma was excised before its aetiology was recognized. The histological findings are of interest.

Case 1

On 25 July 1969 a 10-year-old boy presented with a swelling over his right triceps which had been gradually decreasing, but he complained of pain over the nodule and over the scapula. He had had an injection of purified toxoid aluminium hydroxide vaccine on 29 April 1969 and another on 12 June. On examination a firm tender subcutaneous nodule was present over the midpoint of the right triceps. It was not attached to the skin or bone nor inflamed. On excision it was solid, brownish in colour, measured 17 by 11 by 6 mm and appeared to be encapsulated. The cut surface showed a cicatricial centre containing yellowish creamy material (Fig. 1).

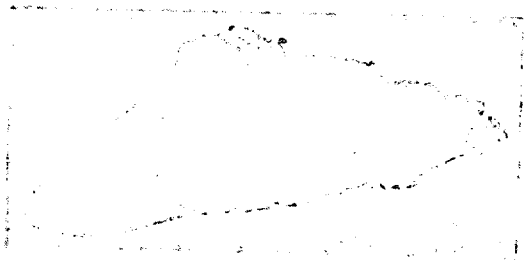

FIG. 1—Naked-eye appearance of a section of the granuloma in Case 1. (H. and E. × 4.)

Haematoxylin and eosin staining showed a centre of eosinophil granular necrotic tissue heavily invaded by polymorph granulocytes. Around the necrotic centre was a narrow zone of epithelioid histiocytes arranged in a loose palisade manner. Many had coalesced to form multinucleated giant cells. Peripherally the histocytes were

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obscured by a dense layer of lymphocytes, plasma cells, and eosinophil granulocytes. The pseudocapsule consisted of compressed collagen fibres infiltrated by inflammatory exudate. Collections of histiocytes and mononuclear leucocytes were also found outside the pseudocapsule. On careful search of several sections deeply basophilic, structureless material was seen in a few giant cells displacing the nuclei to the periphery. The nuclei were usually pyknotic and formed a wreath of rod-like structures outlining the cell boundary. The stored material appeared deep mauve with van Gieson's stain and gave a positive P.A.S. reaction. No iron pigment was present nor were any organisms seen on Gram or Ziehl-Neelsen staining. Aluminium salts were not looked for. Reticulin stain showed destruction of the reticulin fibres in the centre of the lesion. Most of the histiocytes contained P.A.S.-positive material.

Case 2

On 9 January 1970 a 7-year-old healthy girl presented with a small symptomless subcutaneous lump on the outer surface of her upper arm which had been present for about nine months and had been slowly decreasing in size. She had had a triple vaccine booster in December 1968.

On excision the lump (16 by 9 mm) discharged some milky white fluid. Macroscopically it was similar to that in Case 1, but there were important microscopical differences. The central necrotic focus showed no polymorph reaction and there were few lymphocytes and plasma cells. The histological picture was dominated by epithelioid histiocytes and numerous multinucleated giant cells. Many of the latter contained deeply basophilic inclusions which filled the cytoplasm and displaced the nuclei peripherally. The giant cells were mostly elongated or oval, with a frame of pyknotic rod-like nuclei outlining the cell boundary. In the same area there were also many elongated, slit-like or oval empty spaces outlined by rows of rod-shaped nuclei. In some of these spaces remnants of basophilic amorphous material were adherent to the "frame" (Figs. 2 and 3). Much of the basophilic material appeared extracellularly in the necrotic centre.

On van Gieson's staining the stored material was pinkish purple. It gave a positive P.A.S. reaction, and the von Kossa reaction for calcium was negative. Mallory and Parker's haematoxylin method for lead and copper (Culling, 1963) showed light grey granules in the stored material which faded with time. The acid solochrome cyanin method (Pearse, 1960) showed aluminium as a pinkish purple colour, while it was definitely pink by the aluminon method for aluminium hydroxide (King *et al.*, 1955). The physical chemistry department of Glaxo Laboratories detected aluminium hydroxide on x-ray crystallography. It was stressed, however, that the concentration was so low that without previous knowledge it might have been missed.

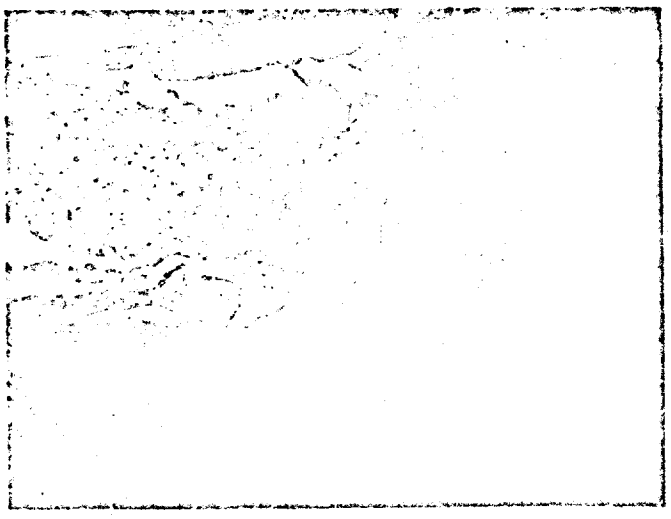

FIG. 2—Microphotograph showing aluminium hydroxide inclusions in giant cells and slit-like spaces outlined by nuclei (arrowed); Case 2. (H. and E. × 160.)

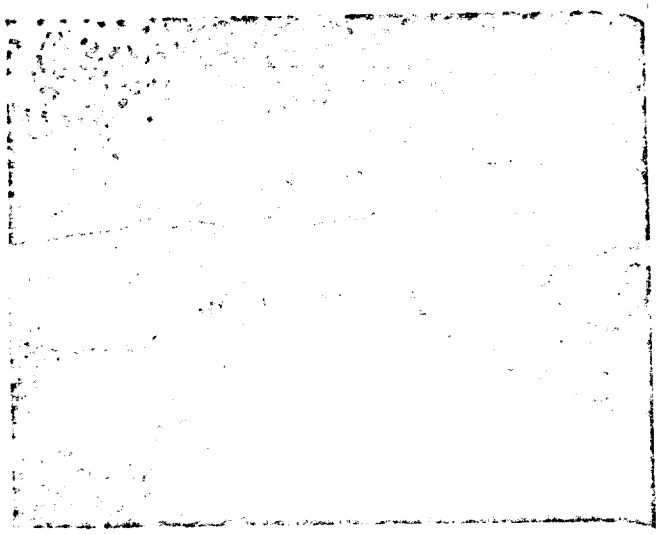

FIG. 3—Microphotograph showing aluminium hydroxide inclusions in giant cells (arrowed); Case 2. (H. and E. × 400.)

Comment

The appearance of the material stored in the granuloma in the second case and the site of the swelling in both cases suggested a metal salt adjuvant used in a vaccine as a possible cause of the reaction. According to information from Glaxo Laboratories (personal communication) the triple vaccine has been combined with aluminium hydroxide adjuvant since 1968 after a gap in its use between 1955 and 1968. Diphtheria and tetanus toxoid have always contained aluminium hydroxide.

We found no British report of excision of a granuloma caused by a vaccine adjuvant. The Medical Research Council's Committee on Clinical Trials of Influenza Vaccine (1955) reported swellings in 14 volunteers out of 399 at three months. The swellings gradually decreased in size. From Sweden, Orell (1962) reported on 15 subcutaneous lesions from the upper arms of healthy patients after mass influenza vaccination. On testing each constituent of the influenza vaccine by inoculating adult guinea-pigs he noted that aluminium oxide adsorbed influenza vaccine produced the characteristic histological appearance, but that it could also be produced by suspension of aluminium oxide in saline with gelatine and phenol (as used in the vaccine) and even by a suspension in saline without any addition. Orell concluded that in the causation of the granuloma the particle size of aluminium oxide was important, since injection of commercial aluminium oxide did not provoke granuloma formation in his experiments.

Voss and Tolki (1960) reported histological findings similar to our cases in a granuloma removed about one year after experimental vaccination with an aluminium oxide adsorbed antiviral vaccine. They claim to have demonstrated aluminium oxide crystals "staining orange with Azan or in the form of Azan-blue-protein complex." Lenz (1966) observed a similar granuloma caused by tetanus toxoid administered by the jet-injection method.

Our two cases are of interest in that conclusive evidence of the presence of aluminium salts was found in one case, and strongly suggestive histological evidence in the other.

We are indebted for the clinical data to Mr. G. F. Walker and Mr. D. M. Forrest, under whose care the patients were admitted. The physical chemistry department of Glaxo Laboratories Ltd., Greenford, Middlesex, performed the x-ray crystallography by kind arrangement with Mr. W. J. Watling, of the medical department. Mr. G. Anderson did the histochemical preparations, and Mr. N. G. Le Page produced the photographs.

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#6

ALUMINUM PHOSPHATE IN THE THERAPY OF PEPTIC ULCER

EFFECT OF ALUMINUM HYDROXIDE ON PHOSPHATE ABSORPTION

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CHICAGO

Our use of an aluminum phosphate gel in the therapy of peptic ulcer resulted from observations on the effect of aluminum hydroxide gel on the absorption of phosphates from the intestine. In a publication [1] on the effect of aluminum hydroxide gel on the well-being and longevity of Mann-Williamson dogs, Fauley, Ivy, Terry and Bradley suggested that the unfavorable effects of this chemical in such animals might be due to an interference with the absorption of phosphates. The present report (*a*) includes data which demonstrate that aluminum hydroxide gel interferes with phosphate absorption and (*b*) presents the results of the use of aluminum phosphate gel in the treatment of experimental and clinical peptic ulcer.

Experimental ulcers were produced in Mann-Williamson dogs. The preparation of such an animal includes performing a gastrojejunostomy and diverting the pancreatic juice and bile into the terminal portion of the ileum (last 20 to 25 cm.). Of 42 dogs so operated on and fed a diet of meat, bread, milk and cod liver oil (8 cc. daily), jejunal ulcer developed in all and all died in seventeen weeks (average, eleven weeks). This experimentally induced ulcer is analogous to the ulcer that sometimes occurs after gastrojejunostomy in man. The diversion of pancreatic juice and bile to the terminal portion of the ileum in the dog markedly increases the incidence of jejunal ulcer. Much experimental

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

Read before the Section on Pathology and Physiology at the Ninety-First Annual Session of the American Medical Association, New York, June 13, 1940.

1. Fauley, G. B.; Ivy, A. C.; Terry, L., and Bradley, W. B.: An Attempt to Prevent Post-Operative Jejunal Ulcer by Aluminum Hydroxide Therapy, Am. J. Digest. Dis. **5**:792, 1939.

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67:563-572 (19[illegible]

work has shown that at least three factors are concerned in the development of this experimental ulcer;[2] the most important one is the acid factor; another is the nutritional factor, since the diversion of pancreatic juice and bile to the lower part of the ileum disturbs intestinal digestion, as well as the neutralization of acid chyme, and the third factor is the increased sensitivity of the jejunal mucosa to gastric chyme as compared with that of duodenal mucosa.

When aluminum hydroxide was given to Mann-Williamson dogs, either four times daily or at hourly intervals from 8 a. m. to 8 p. m., with complete control of gastric acidity for that period, the development of ulcer was not prevented.[1]

After these animals received aluminum hydroxide for three or four weeks anorexia developed. It was thought that the anorexia might counteract any beneficial effect the aluminum hydroxide gel might otherwise exert. Since anorexia is a conspicuous symptom of phosphorus deficiency[3] and since soluble aluminum compounds in the diet may produce rickets and a low level of phosphorus in the blood,[4] it seemed probable that these animals had a phosphorus deficiency.

ALUMINUM HYDROXIDE AND PHOSPHORUS METABOLISM

The following data demonstrate the effect of aluminum hydroxide on the absorption of phosphates from the intestine. The preparation of aluminum hydroxide was the same as that used in previous studies.[5]

2. (*a*) Ivy, A. C., and Fauley, G. B.: Factors Concerned in Determining the Chronicity of Ulcers in the Stomach and Upper Intestine, Am. J. Surg. **11**:531, 1931. (*b*) Fauley, G. B., and Ivy, A. C.: The Prevention of Post-Operative Jejunal Ulcers by Diet and Fundusectomy, Surg., Gynec. & Obst. **63**: 717, 1936. (*c*) Morton, C. B.: Observations on Peptic Ulcer, Ann. Surg. **85**:207, 229 and 879, 1927. (*d*) Orndorff, J. R.; Fauley, G. B., and Ivy, A. C.: The Prophylactic Value of Gastric Mucin in the Therapy of Post-Operative Jejunal Ulcer: An Experimental Study, Am. J. Digest. Dis. **3**:26, 1936. (*e*) Mathews, W. B., and Dragstedt, L. B.: The Etiology of Gastric and Duodenal Ulcers, Surg., Gynec. & Obst. **55**:265, 1932. (*f*) McCann, J. C.: Experimental Peptic Ulcer, Arch. Surg. **19**:600 (Oct.) 1929.

3. Kleiber, M.; Goss, H., and Guilbert, H. R.: Phosphorus Deficiency Metabolism and Food Utilization in Beef Heifers, J. Nutrition **12**:121, 1936.

4. Cox, G. J.; Doods, M. L.; Wigman, H. B., and Murphy, F. J.: The Effect of High Doses of Aluminum and Iron on Phosphorus Metabolism, J. Biol. Chem. **92**:11, 1931. Jones, J. H.: The Metabolism of Calcium and Phosphorus as Influenced by the Addition of Salts of Metals Which Form Insoluble Phosphates, Am. J. Physiol. **124**:230. 1938. Deobald, H. J., and Elvehjem, C. A.: The Effect of Feeding High Amounts of Soluble Iron and Aluminum Salts, ibid. **111**: 118, 1935. McKenzie, K.: The Biochemistry of Aluminum, Biochem. J. **24**:1433, 1930; **25**:287, 1931.

5. Ivy, A. C.; Terry, L.; Fauley, G. B., and Bradley, W. B.: The Effect of Administration of Aluminum Preparations on the Secretory Activity and Gastric Acidity of the Normal Stomach, Am. J. Digest. Dis. **3**:879, 1937.

The Effect of Aluminum Hydroxide Gel on the Excretion of Phosphorus in the Urine and Feces of Dogs.—The data in table 1 show definitely that aluminum hydroxide gel given to normal dogs decreases the excretion of phosphorus in the urine and increases the excretion of phosphorus in the feces. They illustrate further that when a diet adequate in phosphorus and calcium is fed phosphorus is retained in the body, but that when aluminum hydroxide in relatively large doses is administered with such a diet no phosphorus is retained. However,

TABLE 1.—*Effect of Aluminum Hydroxide Gel on Excretion of Phosphorus in the Urine and Feces of Dogs*

Dog No.	Procedure	Average, 3 Day Periods: Urinary P, Gm.	Fecal P, Gm.	Total P, Gm.	Comment	Dog No.	P in 3 Day Sample of Urine, Gm.
	Diet A,* Containing 3.9 Gm. of P and 3.6 Gm. Ca in 3 Days						
1†	No $Al(OH)_3$	1.18	1.61	2.79	P retained	3	1.68
		1.53	2.40	3.73	0.44 Gm.		1.08
		1.21	2.72	3.93			
		1.47	1.91	3.38			
	Average	1.30	2.16	3.46	.....	...	1.08
	With 130 cc. 5% $Al(OH)_3$	0.48	3.48	3.96	No P retained	3	0.68
		0.29	1.70	4.99			0.32
		0.37	2.56	3.33			
	Average	0.38	3.71	4.09	.....	...	0.50
	Diet B, Containing 3.0 Gm. of P and 0.216 Gm. Ca in 3 Days						
2	No $Al(OH)_3$	1.34	0.42	1.76	P retained	4	2.34
		2.56	0.46	3.02	0.5 Gm.		2.56
		1.44	0.47	1.91			2.10
		2.54	0.76	3.30			2.08
	Average	1.97	0.53	2.50	.....	...	2.27
	With 130 cc. 5% $Al(OH)_3$	0.78	1.53	2.31	P retained	4	0.65
		0.57	1.21	1.78	0.57 Gm.		0.65
		1.32	2.07	3.39			0.85
		0.64	1.20	1.84			
		0.91	1.95	2.86			
	Average	0.84	1.59	2.43	.....	...	0.72

* Diet A contained meat, cereal, vitamins and bone meal; diet B contained beef hearts alone.
† Dog 1, on diet A, illustrates the effect of aluminum plus calcium on the diminution of phosphorus absorption; dog 2, on diet B, illustrates the effect of aluminum alone.

when the diet is lean meat, aluminum hydroxide gel in the dose used does not materially disturb the retention of phosphorus. It may be concluded that when an ordinary diet is fed aluminum hydroxide gel interferes with the absorption of phosphates in the dog.

The Influence of Aluminum Hydroxide Gel on the Urinary Excretion of Phosphorus in the Normal Adult Human Subject Receiving a "Light Ulcer Diet."—Four men were placed on a "light ulcer diet" containing approximately 2 Gm. of phosphorus and 2 Gm. of calcium daily. They took the diet for twelve days. During the first five days no aluminum was administered, and an average of 1.1 Gm. of phosphorus was excreted

daily in the urine (table 2). During the next seven days a total of 240 cc. of a 5 per cent aluminum hydroxide gel, divided into three doses, was consumed daily with the meals, and an average of 0.44 Gm. of phosphorus was excreted daily. Thus, the dose of aluminum hydroxide used reduced the excretion of phosphorus in the urine an average of 60 per cent when a light ulcer diet relatively high in phosphorus (2 Gm.) and calcium (2 Gm.) was ingested.

The Influence of Aluminum Hydroxide Gel on Urinary and Fecal Excretion of Phosphorus in a Child with Marble Bone Disease on a Low Phosphorus Diet.—A 5 year old child with marble bone disease was placed on a low phosphorus diet accompanied by aluminum hydroxide therapy with the idea that the progress of the disease might at least

TABLE 2.—*Influence of Aluminum Hydroxide Gel on the Urinary Excretion of Phosphorus in Normal Adult Subjects Receiving a Light Ulcer Diet* *

Day	Subject; Gm. P per 24 Hr. 1	2	3	4	Comment
1	0.56	1.26	0.96	0.78	Diet alone containing 2 Gm. P by calculation
2	0.95	1.24	1.49	0.92	
3	1.19	1.53	1.15	1.08	
4	1.01	0.99	1.09	1.01	
5	1.13	0.98	1.13	1.37	
Average	0.97	1.20	1.20	1.05	1.10
6	0.68	0.87	0.73	0.56	Diet plus 240 cc. of 5% $Al(OH)_3$
7	0.36	0.60	0.45	0.30	
8	0.40	0.56	0.42	0.29	
9	0.38	0.51	0.85	0.34	
10	0.39	0.37	0.28	0.28	
11	0.25	0.28	0.64	0.13	
12	0.12	0.35	0.62	0.27	
Average	0.37	0.51	0.57	0.32	0.44

* Whole milk, 1,000 cc.; 18 per cent cream, 400 cc.; butter, 50 Gm.; white bread, 60 Gm.; gelatin, 100 Gm. (dry weight); orange juice, 100 cc.; chicken, 60 Gm.; dry cereal, 20 Gm., and potatoes, 200 Gm.

be retarded. Patients with this disease have an extraordinary tendency to retain phosphorus. Inspection of the data in table 3 shows that the child on the low phosphorus diet retained phosphorus even when 4 cc. of a 5 per cent aluminum hydroxide gel was given four times daily. When the dose of aluminum hydroxide was increased to 30 cc. four times daily the phosphorus balance became negative. The results show that even in marble bone disease a negative phosphorus balance may be produced by aluminum hydroxide when the diet is low in phosphorus.

The Extraction of Phosphorus from the Intestinal Mucosa by a Mixture of Aluminum Hydroxide Gel and Hydrochloric Acid.—The following typical protocol of an experiment illustrates that in the absence of bile and pancreatic juice a mixture of 5 per cent aluminum hydroxide gel and tenth-normal hydrochloric acid extracts phosphorus from the

body fluids: A. A cannula was inserted and the small intestine was irrigated with warm physiologic solution of sodium chloride. B. Two hours later the intestine was again irrigated and the solution analyzed for phosphorus: 3.62 mg. of phosphorus was obtained. C. A mixture of 100 cc. of 5 per cent aluminum hydroxide and 200 cc. of tenth-normal hydrochloric acid was introduced into the intestine. D. Two hours later the mixture was removed and the intestine was irrigated with warm physiologic solution of sodium chloride. Forty-one milligrams of phosphorus was obtained. When 100 cc. of a 5 per cent aluminum hydroxide gel alone was used 16 mg. of phosphorus was obtained.

TABLE 3.—*Influence of Aluminum Hydroxide Gel on Urinary and Fecal Excretion of Phosphorus in a Five Year Old Child with Marble Bone Disease on a Low Phosphorus Diet* *

Phosphorus Intake 0.2 Gm. Daily, or 1.0 Gm. in 5 Days

Five Day Periods	Phosphorus in Gm. for 5 Day Period			Procedure; Phosphorus Retention
	Urine	Feces	Total	
1	0.55	0.31	0.86	Low P diet, 1 Gm. in 5 days
2	0.55	0.14	0.69	
Average	0.55	0.225	0.775	P retention, 0.225 Gm.
1	0.35	0.55	0.90	Low P diet plus 1 drachm (3.7 cc.) 5%
2	0.18	0.99	1.17	$Al(OH)_3$ q. i. d.
3	0.044	0.60	0.644	
Average	0.191	0.71	0.901	P retention, 0.1 Gm.
1	0.011	1.40	1.411	Low P diet plus 1 ounce (29.57 cc.) 5%
2	0.006	1.50	1.506	$Al(OH)_3$ q. i. d.
Average	0.0085	1.55	1.558	P loss, 0.558 Gm.

* Dr. W. M. Clifton and the staff of the Children's Memorial Hospital cooperated in the metabolic study on the child with marble bones.

These results show that aluminum hydroxide alone or a mixture of aluminum hydroxide and aluminum chloride in the intestines in sufficient concentration may extract phosphorus from the mucosa.

Comment.—The results of the foregoing experiments show, as might be predicted, that aluminum hydroxide interferes with the absorption of phosphates and under certain conditions can cause a negative phosphorus balance. A phosphorus deficiency is not to be expected until the soluble aluminum salts, aluminum chloride for example, are in excess of one-half the total phosphorus in the diet. When this obtains in rats, chicks, guinea pigs and rabbits, the inorganic phosphorus in the blood is likely to be reduced.[4] Conditions that would predispose to the development of a negative phosphorus balance on aluminum hydroxide gel therapy are not likely to be present for a prolonged period in the patient with ulcer. The ordinary ulcer diets contain adequate phosphorus. Still the

possibility has to be considered, and it should be carried in mind that anorexia, though not pathognomonic of phosphorus deficiency, is a conspicuous symptom of this condition.

In order to test aluminum hydroxide therapy under controlled conditions in laboratory animals, the experimental conditions should simulate as closely as possible the actual conditions in man; otherwise the results apply only to the experimental animals and cannot be applied interpretatively to man. In the Mann-Williamson dog a postoperative jejunal ulcer develops when the alkaline juices enter the lower part of the ileum. This renders the Mann-Williamson dog more susceptible to the production of a phosphorus deficiency by aluminum hydroxide gel than a patient, unless the patient has a gastroenterostomy associated with a high grade deficiency of pancreatic juice and bile, or has relative hypersecretion of gastric juice, which will change much of the aluminum hydroxide gel into aluminum chloride. Aluminum hydroxide forms aluminum chloride on reacting with hydrochloric acid, and the aluminum chloride will react with soluble phosphate to form insoluble aluminum phosphate. The absence of the alkaline pancreatic juice from the upper part of the intestine might favor the formation of aluminum phosphate, since the aluminum would probably exist longer in the form of the chloride rather than undergo rapid conversion to the less reactive, insoluble aluminum hydroxide. Further, the Mann-Williamson dog, like some patients with jejunal ulcer, usually has an increased rate of passage through the alimentary canal and loose stools, which predisposes to an inadequate absorption of minerals.

In view of the foregoing considerations, it was pointed out in the paper by Fauley, Ivy, Terry and Bradley [1] that their results with aluminum hydroxide gel in the Mann-Williamson dog are not necessarily applicable to the ordinary human patient with peptic ulcer. It should also be evident that in order to test the theoretic therapeutic advantages of "aluminum" therapy in our experimental animals, it would be necessary to use an aluminum phosphate gel.

PREVENTION AND HEALING OF ULCER WITH ALUMINUM PHOSPHATE GEL

The Aluminum Phosphate Gel Used.—The aluminum phosphate was prepared in the form of a gel by precipitating aluminum chloride with dibasic sodium phosphate (Na_2HPO_4), adjusting the p_H, washing free of salt and adjusting the gel to a concentration by chemical analysis, of 4 Gm. of aluminum phosphate per hundred cubic centimeters of solution.[6] The p_H of the gel used was about 6.5. One hundred cubic centimeters of the 4 per cent gel buffered 100 cc. of tenth-normal hydrochloric acid at

6. The gel was prepared in quantity for us by Wyeth & Brother, Inc.

37 C., a p_H of 4.0 (Töpfer's indicator) being used as the end point of the titration. More accurately, 30 cc., or 2 equivalents, of the gel raises the p_H of 150 cc. of tenth-normal hydrochloric acid from 1.0 to 2.4. The antacid value of the 4 per cent aluminum phosphate gel (4 per cent aluminum phosphate) is slightly less than one-half that of the 4 per cent aluminum hydroxide gel (4 per cent aluminum oxide, Al_2O_3). The antacid value of various compounds is shown in figure 1.

The administration of 120 cc. daily of the aluminum phosphate gel to 2 dogs caused a slight increase in the urinary excretion of phosphates.

Prevention of Ulcer.—Both the treated and the control animals received a daily diet consisting of 200 Gm. of raw ground pancreas,

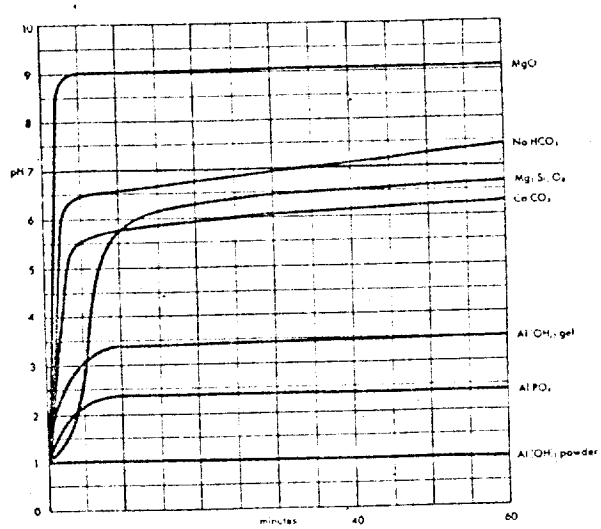

Fig. 1.—Reaction rate of 2 equivalents of various antacids and 150 cc. of tenth-normal hydrochloric acid at 37 C. with stirring and use of the glass electrode.

200 Gm. of raw ground liver, 100 cc. of milk, 450 Gm. of a commercial dog food (diet A) and 8 cc. of cod liver oil. The diet was fed ad libitum twice daily, at 9 a. m. and 4 p. m.

Sixteen control animals died with ulcer in from two to five months after the operation (Mann-Williamson, with an end to side anastomosis), the average time of survival being seventeen weeks.

Twenty-three treated animals received 100 cc. of the aluminum phosphate at 7 a. m., 12 noon, 5 p. m. and 10 p. m. Ulcer developed in only 3, or 13 per cent, of the animals. If one omits the 3 animals which died without ulcer under five months, then ulcer developed in 3, or 15 per cent, of 20 animals (table 4).

Comment.—This is a striking result, the best that we have obtained with any therapy.

Healing of Ulcer.—The aluminum phosphate therapy was discontinued in the case of 2 animals in which an ulcer had not developed (verified by exploration) ten months after the Mann-Williamson operation. Loss in weight and tarry stools developed in 1 of these animals in nine weeks, and in the other in fourteen weeks. On restoration of therapy, the ulcer was found (by exploration) to be healed in sixteen weeks in the first dog and in eleven weeks in the second dog. This procedure was repeated twice on the same dogs. With the third recurrence of ulcer on withdrawal of aluminum phosphate, the ulcer was found healed in four weeks (etherization and autopsy) in 1 dog and in

TABLE 4.—*Results of the Use of Aluminum Phosphate Gel on Mann-Williamson Dogs*

No. of Dogs	Time in Months	Ulcer	Cause of Death
16 control animals	2 to 5, average 17 weeks	All	
	Twenty-Three Animals Treated with $AlPO_4$		
3 animals dying with ulcer *	2, 5.5, 9	All	Perforation
3 animals dying under 5 months	4, 4, 4.5	None	Intussusception; intra-abdominal hernia; meningitis
7 animals dying after 5 months	6, 6, 6.5, 6.5, 9, 11, 12	None	In 4, pneumonia after an exploratory operation or distemper;† in 2, intra-abdominal hernia or adhesions; in 1, abscess of the liver
4 animals.....	24	None	Etherized ‡
2 animals.....	21	None	Etherized ‡
1 animal.....	19	None	Etherized ‡
1 animal.....	17	None	Etherized ‡
1 animal.....	16	None	Etherized ‡
1 animal.....	13.5	None	Died after an exploratory operation; intestinal obstruction

‡ All these animals in excellent health, 1 to 3 lb. (0.5 to 1.3 Kg.) above preoperative weight

* Ulcer developed in only 3 of the 23 animals treated with aluminum phosphate gel.

† An exploratory operation was performed to prove the absence of ulcer. For example, such an operation was performed on dog 11 ten months after the initial operation. No ulcer was found. Treatment was withdrawn, and tarry stools appeared nine weeks later. Treatment was started, and the ulcer was pronounced clinically healed in four weeks. The dog died two weeks later from obstruction due to adhesions. The ulcer was healed.

five weeks (exploration) in the other dog. These results, along with those in 8 other dogs, are shown in table 5. Healing was demonstrated in all dogs by exploration or by autopsy. Spontaneous remissions of an ulcer in Mann-Williamson dogs have not been observed.

Gastric Analyses.—An analysis of the gastric contents of the treated animals was made at 11 a. m. on a number of occasions. Free acid was uniformly found. Thus, gastric acidity was not completely controlled.

Nutrition.—The general nutrition of the animals was always excellent, except when the therapy was withdrawn in the experiments on healing. All of the animals gained weight under therapy and while on treatment were heavier than they were before operation.

Anemia.—After the animals had received aluminum phosphate gel for several months, anemia was observed (table 6), although there was

no evidence of the presence of ulcer. It was suspected that the anemia was due to iron deficiency, although there is no direct evidence showing that excessive phosphates in the diet actually interfere with the absorption of iron.[7] One would not necessarily expect aluminum phos-

TABLE 5.—*Results of Aluminum Phosphate Gel Therapy on the Healing of Ulcer* *

Dog † No.	No Therapy, Ulcer Developed, Weeks	Therapy, Ulcer Healed, Weeks	No. Therapy, Ulcer Developed, Weeks	Therapy Ulcer Healed, Weeks	Comment
1	9	16	6	4	Killed at 20 months; scar found
2	14	11 (exploratory operation)	7	5	Exploratory operation; no ulcer at 24 months
3	16	12	Exploratory operation 1 month later; scar found		
4	11	10	Exploratory operation 1 month later; scar found		
5	6	8	Exploratory operation 6 weeks later; scar found		
6‡	5	10	Exploratory operation; healed scar		
7	9	5	Exploratory operation at 1 month; healed scar found		
8	8	8	Exploratory operation 6 weeks later; scar found		
9	9	7	Exploratory operation 2 months later; scar found		
10	8	8	Died of obstruction at 12.5 months; ulcer healed except for an area 1 by 2 mm.		

* The dogs did not have an ulcer nine to eleven and a half months after the beginning of the experiment, as shown by exploratory operation. The presence of ulcer was diagnosed by loss of weight and by the appearance of anorexia and tarry stools. The healing of ulcer was diagnosed clinically by the disappearance of tarry stools and anorexia and by the return to the original weight. It was diagnosed anatomically by exploration or by autopsy.

† The 20 and 24 months referred to in the case of dogs 1 and 2 means that the dogs were observed for that period and then autopsies were performed or they underwent exploratory operation and no ulcer was found. Dogs 3 to 9 are still alive at the end of a period of from 18 to 24 months.

‡ In this dog on withdrawal of therapy a second time an ulcer developed which refused to heal (exploration) completely. The dog refused to eat raw liver and pancreas, which was then fed by force. The ulcer healed in six weeks.

TABLE 6.—*Presence of Anemia and Response to Parenteral Iron Therapy in Mann-Williamson Dogs Receiving Aluminum Phosphate*

	No. of Dogs	Red Blood Cells	Hemoglobin, Gm./100 Cc.	Hematocrit Reading
Normal for dogs before operation	30	Range 5,760,000-8,000,000 Average 6,900,000	Range 12-16 Average 14.5	Range 41-55 Average 47.5
Before iron therapy.....	12	Range 3,300,000-5,700,000 Average 4,700,000	Range 9.1-13 Average 11.0	Range 25-41 Average 35.0
One to 2 months after iron therapy	11	Range 4,800,000-7,260,000 Average 6,330,000	Range 10.2-14.3 Average 12.7	Range 34-46 Average 41.4
Final, 2 to 4 months on iron therapy	11	Range 5,470,000-8,000,000 Average 6,800,000	Range 11.3-16.0 Average 13.4	Range 35-48 Average 42.6

phate to interfere chemically with the absorption of iron, yet this should be determined by actual experiment.

Since we could not afford to lose time in the management of our animals, 35 mg. of iron in the form of a dialyzed preparation of ferric

7. Brock, J. F., and Diamond, L. K.: Rickets in Rats by Iron Feeding, J. Pediat. **4**:442, 1934. Day, H. G., and Stein, H. J.: The Effect upon Hematopoiesis of Variations in the Dietary Levels of Calcium, Phosphorus, Iron and Vitamin D, J. Nutrition **16**:525, 1938.

oxide (a 0.5 per cent solution[8]) was given intravenously each week. The hemoglobin content and red cell count returned to control levels (table 6).

It should be noted that the stools of the dogs given aluminum phosphate gel are firm, whereas the stools of untreated Mann-Williamson dogs are usually mushy.

Comment.—We have used various therapeutic agents, such as alkalis, gastric mucin, various diets and aluminum hydroxide gel, on Mann-Williamson dogs. None has yielded such decisively favorable results as aluminum phosphate gel. Gastric mucin is definitely better than alkalis and aluminum hydroxide gel, as used by Orndorff, Fauley and Ivy,[24] but is inferior to aluminum phosphate gel. The adjunctive role played by the diet we use is illustrated by dog 6, table 5. An unfavorable observation is the fact that aluminum phosphate gel in the relatively large doses used with the animals apparently caused anemia, but this condition may tend to occur when aluminum therapy of any sort is used with Mann-Williamson dogs. This cannot be decided for aluminum hydroxide gel, since this preparation does not prevent ulcers from developing which per se cause anemia. This observation emphasizes the predictable fact that the administration of large doses of mineral salts which react with the acidic or alkaline juices, whether such salts are absorbed or not, interferes with the natural mineral metabolism of the body. However, this disadvantage appears to be reduced to a minimum by the use of a relatively inert substance, such as aluminum phosphate, which buffers acid and has a mildly astringent effect.

The results with aluminum phosphate gel were so favorable that we decided to administer it to patients with peptic ulcer to ascertain if it might be of value in their management.

USE OF ALUMINUM PHOSPHATE GEL IN TREATMENT OF PATIENTS

Duodenal and Gastric Ulcer.—All except 6 of the patients were treated without rest in bed; 1 of these had a profuse hemorrhage, and the others had severe distress which failed to respond to other managements.

An ulcer diet was prescribed during the active stage of the ulcer, but it was not carefully followed by one fifth of the patients (dispensary patients). During the active stage of the ulcer, a dose of 15 cc., or occasionally 30 cc., of aluminum phosphate gel every two hours was prescribed. Later in the course of management, a dose of 50 cc. with the meals and at bedtime, or 30 cc. six times daily with and between meals, was prescribed.

8. Prepared by Wyeth & Brother, Inc.

The type of ulcer treated and the results, except in the 3 cases of gastrojejunal ulcers, are shown in table 7. The diagnosis in all cases was supported by roentgen examinations.

The ulcer craters filled in (fig. 2), with 1 exception. This was the case of a patient whose condition was diagnosed as "subacute perforation" of a duodenal ulcer. This patient was not relieved by any therapy and was subjected to operation. Another failure occurred in the case of a patient with duodenal ulcer who had had many recurrences. In this instance, night distress was not controlled. He responded to rest, mucin and aluminum phosphate gel. Another patient with duodenal

TABLE 7.—*Results of Aluminum Phosphate Therapy in Human Patients*

Patients with duodenal ulcer: total 32; bleeding 24; hemorrhage 2; crater 6
Patients with gastric ulcer: total 2; bleeding 2; hemorrhage 1; crater 2
Dose of $AlPO_4$: 15 cc. every 2 hr. (300 cc. per day), or 50 cc. with meals t. i. d. and at bedtime

Group I. Patients with first attack	
A. Response to $AlPO_4$	5
B. No response to $AlPO_4$	6
*C. Poor response to other management but active response to $AlPO_4$	2
D. Failure to respond completely to any management	0
Group II. Patients with numerous recurrences	
A. Complete response to other managements and also to $AlPO_4$	15
*B. Complete response to other managements during previous attacks but not during the present attack; complete response to $AlPO_4$	4
*C. Response to a management during one or two attacks; no response to the same management, but active response to a different management during succeeding attacks; response to $AlPO_4$ during present attack	8
D. Incomplete response to all managements, including $AlPO_4$	4

* These results are not to be given undue weight, since the severity of different attacks in the same patient varies. Lack of response to a management in two to five weeks followed by a prompt response to a change in management does not mean necessarily that the second management is better than the first, because continuation of the first management may have been successful if given more time and the psychotherapy of a change or "the new" may be involved.

ulcer had a hemorrhage after fourteen days under treatment with aluminum phosphate and an ambulatory regimen. He then responded promptly to management with rest, restricted diet, mucin and aluminum phosphate. Treatment of a fourth patient with duodenal ulcer was counted a failure because he complained of intermittent atypical distress during several months of therapy. The same distress occurred regardless of the type of management. Since the roentgen signs improved during management, hypochondria was suspected.

An accurate, statistical statement cannot be made regarding the time of relief from distress and the disappearance of blood because of the variability (twenty-four hours to three weeks) in this relatively small series of patients. The hemoglobin content of the blood and the erythrocyte count were followed in the majority of patients. A tendency

to anemia was not observed. A tendency to constipation (hard stools) was noted, and some type of liquid petrolatum was rather frequently administered.

Postoperative Gastrojejunal Ulcer.—Three patients with marginal or jejunal ulcer were available for treatment. The condition of 1 patient, during a third recurrence, was diagnosed as "subacute perforation," with rigidity, an elevated temperature and a high leukocyte count. He did not respond to mucin and calcium carbonate therapy for one week, but responded promptly to aluminum phosphate therapy and became ambulatory in ten days. He continued to take aluminum phosphate for six months, after which he stopped treatment, and distress returned a year later. This recurrence responded promptly to aluminum phosphate therapy. The second patient, while on alkaline therapy, was hospitalized for a subtotal gastrectomy. Aluminum phosphate was substituted for

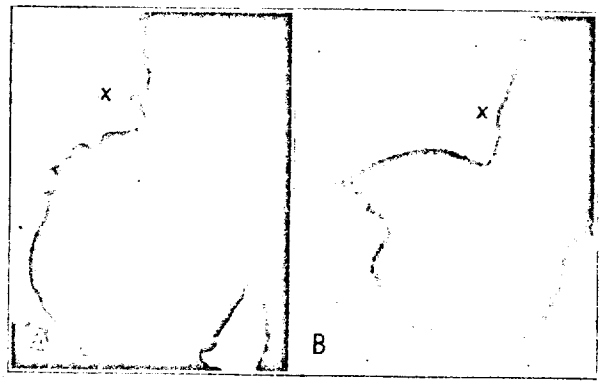

Fig. 2.—The response of a gastric ulcer to aluminum phosphate gel. *A*, gastric ulcer with crater. *B*, disappearance of crater. This picture was made eight weeks after institution of therapy with aluminum phosphate gel combined with a "soft" diet under ambulatory management.

the alkalis, and the patient was sent home after ten days without an operation, which still, six months later, with the patient under aluminum phosphate management, appears to be unnecessary. The third patient, during a third recurrence, responded promptly to aluminum phosphate gel. This patient had responded well to gastric mucin during the previous recurrences. We hope to study other patients of this type because of the similarity of their condition to that in our experimental animals.

Comment.—With reference to table 7, the results in patients in group I C and group II B and C should not be interpreted as indicating that aluminum phosphate therapy is superior to other therapies. It must be kept in mind that the severity of attacks varies even in the same

patient. Lack of response to a management in from two to five weeks followed by a prompt response to a change in management does not necessarily mean that the second management is better than the first. The first management might have been successful if it had been continued longer. Also, the possibility of a psychic factor and of a spontaneous remission has to be considered, factors that are not encountered in our animal experiments. The results in the patients only indicate that aluminum phosphate gel has the promise of being as effective as any other similarly acting medicament in the management of peptic ulcer. This promise is supported, we believe, by the most decisive results of animal experimentation to be found in the literature. As stated before, our experimental results do not necessarily imply that aluminum phosphate gel is superior to aluminum hydroxide gel for the management of peptic ulcer in man except in the presence of a relative or an absolute deficiency of pancreatic juice and bile.

COMMENT

We were particularly interested in testing aluminum hydroxide gel on Mann-Williamson dogs because it was found by Orndorff and his associates[2d] and by McCann[2f] that such dogs usually manifest a continuous secretion of gastric juice, which apparently cannot be controlled by atropine. This observation suggested (Orndorff and his associates[2d]; Ivy and Bachrach[8a]) that the hypersecretion might be due to the elaboration of histamine by the irritated jejunal mucosa in amounts too large to be inactivated by the histaminase in the irritated mucosa. It was thought that an astringent and demulcent, such as aluminum hydroxide, might reduce the irritation and reduce the hypersecretion.[4] In addition, it would serve as an antacid. We believe, as has been suggested by Einsel, Adams and Myers and associates[9] that the astringent and the demulcent properties of aluminum hydroxide or aluminum phosphate, as well as their antacid property, are of therapeutic value. It is also possible that they increase the secretion of mucus. But how they actually operate to produce a favorable effect is still an open question.[10]

8a. Ivy, A. C., and Bachrach, W. H.: An Abnormal Mechanism for the Excitation of Gastric Secretion in the Dog, Am. J. Digest. Dis. **7**:76, 1940.

9. Einsel, I. H.; Adams, W. L., and Myers, V. C.: Aluminum Hydroxide in the Treatment of Peptic Ulcer, Am. J. Digest. Dis. **1**:513, 1934. Adams, W. L.; Einsel, I. H., and Myers, V. C.: Aluminum Hydroxide as an Antacid in Peptic Ulcer, ibid. **3**:112, 1936. Quigley, J. P.; Einsel, I. H., and Meschan, I.: Some Effects Produced in the Normal Stomach by the Ingestion of Moderate and Massive Quantities of Aluminum Hydroxide Gel, J. Lab. & Clin. Med. **24**:485, 1939.

10. Komarov, S. A., and Krueger, L.: The Effect of Aluminum Hydroxide Gel on Gastric Secretion in the Dog, Am. J. Digest. Dis. **7**:170, 1940. Footnote 9.

SUMMARY

It was shown previously that aluminum hydroxide gel has no value in preventing postoperative jejunal ulcer in Mann-Williamson dogs in the presence of a relative deficiency of pancreatic juice and bile. This lack of benefit was ascribed to the production of a phosphorus deficiency. Data presented in this paper show that aluminum hydroxide gel in relatively large doses interferes with the absorption of phosphates in man and dog. It may produce a phosphorus deficiency in the presence of a relative deficiency of pancreatic juice, diarrhea or a low phosphorus diet. It should not produce a phosphorus deficiency in the usual patient with ulcer on the ordinary ulcer diet, which is relatively rich in phosphorus.

Aluminum phosphate gel has antacid, astringent and demulcent properties analogous to those of aluminum hydroxide gel and does not interfere with phosphate absorption. It was used, therefore, to give aluminum gel therapy a fair experimental trial under rigidly controlled conditions and under the most severe test known experimentally, namely, the healing of jejunal ulcer in Mann-Williamson dogs. Aluminum phosphate gel administered with a special diet prevented the development of ulcer in all but 3 of 23 Mann-Williamson dogs. After an ulcer developed, as a result of the discontinuance of aluminum phosphate gel, the administration of the gel caused complete healing of the ulcer in 9 of 10 dogs. This is a striking result. The results of the use of aluminum phosphate gel on a small series of patients with peptic ulcer indicate that the preparation has promise of being as effective as any other similarly acting medicament in the management of peptic ulcer. Our experimental results do not necessarily imply that aluminum phosphate gel is superior to aluminum hydroxide gel for the management of peptic ulcer in man except in the presence of a relative or an absolute deficiency of pancreatic juice, diarrhea or a low phosphorus diet.

ABSTRACT OF DISCUSSION

Dr. Jesse L. Bollman, Rochester, Minn.: When Dr. Ivy gives a paper he leaves little to be discussed. I should like to emphasize the value of the extensive controls for the Mann-Williamson dog, of which Dr. Ivy and associates have spoken. In my experience, students in starting out to perform the Mann-Williamson operation are almost universally unsuccessful in obtaining ulcers. After more experience has been gained, ulcers develop in all the dogs and most of the forms of therapy which at first seemed successful result in failures. I should say that the controls that Dr. Ivy and his co-workers have used—the development of ulcer in the dog after operation, the curing of the ulcer with the therapy described and the cessation of therapy followed by the redevelopment of ulcer—can be taken as absolute controls. This is the first method by which absolute cure of an ulcer produced in a Mann-Williamson dog has been demonstrated. I should like to ask Dr. Ivy a question: In giving substances such as aluminum hydroxide and

aluminum phosphate, which he has shown take from the intestine various inorganic elements, is it not possible that there are a large number of other substances absorbed? Perhaps in these experiments the amount would not be sufficient to be serious, but in continued therapy with highly absorptive colloids I believe that one is going to get into trouble with undesired absorptions. I have particularly in mind the absorption of vitamin B. It could well be absorbed by either the aluminum hydroxide or the aluminum phosphate.

Dr. Hyman I. Goldstein, Camden, N. J.: In all the experiments as carried out on dogs by Dr. Ivy and associates, the element of mental worry and anxiety and the resulting effects on the autonomic vegetative nervous system is entirely absent. One is now not so sure that the factor of hyperacidity is the all-important one from the standpoint of the cause of peptic ulcer. All know that the "go-getter," the hard working fellow, the man who has lost money, the man who is always "on the go," the thinker is the person in whom ulcer of the stomach or duodenum develops.

In recent years one sees a far greater number of duodenal ulcers than gastric ulcers, although the duodenal ulcer develops in an essentially alkaline medium. All have seen many cases in which ulcers healed in patients with marked hyperacidity. Peptic ulcers do not often develop in patients with continued hyperacidity. Some years ago there was considerable interest in attempts to try out other antacid and adsorbent remedies, beginning with the work of Greenwald (1923), Kantor (1923), Mutch (1936, 1937), Mann (1937), Tidmarsh and Baxter (1938), Krämer (1938), Winkelstein, Woldman and Rowland, H. I. Goldstein (1937) and others. Five or six years ago I began to replace the so-called Sippy form of therapy with other measures, including the use of aluminum hydroxide, kaolin (aluminum silicate) and, more recently, magnesium trisilicate. Joannes Scultetus used kaolin about three hundred years ago in the treatment of similar gastrointestinal disturbances, while Aurelius Cornelius Celsus, nearly two thousand years ago, treated ulcers of the stomach and advised an antacid diet! I might mention, too, the old report by Sommer (1696) on "De ardore ventriculi" and his use of soda for hyperacidity, the discussion by Rivinus (1677) entitled "De acido ventriculi fermento," the use by Richter (1794), of "alkaline and absorbent medicines" and a "dieta antacida" and, finally, the employment by Trousseau and Bonnet (1832) of sodium bicarbonate in "painful stomach." The story of ulcer of the stomach and duodenum is an old one, and one must not feel disappointed at one's failure to have at this late date complete and accurate knowledge of the cause of ulcer and its certain cure.

I should like to ask Dr. Ivy whether there is any particular advantage in using aluminum phosphate in place of aluminum hydroxide in clinical practice or such a combination as I have employed during the past three or four years of magnesium trisilicate (50 per cent), kaolin (25 per cent) and aluminum hydroxide (25 per cent) in helping to avoid the chemical changes which he has discussed.

While my own experience has been entirely clinical, and not with Mann-Williamson dogs, the results in my cases have been better after the use of the aforementioned remedies than with the old form of alkaline therapy.

Dr. I. H. Einsel, Cleveland: I have used aluminum hydroxide gel for twelve years. In a large series of cases I have not seen any deleterious effects, except in 2 instances. Both were cases of gastrojejunocolic fistula in which the patients were in an emaciated state due partially to the diarrhea with resulting avitaminosis. They were placed under treatment with the usual dose of aluminum hydroxide gel for several weeks. Instead of improving as one would expect, they became

worse. This necessitated their removal from the aluminum hydroxide therapy. After small feedings of the usual ulcer diet with high vitamin intake they improved.

At the time I could not explain the failure of these patients to respond to the aluminum hydroxide gel therapy, but it can readily be explained by Dr. Ivy's observation that aluminum hydroxide gel causes a disturbance in the phosphorus metabolism under certain physiologic conditions.

In the beginning of aluminum hydroxide therapy there is a slight loss of appetite. I have noticed this many times. Apparently after ten days this loss of appetite disappears, and the patient continues his therapy with no difficulty. This may be caused by the disturbance of phosphorus metabolism by the aluminum hydroxide. The paper by Dr. Ivy and his associates has been of great interest to me.

Dr. A. C. Ivy, Chicago: With regard to the effect of aluminum gels on absorption, there is one point that I neglected to mention. In our animals receiving relatively large doses of aluminum phosphate gel there appeared iron deficiency anemia, or anemia that we interpreted as iron deficiency anemia on the basis of therapeutic results. We injected colloidal iron intravenously, 35 mg. per week, and the anemia was controlled.

We cannot state absolutely that aluminum phosphate interferes with the absorption of iron, but there are reasons why large quantities of any phosphate may do so. As a matter of fact, when minerals which react with the acid and the alkaline juices are introduced in large quantities into the alimentary tract, one can expect in the course of time to produce some change in mineral metabolism.

It is possible for aluminum phosphate or aluminum hydroxide to interfere with the absorption of vitamins. Our animals, however, received 200 Gm. of raw ground pancreas and 200 Gm. of raw ground liver daily, so they were adequately supplied with vitamins. If the vitamin content of the diet is low, then these gels may interfere significantly with the absorption of vitamins. I know how important the anxiety factor is in the production of peptic ulcer in human beings. I believe that the most direct attack on the etiologic agent of peptic ulcer in man, in the majority of cases, is to teach the patient how to eat and how to live; he must learn to relax and to forget his worries. This is frequently difficult. The ulcer that we obtain in the Mann-Williamson dog, in my opinion, is analogous to the ulcer that occurs postoperatively in man, after gastrojejunostomy, and our results should be most applicable to cases of that type. Magnesium trisilicate is a better and a cheaper "antacid" than aluminum phosphate gel, but I suspect that in our experimental animals we shall not obtain as good results with magnesium trisilicate because it does not possess a mildly astringent property. We plan to test the trisilicate on the experimental animal.

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Antitumor Activity and Toxicity of Salts of Inorganic Group IIIa Metals: Aluminum, Gallium, Indium, and Thallium

(Walker 256 carcinosarcoma/reticulum cell sarcoma/lymphosarcoma/mammary carcinoma/leukemia)

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ABSTRACT **The toxicity and antitumor activity of salts of the Group IIIa metals aluminum, gallium, indium, and thallium were determined. With the (lethal dose)$_{50}$ as a measure, the decreasing order of toxicity was $TlCl_3 \geq In(NO_3)_3 > Ga(NO_3)_3 > Al(NO_3)_3$. All four metals exhibited antitumor activity, but when the tumor was inoculated by a route different from that of the drug, only Ga^{+3} and, to a lesser extent, In^{+3} inhibited tumor growth. $Ga(NO_3)_3$ was found to inhibit the growth of three out of four rodent solid tumors. Gallium therefore has potential therapeutic usefulness for treatment of solid tumors in man.**

Since gallium is a bone-seeking element (1–3), radionuclides of this element have been of clinical interest as diagnostic screening agents of bone tumors. In 1969 Edwards and Hayes (4) noted that carrier-free gallium-67 citrate was localized in addition in nonosseous tumors in patients with Hodgkin's disease and other lymphomas. Deposition of this radioactive salt was also observed in patients with other "soft-tissue", slow-growing tumors (4–8), such as poorly differentiated thyroid adenocarcinoma, reticulum cell sarcoma, lymphosarcoma, and carcinomas of the lung, breast, colon, and kidney. These unexpected observations prompted studies on the concentration of this gallium radionuclide in various experimental animal tumors. Seven nonosseous rat and mouse carcinomas and sarcomas showed variable but distinct localization of ^{67}Ga, in contrast to deposition in normal tissues (9). In addition, the Cancer Chemotherapy National Service Center has found that gallium nitrate possessed antitumor activity against Walker 256 carcinosarcoma in rats. These observations prompted us to examine the antitumor activity of gallium and metal salts chemically related to gallium.

In increasing order of atomic numbers and atomic weight, the elements of Group IIIa of the periodic table are boron (B), aluminum (Al), gallium (Ga), indium (In), and thallium (Tl). The +3 oxidation state of these elements as cations is the most stable form in aqueous solution, except for thallium +3 which is slowly converted to the +1 form. The +3 forms of these elements were used in our experiments. We were unable to obtain a stable cationic form of +3 boron, and the only stable soluble form of +3 Tl was the trichloride.

The experiments reported here were designed to determine the toxicity of various Group IIIa salts in two species, the CDF_1 mouse and the Sprague–Dawley rat; to test nontoxic doses of these salts for antitumor activity against two experimental rodent tumors with a high clinical predictability value (10); to attempt to correlate the *in vivo* activity of these salts and their *in vitro* action in tissue culture; and to assess the antitumor activity of the active salt against various rodent solid tumors.

MATERIALS AND METHODS

Adult male CDF_1 mice (NIH colony), 20–27 g, and adult female Sprague–Dawley rats (NIH colony), 120–150 g, were randomly divided into groups and used for all *in vivo* experiments.

Tumors

The mice were inoculated intraperitoneally with 0.1 ml of a 1:1000 dilution of leukemia L1210 ascitic fluid in Locke's solution. The rats received an intraperitoneal or subcutaneous injection of a 1:100 dilution of ascitic Walker 256 carcinosarcoma fluid in Locke's solution. Three solid tumors were transplanted subcutaneously with a trocar in mice. These tumors were lymphosarcoma P1798, reticulum cell sarcoma A-RCS, and mammary carcinoma YMC.

Drugs

Compounds tested were aluminum nitrate [$Al(NO_3)_3 \cdot 9H_2O$], gallium nitrate [$Ga(NO_3)_3 \cdot 9H_2O$], indium nitrate [$In(NO_3)_3 \cdot 5H_2O$], and thallium chloride [$TlCl_3$]. All drugs were dissolved in 0.9% saline and were given in a volume of 0.01 ml/g body weight.

Toxicity studies

Mice were randomly divided into groups of 10; rats into groups of 8. Each animal was injected intraperitoneally with drug daily for 10 consecutive days. The number of deaths occurring within the 30-day observation period were noted. The LD_{50}, with its 95% confidence interval, the slope of the log dose percentage mortality curve, and the LD_{10} were calculated by the method of Litchfield and Wilcoxon (11).

In vivo antitumor activity studies

Tumor-bearing mice were randomly divided into groups of 10; Walker 256 rats, into groups of 6. Beginning 24 hr after

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TABLE 1. *Toxicity of Group IIIa salts in mice and rats*

Compound	CDF$_1$ male mice*		Sprague–Dawley female rats*	
	LD_{50} (95% CI)† (mg/kg)	LD_{10} (mg/kg)	LD_{50} (95% CI)† (mg/kg)	LD_{10} (mg/kg)
$Al(NO_3)_3 \cdot 9H_2O$	320 (282–374)	213	327 (283–378)	240
$Ga(NO_3)_3 \cdot 9H_2O$	80.0 (67.2–95.2)	63.0	67.5 (59.5–76.6)	50.0
$In(NO_3)_3 \cdot 5H_2O$	7.45 (5.87–9.46)	4.22	5.55 (4.66–6.60)	3.85
$TlCl_3$	6.90 (6.52–7.30)	6.00	5.66 (5.25–6.10)	4.85

The observation period totaled 30 days from first injection.

* Animals injected intraperitoneally daily for 10 days.

† LD_{50} and 95% confidence interval (CI) for the LD_{50} as determined by method of Litchfield and Wilcoxon (11).

tumor inoculation each animal was injected intraperitoneally or subcutaneously with drug for 10 consecutive days. The doses of each drug tested were equal to or less than the LD_{10} for the drug in that species. Control animals received an appropriate amount of saline, the pH of which had been adjusted with 0.1 N HCl to that of the drug solution. Each dose of each drug was administered in at least two separate experiments for each tumor. An estimate of the volume of the subcutaneous Walker tumor was made by three-dimensional measurement of the tumor with calipers 10 days after the initiation of daily drug treatment for the Walker 256 carcinosarcoma and 20 days after it for the other solid tumors. Control (saline-treated) mice and rats bearing the ascitic form of L1210 and Walker 256 consistently had median survival times of 8 days. The percentage increase in median survival time above the control was calculated for the drug-treated groups. Those animals surviving for 4 months were killed and examined for tumors. Animals without tumors were designated as long-time survivors.

Antitumor activity in tissue culture

L1210 and Walker 256 (W256) cells were maintained in static culture in R.P.M.I. no. 1630 media. The media were supplemented with 20% (L1210) or 10% (W256) fetal calf serum. Stock bottles, after 5 days of growth, were counted and diluted so that there were approximately 10^6 cells/ml. A total of 5 ml of cells was used in each experiment (12). Drugs were filtered through a Millipore filter immediately before use. A total of 0.1 ml of the drug solution or saline alone was added to 5 ml of these cells. The pH of the saline to be added to each control bottle was adjusted with 0.1 N HCl so that it was identical with that of the drug solutions. Cell counts were made every 24 hr for 3 days with a Coulter counter.

RESULTS

Toxicity studies

In Table 1 the toxicity of four Group IIIa salts in CDF$_1$ mice are tabulated according to the element's position in the periodic table. Note that as one moves down the periodic table, the LD_{50} decreases, that is, the toxicity increases. The order of toxicity is slightly altered when the LD_{10} values are compared: the LD_{10} for thallium chloride is greater than that for indium nitrate, in part because the LD_{50} values for these two compounds are quite similar and the slopes of their respective log dose–percentage mortality curves are markedly different. Thus, indium nitrate is more toxic at doses below its LD_{50} than thallium chloride is below its LD_{50}.

In female Sprague-Dawley rats, the LD_{50} decreases and the toxicity increases as the atomic number of Group IIIa metals is increased. The LD_{50} values for thallium and indium are not statistically significantly different; the LD_{10} values are again reversed for thallium and indium, and for the same reasons.

In vivo antitumor activity against ascitic tumors

Table 2 shows that all four salts increased the median survival time of Sprague-Dawley rats carrying the ascitic form of Walker 256 carcinosarcoma. The number and percentage of long-term survivors (defined as rats that lived for 4 months after tumor inoculation and showed no evidence of tumor at autopsy) was highest for aluminum nitrate (Table 2).

The antitumor activity *in vivo* of these four salts against

TABLE 2. *Antitumor activity of Group III salts against ascitic Walker 256 carcinosarcoma*

Compound	Doses tested (mg/kg)	Optimum dose (mg/kg)	Increase in median survival time (%)	Number (%) of long-term survivors
$Al(NO_3)_3 \cdot 9H_2O$	50–400	150	300	5/12 (42)
$Ga(NO_3)_3 \cdot 9H_2O$	12.5–100	60	158	2/6 (33)
$In(NO_3)_3 \cdot 5H_2O$	0.5–5	1	124	0/18 (0)
$TlCl_3$	2–5	4	769	3/12 (25)

Sprague-Dawley female rats were injected intraperitoneally daily for 10 days, beginning 1 day after tumor transplant.

TABLE 3. *Cytotoxic activity of Group III salts against Walker 256 carcinosarcoma and leukemia L1210 cells in tissue culture*

Compound	W256 Carcinosarcoma		Leukemia L1210	
	ID_{50}*	Slope†	ID_{50}*	Slope†
$Al(NO_3)_3 \cdot 9H_2O$	70	4.90	250	16.5
$Ga(NO_3)_3 \cdot 9H_2O$	58	3.85	100	7.50
$In(NO_3)_3 \cdot 5H_2O$	35	2.70	700	3.50
$TlCl_3$	6.0	3.70	8.2	5.80

* Concentration of drug necessary for 50% growth inhibition (normality $\times 10^6$) 72 hr after administration to culture as determined by the method of Litchfield and Wilcoxon (11).

† Slope of dose-response curve (Δ% growth inhibition/Δ normality).

TABLE 4. *Comparison of in vitro and in vivo antitumor activity of Group III salts against Walker 256 carcinosarcoma and leukemia L1210*

Compound	Dose *In vitro* (N)*	Dose *In vivo* (mg/kg)†	W256 Carcinosarcoma % GI‡	W256 Carcinosarcoma % MST§	Leukemia L1210 % GI	Leukemia L1210 % MST
$Al(NO_3)_3 \cdot 9H_2O$	7.6×10^{-4}	200	53	156	11	0
	3.8×10^{-4}	100	40	139	0	0
$Ga(NO_3)_3 \cdot 9H_2O$	2.1×10^{-4}	60	38	138	0	0
	1.4×10^{-4}	40	30	117	0	0
$In(NO_3)_3 \cdot 5H_2O$	1.1×10^{-5}	3	21	21	0	0
	0.4×10^{-5}	1	18	124	0	0
$TlCl_3$	1.8×10^{-5}	4	60	769	13	0
	0.9×10^{-5}	2	38	100	10	0

* If one assumes distribution of injected drug to total body water, this would be the concentration of drug in the body tissues.
† Daily for 10 days.
‡ Percentage growth inhibition (GI) of cells in tissue culture 72 hr after drug addition.
§ Percentage increase in median survival time (MST) above that of control animals carrying the ascitic form of these tumors *in vivo*.

the ascitic form of leukemia L1210 in mice was negligible. Doses at or below the LD_{10} had no effect on median survival time and did not result in any long-term survivors.

Antitumor activity in tissue culture

To determine the correlation between *in vivo* and *in vitro* antitumor activity of these compounds, we measured their growth inhibitory action on both *in vivo* susceptible (W256) and *in vivo* nonsusceptible (L1210) cells in tissue culture. The concentration of each drug necessary for 50% inhibition of cell growth and the slopes of their dose-percentage inhibition curves for these two cell lines are presented in Table 3. Before any comparisons are made, it is necessary to point out that for any drug the two dose-response curves (W256 and L1210) do not intersect until more than 90% growth inhibition is achieved in both systems. Therefore, any qualitative relationship that exists at one drug concentration also exists at all others that yield less than 90% inhibition. For each drug the ID_{50} and slope are smaller in the Walker 256 system than they are in the L1210 system.

The concentration of each compound in the culture medium listed in Table 4 is equivalent to that in the intact animal at the described intraperitoneal dosage level if these salts are distributed to total body water. The decreasing order of growth inhibition of these salts against the Walker 256 cells, Tl > Al > Ga > In, is identical to the order of decreasing percentage median survival times of rats carrying the tumor. Drug concentrations at these levels (approximately the LD_{10}) had little or no effect on L1210 cells in tissue culture and did not increase the percentage median survival time of mice carrying ascitic L1210. These data suggest a reason for the lack of *in vivo* activity of these salts against L1210, i.e., the dose necessary for L1210 growth inhibition is toxic to the animal.

Effect of route of tumor inoculation and drug administration on antitumor effect of Group III salts against Walker 256 carcinosarcoma

When the same doses of Group III salts effective against the ascitic Walker 256 carcinosarcoma were administered to rats carrying the subcutaneous form of this tumor, the results outlined in Table 5 were obtained. In this situation

TABLE 5. **Effect of route of Walker 256 carcinosarcoma inoculation and drug administration on antitumor activity of Group III salts*

Compound	Dose (mg/kg)	Drug intraperitoneal, tumor subcutaneous % inhibition of control tumor size	Drug subcutaneous, tumor intraperitoneal Increase % MST
Saline control		0	0
		(9,810 ± 1,150 mm³)*	(8.0 ± 0.14 days)†
$Al(NO_3)_3 \cdot 9H_2O$	100	6	0
	150	0	0
	200	0	0
$Ga(NO_3)_3 \cdot 9H_2O$	50	61	25
	60	92	50
$In(NO_3)_3 \cdot 5H_2O$	1	35	19
	2	39	44
$TlCl_3$	3	0	13
	4	0	0

* Mean saline control tumor volume ± SE.
† Median saline control survival time (MST) ± SE.

TABLE 6. *Gallium nitrate inhibition of solid tumors transplanted subcutaneously**

Tumor	Optimal dose (mg/kg)	% inhibition control tumor size
Walker 256 carcinosarcoma	60	92
Reticulum cell sarcoma A-RCS	50	94
Mammary carcinoma YMC	50	24
Lymphosarcoma P1798	30	98

* Animals injected intraperitoneally daily for 10 days, beginning 1 day after tumor transplant. Three-dimensional measurements of these tumors were taken 20 days after transplant (10 days for Walker 256 carcinosarcoma).

the drugs do not immediately come into contact with the tumor cells, but must pass to the tumor via the blood stream. Gallium and indium, but not aluminum or thallium, inhibited growth of the tumor. Apparently, insufficient concentrations of Al and Tl reach the solid tumor to inhibit growth. When the tumor was inoculated intraperitoneally and the drug injected subcutaneously, the same order of activity and inactivity resulted.

Effect of gallium nitrate against various solid tumors transplanted subcutaneously

Because of the high growth inhibitory activity of gallium nitrate against the subcutaneous Walker tumor, we tested this salt for activity against three other subcutaneous solid tumors. The salt inhibited the growth of a reticulum cell sarcoma and a lymphosarcoma by more than 90% (Table 6), but had little effect on the growth of a mammary carcinoma in the same strain of mouse (CDF_1) in which the reticulum cell sarcoma is also carried.

DISCUSSION

The use of metals and their salts as chemotherapeutic agents was advocated by Paracelsus as early as the 16th century. However, it has only been recently that the possible usefulness of inorganic compounds as cancer chemotherapeutic agents has been explored. Rosenberg *et al.* (13) have commented on the antitumor activity of various Group VIII metal salts, including those of platinum and ruthenium. Our studies have shown that all four of the Group III metal salts tested were cytotoxic in tissue culture against Walker 256 carcinosarcoma and leukemia L1210 cells. However, at concentrations comparable to those that could be obtained *in vivo* without lethality, these four Group III salts retained their inhibitory activity against the Walker cells but showed no growth inhibitory action against leukemia L1210 cells in culture. When these compounds were injected intraperitoneally into animals carrying the ascitic form of W256 and L1210, the order of antitumor activity against W256 was identical to that seen in tissue culture, namely Tl > Al > Ga > In. None of the four compounds produced any activity against ascitic leukemia L1210 when the drug was injected intraperitoneally. A possible explanation for the lack of *in vivo* activity of these four salts against leukemia L1210 is that the concentration necessary for growth inhibition in tissue culture is toxic when injected intraperitoneally into the mouse.

When the drugs were not injected directly into the body compartment occupied by the tumor, the order of effectiveness against the Walker carcinosarcoma changed. Only $Ga(NO_3)_3$ and $In(NO_3)_3$ are effective when injected at a site differing from that of the tumor. Gallium is the most effective in this regard, apparently because the ion is sequestered by the tumor from the blood stream.

The anionic component of the metallic salt did not influence the antitumor activity. Thus, the chlorides and sulfates of aluminum, gallium, and indium were equivalent in activity to their respective nitrates. Preliminary experiments have shown that not only gallium, but also indium and in some cases aluminum salts prevent the growth of various experimental solid tumors. We are currently continuing our studies of the antitumor activity of these and other inorganic salts against various transplantable rodent tumors and are attempting to correlate their uptake with their antitumor activity. Not only are gallium salts taken up by solid tumors, but they have now been shown to possess antitumor activity. Gallium therefore has potential therapeutic usefulness for treatment of solid tumors in man. Perhaps an even greater benefit to the patients with gallium-sequestering solid tumors could be derived by attaching other cytotoxic agents (i.e., nitrogen mustards) to one or more of the ligand bonds available on the gallium atom. We are also currently investigating this possibility.

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INTESTINAL OBSTRUCTION CAUSED BY COLLOIDAL ALUMINUM HYDROXIDE

W. Paul Havens, M.D., Philadelphia

Colloidal aluminum hydroxide has been widely used for treatment of peptic ulcer since Crohn reported its value as antacid in 1929. There has been little information available concerning any untoward effects. Kraemer[1] described from constipation and the occurrence of fecal impaction in cases, but the majority of observers have not mentioned complications.

The following case illustrates the danger which exists, the unfortunate result which may occur when colloidal aluminum hydroxide is used in the treatment of certain cases of bleeding duodenal ulcer.

M. F., a Negro woman aged 59, a widow, was admitted to the hospital complaining of generalized weakness and intermittent pain in the lower part of the abdomen of two weeks duration.

There was moderate enlargement of the heart, and the blood pressure was 180 systolic and 110 diastolic. She was treated for hypertensive cardiovascular disease for three weeks. At the end of this time an episode occurred which suggested sudden internal hemorrhage. She became weak and pressed and the mucous membranes appeared pale. The blood pressure fell to 110 systolic and 60 diastolic, and the pulse rate rose to 138. The hemoglobin content and the red cell count, which had been 80 per cent and 3,850,000 respectively, fell to 30 per cent and 1,000,000. Several unformed stools were tarry. She was given frequent feedings of gelatin water and gruel with 8 cc. of colloidal aluminum hydroxide every two hours for three and one-half days. A tube was then placed in the stomach, and a constant drip of two parts water and one part colloidal aluminum hydroxide was allowed to flow in at a rate of 2,000 cc. in twenty-four hours. To this was added 160 cc. of corn syrup (karo) for two days. The patient remained in poor condition in spite of frequent transfusions of blood. Bleeding from the bowel continued and she failed to gain strength. Although she was given liquid petrolatum only, enemas were effective at least every other day and frequent examinations for fecal impaction gave negative results. Because of restlessness, morphine sulfate 10 mg. was administered every four hours for a few days. After seven days of treatment with the constant drip the abdomen became distended and no peristalsis was heard. A diagnosis of intestinal obstruction was made. Suction was applied to the tube in the stomach, and 1,900 cc. of greenish yellow fluid was obtained, which reduced the distention slightly. An enema caused the evacuation of a moderate amount of tarry material. Her condition became progressively worse and she died a few hours later.

At necropsy the characteristic changes of arteriosclerotic cardiovascular-renal disease were noted. The heart was slightly enlarged and there were moderate atherosclerotic changes in the arteries. Immediately adjacent to the pyloric sphincter, the duodenum there was an ulcer measuring 3 by 1.2 by 0.4 cm. The jejunum and proximal two thirds of the ileum were distended with gas. Beginning at about the midportion of the ileum, the lumen contained a semisoft putty-like material which in the distal portion became more inspissated, until firm cases of the bowel were formed which caused total obstruction. Immediately distal to the ileocecal valve was a large gl[illegible] mass of the same material mixed with inspissated [illegible] approximately 7 by 8 cm., forming a partial cast of the cecum. Lumps of similar material about 2 by 6 cm. in size were present in the remainder of the cecum and transverse colon. Beginning at about the midportion of the transverse colon several [illegible] reddish black masses measuring approximately 2 by 5 cm. were present, and these became larger in the descending colon, [illegible] moid flexure and rectum.

COMMENT

Reports on the constipating effect of colloidal aluminum hydroxide and the occurrence of fecal impaction following its use are infrequent. The preparation used (amphogel) contains

From the Department of Medicine, Jefferson Medical College H[illegible]

1. Kraemer, Manfred: The Use of Hydrated Magnesium Tr[illegible]cate in Peptic Ulcer, Am. J. Digest. Dis. 5:422 (Sept.) 19[illegible]

about 5 per cent aluminum hydroxide, which, combined with the astringent effect produced, may favor constipation, especially when there is blood in the intestine. It is possible that the frequent use of liquid petrolatum and enemas by the vigorous young adult may lessen the danger of this complication, but there appears to be a danger in administering aluminum hydroxide to older or very ill patients, whose energy is depleted and whose intestinal tract may lack normal tonus. As a further precaution, morphine sulfate, because of its alleged depressant action on the bowel, should be used only with caution. It is possible that the gelatin given together with the aluminum hydroxide aided in forming firm masses.

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THE METABOLISM OF CALCIUM AND PHOSPHORUS AS INFLUENCED BY THE ADDITION TO THE DIET OF SALTS OF METALS WHICH FORM INSOLUBLE PHOSPHATES

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As early as 1908 Stoeltzner (1) observed that the addition of strontium salts to the diet produced a rachitic-like condition in animals. Lehnerdt (2) confirmed and extended these results and Shipley, Park, McCollum, Simmonds and Kinney (3) reported in 1922 that cod liver oil was ineffective in preventing the so-called strontium rickets. Hess (4) later stated that animals were not protected against the changes produced by strontium by "liberal amounts" of irradiated ergosterol. More recently Guyatt, Kay, and Branion (5) found that beryllium carbonate in amounts of approximately 0.5 per cent or more of the diet produced very severe rickets in rats. Cod liver oil and irradiated ergosterol were ineffective as preventive agents. These workers have concluded that beryllium acts by uniting with phosphorus in the intestinal tract to form an insoluble phosphate with the consequent loss of phosphorus to the body and the development of low-phosphorus rickets. Jones (6) subsequently found that dogs given a diet containing beryllium carbonate develop rickets which was prevented by neither cod liver oil nor ultra violet light. Deobald and Elvehjem (7) were able to produce rickets in chickens by giving either aluminum or iron salts. They agree with Guyatt et al. in respect to the manner in which these metals act. Sobel, Goldfarb and Kramer (8) on the other hand have concluded that beryllium has a direct inhibiting effect on the process of calcification.

With the possible exception of the effect of liberal iron feeding on the development of infantile rickets, as pointed out by Deobald and Elvehjem, it is doubtful if clinical rickets is ever caused by an excess of these metals. Their mode of action, however, is of experimental interest, and furthermore they serve as a useful tool in studying the metabolism of calcium and phosphorus. In the present paper are recorded a number of observations made during the past few years and dealing with various phases of this subject. As salts of different metals have been used the more inclusive term of metal rickets has been adopted to describe this condition.

EXPERIMENTAL. *A. Influence of large doses of irradiated ergosterol on metal rickets.* Since it was previously shown in this laboratory (9), by using diets containing at most only traces of calcium, that this element can be transferred from the ossified portion to the growing parts of the bone, it was thought desirable to conduct a similar experiment using a diet free of phosphorus. As the preparation of such a diet is difficult an attempt was made to substitute a diet in which the phosphorus was made unavailable by the addition of metals which form insoluble phosphates. All workers are agreed that even in the presence of liberal quantities of vitamin D little or no calcification takes place in the growing bone when diets containing any of these metals are being fed. Sobel and associates (8) refer to this condition as incurable rickets. In all of these experiments, however, comparatively small doses of vitamin D were given. The following experiments were planned to test the ability of large amounts of irradiated ergosterol to cure or prevent metal rickets and to determine if the phosphorus came from the food or was transferred from one part of the bone to another in the event ossification took place.

Preliminary experiments, in which either aluminum acetate or beryllium carbonate was used as the metal, gave definite evidence that liberal amounts of vitamin D as irradiated ergosterol could produce healing of metal rickets. The prevention of rickets under similar circumstances was then studied in greater detail. A total of 31 young rats was placed on diet 502 which is the Steenbock (10) stock diet[1] modified to contain 2 per cent yeast and to which was added 1 per cent of beryllium carbonate. Fifteen of these animals were given 5000 I. U. of vitamin D per day as irradiated ergosterol while the remaining 16 rats received no vitamin supplement. The administration of the activated ergosterol was begun 2 days after the initial feeding of the beryllium-containing diet. Nineteen of these rats were on experiment for 23 days and 12 for 16 days. At the end of each period the animals were killed by bleeding and the sera were analyzed for calcium and inorganic phosphate (Clark and Collip, 11; Gunther and Greenberg, 12). The right femur of each animal was removed for ash analysis and the wrist bones were examined by the line test technique. Besides the above 31 animals an additional 6 were given the stock diet without beryllium, 3 of which were given daily 5000 I. U. of vitamin D as above. In table 1 are given in detail the results from the 6 animals receiving the stock diet and 6 receiving beryllium. Of the latter, 3 received irradiated ergosterol and 3 did not. These 12 rats were all on experiment for 23 days and were from either one of two litters. A summary of all the results obtained from the 31 animals is also given in the form of averages in table 1. As the initial weights of most of the animals were between

[1] The casein used in these diets was kindly furnished by Smith, Kline and French, Inc., Philadelphia.

40 and 50 grams only the gains in weight are given. Although the amount of vitamin administered was far above the therapeutic dose, it was below the toxic level as growth on the stock diet was not lessened by the addition of the vitamin, but the calcium of the serum appears to have been raised slightly while the serum phosphate remained unchanged. There is also no particular difference in the degree of calcification between the 2 groups on the stock diet (with and without vitamin D) as shown by the amount and percentage of bone ash. By comparison of the animals on the stock

TABLE 1

Effect of irradiated ergosterol on femur-ash content of beryllium-fed rats

RAT NUMBER	DIET	IRRADIATED ERGOSTEROL I.U.*	GAIN IN WEIGHT	SERUM		FEMUR-ASH	
				Ca	PO_4-P	Weight	Per cent
			grams	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm.*	
1	Stock	0	69	10.7	8.8	84.3	50.3
2	Stock	0	78			94.4	51.8
3	Stock	0	88	11.7	9.7	92.8	50.1
4	Stock	5000	83	12.3	8.9	100.2	52.3
5	Stock	5000	86			97.9	51.6
6	Stock	5000	99	12.5	10.4	98.3	50.5
7	502	0	30			25.6	28.2
8	502	0	18	11.5	0.9	19.5	25.4
9	502	0	27			19.0	21.1
10	502	5000	29			30.7	31.2
11	502	5000	43	14.7	3.0	35.1	32.6
12	502	5000	50			34.9	30.6
Averages of all animals receiving beryllium							
NUMBER OF RATS							
16	502	0	22.9	11.4	2.5	21.3	25.5
15	502	5000	37.1	14.1	3.5	30.7	31.8

* International units of vitamin D as irradiated ergosterol given per animal per day.

diet with those on the beryllium-containing diet it is clear that the addition of the metal greatly decreased the rate of growth. The rate on the latter diet, however, was increased slightly (statistically significant) by the administration of irradiated ergosterol. Also, the giving of the vitamin D supplement to the animals on the beryllium-containing diet produced a slight rise in serum calcium and a considerable rise in phosphate. The average of the serum phosphate for the animals not receiving vitamin D on the beryllium diet is probably somewhat higher than the true value as just previous to the end of the experiment several of these animals were

consuming but little food and consequently losing weight. Under these conditions inanition invariably leads to an increase in the inorganic phosphate of the serum.

Apparently the increased serum phosphate in the animals receiving vitamin D was reflected in the degree of calcification of the bones. Although still far below those on the stock diet the absolute and relative amounts of femur-ash of the beryllium plus vitamin fed animals were definitely greater than those of the animals receiving only beryllium. Clinical examination of the wrists previous to killing the animals showed a marked difference between these 2 groups. The wrists of the rats not receiving the vitamin were much enlarged while the others appeared normal. Examination by the line test showed a wide uncalcified area at the metaphyses of the bones of the animals receiving no irradiated ergosterol, whereas this area appeared practically normal in the vitamin D fed animals. Similar results in all respects were obtained with 22 animals which were fed aluminum sulfate and with 6 animals given strontium carbonate.

In addition to the above experiments the effect of increasing the daily dose of vitamin D and the length of time it was given were studied. Although the larger amounts of irradiated ergosterol considerably increased the levels of calcium and phosphate in the serum the amount of calcification was still definitely below normal. Thus the averages for 3 rats which received, in addition to diet 502, 25000 I. U. of vitamin D per day for a period of 6 weeks were as follows: Serum calcium—15.6 mgm.; serum phosphorus—7.6 mgm.; femur ash—43.8 mgm. and 37.1 per cent. From these data it appears that large amounts of vitamin D can increase the calcification at the metaphyses when a diet which contains a metal which forms an insoluble phosphate is being fed. Furthermore, since there is an increase in both the absolute and relative amounts of bone ash it would seem that at least part of the phosphorus concerned with this calcification has come from the food. However, even when amounts of vitamin D only slightly below the toxic level are given the rate of ossification is far below normal. It is impossible to say if the vitamin increases the absorption of the phosphorus under these conditions or assists in a more economical utilization of that which is absorbed. In any case these experiments fail to determine if in times of phosphorus-want there can be a transfer of phosphorus from one part of the bone to another as appears to be true for calcium.

B. Influence of the addition of metals which form an insoluble phosphate on the toxicity of irradiated ergosterol. Numerous reports have appeared concerning the relation of the amounts of phosphorus in the diet to the toxicity of irradiated ergosterol. If the toxicity of large doses of vitamin D can be augmented by increasing the phosphorus of the diet, then it might

be possible to decrease the toxicity by making the phosphorus which is there unavailable. With this in mind the toxicity of irradiated ergosterol was compared with and without the addition of metal. Aluminum sulfate, aluminum acetate and beryllium carbonate were each used for the added salt, and the activated ergosterol was given in daily doses of 2000, 2500, 3000 and 5000 I. U. per 10 grams of body weight. In all, about 120 adult rats were employed in these studies. The approximate minimum lethal dose and the survival time on various levels of fatal doses were determined. There was no consistent difference in any case between the animals receiving one of the salts and the controls on the stock diet. The M.L.D. was found to be between 2000 and 2500 I. U. per 10 grams of body weight. The serum calcium and phosphate of a number of animals which had received 2000 I. U. per 10 grams of body weight for a period of 3 months on the stock diet were compared with similarly treated animals on diet 502 (stock + 1.0 per cent $BeCO_3$). The averages for 6 animals on each of these diets were respectively: calcium 14.5 and 15.0; phosphorus 5.5 and 8.1. It is apparent that there was no difference in the levels of calcium but the phosphate of the animals receiving the beryllium appears to have been definitely below the controls. However, in another experiment conducted in a similar manner but for only two weeks instead of three months there was no difference in either the calcium or the phosphate of the serum of the animals fed ration 502 and those fed the stock diet. Ash determinations on the femurs of the animals which were on experiment for three months indicated that there had been some loss of bone salts during this time, but there was no difference in this respect between the animals receiving both beryllium and irradiated ergosterol and those receiving only the vitamin preparation. The average for each group was 52.1 and 52.6 per cent which compares with a normal of about 60 per cent. Apparently in these experiments the toxicity of irradiated ergosterol was not reduced by making the phosphorus of the diet unavailable by adding to the diet metals which form insoluble phosphates.

C. Effect of change in serum phosphate on serum calcium. There is considerable evidence to indicate that under certain conditions there is a reciprocal relation between serum calcium and inorganic phosphate. However, it appears that this is not always the case. In a previous publication (13) this subject was discussed, and it was stated, without presenting the data, that it was possible to materially decrease the level of serum inorganic phosphate by feeding aluminum salts with little or no rise in calcium. Since then these experiments have been repeated using beryllium carbonate as the salt. Fourteen adult female rats were taken from the regular stock colony. Two were bled as controls and the remaining 12 were given ration 502. Two were sacrificed at weekly intervals and the serum analyzed for calcium and phosphate. The results, which

are presented in table 2, show clearly that it is possible for the inorganic phosphate of the serum to fall to very low levels without any detectable rise in serum calcium.

It has been frequently observed that if young animals are placed on a high calcium-low phosphorus rachitogenic diet for some time and then transferred to a similar diet from which the added calcium has been omitted there is a precipitous fall in calcium and a pronounced rise in the inorganic phosphate of the serum. Frequently this change in the composition of the serum is accompanied by tetany. In these cases the most obvious change in the diet is a reduction in the amount of calcium. However, the decrease in calcium has the effect of increasing the amount of phosphorus

TABLE 2

Influence of lowered serum phosphate on serum calcium

RAT NUMBER	TIME ON DIET*	SERUM	
		Calcium	PO_4-P
	weeks	*mgm. per 100 cc.*	*mgm. per 100 cc.*
13	0	11.3	5.6
14	0	10.9	5.9
15	1	11.2	1.6
16	1	11.5	1.6
17	2	11.5	1.5
18	2	11.3	1.3
19	3	11.1	1.1
20	3	10.9	1.4
21	4	9.9	1.1
22	4	11.0	1.4
23	5	10.7	1.0
24	5	10.9	0.9
25	6	10.9	0.5
26	6	11.8	0.9

* Stock ration + 1.0 per cent beryllium carbonate.

as a larger portion of that present is absorbed. Thus it is difficult to conclude if the change in the blood is due to a lowered intake of calcium, an increase in the absorbed phosphorus or both. By adding beryllium carbonate to a high calcium-low phosphorus rachitogenic diet such as the Steenbock-Black diet 2965 (14) it would be possible to reduce the calcium after the development of rickets without increasing the availability of the phosphorus. When such a diet was fed to young rats it was found possible to suddenly omit the added calcium carbonate without any appreciable change in the levels of serum calcium and phosphorus and consequently with no indications of tetany.

In another series of experiments aluminum salts, either the sulfate or

acetate, were fed in addition to the stock diet for a period of approximately 3 weeks. The metal was then omitted from the diet. Of 22 young rats so treated a few were observed in tetany, and invariably there was a pronounced rise in phosphorus and a definite drop in calcium. Thus it appears that under these conditions the primary effect is on phosphate and not on calcium. As stated above usually it is necessary to work with young animals and to keep them on the high calcium-low phosphorus diet for some time before the change is made if tetany is to result. During this time rickets develops and when the phosphate of the serum is increased there is a rapid deposition of calcium phosphate in the rachitic bones. The calcium is drawn so rapidly from the blood that it is impossible to maintain the normal level. It seems that a sudden increase in serum phosphate coupled with a rachitic condition is the primary factor in the production of the hypocalcemia and the tetany which frequently follows.

D. Effect of aluminum sulfate on the femur-ash content of adult rats. As metals which form an insoluble phosphate can prevent calcification by producing a type of phosphorus deficiency, it is possible that under similar circumstances bones of adult rats might show a loss of mineral constituents. To determine to what extent this may take place 6 adult female rats were placed on the stock diet referred to in section A to which was added 5 per cent $Al_2(SO_4)_3 \cdot 18H_2O$. Six female rats of approximately similar age and history were given the stock diet without the aluminum salt. Three months later when the animals were killed the sera gave the following averages for calcium and inorganic phosphorus: aluminum-fed animals 11.8 and 2.3 and control animals 12.6 and 4.7 mgm. per cent respectively. The averages for the femur ash of the rats receiving the aluminum were 146 mgm. and 53.5 per cent and for the controls the values were 223 mgm. and 62.0 per cent. It is clear that the aluminum sulfate produced a definite decrease in the amount of mineral constituents in the bones. This was in all probability due to the marked decrease in serum phosphate which tended to shift the inorganic salts from the bone to the blood. Here again, however, there was no increase in the level of serum calcium in spite of the fact that considerable quantities of calcium must have passed from the bones into the blood. Judging from the comparative amounts of ash in the femurs of each group at least one-third of the ash constituents of the skeleton were lost during the three-month period in which the aluminum sulfate was being given.

SUMMARY

Four per cent basic aluminum acetate, 5 per cent $Al_2(SO_4)_3 \cdot 18H_2O$, 1 per cent $BeCO_3$ and 3 per cent $SrCO_3$ added to a stock diet were each found to produce a marked rickets in rats with an accompanying low serum phosphate. Large doses of irradiated ergosterol either prevented or cured

the rachitic condition as determined by calcification at the metaphyses of the long bones. The inorganic phosphate of the serum was increased as were also the absolute amount and percentage of femur ash. Doses of irradiated ergosterol slightly below the toxic level, however, failed to produce normal calcification as compared to animals on the stock diet.

The addition to the diet of metals which form an insoluble phosphate did not decrease the toxicity of large doses of irradiated ergosterol.

The presence of 1 per cent of $BeCO_3$ in the diet of adult rats produced a very low level of inorganic phosphate in the serum within one week. The serum calcium failed to show a reciprocal rise at any time during the period of 6 weeks while the phosphorus remained at a concentration of approximately 1 milligram per 100 cc. If beryllium or aluminum salts were added to a high calcium-low phosphorus rickets-producing diet and the diet fed until rickets developed the calcium could be dropped out without any material change in the calcium and phosphate levels of the blood and without the occurrence of tetany.

When adult rats were placed on a diet containing 5 per cent aluminum sulfate for a period of 3 months there was a marked decrease in serum phosphate and, judging from ash analyses of the femurs, nearly $\frac{1}{4}$ of the mineral constituents of the skeleton were lost. There was no rise in serum calcium.

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The Carbohydrate Metabolism Accompanying Intoxication by Aluminium Salts in the Rat

The effect of aluminium on mammal organism is not yet fully understood. It was found that intoxication with aluminium salts is accompanied by changes in metabolism of phosphorus compounds[1,2]. The common cause of serious disturbances is probably the formation of insoluble aluminium phosphate in the intestinal tract. This leads to the enhanced excretion of phosphorus from the organism and its negative balance. It was also found that, after peroral application of aluminium salts, the incorporation of intragastrically applied ^{32}P into the blood, liver, brain, kidney, spleen, muscle tissue and femur was decreased. Similarly the excretion of ^{32}P in the urine was lowered, while its excretion by the faeces was enhanced[3]. In the same paper it was confirmed that, during intoxication with aluminium salts, the levels of ATP decrease, while the levels of ADP and AMP increase.

It can therefore be supposed that application of increased doses of aluminium salts will influence the metabolism of carbohydrates. The findings of inhibition of glucose absorption in intestinal tract with aluminium salts[4] seems to confirm this view. We therefore studied some parameters of glycide metabolism in rats after the application of increased doses of aluminium chloride.

Two groups of white male rats, strain Wistar, weighing 175 ± 10 g were given the basal Larsen diet and water ad libitum, the control group received no aluminium, but the experimental group were given 200 mg aluminium/kg body weight incorporated into normal diet daily.

The Larsen diet consisted of: 622.6 g wheaten flower, 108.8 g dried milk, 163.3 g caseine, 32.7 g dried trefoil, 16.45 g calcium carbonate, 47.2 g margarine, 7.0 g fish liver oil, 2.4 g sodium chloride and tracer elements added.

Aluminium was added to the diet in the form of aluminium chloride. The extent of aluminium absorbed is about 10%. The experiment lasted 18 days. The weight of the rats under aluminium application decreased significantly ($P < 0.001$) compared with the control group, but neither the groups appeared sick. The last portion of diet and also of aluminium was given 24 h before decapitation of the animals.

The blood glucose was estimated according to SOMOGYI[5], liver and muscle glycogen according to GOOD[6], pyruvic acid in blood and liver following FRIEDEMANN and HAUGEN[7], lactic acid in blood, liver and muscle according to BARKER and SUMMERSON[8] and coenzyme A levels in liver according to HANDSCHUMACHER et al[9].

The results are summarized in the Table. The most pronounced change was the decrease of glycogen concen-

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Influence of aluminium intoxication on experimental rats

Values estimated	Control group	Experimental group	Significance
No. of rats	8	8	
Blood glucose mg%	109.60 ± 4.25	103.30 ± 2.23	$P > 0.05$
Glycogen			
Liver g%	2.530 ± 0.085	0.309 ± 0.030	$P < 0.001$
Muscle g%	0.442 ± 0.028	0.271 ± 0.023	$P < 0.001$
Lactic acid			
Blood mg%	13.72 ± 0.76	13.55 ± 0.66	$P > 0.05$
Liver mg%	11.89 ± 0.75	15.36 ± 0.72	$P < 0.01$
Muscle mg%	32.36 ± 3.50	51.61 ± 2.80	$P < 0.001$
Pyruvic acid			
Blood mg%	0.265 ± 0.012	0.366 ± 0.019	$P < 0.001$
Liver mg%	0.296 ± 0.002	0.400 ± 0.021	$P < 0.001$
Coenzyme A liver	17.27 ± 1.03	5.05 ± 0.27	$P < 0.001$

tration in liver, while its level in muscle was decreased only slightly though still significantly ($P < 0.001$). The glucose and lactate level in blood did not change significantly. However, the level of lactic acid in liver and muscle was increased. The level of pyruvic acid increased too, mostly in liver. The coenzyme A values are expressed in mg of acetylated 4-aminoazo-benzene related to 100 g of fresh liver tissue. These levels are strongly decreased in the experimental group ($P < 0.001$).

These results seem to point to a disturbance of glycide metabolism accompanying increased supply of aluminium salts. The most pronounced changes were the decrease of glycogen concentration in liver and increase of pyruvic and lactic acids levels in the same tissue. These changes have perhaps a common cause, i.e. the disturbance in phosphorus metabolism provoked by excess doses of aluminium salts. Therefore it can be assumed that there is decreased glucose absorption from the gut. The results correlate well with the other experiments in which the incorporation of ^{32}P into various phosphate fractions under the influence of aluminium was studied. The incorporation of ^{32}P into phospholipids, RNA and DNA in experimental animals was found to be significantly lowered[3].

Zusammenfassung. Bei Intoxikation von Versuchsratten durch Aluminiumsalze wurden Störungen im Glyzid-Metabolismus festgestellt. Er ergab sich ein Glykogenrückfall in Leber und Muskelgewebe und eine Erhöhung des Brenztraubensäure- und Milchsäurespiegels. Es wird angenommen, dass die beobachteten Veränderungen mit Störungen des Phosphormetabolismus und der Phosphorilationsreaktionen durch die Aluminiumtoxikation zusammenhängen.

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EVIDENCE FOR A PHOSPHORUS-DEPLETION SYNDROME IN MAN

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Abstract Antacids can impair phosphorus absorption in man. The long-term results of such impaired absorption are not fully appreciated despite knowledge of the vital role of phosphorus in life processes and the serious results of its depletion in animals. To determine depletion in man, studies were performed in three normal subjects and three patients with parathyroid dysfunction during prolonged treatment with antacids.

It was found that a syndrome of phosphorus depletion characterized by hypophosphatemia, hypophosphaturia, increased gastrointestinal absorption of calcium, hypercalciuria, increased resorption of skeletal calcium and phosphorus, and debility, with anorexia, weakness, bone pain and malaise, can be produced by prolonged treatment with nonabsorbable antacids such as magnesium-aluminum hydroxides.

THE clinical and pathological features of selective phosphorus depletion in man have received little attention — indeed, it has been stated that such an entity does not occur.[1,2] This view may stem from the fact that a selective deficiency of phosphorus is not likely to result from malnutrition alone because of its widespread distribution in ordinary foodstuffs.[3] In contrast to that of vitamin B_{12} and calcium, for example, absorption of phosphorus is not known to depend on specific substances comparable to intrinsic factor or vitamin D. Also, long-term research studies have not, to our knowledge, been done, probably because a synthetic, phosphorus-deficient diet is unpalatable.

Since phosphorus plays a crucial part in the vital processes of all living cells, its depletion should have definite pathologic sequelae. In fact, studies in animals indicate that deficiency may indeed result in a variety of disorders ranging from anorexia and weakness, to stunted growth, bone disease and even to rapid death.[4-16]

It has long been known that nonabsorbable antacids containing magnesium-aluminum hydroxides can limit gastrointestinal absorption of phosphorus.[15] To avert such depletion and its possible consequences — aluminum phosphate gel was introduced into clinical use for peptic ulcer therapy — particularly in patients with diarrhea.[16] This compound, however, has been largely displaced by various preparations of aluminum and magnesium hydroxides, marketed under a wide variety of trade names (Table 1). Subsequently, studies involving depletion of phosphorus by these agents were carried out, but usually were short ones, and were directed not toward a study of phosphorus deficiency per se but rather toward solution of diagnostic problems, as in hyperparathyroidism,[17] or toward therapy, as in the prophylaxis of kidney stones[18,19] and the hyperphosphatemia of chronic renal failure and hypoparathyroidism.[20]

Bloom and Flinchum[21] renewed interest in this problem by reporting a case of osteomalacia thought to result from phosphorus depletion caused by antacids. We have previously reported on a patient

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TABLE 1. *Partial List, by Trade Name, of Antacids Containing Magnesium or Aluminum Hydroxides or Both.*

Alkets	Co-Gel	Marblen	Silain-Gel
Aludrox	Gelusil	Mylanta	Trevidal
Aluscop	Kudrox	Oxaine	Trisogel
Alzinox	Maalox	Riopan	WinGel
Amphojel	Magnatril	Robalate	

whose illness was characterized by osteomalacia, debility and striking alterations in calcium and phosphorus metabolism, thought to result from prolonged phosphorus depletion resulting from excessive quantities of nonabsorbable antacid.[22] The present study was designed to gain a better understanding of the results of prolonged phosphorus depletion in normal subjects and in patients with parathyroid dysfunction. Because one of the striking features of this syndrome is the rapid onset of total renal conservation, studies were carried out to examine renal handling of phosphorus before and during depletion with antacids.

We conclude from our studies that prolonged, excessive ingestion of certain antacids produces a syndrome of clinical importance in man and that this syndrome is attributable to depletion of phosphorus.

METHODS

Three normal volunteers, two patients with hypoparathyroidism and one patient with pseudohypoparathyroidism were studied during confinement on an air-conditioned metabolic ward. Informed consent was obtained in all. Phosphorus metabolism was studied by renal clearance technics and by balance studies. When following a metabolic regimen, the subjects were given constant dietary and fluid intakes. Stools were collected in four-day pools with the aid of carmine markers, and urine in one-day pools. Blood, urine, stools and aliquots of the metabolic diets were analyzed for calcium[23] and phosphorus.[24]

Antacid treatment usually consisted of a combination of magnesium-aluminum hydroxide, 60 ml by mouth four times a day, and aluminum hydroxide, 30 ml by mouth four times a day. Although no diarrhea or constipation resulted from this combination, several patients had nausea initially.

In the normal subjects (S.T.B. and J.D.) phosphorus clearance was studied at identical times of the day before and during phosphorus depletion. Standard clearance technics were employed: priming and sustaining infusions of inulin and para-aminohippurate (PAH) were used. Bladder urine was collected by catheterization, with water and air rinses. After a one-hour equilibration period, collection was made for three 20-minute clearance periods.

Calcium absorption was estimated from the long-term balance data. In M.J.G. 5 μc of labeled calcium chloride ($^{47}CaCl_2$) (Oak Ridge National Laboratories) was given intravenously during control periods and during phosphorus depletion. Stools, urine and blood were analyzed for radioactivity as previously described.[25] "Endogenous fecal calcium" and gastrointestinal absorption of calcium were then determined by the method of Aubert and Milhaud.[26]

Results of Phosphorus Depletion in Hypoparathyroidism

CASE 1. M.L., a 47-year-old woman, had well documented postoperative hypoparathyroidism (Fig. 1).

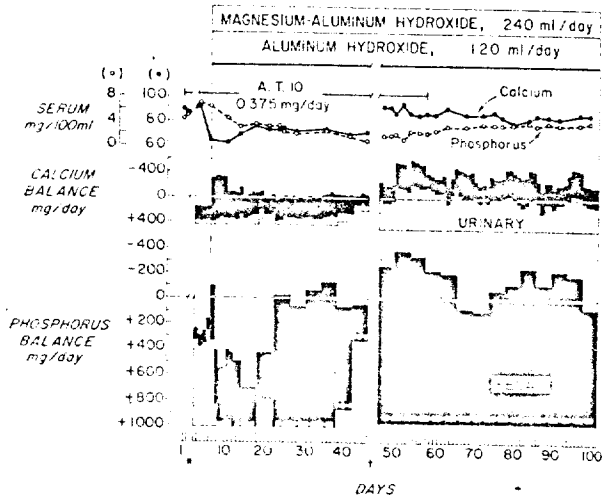

FIGURE 1. *Results of Prolonged Phosphorus Depletion in Case 1. The intake of calcium and phosphorus is plotted from the zero line downward, and the output from the intake line upward. A positive balance is indicated by a clear area below the zero line, and a negative balance by the stippled or dark area above the zero line. Stool data are represented by the black, and, urinary data by the stippled areas.*

**Time when oral calcium supplementation was stopped before the start of the study.*

†Uncharted 28-day period of ad libitum *dietary intake between metabolic day 47 and 48 during which magnesium-aluminum hydroxide and A.T. 10 were continued.*

The results of the long-term antacid treatment extending over 130 days of study were as follows: fecal excretion of phosphorus rose sharply to 1000 to 1200 mg per day. Urinary excretion declined rapidly to reach undetectable values as early as the 6th day of therapy, and remained zero throughout the study. By as early as the 20th day of therapy (metabolic day 23 in Fig. 1) there was a negative phosphorus balance. The urinary calcium rose to 500 to 700 mg per 24 hours, nearly a 10-fold increase, and high values persisted after omission of A. T. 10. Despite this hypercalciuria, there was no increase in urine volume or in thirst.

Fecal calcium increased initially with treatment and then declined to control values. Hyperabsorption was not evident, despite a markedly negative calcium balance largely attributable to the hypercalciuria.

Within 12 days of treatment the serum calcium rose to the range of 7 to 8 mg per 100 ml, where it remained for some 30 days before rising further to the range of 9 to 9.5 mg per 100 ml during a 28-day period of *ad libitum* diet during which magnesium-aluminum hydroxide was continued († in Fig. 1). Despite omission of A.T. 10 on metabolic day 58, the serum calcium remained in the normal range until the study was stopped on metabolic day 102 (study day 130).

The serum phosphorus declined progressively. When it had reached 1 mg per 100 ml the patient began to complain

of weakness, bone aches, anorexia, malaise and diffuse numbness. There was no clinical evidence of tetany, and the serum calcium was normal. On day 46 the patient had diffuse muscular weakness and a fine to coarse intention tremor of the upper extremities that was seemingly related to her diffuse weakness. The serum phosphorus then rose spontaneously over 8 days to reach 3 mg per 100 ml, and the symptoms subsided considerably; the level then fell again during the period of *ad libitum* diet (‡ in Fig. 1, and thus not charted). When it reached 0.7 mg per 100 ml all symptoms returned. The serum phosphorus then rose gradually and stabilized at 4 mg per 100 ml, and the tremor and numbness subsided completely whereas the other complaints continued, although to a lesser degree.

After cessation of magnesium-aluminum hydroxide (not shown) the serum phosphorus rose in 3 days from 4 to 9 mg per 100 ml. The serum calcium declined from 8.5 to 6.1 mg per 100 ml. The urinary calcium declined rapidly in 3 days from 400 to 40 mg per day whereas the urinary phosphorus rose from 0 to 700 mg per day. The anorexia, malaise and weakness diminished gradually over a 2-week period.

Case 2. A.S., a 40-year-old woman, had well documented postoperative hypoparathyroidism (Fig. 2).

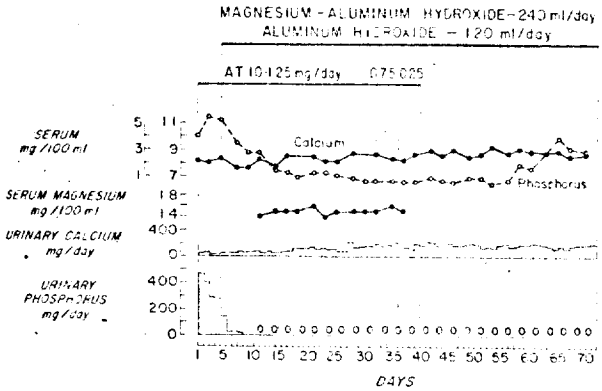

Figure 2. *Results of Phosphorus Depletion in Case 2.*

The diet was constant throughout the study. Calcium intake was 750 mg per day.

The urinary phosphorus fell to undetectable values on the 5th day of antacid treatment, and remained essentially zero throughout the study. Despite omission of A.T. 10 the urinary calcium rose progressively to reach values 5 times those of the control period. There was no increase in thirst or in urine volume.

Despite withdrawal of A.T. 10 (days 32 to 40) the serum calcium remained in the range of 8.5 to 9.5 mg per 100 ml. The serum phosphorus declined, to reach 0.8 mg per 100 ml on day 28. At that time the previously vague symptoms of weakness, anorexia, bone pains and malaise suddenly increased. These symptoms remained moderately severe until the serum phosphorus rose spontaneously to values above 1.5 mg per 100 ml (day 54). Although all the symptoms then diminished, they did not disappear entirely, even after the serum phosphorus had risen to normal (3 to 4 mg per 100 ml on days 65 to 70).

After therapy was stopped (not shown) the changes were similar to those in Case 1.

Results of Phosphorus Depletion in Pseudohypoparathyroidism

Case 3. A.M., a 13-year-old girl, had well documented pseudohypoparathyroidism (Fig. 3 and 4).

With a constant metabolic diet, the serum calcium ranged from 7.5 to 8.4 mg, and the serum phosphorus from 8 to 8.2 mg per 100 ml. These values did not change with addition of calcium lactate, 9 gm per day, for 15 days (not charted). Supplemental calcium was then omitted, and aluminum hydroxide was given (Fig. 3), with no effect on serum calcium and phosphorus during the 16 days of treatment. However, the urinary phosphorus declined to 160 mg per day by the 1st day of treatment, and continued to decline. Urinary calcium remained negligible.

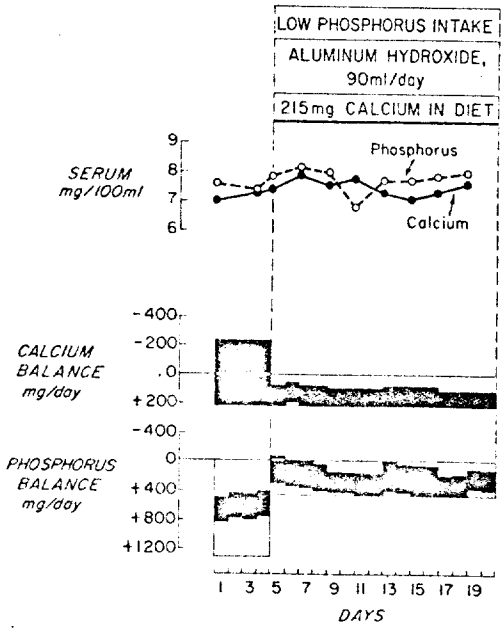

Figure 3. *Results of a Low Phosphorus and Low Calcium Intake during Aluminum Hydroxide Therapy in Case 3.*

Balance data are plotted as described in Figure 1. Stool data are in black, and urine data in white.

Fecal calcium fell from about 400 to 100 mg per 24 hours by day 16, indicating an increase in calcium absorption. Fecal phosphorus increased almost to intake values.

Subsequently (Fig. 4), supplemental calcium lactate was added to the diet during aluminum hydroxide treatment. The serum calcium then rose gradually to 9.0 to 9.5 mg per 100 ml during 21 days of therapy. The serum phosphorus declined from 8 mg to a range of 4.5 to 5.0 mg per 100 ml over the same period.

Results of Phosphorus Depletion in a Normal Subject

M.J.G., a 29-year-old white woman, entered the study as a normal volunteer (Fig. 5).

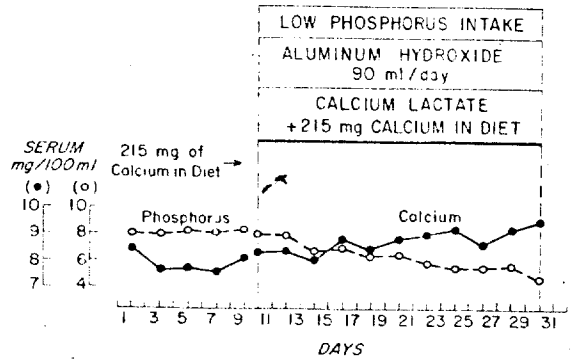

Figure 4. *Results of a High Calcium, Low Phosphorus Intake during Aluminum Hydroxide Therapy in Case 3.*

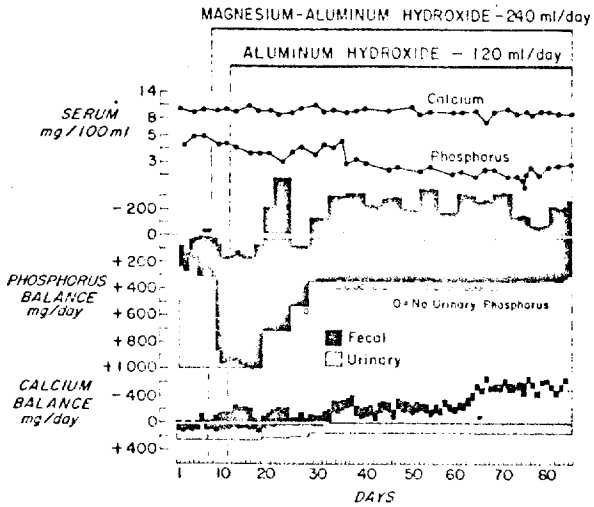

FIGURE 5. *Results of Prolonged Phosphorus Depletion in a Normal Subject (M.J.G.).*

Balance data are plotted as described in Figure 1. The calcium and phosphorus intakes were reduced progressively in accord with the patient's wish to avoid weight gain, which was occurring early in the study.

During antacid treatment the urinary phosphorus declined, to undetectable values by the 6th day. The urinary calcium rose 5-fold, to a range of 600 to 750 mg per day. Urine volume did not change, and there was no increase in thirst.

Fecal phosphorus rose to exceed intake by 200 to 300 mg per day by the 11th day of therapy (metabolic day 19, Fig. 5); about 12 gm of phosphorus was lost during the study.

Fecal calcium fell from control values of about 200 mg to 75 to 90 mg per day by the end of the study. That this decline represented an actual increase in calcium absorption was also shown by the studies with $^{45}CaCl_2$, which indicated 75 per cent absorption on the 73d day of phosphorus depletion as compared to a 45 per cent absorption during control periods.

The serum calcium remained in the normal range. Alkaline phosphatase (not shown) rose from 5 to 10 King-Armstrong units per 100 ml.

The serum phosphorus fell, to 1.1 mg per 100 ml on metabolic day 75, when the subject complained of such severe weakness and anorexia that she remained in bed. Despite a spontaneous return to the range of 2.5 to 3.0 mg per 100 ml over the next few days, the weakness and anorexia persisted, although to a lesser degree. The subject was bedridden for only 1 day (day 75).

After cessation of treatment (data not shown), there was a rapid regression of all the abnormalities described during phosphorus depletion.

Renal Handling of Phosphorus in Normal Subjects before and during Phosphorus Depletion

Table 2 describes the results in S.T.B., an 18-year-old normal woman, and J.D., an 18-year-old normal woman.

During depletion in these subjects, the fractional reabsorption of filtered phosphorus (percentage of tubular reabsorption of phosphorus) increased despite increased or unchanged filtered loads. Inulin and PAH clearances were similar before and during treatment.

DISCUSSION

When we induced phosphorus depletion by rendering phosphorus nonabsorbable, a number of striking events occurred. These included a rapid, profound decrease of urinary phosphorus, hypercalciuria, hypophosphatemia, increased resorption of skeletal calcium and phosphorus increased gastrointestinal absorption of calcium and a state of debility characterized by weakness, anorexia and malaise. The present study indicates that these changes can occur in the absence of the parathyroid glands.

Renal Conservation of Phosphorus

The rapid onset of hypophosphaturia is comparable in magnitude and rapidity to the renal conser-

TABLE 2. *Phosphorus Clearances before and during Phosphorus Depletion.**

SUBJECT	PERIOD	INULIN CLEARANCE	PAH CLEARANCE	SERUM PHOSPHORUS	PHOSPHORUS FILTERED	PHOSPHORUS REABSORBED	PHOSPHORUS EXCRETED	TUBULAR REABSORPTION OF PHOSPHORUS
		ml/min	*ml/min*	*mg/100 ml*	*mg/min*	*mg/min*	*mg/min*	*%*
S.T.B.:								
Before treatment†	1	141	778	4.3	6.06	5.50	0.56	91
	2	113	623	4.2	4.74	4.16	0.58	88
	3	163	594	4.0	6.52	5.93	0.59	91
During treatment†	1	160	712	4.6	7.36	7.24	0.12	98
	2	132	488	4.9	6.46	6.32	0.14	98
	3	137	649	4.6	6.30	6.10	0.20	97
J.D.:								
Before treatment†	1	71	365	3.8	2.70	2.10	0.60	78
	2	70	444	3.6	2.52	2.00	0.52	79
	3	54	303	3.6	1.94	1.34	0.60	69
During treatment†	1	58	336	3.6	2.09	2.08	0.01	99
	2	74	355	3.6	2.66	2.61	0.05	98
	3	68	300	3.5	2.38	2.33	0.05	98

*Dietary phosphorus similar before & during phosphorus depletion.

†Treatment with magnesium aluminum hydroxide, 360 ml/day.

vation of sodium during sodium depletion. Conservation of phosphorus, however, unlike that of sodium, is not known to be mediated by hormonal mechanisms. The clearance results indicate that it does not depend upon a decrease in the filtered load of phosphorus (Table 2). The nature of the stimulus to the rapid and profound increase in phosphorus reabsorption is not clear; it could conceivably result from a humoral stimulus from the bowel evoked by the sudden cessation of phosphorus absorption. It is also possible that intracellular concentration itself governs renal conservation of phosphorus.[27]

Hormones other than parathyroid hormone are known to influence renal handling of phosphorus. Thus, growth hormone decreases clearance, and thyrocalcitonin may facilitate it.[28,29] Conceivably, appropriate changes in activity of one or combinations of these hormones could result in the changes that we observed. As yet, however, there is no evidence that any aspect of phosphorus metabolism acts as a stimulus to the production of these hormones.

Whatever its nature, the mechanisms leading to renal conservation of phosphorus must be extremely sensitive since "complete" conservation occurs with losses that are extremely small in relation to total body phosphorus — for example, after losses of approximately 1 gm out of a total body content of approximately 700 gm.

Increase in Urinary Calcium

The marked increase in urinary calcium in the normal subject (M.J.G.) and in the patient with hypoparathyroidism (M.L.) occurred without any abnormal elevation in serum calcium. (The absence of any increase in urinary excretion despite return of the serum level to normal in the patient with pseudohypoparathyroidism may reflect the tendency of growing children to conserve calcium rather than any feature of pseudohypoparathyroidism itself). The source of the increment of urinary calcium includes both the gastrointestinal tract and the bones, since output greatly exceeded intake in M.L. (Case 1) and in M.J.G. Whereas magnesium is thought to share a common renal tubular reabsorption mechanism with calcium it is unlikely that any small amounts of magnesium absorbed from the magnesium-aluminum hydroxide could have produced the degree of hypercalciuria observed.[30-32] In A.S. (Case 2), repeated measurements of serum magnesium showed no change while she was receiving magnesium-aluminum hydroxide. Increases in urinary calcium may also reflect urinary sodium[33] or result from increases in filtered load. Whereas urinary sodium, ultrafilterable calcium and glomerular filtration rate were not measured in this study, it has been shown that these variables are not responsible for the increase in urinary calcium during phosphorus depletion and that administration of phosphorus alone can reverse the hypercalciuria.[34] Its use for the management of hypercalciuria is well recognized clinically.[35] It is conceivable that the mechanism for renal tubular reabsorption of calcium may depend upon (? cellular) phosphorus.

Despite hypercalciuria in M.J.G. and M.L., neither subject showed an increase in thirst or in urine volume or an unusual loss of weight. Thus, these subjects did not constitute a counterpart of the large increase in thirst and urinary volume reported for phosphorus-depleted pigs.[10] Their urinary concentrating ability did not decrease as is sometimes found with hypercalciuria, even without hypercalcemia.[36] Whatever its mechanism, the marked hypercalciuria, if continued long enough, could conceivably lead to urolithiasis, nephrocalcinosis, and kidney damage.

Changes in Serum Phosphorus

The serum phosphorus invariably decreased during treatment, most notably in the subjects with hypoparathyroidism. This decline was slow and progressive until very low values were achieved. In the long-term studies all subjects experienced an increase in their state of "debility" (as discussed below) at about the low point of serum phosphorus, and this symptom tended to remit somewhat as the serum level gradually rose.

It has been suggested that as the body is depleted of phosphorus, cellular rather than bone phosphorus preferentially regulates the serum level until some value is reached at which this mechanism "breaks down" and bone phosphorus then acts as the source of serum phosphorus.[37] It is possible that the gradual, spontaneous increases in the serum level from the low points resulted from more rapid dissolution of bone, but urinary calcium showed no increase while the serum phosphorus was increasing. If bone dissolution did take place, the parathyroid glands were not necessary for such a response.

The balance data indicate an increase in absorption of calcium in the normal subjects (M.J.G.) and in A.M. (Fig. 5 and 3, respectively) as shown by the decreases in fecal calcium as the studies progressed, and as confirmed by the results with ^{47}Ca-labeled chloride in M.J.G. There was no clear increase in calcium absorption in M.L. It is of great interest that the serum level of calcium rose to normal and remained normal for extended periods without vitamin D in the 2 patients with hypoparathyroidism during phosphorus depletion. In the patient with pseudohypoparathyroidism it was necessary both to increase dietary calcium and to limit availability of phosphorus to effect an increase in serum calcium. This is in agreement with earlier observations.[38,39] It is of interest that hypercalcemia never occurred.

Markedly negative calcium balance was produced in M.J.G. and M.L. Clearly, some of the excreted calcium must have been released from the bones. Thus, skeletal resorption must have been taking

place, releasing both calcium and phosphorus into the bloodstream. A negative calcium balance, if continued long enough, could conceivably result in bone disease, presumably osteomalacia.[22]

Clinical Syndrome of Phosphorus Depletion

All subjects treated with antacids for prolonged periods manifested a state of debility characterized by weakness, anorexia and malaise. The severity of these symptoms correlated best with the decrease in serum phosphorus. As the serum level subsequently rose, the symptoms tended to subside somewhat, but they never remitted entirely during treatment. In A.S., severe bone pain and joint stiffness developed and persisted throughout the study. M.L., in addition, manifested an intention tremor of the hands that occurred on two occasions when the serum phosphorus had declined to below 1 mg per 100 ml and remitted as the serum level rose.

Presumably, this state of debility resulted from phosphorus depletion. Anorexia and weakness (which may progress to death) are prominent features of depleted animals.[4-16] It is possible, of course, that vital compounds other than phosphorus of which we are unaware are being lost during these experiments, or that some other effect of the antacids themselves is responsible. It is known that antacids may bind antibiotics such as tetracycline; possibly, they bind several gastrointestinal enzymes.[40] However, it is generally believed that such antacids do not have systemic effects.[40] There was no sign of weight loss or of other malabsorption in any of our subjects.

It is possible that the debility in some patients with hyperparathyroidism or osteomalacia is attributable to phosphorus depletion. Repletion is an accepted therapeutic modality in each of these disorders.[41-43] Phosphorus depletion results in decreases of blood adenosine triphosphate (ATP) stores in chickens, and it is possible that a similar process occurs in man.[44]

In two recent reports osteomalacia was attributed to prolonged depletion of phosphorus produced by excessive ingestion of nonabsorbable antacids.[21,22] One of these patients had, in addition to osteomalacia, abnormalities characteristic of the phosphorus-depletion syndrome — that is, hypophosphaturia, hypercalciuria, hypophosphatemia, increased absorption of calcium and debility.[22] It is not clear whether depletion produced in some other way would result in similar changes. A diet deficient in phosphorus alone is not selected spontaneously by any population group. The present study suggests that such a syndrome in man does indeed exist. The widespread use of nonabsorbable antacids for the treatment of peptic ulcer makes recognition of the syndrome clinically important. For example, a patient with an ulcer receiving antacids can show a low serum phosphorus and high urinary calcium, findings that suggest a diagnosis of parathyroid adenoma. Awareness of this syndrome might thus prevent unnecessary surgical exploration. Similarly, use of antacids as prophylaxis against peptic ulcer in patients receiving corticosteroids may cause phosphorus depletion. In this situation bone disease may result both from the steroids and from the antacids.

The frequency of this syndrome in man is not known. Greater awareness may result in greater recognition. It is likely that the sporadic intake of antacids by most patients and the high phosphorus content (milk) of most diets used for the therapy of peptic ulcer prevent the frequent development of these changes.

Therapy is simple since it involves only the provision of adequate dietary phosphorus. In a patient with hypophosphaturia, determination of urinary phosphorus should readily establish or rule out the syndrome as produced by antacids: a level of over 300 mg per day indicates that the amount contained in the diet is adequate.

In immobilized patients with fractures, however, supplementation of the diet with phosphorus is alleged to result in more rapid callus formation and healing.[45] If antacids are given to such patients, supplementation should be increased accordingly.

Recently, a large dietary intake of phosphorus has achieved favor in the therapy of certain types of kidney stones.[46] It is thought that the phosphorus facilitates formation of a phosphorus-rich compound normally occurring in urine to solubilize apatite.[47] The effect of depletion on the excretion of this factor is unknown; theoretically, it is of great importance.

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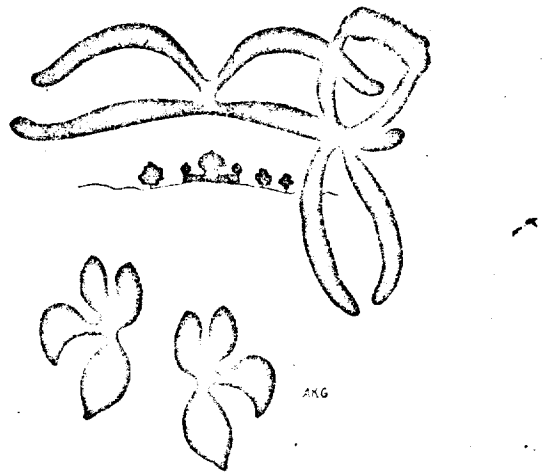

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National Academy of Sciences - National Research Council

Annual Poundage Reported per Substance
by NAS and FEMA User Firms

(Preliminary data tables available
at Food and Drug Administration)

SKORYNA AND WALDORN Edward: Intestinal Absorption of Metal Ions (Pergamon Press: NY 1971)

ALUMINUM, ITS ABSORPTION, DISTRIBUTION, AND EFFECTS ON PHOSPHORUS METABOLISM

RUDOLF ONDREIČKA, JOZEF KORTUS and EMIL GINTER
Institute of Human Nutrition Research, Bratislava, Czechoslovakia

As ALUMINUM is used extensively in the food industry, it becomes increasingly desirable to determine its biological effects. However, in spite of considerable effort to understand and explain the effect of aluminum and its compounds on living organisms, many questions have not yet been completely solved.

The metal aluminum has atomic number 13 and atomic weight 26·97. It is very light with a specific gravity of 2·7; melting point 660·2°C. In its elementary form it is extremely resistant to atmospheric corrosion as well as to corrosion caused by common chemicals. Solid structural alloys are considerably less resistant; nevertheless, even in their case a high grade of resistance to corrosion can be attained by a suitable method of founding and subsequent technological treatment.

The physical and chemical characteristics of aluminum and its compounds help to explain some of its important actions and reactions in a living organism. Aluminum combines with acids and bases, producing salts and aluminates. Due to the high charge and small size of the Al^{3+} ion, negatively charged groups such a Cl^- and OH^- ions very readily form strong bonds; aluminum chloride and aluminum hydroxide behave as covalent compounds and their properties are different from similar compounds of typical metals. The solubility of individual aluminum compounds as determined by Seidell[1] is listed in Table 1. Some properties of aluminum compounds that may be introduced into the living organism from the environment will be described as they may have some importance in their biological effects.

Soluble and Insoluble Compounds of Aluminum

Aluminum chloride forms a hydrate, $AlCl_3 \cdot 6H_2O$ (m.w. 241·43), or $Al(H_2O)_6Cl_3$ in the presence of water; the final solution has an acidic reaction. Owing to the acidic properties of the solution, aluminum chloride is used for the preparation of antiseptics and deodorizing agents.

When sodium hydroxide solution is added to a solution of aluminum chloride at a temperature of 25°C, aluminum hydroxide starts to precipitate as the pH is increased to 3. Precipitation ceases after pH 7; further addition of alkali results in solution of the precipitate.

Binary salts of aluminum chloride with metallic chlorides and with various organic compounds such as ketones, esters, amines, nitro-compounds are known.

TABLE 1. SOLUBILITY OF VARIOUS ALUMINUM COMPOUNDS IN WATER

Compound	Temperature °C	Solute	
$AlCl_3 \cdot 6H_2O$	25	31·6 $AlCl_3$	(as $AlCl_3 \cdot 6H_2O$)
	40	32·1 $AlCl_3$	
	100	32·9 $AlCl_3$	
$Al(NO_3)_3 \cdot 18H_2O$	25	39·5–43·5 $Al(NO_3)_3$	(as $Al(NO_3)_3 \cdot 9H_2O$)
	40	43·0–47·0 $Al(NO_3)_3$	
	90	60·5 $Al(NO_3)_3$	(as $Al(NO_3)_3 \cdot 9H_2O + Al(NO_3)_3 \cdot 3H_2O$)
$Al_2(SO_4)_3 \cdot 18H_2O$	20	26·7 $Al_2(SO_4)_3$	(as $Al_2(SO_4)_3 \cdot 18H_2O$)
	40	31·4 $Al_2(SO_4)_3$	
	100	47·1 $Al_2(SO_4)_3$	
$Al(OH)_3$	18	0·94–1·04 Al_2O_3	(as $Al(OH)_3$)
	29	0·98 Al_2O_3	
$AlNa(SO_4)_2$	25	43·1 $Na_2Al_2(SO_4)_4$	
	38	133·7 $AlNa(SO_4)_2 \cdot 12H_2O$	
$AlNa(SO_4)_2 \cdot 24H_2O$	25	131·8 $Na_2Al_2(SO_4)_4 \cdot 24H_2O$	

g/100 g H_2O — g/100 g saturated solution

Hydrated aluminates or aluminum hydrogels are precipitates with the formula $Al_2O_3 \cdot nH_2O$, and are formed by a reaction of aluminum salt solutions with OH^- ions. They are not known in crystalline form as definitive hydroxides, being obtained either in amorphous form, colloidal aluminum hydrogel, or as soluble hydrates in the form of aluminum hydrosol. The aluminum hydrogels, when freshly precipitated, readily dissolve in acids. Due to the effect of aging they become less water-soluble after a few days.

Aluminum hydroxide $Al(OH)_3$ (m.w. 77·99), is insoluble in water at pH 4 to 8, eventually up to 10·5. When freshly precipitated it is readily soluble in cold HCl and in H_2SO_4 and in solutions of permanent bases. Its solubility deteriorates on drying. The OH^- in aluminum hydroxide is so strongly bound to aluminum, that H^+ is released into water resulting in an acidic reaction of the solution. When precipitating aluminum hydroxide from a solution containing suspended or dissolved substances, a part of these substances will be absorbed by the precipitate. Freshly precipitated hydroxide suspended in water will not dissolve after introduction of gaseous CO_2; on the contrary the gas is bound to the solid component of the system. Aluminum chloride, however, is partially soluble in a solution of carbonic acid and can be repeatedly precipitated by boiling or by exposure to the air.

Aluminum phosphate. Aluminum forms a series of phosphates—orthophosphate $AlPO_4$, primary and secondary orthophosphate $Al(H_2PO_4)_3$ and $Al_2(HPO_4)_3$, and various combinations, pyrophosphate $Al_4(P_2O_7)_3$, dialuminumpyrophosphate $Al_2H_6(P_2O_7)_3$ and metaphosphate $Al(PO_3)_3$. Aluminum phosphate is relatively insoluble in water, but is readily dissolved in solutions of aluminum salts or aluminates between pH 3·9–5·5 and 8·6. Calcium hydroxide increases the solubility of phosphoric acid derivates of aluminum while organic acids tend to prevent the precipitation of the aluminum phosphate from solution.

Aluminum sulfate, $Al_2(SO_4)_3 \cdot 18H_2O$ (m.w. 666·40), is used to prepare some aluminates. It is soluble in water; strong alkali causes the formation of $Al(OH)_3$.

Aluminates. Aluminum salts tend to form binary salts, for example, aluminum sulfate forms a series of salts of the type $R'_2SO_4 \cdot Al_2(SO_4)_3 \cdot 24H_2O$ or $R'Al(SO_4)_2 \cdot 12H_2O$, where R′ represents a univalent radical. Due to the relatively low stability of trivalent forms of some elements it seems to be a rule that the greater the molecular weight of a univalent radical, the greater the stability of the aluminate and vice versa, the greater the molecular weight of a trivalent metal, the lesser the stability of aluminate.

The reactivity and solubility of aluminum changes within the pH range from 2 to 7·4 under the effect of enzymes, catalysts and protective substances present in the digestive tract and also in food. As with most other common metals, aluminum is capable of forming complex compounds with proteins, and other substances of biological importance. These properties must be borne in mind in assessing the biological activity of aluminum, particularly in problems concerning its absorption and subsequent tissue distribution.

Aluminum Content of Foodstuffs

Aluminum is present in human nutrients in varying amounts. Its content in foodstuffs of plant origin depends on the content of the aluminum in the soil. Easily soluble aluminum compounds do not occur in nature; soil solutions contain in the order of 10^{-10}% aluminum. In natural waters, the content is under 10^{-30}%.[4] For this reason, aluminum, in spite of its extensive distribution, accumulates only in insignificant amounts in living organisms. Early determinations of the aluminum content of foodstuffs must now be considered as being unduly high, due mainly to the lack of adequate techniques and also to possible contamination of reagents, equipment and even of the samples. According to Calvery,[4] the most probable evaluation of the aluminum in foodstuffs falls within 10–30 mg Al per kg fresh weight. Foodstuffs of plant origin are higher, especially from some species of subtropical plants (Theaceae, Euphorbiaceae, Caryophyllaceae). Many plants accumulate a considerable amount of aluminum. For example, Lycopodiaceae contain as much as 70% of Al_2O_3 in ash.[5] Some plants derive their typical coloration from aluminum which is therefore in higher concentrations in these parts. In foodstuffs containing high amounts of anthocyanine dyes, aluminum is increased but other plant dyes may also help to accumulate this metal.

The aluminum content of foodstuffs of animal origin is lower and may be 50 or 100 times less than in foodstuffs of plant origin.[6] This can be explained by the low solubility of aluminum compounds in the digestive tract, and in the ability of these compounds to form complexes within the digestive tract which results in low absorption of aluminum.

Aluminum concentration in foodstuffs of animal origin fluctuates within the limits of 1·6–16 mg per kg.[7] Relatively large amounts were found in the bowels. Liver, heart, brain, stomach and intestinal wall show a higher content of aluminum compared with other tissues.

As a result of using aluminum containers and equipment in foodstuffs technology, a certain amount of aluminum may be incorporated into the final products. Aluminum equipment or containers are only minimally attacked by pure water. According to Truffert,[6] the solubility of aluminum is greatly increased by alkaline or acidic solutions. The amounts of aluminum taken up during culinary treatment of neutral foodstuffs is insignificant. Acidic foodstuffs produce a certain degree of aluminum dissolution, but the effect of acid is suppressed by the presence of sugar.[8] Alkaline foodstuffs, or those turning alkaline by the addition of soda, also attack aluminum containers.

TABLE 2. ALUMINUM CONTENT OF FOODS

	Mean†	Mg Al/100 g wet weight
Vegetables	10·42	Min. value—3·1 green pepper Max. value—49 lentils
Fruits	12·11	Min. value—0·6 apricot Max. value—50·6 dried pear
Cereals and cereal products	12·38	Min. value—0·7 roll Max. value—29·5 rice
Meat and meat products	0·73	Min. value—0·6 pork back Max. value—1·1 sausage

† Means of twenty individual types in each category. A photometric method with ammonium aurintricarbonate was used to determine Al content.

The complex physicochemical system represented by the composition and structure of food, generally helps towards the inhibition of the chemical effect of acids, alkali and salts on aluminum containers and equipment. From all available data, it is evident that, apart from some exceptional cases, the concentration of aluminum in foodstuffs, after preparation in aluminum containers, falls well within the same limits of the concentrations determined in the raw materials. The few exceptions to this rule are of only little physiological significance.

The foregoing data were obtained from samples prepared in the laboratory. Sample analysis of products from household or industrial sources give a more comprehensive range of data.

TABLE 3. ALUMINUM CONTENT OF FOODS COOKED AND STEWED IN ALUMINUM (Al) AND STAINLESS STEEL (Ss) UTENSILS

Boiled foods	Boiling conditions			Mg Al/100 g dry weight		Stewed foods	Stewing conditions			Mg Al/100 g dry weight	
	pH	Temp. (°C)	Time (min)				pH	Temp. (°C)	Time (min)		
Ox-tail soup	5·5	98	125	Ss Al	0·27 0·49	Fish soup	5·5	97	45	Ss Al	0·55 1·86
Tomato sauce	5	95	15	Ss Al	3·17 3·87	Stewed cabbage	5·5	97	60	Ss Al	0·15 3·05
Beans side-dish	7	96	155	Ss Al	2·62 3·47	Stewed carrots	6·5	96	30	Ss Al	27·39 42·07
Lettuce side-dish	5·5	97	15	Ss Al	2·45 3·66	Segedin goulash	5·0	98	90	Ss Al	0·03 0·05
Sauerkraut soup	5	97	30	Ss Al	0·32 0·71	Beef meat with onion	5·5	98	180	Ss Al	0·06 0·67

All facts seem to indicate that factors influencing the aluminum content in human nutrition are manifold and variable. It is apparent that considerable fluctuations occur in the intake of numerous groups of people living on differing dietary regimes.

Aluminum Metabolism

In early studies, before sensitive analytical methods were developed for determination of aluminum, it was erroneously concluded that aluminum is not absorbed by the animal organism, and is quantitatively excreted in feces. However, more recent investigations have revealed that a small part of ingested aluminum is absorbed; this is confirmed by its presence in all tissues, blood and urine.[2, 9–12] A relatively small number of papers deal with the retention and mechanism of elimination of absorbed aluminum.[13, 14] Our results showed[15] that by increasing the aluminum content in food two-fold, fecal elimination is significantly increased; retention of aluminum and tissue level are not significantly affected. On the other hand, considerably increased doses of aluminum sulfate, 200 mg Al per 1 kg, caused a significant increase in retention of aluminum in rats; increased levels also appeared in urine. Tissue concentrations were increased: the most pronounced accumulation of aluminum was observed in liver, testes and bones, while in other organs the aluminum level was not significantly changed. The data obtained indicate that the animal organism is able to accommodate a slightly increased intake of aluminum compounds by increased fecal elimination, so that there is no significant increase in aluminum retention. On the contrary, an extreme increase of aluminum intake results in increased absorption; as a result a part of the absorbed aluminum will be deposited in some tissues, especially in skeleton and liver, and a part is eliminated by urine.

Absorption and retention of aluminum compounds from food also depends on the presence of other substances in the food, particularly the effect of fluorides.

In the course of balance experiments performed on rats we have found that, following the administration of 1 mg of fluorine per animal, elimination of aluminum by urine and feces was significantly increased and its retention in the organism was decreased. Similarly

TABLE 4. ALUMINUM LEVELS IN SOME RAT TISSUES AND IN BLOOD AFTER ADMINISTRATION OF INCREASED DOSES OF Al^{3+} AND F^-

Tissue	Larsen diet	Larsen diet + 50 mg Al	Larsen diet + 1 mg F	Larsen diet + 50 mg Al + 1 mg F
Liver	1·55 ± 0·25	2·99 ± 0·27	1·46 ± 0·11	1·90 ± 0·21
Brain	0·71 ± 0·08	1·07 ± 0·15	0·66 ± 0·09	0·77 ± 0·11
Kidneys	0·86 ± 0·08	0·90 ± 0·10	0·73 ± 0·10	0·85 ± 0·09
Spleen	1·46 ± 0·22	1·48 ± 0·10	1·25 ± 0·12	1·43 ± 0·21
Heart	1·07 ± 0·12	1·27 ± 0·12	1·00 ± 0·14	1·05 ± 0·12
Lungs	0·40 ± 0·05	0·42 ± 0·08	0·42 ± 0·06	0·41 ± 0·06
Testes	0·17 ± 0·05	0·47 ± 0·06	0·17 ± 0·02	0·21 ± 0·04
Colon	0·68 ± 0·10	0·62 ± 0·04	0·59 ± 0·06	0·60 ± 0·09
Muscle	0·03 ± 0·008	0·04 ± 0·002	0·04 ± 0·007	0·04 ± 0·005
Blood	0·65 ± 0·11	1·10 ± 0·08	0·79 ± 0·06	0·82 ± 0·10
Femur	70·18 ± 0·98	91·16 ± 0·65	44·08 ± 0·54	75·42 ± 0·80

we have observed that with increasing doses of fluoride and prolonging the time of administration aluminum in bone tissue was also decreased. In one series of experiments with rats, we determined the effect of simultaneously dosing Al (50 mg/kg) and fluorine (1 mg F^- perorally per animal) for 24 days. The level of aluminum (Table 4) in all organs and tissues, with exception of the intestines, is higher following the administration of aluminum alone. Administration of fluoride resulted in decreased aluminum levels in most organs. After simultaneous administration of fluoride and aluminum ion, the level of aluminum in individual organs does not show any substantial difference compared with the level of this element in control group tissues.

The results point out the antagonistic relationship between fluoride and aluminum. We suggest that a mutual reaction occurs between them in the organism, resulting in the formation of a readily soluble complex $(AlF_6)^{-3}$, which is more soluble than calcium fluoride, with the result that aluminum is not retained in the organism.

The physiological requirement of aluminum by the organism is still open to discussion, in particular the influence of aluminum on growth, or on fertility. Individual authors differ in their views. Some maintain that the growth of experimental animals is favored by the presence of aluminum, while the others do not share this view.[16-19]

Toxicity of Aluminum

Up to the present time there has been no consensus on the toxicity of aluminum compounds. According to some authors "aluminum compounds are not harmful, even after administration over a long period of time".[20] On the contrary there are some reports of various toxic effects induced in experimental animals by the administration of high doses of aluminum compounds (ref. 13 and others).

The elucidation of toxic effects of aluminum salts is further complicated by the influence of the accompanying acid radical and degree of solubility. In general, the lethal dose LD_{50} for the most common aluminum compounds falls within the range of 1–5 g/kg of body weight after peroral administration.[21, 22] In our study on the acute toxic effect of aluminum chloride after peroral administration we found[15] that the LD_{50} for mice, calculated on the aluminum content, was $0{\cdot}77 \pm 0{\cdot}12$ g Al per kg of weight. The toxic effect of aluminum in aluminum sulfate is slightly lower, probably because of its different physicochemical properties. These substances therefore may be classified as mildly toxic substances when given in single doses. The toxicity of aluminum salts after intravenous administration is considerably higher.[22]

There is scant information only, available about chronic toxicity of aluminum compounds. The toxic effect of these substances depends on the mode of administration. By admixture with food, retardation of growth is the only toxic effect, for which high doses are required. Even after doses as high as 2 g per kg no pathological changes, apart from growth retardation, were observed on individual organs.[23] However, it is probable that some metabolical disturbances do result from such doses. Of some importance is the question of the potential toxic effect of prolonged administration of slightly increased doses of aluminum salts on living organisms. This area has been only studied superficially until now. Our experimental results[15] indicated that relatively low doses of aluminum chloride (19·3 mg Al per 1 kg of weight) administered in drinking water showed retardation of growth

in the second and third generation (Fig. 1). The results which we obtained are not in agreement with the opinions of previously published papers, in which perorally administered aluminum compounds are not ascribed any toxic effects. The fact that the blood picture and also the morphology of various tissues of these animals showed no significant deviation from the norm is in agreement with the results of other authors[23] and, at the same time, points out the necessity of seeking for more sensitive indicators of metabolic disturbances which may be brought about as a result of the chronic uptake of relatively small amounts of aluminum compounds.

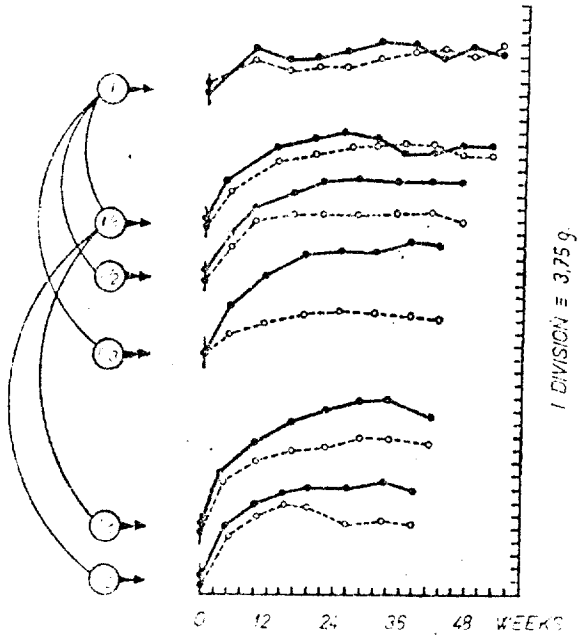

FIG. 1. Weight increases of normal (full lines) and of aluminum trichloride treated (dotted lines), white mice: I, original generation; II 1, 2, 3, first, second, and third litter of the second generation; III 1, 2, first and second litter of the third generation.

Aluminum and Metabolism of Phosphorus

The mechanism of the toxic effect of aluminum salts has not hitherto been completely understood. It can be predicted that aluminum compounds will react with phosphates in the gastrointestinal tract to form slightly soluble aluminum phosphates. Reduced to simple terms, we can present the reaction:

$$Na_2HPO_4 + NaH_2PO_4 + 2AlCl_3 \rightarrow 2AlPO_4 + 3NaCl + 3HCl$$

The result of these reactions is the formation of non-absorbable phosphorous compounds, which will produce increased phosphorus elimination by feces, depriving the organism of its phosphorus. We would like to point out that aluminum compounds are only slightly ionized in aqueous solution and therefore only a part of the aluminum salts present in the gastrointestinal tract is probably able to participate in the above mentioned reactions.

Smith[24] presents data from various working groups, showing that an intake of 100–150 mg of aluminum (as potassium–aluminum sulfate) does not influence the urinary or fecal elimination of phosphorus in human subjects. If the aluminum intake was increased above 200 mg, increased fecal elimination of phosphorus was observed. Such increased elimination was compensated for by less phosphorus in the urine. The effect of aluminum sulfate and hydroxide were approximately the same, while aluminum chloride affected transfer of phosphorus from urine to feces to a lesser degree. Kirsner[25] mentions that administration of 650 mg of aluminum/24 hours to humans in a form of aluminum phosphate gel increases the phosphorus content in feces, while elimination of phosphorus by urine remained unchanged. According to Schmidt,[26] aluminum can also be bound with organic compounds of phosphorus in the gastrointestinal tract.

Experiments with animals using doses of aluminum considerably greater than those used in human studies, uniformly confirm that there is interaction of aluminum salts with phosphorus compounds in the gastrointestinal tract. Leary[27] shows that administration of high doses of aluminum hydroxide (more than 100 mg of Al per 1 kg of weight) caused the disappearance of phosphorus in bodies of young dogs, fed on a low P diet. In rats a diet containing 5% of aluminum sulfate resulted in diversion of phosphorus from urine to feces, a decreased inorganic P in serum and a decrease of phosphorus level in femoral bone.[16] The most significant influence on the phosphorus balance of rats appears in the first stage of an increased intake of aluminum; later on, the organism is partially accommodated to the changed conditions.[25] Various authors mention the possibility of developing acute rachitis resistant to vitamin D as a result of given high doses of aluminum compounds to chicks.[13] Pragay[29] found a decrease in the level of inorganic phosphorus by chicks, intoxicated by aluminum chloride.

Balance studies performed with two types of laboratory animal also confirm the interaction of aluminum with phosphorus in the gastrointestinal tract.[15] A two-fold increase of

TABLE 5. INFLUENCE OF ALUMINUM TRICHLORIDE INTOXICATION ON THE TISSUE INCORPORATION AND EXCRETION OF ^{32}P IN RATS

Sample	Control (cts/min)	Single oral dose of 188 mg/kg (cts/min)	Statistical significance
Blood	982 ± 73	77 ± 11	$P < 0{\cdot}001$
Liver	6891 ± 936	342 ± 52	$P < 0{\cdot}001$
Spleen	3934 ± 306	140 ± 29	$P < 0{\cdot}001$
Kidneys	4644 ± 489	327 ± 69	$P < 0{\cdot}001$
Brain	816 ± 75	190 ± 61	$P < 0{\cdot}001$
Muscle	1986 ± 165	33 ± 10	$P < 0{\cdot}001$
Femur	23,390 ± 2,040	950 ± 280	$P < 0{\cdot}001$
Feces/24 hours	1,799,000 ± 378,000	1,974,000 ± 565,000	$P > 0{\cdot}05$
Urine/24 hours	1,195,000 ± 197,000	20,750 ± 4190	$P < 0{\cdot}001$

Average values ± S.E. are given.

For blood the count rates are expressed per 100 μl, for tissues per 100 mg, and for feces and urine, the total excreted in 24 hours.

the aluminum content of food caused a reduced positive phosphorus balance in mice. Addition of 2665 ppm aluminum sulfate to their food resulted in a negative phosphorus balance in rats. A convincing proof of the interaction of aluminum salts with phosphate in the gastrointestinal tract was obtained by studying the distribution of ^{32}P after intragastric administration of labeled phosphate $Na_2H^{32}PO_4$ and aluminum chloride to rats. Results, summarized in Table 5, showed that a single dose of aluminum chloride (188 mg Al per 1 kg of live weight) caused a highly significant, decreased elimination of ^{32}P in urine while incorporation of ^{32}P into a whole range of tissues was also rapidly decreased.

On the basis of these results it seems safe to assume that increased doses of aluminum compounds results in the formation of insoluble non-absorbable phosphate–aluminum complexes in the gastrointestinal tract. Further experiments provided evidence that the administration of high doses of aluminum salts affects P metabolism in regions apart from the area of absorption. After intraperitoneal administration of $Na_2H^{32}PO_4$ we found that both chronic and acute peroral intoxication by aluminum chloride causes a decrease in incorporation of ^{32}P into the phospholipid fraction, as well as into ribo- and deoxyribonucleic acids in various tissues (Table 6). The intake of phosphate-^{32}P into tissues was not a limiting factor in the processes studied, since specific activity of the acid-soluble phosphorus fraction was not, in this case, significantly affected.

Since phosphorus incorporation into phospholipid and nucleic acids depends on the activity of the phosphorylating mechanisms, the data indicated that intoxication by aluminum salts disturbs the activity of these mechanisms. An important component of the phosphorylating mechanisms is adenosinetriphosphate. A study of the concentration of adeno-

TABLE 6. INFLUENCE OF CHRONIC AND ACUTE ALUMINUM TRICHLORIDE INTOXICATION ON THE INCORPORATION OF ^{32}P INTO TISSUE FRACTIONS IN THE LIVER, SPLEEN, AND KIDNEYS OF RATS

Fraction	Specific activity (counts/min/100 μg P)			Significance against control	
	Control	Chronic intoxication	Acute intoxication	Chronic intoxication	Acute intoxication
Liver					
Acid-soluble P	1944 ± 96	1805 ± 81	2244 ± 132	$P > 0.05$	$P > 0.05$
Lipid P	2139 ± 108	1720 ± 132	1625 ± 61	$P < 0.05$	$P < 0.01$
RNA	1234 ± 65	928 ± 40	870 ± 207	$P < 0.01$	$P > 0.05$
DNA	176 ± 16	172 ± 23	95 ± 15	$P > 0.05$	$P < 0.01$
Spleen					
Acid-soluble P	2395 ± 177	1997 ± 112	1736 ± 356	$P > 0.05$	$P > 0.05$
Lipid P	1294 ± 176	986 ± 55	744 ± 271	$P > 0.05$	$P > 0.05$
RNA	1671 ± 504	1242 ± 119	358 ± 131	$P > 0.05$	$P < 0.01$
DNA	2285 ± 1324	1750 ± 227	252 ± 62	$P > 0.05$	$P < 0.01$
Kidneys					
Acid-soluble P	1994 ± 57	1801 ± 83	2238 ± 95	$P > 0.05$	$P > 0.05$
Lipid P	1743 ± 194	1190 ± 77	1013 ± 61	$P < 0.05$	$P < 0.05$
RNA	834 ± 65	696 ± 57	602 ± 69	$P > 0.05$	$P < 0.05$
DNA	128 ± 28	334 ± 152	44 ± 8	$P > 0.05$	$P < 0.05$

Average values ± S.E. are given.

TABLE 7. INFLUENCE OF CHRONIC AND ACUTE INTOXICATION BY ALUMINUM CHLORIDE ON THE LEVEL OF ADENOSINE MONO-, DI-, AND TRIPHOSPHATES IN RAT BLOOD

Acid	Control	Chronic intoxication	Acute intoxication	Significance against control	
				Chronic intoxication	Acute intoxication
AMP (mg/100 ml)	2·95 ± 0·19	3·18 ± 0·60	3·48 ± 0·27	$P > 0·05$	$P > 0·05$
ADP (mg/100 ml)	3·69 ± 0·19	4·50 ± 0·42	4·39 ± 0·11	$P > 0·05$	$P < 0·01$
ATP (mg/100 ml)	8·33 ± 0·41	7·56 ± 0·95	5·52 ± 0·44	$P > 0·05$	$P < 0·001$

Average values ± S.E. are given.

sine mono-, di-, and triphosphate in rats' serum showed[15] that chronic and acute intoxication by aluminum chloride causes a decrease of adenosine triphosphate and an increase of adenosine di- and monophosphate (Table 7). A significant decrease in the ATP/ADP ratio indicates that in the system

$$ATP \rightleftharpoons ADP + \text{inorg. P} \rightleftharpoons AMP + \text{inorg. P}$$

the equilibrium is shifted to the right as a result of intoxication by aluminum. The mechanism of this phenomenon is not known but it may be connected with a decrease in the level of inorganic phosphate in the blood of animals intoxicated by aluminum compounds.[16, 29] Decreased production of ATP could endanger the course of a whole series of phosphorylation reactions as, for example, in the synthesis of phospholipids and nucleic acids.

All these data indicate the presence of an intense disturbance of the metabolism of phosphorous as a result of intoxication by aluminum compounds. This effect is limited not only to the absorption stage, but it also affects phosphorylation mechanisms. Since the metabolism of carbohydrates is closely interrelated with these mechanisms, one may expect that aluminum intoxication will also give rise to disturbances of carbohydrate metabolism. We have found[30] that acute intoxication by intragastrically administered aluminum chloride (122 mg Al per 1 kg of body weight) causes a decrease in concentration of liver glycogen to as low as one-fourth of control values; aldolase activity of the blood serum was increased by more than twice the normal values and definite hyperglycemia was observed in some animals. It is interesting that the total character of metabolic changes in aluminum-intoxicated rats rather resembles disturbances in carbohydrate metabolism occurring as a result of the action of hepatotoxic substances. Long-term administration of aluminum chloride to rats at a lower dose rate (69 mg Al per 1 kg of weight) affects the metabolism of carbohydrates to a lesser degree. A trend towards decreased hepatic and muscular glycogen can be seen. By increasing the dose of aluminum salt three times (that is to 200 mg Al per 1 kg of weight), the effect is much more pronounced:[31] liver glycogen decrease to nearly one-tenth of normal level; skeletal muscle glycogen is also significantly decreased. The concentration of lactic acid is increased in both liver and muscle. Similarly the concentration of pyruvic acid in blood serum and liver increases very significantly. These changes are probably connected with a decrease of coenzyme A activity in rat liver to less than one-third of values determined in normal animals.

The changes observed in the carbohydrate metabolism could also be due to the low non-specific intake of food of animals intoxicated by aluminum. The weight of these animals falls significantly as a result of administering high doses of aluminum chloride and it has recently been found that aluminum salts inhibit absorption of glucose from the intestinal tract.[32]

In conclusion one may state that both acute and chronic intoxication by inorganic aluminum compounds causes primary disturbances to the normal absorption of phosphorous compounds in the gastrointestinal tract. These can result in a negative P balance and a decrease in the level of phosphate in blood and tissues. As a result of increased doses of aluminum salts, disturbances in the metabolism of phosphorous compounds appear in other tissues, especially in the phosphorylation processes.

Significant changes in carbohydrate metabolism have been observed to accompany aluminum intoxication, probably arising secondarily to the changes in P absorption.

Summary

The chemical and physical properties of aluminum and its compounds help to explain to some extent their actions and reactions in the body. Aluminum is present in natural diet, ranging from extremely low concentrations, particularly in foods derived from animals, to relatively high values in certain plant products. The amounts of aluminum that may be added to food by utensils made of aluminum in the process of cooking are negligible. The concentration of aluminum in foods after being cooked in aluminum vessels fall well below the concentration range found in the raw materials.

In earlier papers, it has been stated that aluminum is not absorbed and is excreted quantitatively in the feces. More recent work has indicated that a small fraction of aluminum is absorbed and metabolized. The concentration of aluminum in the body is a function of the amount of aluminum in the food. The retention of aluminum depends on the composition of the food, e.g. a high fluoride content decreases the retention of aluminum.

There is a conflict of opinion regarding the toxicity of aluminum compounds. Our own studies, based on oral LD_{50} values, resulted in classifying aluminum chloride and sulfate as compounds of low acute toxicity. The intravenous toxicity of aluminum salts is much higher. In chronic exposure of mice to relatively small doses of aluminum chloride in drinking water (19·3 mg Al per kg per day) caused a decrease in growth in the second and third generations.

The toxic effects of aluminum salts are connected with the disturbance of phosphorus metabolism. A high aluminum intake caused a negative phosphorus balance in the rat; an increased output of phosphorus was found in the feces. Decreased absorption of phosphorus has also been demonstrated by following the distribution of intragastrically administered $Na_2HP^{32}O_4$.

Experimental chronic and acute poisoning by aluminum chloride caused a decrease in the incorporation of intraperitoneally injected $Na_2HP^{32}O_4$ into the phospholipids and nucleic acids of various tissues in the rat. It also causes a fall in the adenosinetriphosphate acid-levels in plasma, and a rise in the adenosinediphosphate levels. Further studies are necessary to determine the mechanisms of absorption of aluminum and the nature of the effects on phosphorylation processes in the tissues.

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Chronic Toxicity of Aluminium in Rats and Mice and its Effects on Phosphorus Metabolism

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In mice the oral LD_{50} values of aluminium chloride and aluminium sulphate were 0·77 ± 0·12 and 0·98 ± 0·09 g. Al kg. (± S.E.) respectively.

Mice and rats were fed on a normal diet containing about 170 p.p.m. of aluminium. Doubling this concentration caused a decrease in growth in the second and third generations of mice. A high intake of aluminium sulphate (2,835 p.p.m.) caused a 20-fold increase of aluminium retention in white rats. Aluminium accumulated in various tissues, especially in the skeleton, liver, and testes. A high rate of intake (2,665 p.p.m.) caused a negative phosphorus balance in the rat, with an increased output of phosphorus in the faeces. The lower absorption of phosphorus was also demonstrated with $Na_2H^{32}PO_4$. Chronic and acute poisoning by aluminium chloride caused, after intraperitoneal $Na_2H^{32}PO_4$, decreased incorporation of ^{32}P into the phospholipids and nucleic acids of various tissues in the rat. It also caused a fall in the adenosinetriphosphate acid-levels in plasma, and a rise in the adenosine diphosphate level.

The results suggest that the toxic effects of aluminium salts result both from decreased absorption of phosphorus and from interference with phosphorylation processes in the tissues.

Modern applied research into possible applications of aluminium has resulted in the introduction of many inorganic and organic compounds of the most diverse and common use. There is, however, no general consensus of opinion on the toxicity of aluminium salts. On the one hand it has been stated: 'aluminium compounds are harmless when ingested, even when the administration is continued over long periods' (Quarterly Bulletin of the Association of Food and Drug Officials of the United States, 1955); on the other hand, there is evidence that various toxic effects are induced in experimental animals by the administration of large amounts of aluminium compounds (Seibert and Wells, 1929; Stenberg and Naumova, 1952; Vojnar, 1953; Hara, Seki, Usui, Nakakawaji, and Mori, 1959a).

The original conclusion that aluminium given by mouth was wholly excreted has been proved to be wrong. More recent papers agree that various aluminium compounds given in large doses are partly absorbed and transported into the tissues (Perry, Tipton, Schroeder, and Cook, 1962; Kortus, Dibák, and Kotuliak, 1963). The toxicity of aluminium salts in the diet has been studied only at very high doses. Not even with doses as large as 2,000 parts Al per million have pathological changes in individual organs been observed. The only effect is growth retardation (Hara *et al.*, 1959b). Similar results have been obtained by Vozár (1959).

Aluminium in the diet has been shown to interact with phosphate in the alimentary tract and to affect its metabolism (Ivy and Gray, 1939; Jones, 1942).

The aims of this work were to study the effects of moderately low doses of aluminium on the growth of animals in succeeding generations, and to compare the effects of chronic low and acute high doses of aluminium on the retention and fate of aluminium and on the retention and fate of phosphorus with special reference to phosphorus compounds of fundamental metabolic importance.

Materials and Methods

Animals Rats were of the Wistar strain, weighing 150 to 250 g. Mice were of the 'Dobrá Voda' strain from the Slovakian Academy of Science, Dobrá Voda, average weight 200 g. Male animals were used for all but experiments on succeeding generations, in which a male female ratio of 0·4 was maintained. Animals were fed on the Larsen diet (Fábry, 1955). This normal diet contained 160 to 180 parts per million (p.p.m.) of aluminium and about 6,000 p.p.m. of phosphorus.

Aluminium All doses are expressed in terms of aluminium. The chloride was mostly used, but the sulphate was used in some experiments.

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Acute Toxicity Studies The aluminium salt was given orally by probe.

Chronic Toxicity Studies The aluminium salt was given in the drinking water or admixed in the diet. Weights of animals and daily food and water intake were checked at intervals.

Balance Studies Animals were kept in metabolic cages allowing separate collection of urine and faeces for 24 hrs. during which no food was given. They were then decapitated. Aluminium in the urine, faeces, and tissues was estimated by Nikitina's (1956) procedure, and phosphorus by Travina's (1955) method.

In tracer studies rats were given by mouth $Na_2H^{32}PO_4$ (10 mg. ml. in 0·9%, NaCl) obtained from the Institute of Nuclear Research, Řež, Czechoslovakia at the beginning of the period. ^{32}P was either estimated after digestion of the tissues with perchloric acid or in tissue fractions: acid soluble; phospholipid; ribonucleic and deoxyribonucleic acids. These were separated as described by Davidson, Frazer, and Hutchison (1951). ^{32}P estimations were carried out by end-window counting on aluminium discs using a Geiger-Müller tube.

Adenosine mono-, di-, and tri-phosphates in the blood serum of rats were estimated by Boehringer's enzymic methods (Bücher, 1953).

Results

Oral LD_{50} Values of Aluminium in Mice As aluminium chloride was used in most chronic toxicity experiments its acute LD_{50} value was determined and compared with that of the sulphate. After approximate LD_{50} values had been found, more precise values were determined by Roth's nomographic procedure (1961). The LD_{50} value of the chloride was found to be 0·77 ± 0·12 g. Al/kg., and of the sulphate 0·98 ± 0·09 g. Al/kg. (means ± S.E.). These values are not significantly different. They place aluminium salts in the category of compounds with low acute toxicities according to the criteria of Hodge and Sterner, as quoted by Simonnet (1956).

Chronic Toxicities in Mice The chronic toxicity was studied in a reproduction experiment on white mice. Ten mice received aluminium chloride in their drinking water, receiving on the average 19·3 mg. Al/kg. day. They were compared with 10 control mice. The experiment lasted 180 to 390 days, during which weight increases, number of litters, and number of off-spring were recorded.

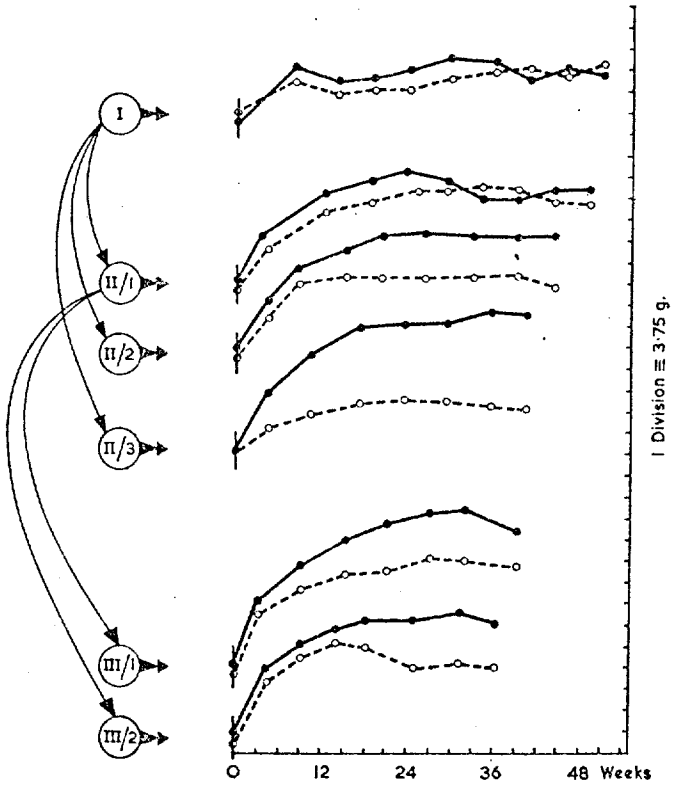

FIG. 1. Weight increases of normal (full lines) and of aluminium trichloride treated (dotted lines) white mice: I, original generation; II 1, 2, 3, first, second, and third litter of the second generation; III/1, 2, first and second litter of the third generation.

The weanlings were treated from 4 weeks of age like their parents. At the end of the experiment the animals were killed by decapitation, the blood was examined for changes in the red cell count, and the liver, spleen, and kidneys were examined histologically.

There were no significant differences in the numbers of litters or off-spring between the treated and control mice. Growth was retarded and was dependent on the intake of aluminium, but the effect did not appear in the first generation or in the first litter. The subsequent litters manifested a very marked growth retardation, as did those of the third generation (Fig. 1). An analysis of variance (Weber, 1964) established that, under the conditions of our experiment, weight variations could be accounted for by aluminium uptake ($P < 0.001$). The differences in the course of weight plots for successive generations and litters were also statistically significant ($P < 0.01$).

The erythrocyte counts and haemoglobin levels in the first and last generations did not differ significantly from those in the controls; and no pathological changes could be found in the tissues examined.

Effects of Dose Rate on Aluminium Balance

Ten mice received the standard diet containing about 170 p.p.m. Al, and 10 a diet containing 355 p.p.m., both groups for 40 days. During this period there were no significant differences between the growth curves nor between the intake of water, but the food intake was significantly less in the group receiving the higher dose. The aluminium balance was studied over the last six days (Table I). Intake and faecal excretion of aluminium were sig-

TABLE I

ALUMINIUM BALANCE IN MICE KEPT ON DIETS WITH DIFFERENT ALUMINIUM CONTENTS

	Aluminium (mg.)		*Statistical Significance*
	Control (170 p.p.m. Al)	*$AlCl_3$ (355 p.p.m. Al)*	
Intake	3·87 ± 0·29	7·75 ± 1·00	$P < 0.01$
Excretion			
Faeces	2·47 ± 0·19	5·72 ± 0·71	$P < 0.002$
Urine	0·11 ± 0·03	0·11 ± 0·04	$P > 0.05$
Balance	1·28 ± 0·38	1·91 ± 0·51	$P > 0.05$

The average values ± S.E. for 10 mice over 24 hours are given.

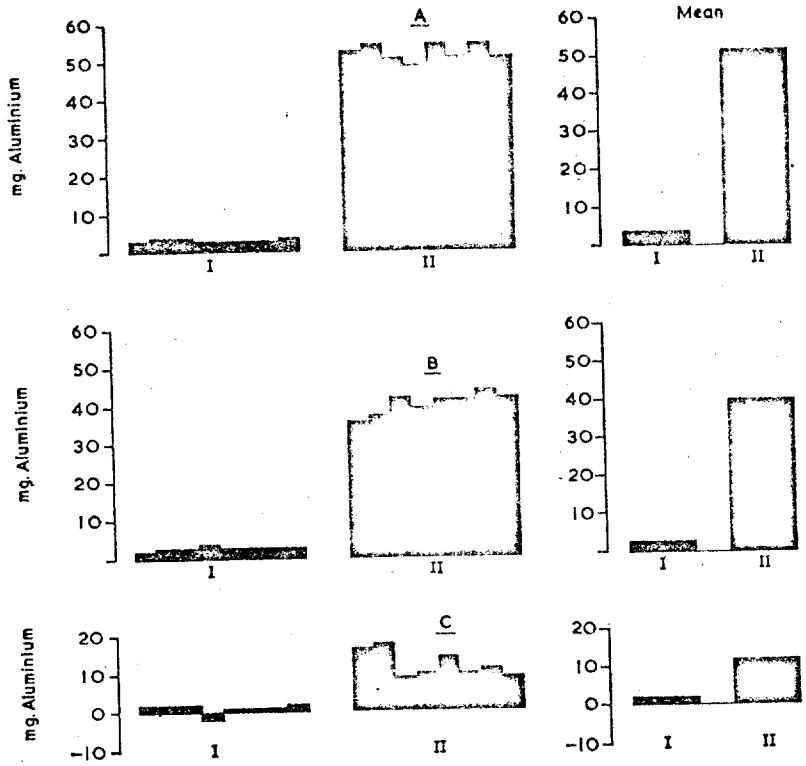

FIG. 2. Influence of high doses of aluminium sulphate on the aluminium balance in rats: A, aluminium intake; B, aluminium excretion; C, aluminium retention; I, control group (170 p.p.m. Al in diet); II, treated group (2,835 p.p.m. Al in diet). All values are expressed in mg. Al.

nificantly higher at the higher dose, whereas urinary excretion and the retention of aluminium were not.

The aluminium balance was also studied in eight rats kept for eight days on the diet containing 160 to 180 p.p.m. Al and then given a diet containing 2,835 p.p.m. Al (as sulphate) for a further eight days. The increased dose rate of aluminium led to a reduction in food intake from 20 to 15 g. rat day and a reduction in average body weight. Aluminium excretion was increased significantly in both faeces and urine ($P < 0.001$) when the higher dose was given (Fig. 2). About 70% of the dose was excreted in the faeces. Retention was increased 20 times.

Increased retention in several tissues from a higher dose was also demonstrated in two groups of eight rats each by analysis of the tissues (Table II).

TABLE II

INFLUENCE OF DOSE RATES ON ALUMINIUM LEVELS IN RAT TISSUES

Tissue	Concentration of Al in Diet: 170 p.p.m.	Concentration of Al in Diet: 2,835 p.p.m.	Statistical Significance
Liver	1·55 ± 0·26	2·99 ± 0·28	$P < 0.001$
Brain	0·71 ± 0·08	1·08 ± 0·15	$P < 0.05$
Kidneys	0·86 ± 0·08	0·90 ± 0·10	$P > 0.05$
Adrenals	41·5 ± 5·2	51·4 ± 5·5	$P > 0.05$
Spleen	1·46 ± 0·23	1·48 ± 0·10	$P > 0.05$
Heart	1·08 ± 0·12	1·27 ± 0·12	$P > 0.05$
Lungs	0·407 ± 0·054	0·428 ± 0·080	$P > 0.05$
Testes	0·170 ± 0·057	0·470 ± 0·060	$P < 0.001$
Colon	0·69 ± 0·10	0·63 ± 0·04	$P > 0.05$
Muscle	0·039 ± 0·008	0·044 ± 0·002	$P > 0.05$
Blood	0·65 ± 0·11	1·08 ± 0·09	$P < 0.01$
Femur	70·2 ± 1·0	91·2 ± 0·7	$P < 0.001$

Aluminium concentrations are given in mg./100 g. fresh tissue. The diet was maintained for 24 days before the rats were killed.

One group received the basic diet containing 160 to 180 p.p.m., the other a diet containing 2,835 p.p.m., both for 24 days.

Influence of Aluminium on Metabolism of Phosphorus Ten mice received the standard diet containing 160 to 180 p.p.m., and 10 a diet containing 355 p.p.m. for 40 days, and the phosphorus balance was studied during the last six days. The higher dose significantly lowered phosphorus retention (Table III), which, on some days, even became negative, *i.e.*, there was net excretion. The concentrations of phosphorus in the liver and femur were not, however, significantly affected by the end of the experiment. The weight plots were also unaffected in spite of some reduction in food intake in animals fed the diet containing increased amounts of aluminium.

TABLE III

PHOSPHORUS BALANCE IN MICE KEPT FOR 40 DAYS ON DIETS CONTAINING DIFFERENT ALUMINIUM LEVELS

	Phosphorus (mg.): Control (170 p.p.m. Al)	Phosphorus (mg.): $AlCl_3$ (355 p.p.m. Al)	Statistical Significance
Intake	218·8 ± 10·3	148·1 ± 20·4	$P < 0.02$
Excretion			
Faeces	150·4 ± 5·4	139·5 ± 28·6	$P > 0.05$
Urine	2·9 ± 0·6	3·7 ± 0·8	$P > 0.05$
Balance	+ 65·6 ± 13·0	+ 4·9 ± 14·4	$P < 0.02$

The average values ± S.E. for 10 mice over 24 hours are given.

More pronounced changes in the phosphorus balance were found in eight rats kept for eight days on the standard diet and for another eight days on the same diet containing an additional 2,665 p.p.m. Al as sulphate. The addition of the aluminium salt to the diet resulted in a lower food intake and consequently to a significantly lower phosphorus intake ($P < 0.001$) and a reduced excretion of phosphorus in the urine ($P < 0.001$). The excretion of phosphorus in faeces was, however, increased ($P < 0.001$). These effects are illustrated in Figure 3.

In the first series of tracer experiments, the influence was studied of a single large oral dose of aluminium chloride, 188·2 mg. kg. body weight, on the absorption of $Na_2H^{32}PO_4$, 0·1 μc/100 g. wt., given orally 15 minutes after the aluminium salt. The experimental and control groups were each of eight rats. The distribution of radioactivity in the urine, faeces, blood, and various tissues was measured. The results are summarized in Table IV.

TABLE IV

INFLUENCE OF ALUMINIUM TRICHLORIDE INTOXICATION ON THE TISSUE INCORPORATION AND EXCRETION OF ^{32}P IN RATS

Sample	Control (cts./min.)	Single Oral Dose of 188 mg./kg. (cts./min.)	Statistical Significance
Blood	982 ± 73	77 ± 11	$P < 0.001$
Liver	6,891 ± 936	342 ± 52	$P < 0.001$
Spleen	3,934 ± 306	140 ± 29	$P < 0.001$
Kidneys	4,644 ± 489	327 ± 69	$P < 0.001$
Brain	816 ± 75	190 ± 61	$P < 0.001$
Muscle	1,986 ± 165	33 ± 10	$P < 0.001$
Femur	23,390 ± 2,040	950 ± 280	$P < 0.001$
Faeces/24 hours	1,799,000 ± 378,000	1,974,000 ± 565,000	$P > 0.05$
Urine/24 hours	1,195,000 ± 197,000	20,750 ± 4,190	$P < 0.001$

Average values ± S.E. are given.

For blood the count rates are per 100 μl., for tissues per 100 mg., and for faeces and urine the total excreted in 24 hours.

See text for further details.

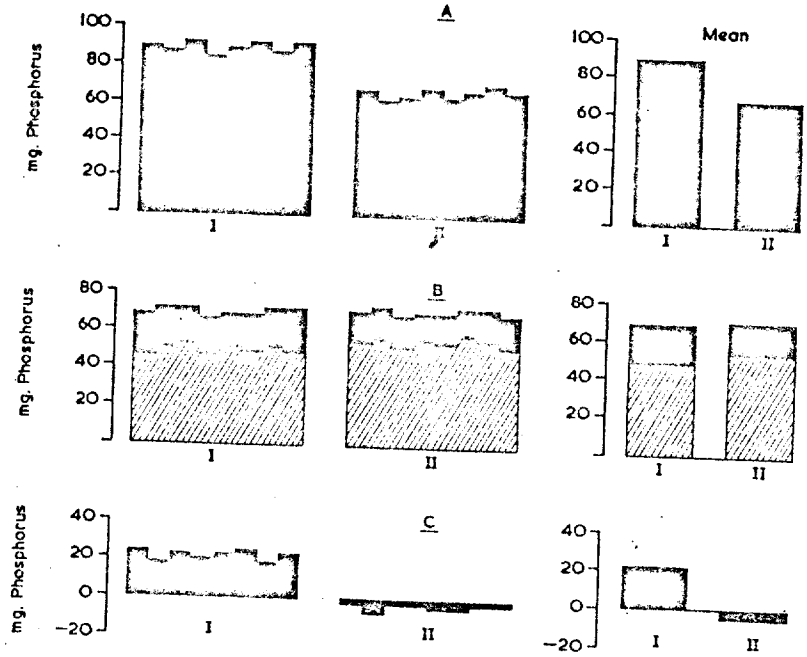

FIG. 3. Influence of high doses of aluminium sulphate on the phosphorus balance in rats: A, phosphorus intake; B, phosphorus excretion (lined area—faecal excretion); C, phosphorus retention. I, control group: II, treated group. All values are expressed in mg. phosphorus.

They show a significant reduction of ^{32}P incorporation into the blood and all the tissues examined. The increase in the excretion of ^{32}P in the faeces is not significant and is less than one expects if there is formation of complexes of aluminium and phosphate anions which are absorbed with difficulty. This finding is obviously influenced by the lower total excretion of faeces during our experiment, since after the administration of large amounts of aluminium chloride constipation occurs. The low level of ^{32}P in the blood, in tissues, and in urine, however, clearly shows reduced phosphorus absorption. Owing to technical difficulties the contents of the digestive tract were not analysed. This omission explains the failure to achieve 100% recoveries of injected ^{32}P.

In the second series of tracer experiments the influence of chronic and acute intoxication by aluminium chloride on the metabolism of ^{32}P was studied. The ^{32}P was injected intraperitoneally at 0·16 μC/100 g. to ensure complete absorption. The experiment was carried out with rats, divided into three groups of six animals. One of the groups served as a control, the second group was given daily oral doses of aluminium chloride (36·5 mg. Al/kg.) for a period of 52 days, and the third group received, after having been fed the standard diet for 52 days, a single large intragastric dose of aluminium chloride of 240 mg. Al/kg. Administration of aluminium did not influence food intake or the weight curves of the animals. The average body weight of the animals of the control group at the end of the experiment was 206 g. and that of the group with chronic aluminium intake was 221 g. $Na_2H^{32}PO_4$ was given (to the last group at the same time as the aluminium) and 24 hrs. later the specific activities of phosphorus fractions isolated from the liver, spleen, and kidney tissues were measured. The results obtained (Table V) prove that, whereas the specific activity of acid soluble phosphorus was not influenced by either chronic or acute intoxication, the incorporation of ^{32}P into phospholipids and ribonucleic and deoxyribonucleic acid fractions was in most cases significantly reduced. The effect of acute intoxication was, as a rule, more pronounced.

In another similar experiment the influence was studied of chronic and acute intoxications by aluminium chloride on the blood serum level of adenosine mono-, di-, and tri-phosphates in 30 rats divided into three groups. All rats were fed the standard diet for 55 days. The first experimental group received additional aluminium chloride throughout the 55 days at 36·5 mg. Al/kg./day. This did not affect the average final body weight significantly (215 g. for controls; 209 g. for the treated rats). The second experimental group received one oral dose of 223 mg. Al/kg. (as the chloride) on the fifty-sixth day. The results are shown in Table VI. They indicate that aluminium chloride treatment causes an increase of adenosine mono- and di-

TABLE V

INFLUENCE OF CHRONIC AND ACUTE ALUMINIUM TRICHLORIDE INTOXICATION ON THE INCORPORATION OF ^{32}P INTO TISSUE FRACTIONS IN THE LIVER, SPLEEN, AND KIDNEYS OF RATS

Fraction	*Specific Activity (counts/min./100 μg. P)*			*Significance against Control*	
	Control	*Chronic Intoxication*	*Acute Intoxication*	*Chronic Intoxication*	*Acute Intoxication*
Liver					
Acid-soluble P	1,944 ± 96	1,806 ± 81	2,244 ± 132	$P > 0.05$	$P > 0.05$
Lipid P	2,139 ± 108	1,720 ± 132	1,625 ± 61	$P < 0.05$	$P < 0.01$
RNA	1,234 ± 65	928 ± 40	870 ± 207	$P < 0.01$	$P > 0.05$
DNA	176 ± 16	172 ± 23	95 ± 15	$P > 0.05$	$P < 0.01$
Spleen					
Acid-soluble P	2,395 ± 177	1,997 ± 112	1,736 ± 356	$P > 0.05$	$P > 0.05$
Lipid P	1,294 ± 176	986 ± 55	744 ± 271	$P > 0.05$	$P > 0.05$
RNA	1,671 ± 504	1,242 ± 119	358 ± 131	$P > 0.05$	$P < 0.01$
DNA	2,285 ± 1,324	1,750 ± 227	252 ± 62	$P > 0.05$	$P < 0.01$
Kidneys					
Acid-soluble P	1,994 ± 57	1,801 ± 83	2,238 ± 95	$P > 0.05$	$P > 0.05$
Lipid P	1,743 ± 194	1,190 ± 77	1,013 ± 61	$P < 0.05$	$P < 0.05$
RNA	834 ± 65	696 ± 57	602 ± 69	$P > 0.05$	$P < 0.05$
DNA	128 ± 28	334 ± 152	44 ± 8	$P > 0.05$	$P < 0.05$

Average values ± S.E. are given. See text for details.

TABLE VI

INFLUENCE OF CHRONIC AND ACUTE INTOXICATION BY ALUMINIUM CHLORIDE ON THE LEVEL OF ADENOSINE MONO-, DI- AND TRIPHOSPHATES IN RAT BLOOD

Acid	*Control*	*Chronic Intoxication*	*Acute Intoxication*	*Significance against Control*	
				Chronic Intoxication	*Acute Intoxication*
AMP (mg./100 ml.)	2·95 ± 0·19	3·18 ± 0.60	3·48 ± 0·27	$P > 0.05$	$P > 0.05$
ADP (mg./100 ml.)	3·69 ± 0·19	4·50 ± 0·42	4·39 ± 0·11	$P > 0.05$	$P < 0.01$
ATP (mg./100 ml.)	8·33 ± 0·41	7·56 ± 0·95	5·52 ± 0·44	$P > 0.05$	$P < 0.001$

Average values ± S.E. are given. See text for other details.

phosphates, and a decrease of adenosine triphosphate. The effects of acute intoxication were more pronounced and are statistically significant.

Discussion

In the introduction to this paper the conflict of opinions regarding the toxicity of aluminium compounds was emphasized. Our own studies resulted in our classifying aluminium chloride and sulphate as compounds of low acute toxicity. This conclusion was based on oral LD_{50} values. The intravenous toxicity of aluminium salts is much higher (Hara *et al.*, 1959a) but is of little relevance as this is not a possible path of aluminium intake in everyday life.

The effects of the chronic intake of only slightly increased amounts of aluminium seem to be considerably more significant. They have been little studied in the past. In the present experiments the effects of low doses of aluminium chloride on the growth rates of three generations of mice were studied. The results show that weight gains were less, but not in the first generation. As retardation of growth was caused by relatively small doses of an aluminium compound, we consider this finding to be important. The results contradict opinions expressed in earlier papers that aluminium exerted no toxic effects at such doses. The lack of significant departures from normal either in the blood count or in the morphology of various tissues of these animals is, however, in accordance with the earlier results of Hara *et al.* (1959b) and underlines the necessity for seeking other, more sensitive indices of metabolic disturbances caused by a chronic intake of small amounts of aluminium compounds.

In the earlier papers, due to the low sensitivity of the analytical methods used, it was stated that aluminium was not absorbed and should be excreted

quantitatively in the faeces. More recent papers, however, have indicated that a small fraction of aluminium is absorbed by demonstrating it in all tissues, in blood, and in urine (Kehoe, Cholak, and Storey, 1940a, 1940b; Heupke, 1950; Campbell, Cass, Cholak, and Kehoe, 1957; Perry *et al.*, 1962; Kortus *et al.*, 1963). We have shown that doubling the aluminium intake caused a significant increase of aluminium output in the faeces but did not significantly influence either its retention or its level in some tissues. On the other hand, a considerable increase in intake by the rat resulted in a large increase in retention and in excretion in the urine. The increased retention of aluminium was also demonstrated by analysis of several tissues. The most pronounced accumulation of aluminium was observed in the liver, testes, and bones, whereas in other organs (kidneys, spleen, lungs, intestines, and muscles) the aluminium levels were not significantly altered. The finding of a very high concentration, 10 times greater than in other soft tissues, of aluminium in the adrenals is interesting. Apparently animals can balance moderate increases in the intake of aluminium by a higher output in the faeces; but an extreme increase in the intake of aluminium results in increased absorption, part being deposited in some tissues, mainly in the skeleton and the liver, and part being excreted in the urine.

The mechanisms of the toxic effects of aluminium salts have not yet been elucidated. Several authors (Leary and Sheib, 1917; Jones, 1938; Kirsner, 1943) agree that the primary effects are caused by the reaction of aluminium with phosphates in the gastro-intestinal system, with the formation of insoluble phosphates of aluminium. The simplified reaction may serve as an example:

$$Na_2HPO_4 + NaH_2PO_4 + 2\,AlCl_3 \rightleftharpoons 2\,AlPO_4 + 3\,NaCl + 3\,HCl$$

As a consequence of such reactions the output of phosphate in the faeces is increased, thus depriving the animal of phosphorus. Our balance studies, carried out with both rats and mice, have fully confirmed this hypothesis. The standard diet contained about 170 p.p.m. Al. Merely doubling this resulted in a sharp decrease in the positive phosphorus balance in mice. Administration of high doses of aluminium caused a considerable negative balance of phosphorus. The results may have been distorted by a lowered food intake. To render them more convincing, pair feeding experiments would be needed. However, an isotope study, in which ^{32}P incorporation was followed after a single dose of aluminium chloride and in which the nutritional state at the time of the experiment was the same in the experimental and in the control group, demonstrated unequivocally that phosphorus absorption was reduced.

Even when the effects on the absorption of phosphorus were eliminated, by intraperitoneal injection, it was found that feeding high doses of aluminium salts influenced the metabolism of phosphorus. Both chronic and acute dosing led to a decreased incorporation of ^{32}P into the phospholipid fraction and into the ribonucleic and deoxyribonucleic acids in several tissues. The controlling factor was not the supply of ^{32}P to the tissues, as the specific activity of the acid-soluble phosphate fraction was in no case significantly decreased. As the incorporation of phosphate into phospholipids and nucleic acids is determined by the activity of the phosphorylation mechanisms, the results obtained suggest the impairment of this activity by aluminium salts. An important component of the phosphorylation process is adenosine triphosphate; accordingly, we demonstrated a decrease in the adenosine triphosphate levels in the blood serum of rats, and increases in adenosine mono- and di-phosphates, in both chronic and acute intoxication. The significant decrease in the ATP ADP ratio suggests that the equilibrium of the system:

$$ATP \rightleftharpoons ADP + \text{inorganic P} \rightleftharpoons AMP + \text{inorganic P}$$

is shifted to the right. We do not know the actual mechanism operating. It may be connected with the decrease in inorganic phosphate in the blood of poisoned animals. Recently such a decrease has been shown by Pragay (1962) in chicken intoxicated by aluminium hydroxide. Our results on ^{32}P incorporation were not influenced by a lowered food intake since the rats of all groups gained weight at very similar rates.

Our results therefore demonstrate that the metabolism of phosphorus compounds is profoundly disturbed by the intake of aluminium salts. Not only is the absorption of phosphorus affected, but so are the phosphorylation mechanisms in the tissues.

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Biochemistry

MUSCULAR DYSTROPHY IN CHICKS CAUSED BY DIETARY ALUMINUM HYDROXIDE GEL. D.A. Emery (Intr. by D.M. Surgenor) Univ. of Buffalo Chronic Disease Res. Inst. Buffalo, N.Y.

This laboratory (J. Appl. Physiol. 11:435 (1957)) has studied a dystrophy in chicks fed on a diet containing reactive $Al(OH)_3$ gel (Amphojel, Wyeth, Inc.). This dystrophy is characterized by an inability to stand or walk. Serum inorganic phosphate, iron, plasma cholesterol and vitamin A are diminished in the dystrophic chicks.

In the present experiments serum of dystrophic chicks showed normal K and Na levels; calcium was slightly elevated (by 30 to 40%). Serum transaminase and plasma creatinine were unchanged. Serum alkaline phosphatase and plasma creatine were consistently elevated (by 30 to 100%).

With the onset of dystrophy transient slight elevation of globulins and persistent decrease of lipoproteins were noted. Actomyosin content of muscle and myosin ATPase activity were decreased. Other changes included increased fragility of erythrocytes. Poor mineralization of bone was demonstrated by X-ray and by histology. Histological examination of muscle revealed swelling, vacuolization, breaks of the fibers, disappearance of cross-striation, fraying of the ends of fibers and fragmentation of nuclei.

These findings will be discussed in comparison with other muscular dystrophies.

Fed. Proc.
21(2):388 (1962)
--Abstract

Z. Hyg. 28: 92-99 (1928)

THE DANGERS OF CERTAIN MINERAL BAKING-POWDERS BASED ON ALUM, WHEN USED FOR HUMAN NUTRITION.

By GEORGES SCHAEFFER, G. FONTÈS, E. LE BRETON, CH. OBERLING, and L. THIVOLLE.

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Recent studies on vitamins have led us to consider as dangerous the suppression of uncooked foods in our nourishment. Such a suppression is actually never complete. If we consider the alimentary habits imposed on man by modern conditions of life, they may be dangerous for other reasons than simply lack of vitamins.

The economical necessities which urge us more and more to spare time and labour have brought us to industrialise the preparation of our foods, and to increase the use of products requiring little or no preparation before cooking: all sorts of conserves, dressings, condensed foods, extracts, the manufacture and canning of which we know nothing of. Such products, we take it for granted, are examined by the Adulteration Department before being sold to the public, and that satisfies us. Can this attitude of the public be reasonably upheld by physiologists, hygienists, and physicians? Certainly not.

The laws suppressing fraud assure us against finding in our prepared foods, bought daily, bacteria capable of causing serious alimentary intoxications, poisons leading to serious accidents, and antiseptics in big doses. Still, this is not sufficient. It is a well-known fact to pathologists that the daily ingestion of slight doses of feebly nocuous substances can lead to serious functional troubles or organic disorders, if it is repeated for a long stretch of time. There is no doubt that the daily consumption of the above-mentioned foods exposes us to the risks of such slow intoxication. Many of the present laws concerning fraud were passed at a time when physiology and the hygiene of alimentation were far from their present state of development. It was almost exclusively toxicologists and chemists who were entrusted with their establishment. Should not biologists also participate in the present-day revision of these laws?

In the following lines we would like to show by a precise example to what social dangers we are exposed through the daily use of products which have no justification from the physiological point of view, but only mean a slight economy of time to cooks and house-keepers. We speak of the artificial leavens or baking-powders which tend more and more to replace yeast in use by bakers for the manufacture of rusks, biscuits and all other pastries.

The great chemist Liebig was the first who thought of using mineral leavens or baking-powders, instead of yeast, and thus suppressing preliminary fermentation. The baking-powders have proved to be successful in Germany, Austria, Switzerland, U.S.A., where a great deal of pastry is made at home. German "Backpulver" and American baking-powders are well known for their efficiency; they reduce the manipulation of the dough and permit immediate cooking, without the preliminary necessity of letting the mixture rest for a certain time at a temperature of 25°.

What is the composition of the powders or mineral leavens? Carbonic acid is present as bicarbonate of soda; it makes the dough rise, and renders it more digestible; the gas is liberated by an acid salt—biphosphate, bitartrate, or a mixture of bitartrate and tartaric acid. In a well-balanced leaven the proportion of the different salts is always so calculated that after the combined action of water and heat on the mixture of flour and baking-powder, the reaction which takes place, once the carbonic acid is liberated, should leave neither an excess of free mineral acidity, nor alkalinity, which might prove nocuous to the organism.

Such mixtures when they were well manufactured were used for a long time without raising any criticism. No country has yet legally prohibited the use of tartaric acid and bitartrate, for this purpose.

But as potassium bitartrate and phosphates are expensive, industry has, for a long time already, been trying to replace them by cheaper products in which the acidity is given by acid salts which are as dangerous as bisulphates or alums of soda, potash or ammonia. Does the repeated absorption of such ingredients raise serious problems of alimentary hygiene?

The use of mineral leavens chiefly constituted by alum and bicarbonate of soda is lawful in the United States, where these baking-powders constitute nearly two-thirds of the total consumption (this consumption amounting actually to several thousand tons a year). In Europe the use of mineral leavens containing alum is virtually forbidden in France, England, Germany, Sweden, Switzerland, Belgium, etc. The introduction of alum into any kind of alimentary product is considered as fraud.

Although Germany could not during the Great War be provided with potassium bitartrate on account of the blockade, her hygienists severely opposed themselves to the production or sale of mineral leavens containing alum, even as an "Ersatz."

We thus see that, on the one hand, nearly all European countries consider the presence of alum or aluminium salts in foods as dangerous and intolerable; while, on the other hand, the United States authorises the production and sale of mineral leavens containing alum in nearly all the states—and yet it is known that in the U.S.A. public hygiene is as strictly controlled as anywhere else. How can one conciliate such divergent opinions on a question of so great an importance in alimentary hygiene?

We may presume that in authorising the consumption of these mineral

products the legislators of the U.S.A. have been governed by the following theoretical ideas: Supposing the leaven to be a well-prepared alum-phosphate baking-powder, that is to say a scientifically calculated mixture of acid calcium phosphate, alum, and bicarbonate of soda, all that remains in the bread when the reaction is finished will be sodium sulphate, acid sodium phosphate, calcium carbonate and aluminium phosphate.

Aluminium phosphate being insoluble will traverse the digestive tract as an inert body. If we take this for granted the question of any possible toxic action of aluminium salts is never raised, since no soluble aluminium salt will, at any moment, exercise its astringent action on the mucous membrane of the digestive tract.

Is the above-mentioned opinion of American hygienists justified? Through a series of experiments which were published in the *Chemical News* in 1888, Maliet brought to light certain defects of manufacture in the baking-powders that were being sold at that time. He furthermore showed that a solution of pepsin of the same hydrochloric acidity as the gastric juice gave aluminium chloride when in contact with alumina, and displaced the phosphoric acid of aluminium phosphate. Aluminium chloride in an acid solution acts like an energetic precipitant of protein matters and the pepsin is carried down with the precipitate, as is always the case when proteins are precipitated in a solution containing ferments.

To these facts, which have been repeatedly confirmed, especially by V. C. Myers, we can add our personal observations[1]. Briefly, these are as follows: The aluminium phosphate formed during the action of the leaven is deprived of its water by the baking of the bread, after which it still contains about 17 per cent. of water. In this state of hydration it is easily dissolved in solutions of hydrochloric acid of the same strength as the gastric juice, thus giving soluble aluminium chloride. We have discovered the formation of this astringent salt in the gastric juice of dogs as well as of men, through tests made *in vitro*, as well as *in vivo*. The solubility of aluminium phosphate rises with the proportion of acid contained in the gastric juice, that is to say with the hyperhydrochloric conditions of the subject.

We have, on the other hand, been able to show that, under the alkaline influence of the pancreatic juice, the reprecipitation of the aluminium chloride to the state of aluminium hydrate, at the level of Vater's ampulla, is incomplete owing to the preventive action of bile.

The absorbing of alum-phosphate baking-powder therefore results in the contact of dilute solutions of *aluminium chloride*, with the *gastric, pyloric and duodenal mucosa*. We consider that this fact alone is sufficient to justify the prohibition of alum leavens.

But the presence in the digestive tract of soluble aluminium salts, apart

[1] See G. Schaeffer, G. Fontès, E. Le Breton, Ch. Oberling, L. Thivolle (1928), "Physiological research on mineral leavens. Leavens containing alum." *Bull. Soc. sci. d'hygiène aliment.* 16, Nos. 1 et 2.

from their possible direct action, raises the question of their absorption, because these salts are considered poisonous for living beings.

Orfila (1814) and Mitscherlich (1840) have seen the effect of intoxication by high doses. This question does not interest us here. Siem (1886) brought to light the results of slow intoxication, but the works that are most important for this discussion are those of Dölcken (1897). This author injected lactate or double tartrate of aluminium and sodium into rabbits and cats, by daily doses of 5 mg. per kg., and thus studied the aluminium intoxication in its chronic manifestations. Anatomo-pathological examinations on cats showed important lesions of the medulla, posterior parts of the column, median parts of the anterior areas, and the tract of Goll. The motor roots of the trigeminal, facial and hypoglossal nerves showed a degeneration of more than half the fibres; there were also many degenerated fibres in the IX, X and XI nerves.

But all these lesions were produced with soluble salts by subcutaneous injections; they are therefore not sufficient to condemn the use of mineral leavens. Before prohibiting the use of these leavens in the name of alimentary hygiene one must examine the following questions:

(1) Whether the aluminium chloride formed in the stomach acts on the digestive ferments?

(2) Whether the frequent contact of this precipitant and astringent salt with the mucous membrane of the digestive tract causes organic lesions?

(3) Whether the daily absorption of aluminium chloride in small quantities provokes certain physiological disorders?

(1) Our personal researches, published elsewhere, have led us to ascertain a marked delay in the gastric evacuation of dogs treated with bread leavened with alum-phosphate baking-powder, as compared with dogs treated with the same bread leavened with baker's yeast. It has furthermore been found that frequent diarrhoea was provoked at the beginning of the treatment, when dogs were given bread containing three times the normal dose of baking-powder[1]. The diarrhoea became rarer as the organism grew more accustomed to the treatment, which lasted two months; however it was sufficient to upset the nutrition equilibrium, as shown by important variations of the animals' weight.

If, instead of alum leaven, a corresponding quantity of aluminium phosphate is used in the bread, the purgative effect is much more violent. By using a quantity of aluminium phosphate equivalent to that contained in one dose and a half of leaven, the bread given to the animals provokes such diarrhoea and sickness that this diet cannot be continued for over a fortnight.

The simplest explanation one can give to such a fact is that bread, for which leaven has been used, contains not only aluminium phosphate, but large quantities of calcium salts, which have a moderating action on the intestinal activity.

[1] The ordinary dose of leaven is of 16 g. for 230 g. of flour. The leaven containing 21 per cent. of burnt alum corresponds after reaction to 1·693 g. of alum phosphate, or to 0·374 g. of alum

(2) After prolonged ingestion of complete and balanced diets, where the energy requirements were supplied by special breads containing alum-phosphate baking-powder, we have observed important lesions of the digestive tract, as well in dogs as in mice. We will not insist on the detail of these researches, but merely state their main results.

Mice were fed for four months on bread containing flour, casein, sugar, fat, milk and salts, proportioned to form a complete food; the vitamins were added as butter, brewer's yeast and green vegetables.

The check Group No. I, composed of 20 couples, was fed on this bread made with yeast; Group No. II received the same, with addition of aluminium phosphate[1]; Group No. III got bread of the same composition, but raised with alum baking-powder[2].

All the specimens of the Groups II and III when submitted to anatomo-pathological examinations showed serious lesions of the digestive tract, being more or less extensive according to the individuals. We will describe a few of the lesions that were observed.

The most common of them is a paracellular necrosis of the superficial epithelium of the stomach and of the tops of certain intestinal villi. The necrosis can be brutal and attack not only the epithelial layer, but also the vascular tissue of the papillary axis. These lesions resemble greatly erosions met with in human pathology. With certain animals (mice no. 4 in Group No. II, for example) one of the lesions is found to be situated on the pylorus, the other a few millimetres away in the duodenum; a loss of superficial substance characterises these lesions: the epithelium is replaced by a necrotic fibrous magma containing a few polynuclear leucocytes. This necrosis can be important; situated in the fundus of the stomach, they often penetrate in depth, leucocytes having penetrated around the necrotic centres. One never sees these lesions with the mice of Group I, fed on the same bread but leavened with yeast. They are specific and due to the formation of soluble aluminium salts in the digestive duct. To make them disappear it is sufficient to introduce into the food an excess of physiological saline mixture, such as that of Osborne and Mendel.

The hydrochloric acid then reacts with other metallic cations than aluminium; the formation of aluminium chloride is thus prevented, and consequently there is no production of lesions. It is to be noticed that in reality when man absorbs bread or biscuit containing alum baking-powders such a mineralisation of the diet is never realised, and consequently cannot exert its protecting action.

Contrary to the mice, four dogs fed for three or four months on bread containing alum-phosphate baking-powder were not found to have any lesions of the stomach or the small intestine. But there were distinct lesions in the large intestine and especially in the descending colon and the sigmoid flexure.

[1] 2·07 g. of aluminium (Al) for 1000 g. of bread.
[2] 4·1 g. of aluminium (Al) for 1000 g. of bread.

The congested, oedematised mucous membrane, covered with mucus, is visible even to the naked eye. Histological slides show intense congestion, together with small, subcutaneous haemorrhage, superficially localised; in some places submucous oedema is noticeable.

(3) Are the soluble aluminium salts formed in the digestive duct absorbed? This can be shown by appropriate methods of chemical estimating which put into evidence the aluminium in the organs of animals subjected to diets based on alum-leavened bread (Gies, Steel).

We have made several determinations of this kind, all of which have given positive results.

But the best proof, we think, that aluminium chloride is absorbed by the digestive duct are the troubles occurring in certain physiological functions with animals that have absorbed leavens containing alum, troubles which never occur with animals fed on the same diet, but without aluminium salts.

A. Let us first mention interference with growth in young animals.

On feeding non-adult chickens and rats with perfectly balanced diets rich in vitamins, but based on bread leavened with alum-phosphate baking-powder, a marked delay of growth is noticed as compared with control animals fed on yeast-leavened bread. This delay in the growth is more marked still if the experiments are made on younger animals.

B. The troubles that occur in the reproductive functions are the most characteristic of all those we have observed. Our experiments have been carried out on mice.

In the first series of researches we have observed the reproduction with the three groups of mice already mentioned. During the four months of treatment the twenty pairs of Group I (bread with yeast) had 300 offspring; the 20 couples of Group II (bread with aluminium phosphate) had 193; and the 20 couples of Group III (bread leavened with alum) had only 71.

In a second series of experiments where four groups of ten couples were used the diet was as follows:

Group I. Bread with yeast to which was added 4 % of the Osborne and Mendel salts mixture ("pain à la caséine" constituting the basis of the diet and completed by vitamins).

Group II. Bread with yeast, containing 13 per cent. of the same saline mixture; otherwise the same diet.

Group III. Bread with alum-phosphate baking-powder, 3 doses, that is to say 4·4 per cent. of aluminium (Al), to which was added moreover 4 per cent. of the Osborne and Mendel saline mixture; otherwise the same diet.

Group IV. The same bread as in Group III, but with one dose of alum-phosphate baking-powder, that is to say 1·3 per cent. of aluminium.

After four months of the treatment the following results were observed:

Group I. 46 litters, giving in all 328 individuals. Death-rate during the first week after the birth, 6 per cent.

Group II. 45 litters, 310 individuals; death-rate during the first week, 10 per cent.

Group III. 35 litters, 192 individuals; death-rate during the first week, 23 per cent.

Group IV. 12 litters, 244 individuals; mortality during the first week, 10 per cent.

These results are so outstanding that they need no comment. They can be explained by an elective, specific action of the aluminium ion on the ovary. This action is diminished, though still very marked, when the formation of aluminium chloride in the stomach is reduced by introducing other salts in the diet. As is shown by Group II in the second series of experiments, one cannot put forth as a cause an action due to an over-mineralisation of the diet.

Anatomo-pathological examination confirms these experimental results in a striking manner. While in the case of male mice having absorbed aluminium the testicles remain normal and the spermatogenesis active, the female mice show characteristic lesions of the ovaries.

Ovaries of the Females belonging to the First Series of Experiments.

In its normal state the ovary *of a mouse* contains a great number of atretic follicles, but their proportion is much greater with the mice of Groups II and III, fed on bread-containing aluminium salts. The oocytes of the cortical layer are very rare. When the rare follicles which seem normal at first sight are carefully examined regressive phenomena are observed. *The lesions are non-existent in all the control mice fed on bread with yeast; whereas they are found in every single female mouse which has absorbed aluminium salts;* they are particularly distinct with the mice of Group III, fed on bread made with alum-phosphate baking-powder.

Ovaries of the Females in the Second Set of Experiments.

One is struck with the distinctly reduced dimensions of the ovaries of all the females fed on the alum breads (II and III); the diameter is hardly half that of the ovaries of the control mice. On the microscopic slides the practically total absence of normal follicles is visible at a glance; a few rare ones are formed in the peripheral region, as well as numerous atretic follicles[1]. From time to time scars of corpora lutea are to be seen on the slides, or else involuting corpora lutea. *This description holds good for the ovaries of all the mice having undergone a diet based on alum-phosphate baking-powders. These symptoms are never met with in the case of the control mice, fed on bread leavened with yeast, containing doses, however important, of synthetic salt mixture. We therefore consider these lesions as specifically due to the action of aluminium salts.*

The photographical reproduction of several microscopical slides is given in the original work, *loc. cit.*

SUMMARY.

We hope that this summary of our researches will be sufficient to make clear the nature of the danger spoken of at the beginning of this article. We see that certain bodies, not poisons in themselves in the usual sense of the word, can provoke physiological disorders and organic lesions, the etiology of which remains generally unnoticed, when they are absorbed daily for months and years. Such products are most certainly responsible for many illnesses, the frequence of which augments with the alimentary conditions imposed upon us by modern civilisation in a manner inexplicable beforehand.

Our object will have been attained if we succeed in attracting the attention of physicians and hygienists to certain problems, the study of which is perhaps not very attractive but the solution of which is of considerable practical interest.

(*MS. received for publication* 11. v. 1928.—Ed.)

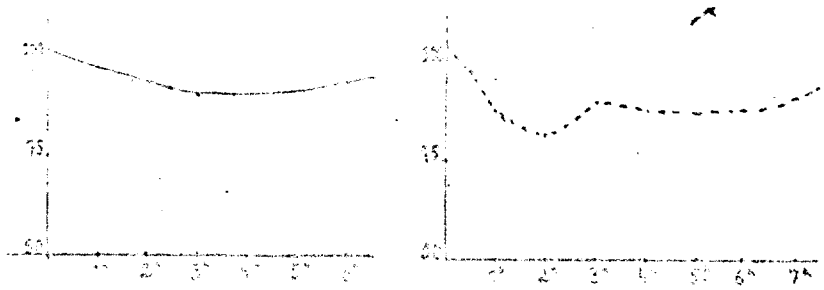
The contrasting influence of weak and strong doses of aluminum salts on insulin caused hypoglycemia and adrenalin caused hyperglycemia
Reviewed by M. Henry Schwab; presented by M. Maurice Javillier

The addition of zinc chloride to an insulin-gelatin complex noticeably prolongs the duration of the hypoglycemic effect of pancreatic hormone and thus extends the range of its therapeutic applications, however only on condition that suitable doses are used, because large doses have an inhibiting effect.

It would be interesting to see if another metal, such as aluminum, possesses the same properties. We have undertaken such a study and have found that with large doses of an aluminum salt (chloride) the hypoglycemic effect of insulin is completely canceled while with weak doses, these effects are both prolonged and intensified.

Our experiments were carried out with rabbits (2-3 kg) previously fasted for at least 12 hours. The insulin-gelatin (one unit of insulin and 2 cc of gelatin in the ratio 1:100) was given by subcutaneous injection at one time with 3.5 mg aluminum (strong dose), and at another time with 0.16 mg of aluminum (weak dose).

The first graph below clearly shows the inhibition of the hypoglycemic effect of the insulin-gelatin after the administration of strong doses of aluminum, whereas the second graph, corresponding to the experiment with weak doses of metal, makes clear the reenforcement and prolongation by weak doses.



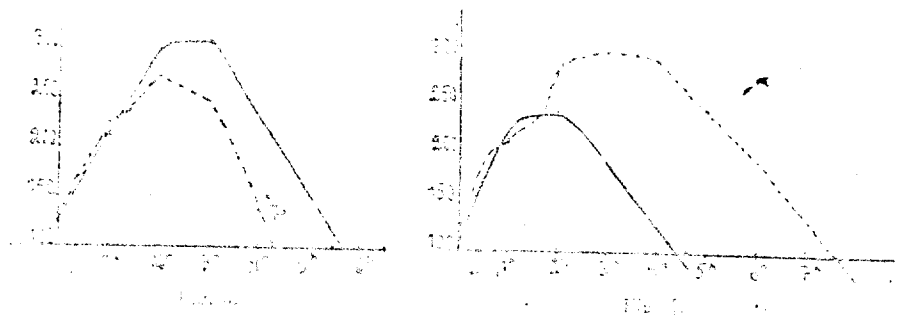
Scott and Fischer have expressed the opinion that metals (zinc) form a chemical or physical-chemical combination with hormones. We have also shown that the addition of zinc to adrenaline can prolong or reenforce hyperglycemic action.

Zinc salts probably can not interfere specifically to chemically combine with two such different natural hormones. We have hypothesized that the unusual effects produced by this salt result from an action on the cellular substrate and probably consists of a modification of the colloidal structure of protoplasm inducing in it a change of its fixed properties.

After having shown that other salts, notably those of aluminum, are capable of similarly modifying the effects of pancreatic hormone, we considered confirming our hypothesis by investigating whether aluminum salts can exert an analogous effect on the hyperglycemic action of adrenaline.

Our experiment was carried out with rabbits (2-3 kg) previously fasted for at least 12 hours. A subcutaneous dose of adrenaline (0.30 mg/kg) and aluminum (as the chloride) in the doses of 0.87 mg/kg and 0.04 mg/kg were injected simultaneously.

As the third graph shows, the hyperglycemic action of adrenaline was inhibited with the strong dose, while with the weak dose, there was clearly a reenforcement and prolongation of the effect of adrenaline on glycemia (graph 4). This is analogous to what we found with aluminum associated with insulin, as well as with zinc.



Thus, the action of aluminum on the duration of the hypoglycemic effects of insulin is similarly seen with regard to the hyperglycemic effects of adrenaline and depending on the dose is manifested differently: reenforcement and augmentation of the duration for the small doses and inhibition for strong doses.

PHARMACOLOGIE. — *Influence opposée de doses faibles et fortes de sels d'aluminium sur l'hypoglycémie insulinique et sur l'hyperglycémie adrénalinique.* Note de M. HENRY SCHWAB, présentée par M. Maurice Javillier.

L'addition de chlorure de zinc au complexe insuline-gélatine (1) permet de prolonger d'une manière notable la durée des effets hypoglycémiants de l'hormone pancréatique et d'étendre ainsi le champ de ses applications thérapeutiques, à condition toutefois de recourir à des doses convenables, car les doses fortes peuvent exercer une action inhibitrice (2).

Il était intéressant de voir si un autre métal, tel que l'aluminium, possède

(1) BROUN et SCHWAB, *Paris-Médical*, 27, 1937, p. 215.

(2) RABINOVITCH, J. S. FOSTER et A. C. CORCORAN, *Canad. M. Ass. J.*, 34, 1936, p. 637.

Comptes Rend. Acad. Sci. 206, 211-213 (1938)

les mêmes propriétés. Nous avons entrepris une telle étude et nous avons constaté qu'avec de *fortes* doses d'un sel d'aluminium (chlorure) les effets hypoglycémiants de l'insuline sont complètement annulés, tandis qu'avec de *faibles* doses, ces effets sont à la fois prolongés et renforcés (³).

Nos expériences ont été effectuées sur des lapins de 2^{kg} à 3^{kg}, maintenus préalablement à jeun pendant au moins 12 heures. L'insuline-gélatine (une unité d'insuline et 2^{cm^3} de gélatine à 1 pour 100) a été additionnée tantôt de $3^{mg},5$ d'aluminium (dose forte), tantôt de $0^{mg},16$ d'aluminium (dose faible) et l'injection effectuée par la voie sous-cutanée.

Le premier des graphiques ci-dessous montre nettement l'inhibition des effets hypoglycémiants de l'insuline-gélatine, après administration de doses *fortes* d'aluminium, tandis que le deuxième graphique, correspondant aux

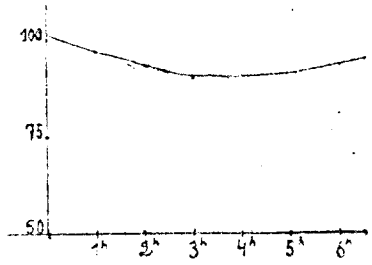


Fig. 1.

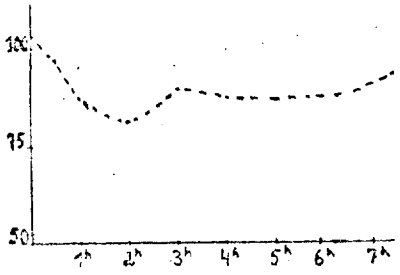


Fig. 2.

expériences faites avec de *faibles* doses d'aluminium, met en évidence le retardement et le renforcement des effets hypoglycémiants de l'insuline-gélatine.

Ces résultats confirment les constatations que nous avons faites avec le complexe insuline-gélatine-zinc, pour lequel on observe également l'inhibition des effets hypoglycémiants de l'insuline par les *fortes* doses de métal, renforcement et retardement par les doses *faibles*.

Scott et Fischer (¹) avaient émis l'avis que le métal (zinc) forme une combinaison chimique ou physico-chimique avec l'hormone. Nous-même avons montré (²) que l'addition de chlorure de zinc à l'adrénaline peut en retarder et en renforcer les effets hyperglycémiants.

(¹) Fazekas et Himwick, *Journ. of Pharmacol. Exp. Ther.*, 58, 1936, p. 260.

(²) *Journ. of Pharmacol. Exp. Ther.*, 54, 1935, p. 206; *J. of Biol. Chem.*, 114, 1936, p. 88; *Proceed.*, 30, 1936, p. xxxviii.

(³) *Comptes rendus*, 205, 1937, p. 628.

Le sel de zinc ne pouvant vraisemblablement pas intervenir spécifiquement pour se combiner chimiquement avec deux hormones de nature aussi différente, nous avons émis l'hypothèse que les curieux effets produits par ce sel résulteraient d'une action exercée sur le substratum cellulaire et consistant vraisemblablement en une modification de la structure colloïdale du protoplasma entraînant un changement dans les propriétés fixatrices de ce dernier.

Après avoir montré que d'autres sels, notamment ceux d'aluminium, sont susceptibles de modifier de la même façon les effets de l'hormone du pancréas, nous avons pensé étayer notre hypothèse en recherchant si les sels d'aluminium ne pourraient exercer une action analogue sur les effets hyperglycémiants de l'adrénaline.

Nos expériences ont été effectuées sur des lapins de 2^{kg} à 3^{kg}, maintenus

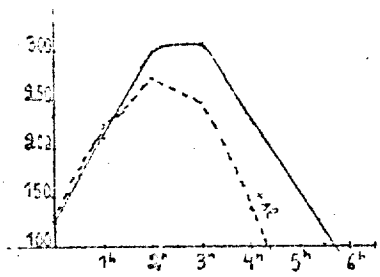


Fig. 3.

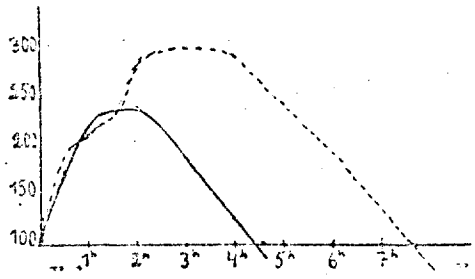


Fig. 4.

à jeun depuis au moins 12 heures. L'adrénaline à la dose de $0^{mg},30$ par kilogramme et l'aluminium à la dose de $0^{mg},87$ par kilogramme et de $0^{mg},04$ par kilogramme (sous forme de chlorure) furent simultanément injectés par la voie sous-cutanée.

Comme le montre notre troisième graphique, il y a, avec les *doses fortes* de chlorure d'aluminium, inhibition de l'action hyperglycémiante de l'adrénaline, tandis qu'avec les *doses faibles*, il y a nettement renforcement et retardement de l'action de l'adrénaline sur la glycémie (graphique 4). Ceci est analogue à ce que nous avons obtenu avec l'aluminium associé à l'insuline, ainsi qu'avec le zinc.

Ainsi l'action qu'exerce l'aluminium sur la durée des effets hypoglycémiants de l'insuline s'observe également en ce qui concerne les effets hyperglycémiants de l'adrénaline et se traduit différemment suivant la dose : renforcement et augmentation de la durée pour les petites doses et inhibition pour les fortes doses.

Arch Path 8: 230-62 (1929)

THE EFFECT OF ALUMINUM ON MAMMALIAN BLOOD AND TISSUES *

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There is comparatively little to be found in the literature concerning the effects of alum on the mammalian organism, when administered either in large doses or in small doses for long periods. We have therefore undertaken a series of experiments to secure information concerning the toxic effects of aluminum, especially in the form of alum (sodium aluminum sulphate). Two methods of attack were used. In the first, aluminum compounds were injected intravenously, and the effects on the blood cells, on the formation of antibodies and on the tissues were noted. These experiments demonstrated that markedly toxic effects resulted when the aluminum ion was put directly in contact with body tissues. To learn whether aluminum administered by mouth can be absorbed sufficiently to produce similar effects, aluminum compounds were fed in small doses over an extended period of time, and finally large single doses were fed at one time. When effects followed ingestion of the aluminum compounds similar to those caused by direct injection, the conclusion seemed warranted that the aluminum ion is able to pass through the alimentary tract and is then able to do damage to the tissues.

It has been contended that aluminum, as it is commonly eaten in the largest amounts, that is, in the form of food prepared with alum baking powder, is in an insoluble form which resists absorption and passes on through the alimentary tract as inert material. Evidence, however, has been brought to light showing that this may not be altogether the case. Steel,[1] in 1911, showed that aluminum in alum baking powders was not rendered wholly insoluble in the baking process and that it was found in the blood of dogs that ate biscuits made with the powder. Kahn[2] also showed that aluminum was absorbed into the blood stream after ingestion of aluminized biscuits. Much was eliminated in the urine and feces, but a considerable amount was distributed in the various tissues of the body. Aluminum was found by

* Submitted for publication, April 16, 1929.

* From the Otho S. A. Sprague Memorial Institute and the Department of Pathology of the University of Chicago.

1. Steel, M.: Am. J. Physiol. **28**:94, 1911.
2. Kahn, Max: Biochem. Bull. **1**:235, 1911-1912.

Balls[3] in the blood of men fed aluminized biscuits. Evidence was submitted by Myers and Killian[4] that from 2 to 20 per cent of the aluminum compounds ingested in baking powder residues was found in soluble form and a smaller proportion in solution in the gastric contents of eighteen subjects. Somewhat similar observations were made on the solubility of aluminum in the duodenal contents of four subjects.

The French investigators Schaeffer, Fontés, LeBreton, Oberling and Thivolle[5] recently found that aluminum phosphate, as it is found in some baking powders, is soluble both in vitro and in vivo in the hydrochloric acid of the gastric juice. Furthermore, it is not reprecipitated in the duodenum by the alkaline pancreatic juice when the chyme is neutralized. Bile prevents this (precipitation) in an as yet undetermined manner.

Mull, Morrison and Myers,[6] using the aurin reaction of Hammett and Sottery,[7] reported finding less than 0.2 mg. aluminum per hundred cubic centimeters in the blood of thirteen hospital patients with miscellaneous diseases and in pig's blood. Recently Myers, Mull and Morrison[8] reported the aluminum content of rat liver, per hundred grams, to be: 0.130 mg. in control rats; 0.161 mg. in rats fed aluminum; 0.063 mg. in rats fed an aluminum-free diet, and 8.2 mg. in rats receiving intraperitoneal injections of aluminum. They found the aluminum content of dog liver per hundred grams, to be 0.150 mg. in control dogs and 0.266 mg. in dogs receiving aluminum. In the autopsy material from man they found the following amounts of aluminum per hundred grams of tissue: in the heart, 0.225 mg.; in the brain, 0.203 mg.; in the liver, 0.074 mg. and in the gallbladder with bile, 0.069 mg. From their data, they concluded[9] that traces of aluminum are present in the tissues normally and that the amount is slightly increased on a diet containing considerable aluminum. The growth curves of animals receiving aluminum, however, compare well with those of the controls, the only difference being that the rats fed aluminum showed a slightly greater initial growth. They further concluded[10] that when aluminum compounds are administred orally to dogs, the absorption of the aluminum is slight.

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3. Balls, A. K.: Dissertation, Columbia University, New York.
4. Myers, V. C., and Killian, J. A.: J. Biol. Chem. **78**:591, 1928.
5. Schaeffer, G.; Fontés, G.; LeBreton, E.; Oberling, C., and Thivolle, L.: Bull. Soc. d'hyg. aliment. **16**:1 and 49, 1928.
6. Mull, J. W.; Morrison, D. B., and Myers, V. C.: Proc. Soc. Exper. Biol. & Med. **27**:476, 1927; J. Biol. Chem. **78**:595, 1928.
7. Hammett, L., and Sottery, C. T.: J. Am. Chem. Soc. **47**:142, 1925.
8. Myers, V. C.; Mull, J. W., and Morrison, D. B.: J. Biol. Chem. **78**:595, 1928.
9. Myers, V. C., and Mull, J. W.: J. Biol. Chem. **78**:605, 1928.
10. Myers, V. C., and Morrison, D. B.: J. Biol. Chem., **78**:615, 1928.

There have been numerous claims that aluminum is not absorbed into the blood stream following oral ingestion, such as the report by Taylor.[11] In a recent study, McCollum, Rask and Becker,[12] with the use of a spectroscopic method for determining aluminum, were unable to demonstrate that aluminum is a constituent of either plant or animal matter, and stated that compounds of this element are not absorbed out of the stomach or intestinal tract when present in the diet. This is not in agreement with the results of Mull, Morrison and Myers and others and can scarcely be accepted until repeated and confirmed.

EFFECTS OF INTRAVENOUS INJECTIONS OF ALUMINUM

Rabbits were used in all the experiments. The effects of aluminum on the number of red and white blood corpuscles, on the relative proportions of the different leukocytes, on the fragility or the resistance of the red cells to hemolysis with saponin and hypotonic salt, on the percentage of hemoglobin, on the body weight and on the histology of the various tissues, as well as the clinical symptoms, were noted.

The investigations in this part, dealing with intravenous injections of aluminum compounds, were divided into two types of experimentation: One dealt with the effects following intravenous injection of small daily doses of different aluminum compounds over an extended period of time. The other dealt with the effects following intravenous injection of a single large dose or a few large doses, in order that we might determine the amount of sodium aluminum sulphate that can be tolerated at one time.

TECHNIC

The red cell count and the total and differential leukocyte counts were made in the usual manner in a Levy counting chamber, before any injections were given and at frequent intervals thereafter. These counts were made before the rabbits were fed their daily ration. Two counts were made in every case reported in this paper and the average recorded. No numbers were accepted that varied more than from 300,000 to 500,000 erythrocytes or 1,000 leukocytes. The percentage of hemoglobin was determined by the Sahli method, and the fragility of the corpuscles was studied by determining the degree of hemolysis with saponin and hypotonic salt solutions.

The hemolytic methods used were those described by Bigland,[13] with modifications. For example, a series of six tubes was set up, containing the following amounts of 0.8 per cent sodium chloride: 1.4, 1.5, 1.6, 1.7, 1.8 and 1.9 cc. Then 1 cc. of a 1 per cent solution of sheep's blood made in saline solution was added to each tube. And, lastly, the following amounts of a 0.01 per cent saponin saline

11. Taylor, A. E.: Alum in Foods, Bull. 103, U. S. Dept. of Agric., 1914.
12. McCollum, E. V.; Rask, O. S., and Becker, J. E.: J. Biol. Chem. 77:753, 1928.
13. Bigland, A. D.: Quart. J. Med. 7:369, 1914.

solution were added: 0.6, 0.5, 0.4, 0.3, 0.2 and 0.1 cc., making a total of 3 cc. in each tube. Each tube was shaken well and allowed to stand at 37.5 C. for two hours and then over night at room temperature. The following day, the amount of hemolysis was read by noting the last tube in which complete hemolysis had occurred. By comparing, in this manner, the number of cubic centimeters of a standard saponin solution required to hemolyze completely a definite amount of blood of a rabbit before treatment, with the amount required after treatment, it was possible to determine whether the fragility of the blood cells was increased or decreased. It was found that the weaker (1 per cent) solution gave more clearcut readings than did a stronger (5 per cent) solution, and still the absolute values obtained in the two cases were identical.

The determination of the fragility of the red cells by means of the hypotonic salt solution was similar in process. A series of nine tubes was set up containing the following amounts of physiologic solution of sodium chloride: 1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8 and 2 cc. To each was added 1 cc. of a 1 per cent solution of whole blood made with saline solution, and then distilled water sufficient to make a volume of 3 cc. The resulting percentage of saline, which was just sufficient to produce complete hemolysis, could be easily calculated and was reported accordingly.

At first, an attempt was made to determine the hemolysis of blood cells washed free from serum, but the excessive bleeding that was necessary to secure adequate amounts for this determination had deleterious effects on the rabbits and greatly complicated the results due to the aluminum solutions. Hence this test was eliminated. The few preliminary experiments performed, however, showed that washed blood cells of normal rabbits are slightly less resistant to hemolysis by saponin than are the blood cells when mixed with their normal serum, a result which confirms the work of M'Neil.[14] There seemed to be no difference in hemolysis with hypotonic salt solution between washed and unwashed cells.

INTRAVENOUS INJECTIONS OF SMALL DAILY DOSES

Rabbits were each given a daily intravenous injection of from 5 to 10 cc. of an aluminum solution containing an amount of aluminum which would be comparable with the amount found by Kahn in the blood stream of dogs that had been fed aluminized biscuits. For example, he found an average of 1.7 mg. of aluminum, determined as Al_2O_3, per hundred grams of blood in three dogs fed aluminized biscuits for from fifty-five to sixty days. Calculated for an average rabbit weighing 3 kg., the daily injection of 0.04 Gm. of sodium aluminum sulphate ($Al_2[SO_4]_3.Na_2SO_4.24H_2O$) or 0.01 Gm. of aluminum chloride ($AlCl_3.6H_2O$) would constitute a comparable amount of aluminum ion in the blood stream.

Both of these solutions were acid: the 0.8 per cent solution of $Al_2(SO_4)_3.Na_2SO_4.24H_2O$ having a p_H of 3.4 and the 0.2 per cent solution of $AlCl_3.6H_2O$ Having a p_H of 3.8. Since acid is known to hemolyze blood cells, it was thought that the anemia produced by the

14. M'Neil, C.: J. Path. & Bact. **15**:56, 1910-1911.

injection of these aluminum solutions might possibly be due to the hydrogen ion concentration of the solutions. Sulphuric acid, therefore, of such strength that its p_H was 3.4, was injected intravenously into two rabbits for the same length of time as the aluminum solutions. Moreover, since sodium aluminum sulphate solutions liberate their acid slowly, a p_H determination does not indicate the amount of acid that is capable of being released, and therefore, a sulphuric acid solution of acidity corresponding to the maximal amount of acidity that could be liberated by the corresponding aluminum solution, namely, a p_H of 2.6, was also injected daily into two rabbits. It is not probable, however, that the tissues were ever subjected to such a high degree of acidity at any one time from the alum injected, for all of the acid would probably not have been released instantaneously, and the buffering action of the blood would have considerably diminished the acidity.

Results with $Al_2(SO_4)_3.Na_2SO_4.24H_2O$.—Five rabbits, no. 1 (3.6 Kg.), no. 2 (3.4 Kg.), no. 10 (2.4 Kg.), no. 15 (2.7 Kg.) and no. 20 (2.5 Kg.), which were given intravenous injections of 0.04 Gm. each of sodium aluminum sulphate ($Al_2[SO_4]_3.Na_2SO_4.24H_2O$) daily, all developed a secondary anemia after a period of from eleven to twenty days (chart 1). The percentage of hemoglobin always decreased considerably. The number of red cells per cubic centimeter showed an ultimate decline in every rabbit, except no. 15, extending as low as 1,870,000 in no. 1. The total numb · of leukocytes per cubic centimeter usually rose, reaching its peak, as high as 25,650, in rabbit 10, about the time that the number of erythrocytes per cubic centimeter was the lowest, and the increase was usually in the number of small mononuclear lymphocytes (chart 2). In order to save space the polymorphonuclear and mononuclear leukocyte curves for all five rabbits have been superimposed on each other and from these a single average curve computed and recorded.

The blood cells became more resistant to hemolysis as treatment continued (chart 3). In other words, a more hypotonic saline solution was required to hemolyze the cells, after treatment of the rabbit with aluminum compounds. This increased resistance of the blood cells to hemolytic agents was also demonstrated (to a slight extent) with saponin, for more saponin was necessary to hemolyze the blood at the end of the experiment than in the beginning.

A study of the blood cell picture (Wright's stain) revealed in all five rabbits a marked development of abnormal cells, similar to those found in lead poisoning. Some nucleated erythrocytes or normoblasts were always found in the later stages. Polychromasia, poikilocytosis and anisocytosis were marked. Stippled cells were found.

In addition to these definite indications of anemia, all five rabbits showed unmistakable symptoms of local aluminum poisoning. The

ears into the veins of which the aluminum solution was injected became edematous, inflamed and then necrotic, so much so that portions of the ears of some of the rabbits sloughed off. This, of course, was due to the irritant effects of the aluminum on the tissue or to thrombosis of the vessels, and was undoubtedly a local reaction. Swellings later

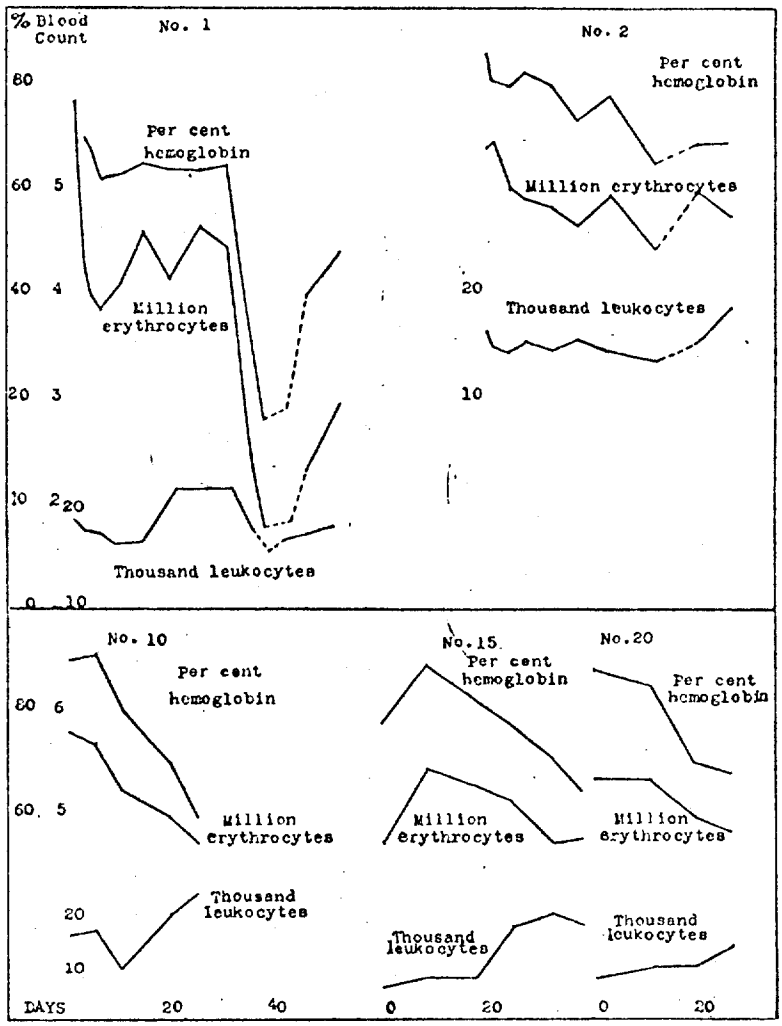

Chart 1.—The individual hemoglobin, erythrocyte and leukocyte curves of five rabbits following daily intravenous injection of 0.04 Gm. each of sodium aluminum sulphate.

appeared on the under side and the back of the neck and in the axillary glands in all five rabbits. The rabbits, as a whole, lost comparatively little weight, in spite of the development of roughened hair and a sickly

appearance. However, no. 1 lost 1⅛ pounds (1.2 Kg.) and gave the most marked reactions of all. The temperatures of nos. 1 and 2 never rose much above normal, while that of no. 10 reached 105.6 F. three days before its death, which was probably due to sepsis.

The toxic effects of injecting aluminum solutions are not surprising when the effects of aluminum on the blood in vitro are seen. When a

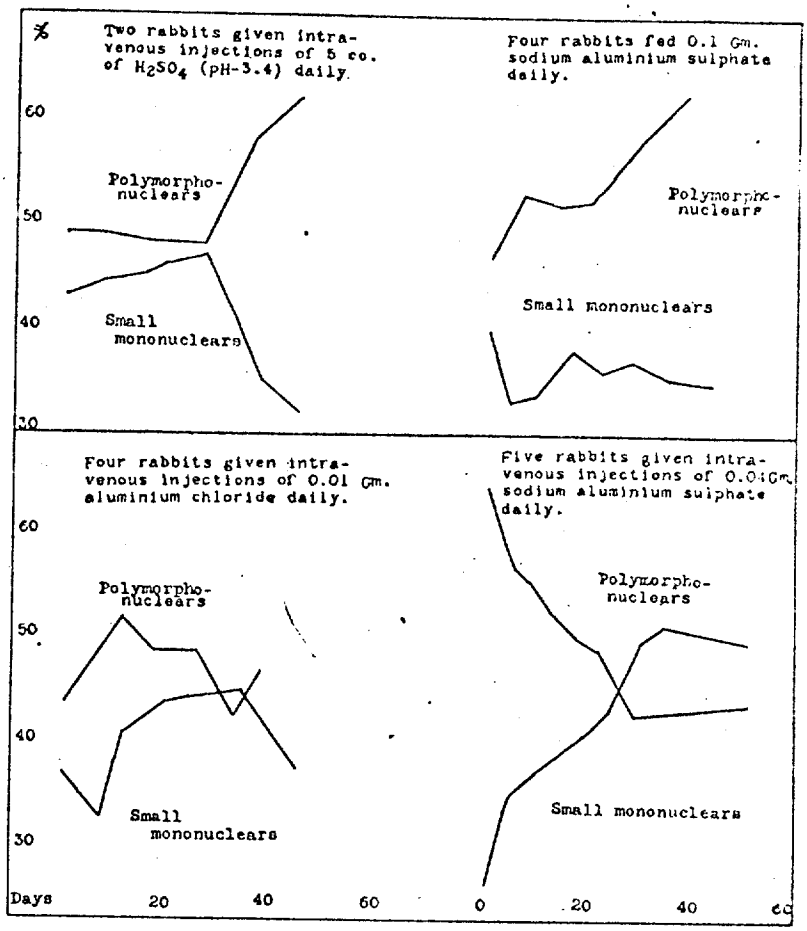

Chart 2.—The average leukocyte curves of rabbits following daily intravenous injection of sulphuric acid; daily intravenous injection of aluminum chloride; daily intravenous injection of sodium aluminum sulphate, and daily oral ingestion of sodium aluminum sulphate.

drop of fresh blood of a normal rabbit was placed in a small amount of a solution of sodium aluminum sulphate of the strength used for injections, almost immediately the hemoglobin was expelled from the corpuscles and decomposed to a brown hematin solution, and a white,

flocculent precipitate formed. When the blood was dropped into the solution of aluminum chloride, a clear brown hematin solution resulted. But when the blood was dropped into a sulphuric acid solution of a *p*H of 3.4, no hemolysis occurred; in fact, the cells became closely packed in the bottom of the tube and remained there for days unhemolyzed.

Results with $AlCl_3.6H_2O$.—Four rabbits, no. 3 (2.7 Kg.), no. 9 (2.9 Kg.), no. 12 (2.7 Kg.) and no. 14 (2.4 Kg.), received intravenous

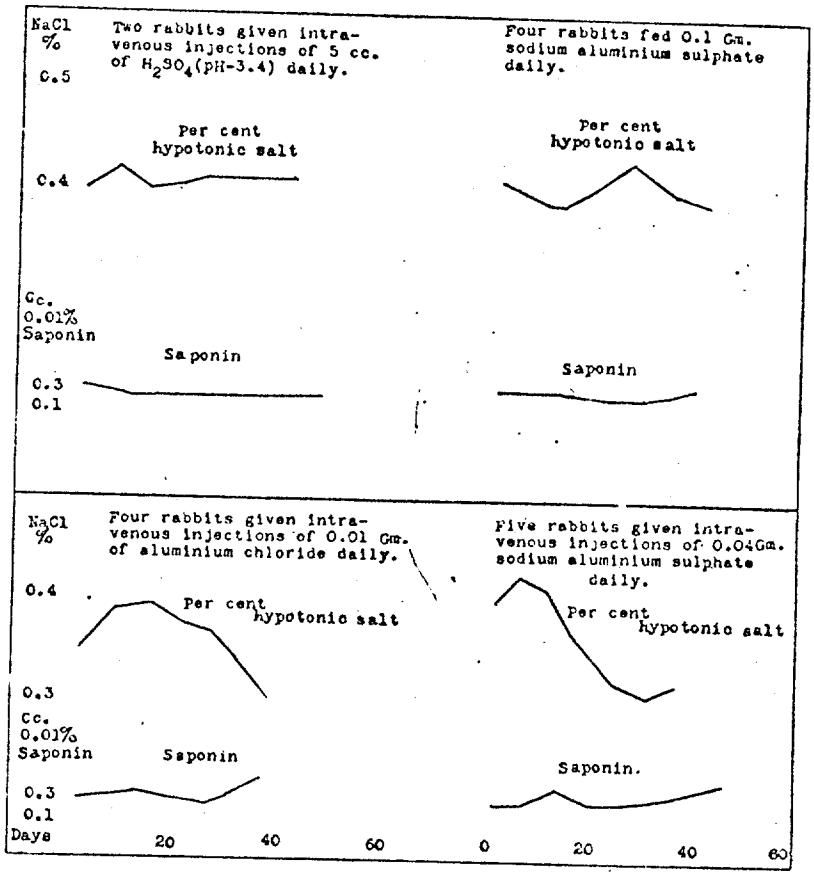

Chart 3.—The average curves for the hemolysis of red cells by hypotonic salt and saponin in rabbits treated with aluminum compounds (controlled by the curves of two treated with sulphuric acid).

injections of 0.01 Gm. each of aluminum chloride ($AlCl_3.6H_2O$) daily (chart 4). In every rabbit there was a marked decrease in homoglobin. In all but one, the resistance to hemolysis increased in the later stages (chart 3). The number of erythrocytes fell somewhat in all except no. 12. At certain times, all showed a definite leukocytosis, and in nos. 3, 12 and 14 the increase seemed to be in the number of the small

mononuclear lymphocytes (chart 2.) Abnormal blood cells, chromatophilic cells, poikilocytes, anisocytes and normoblasts were found in the later stages, as in the blood of rabbits treated with sodium aluminum sulphate ($Al_2[SO_4]_3.Na_2SO_4.24H_2O$). The experiment on rabbit 9 was discontinued sooner than usual because of the development of an

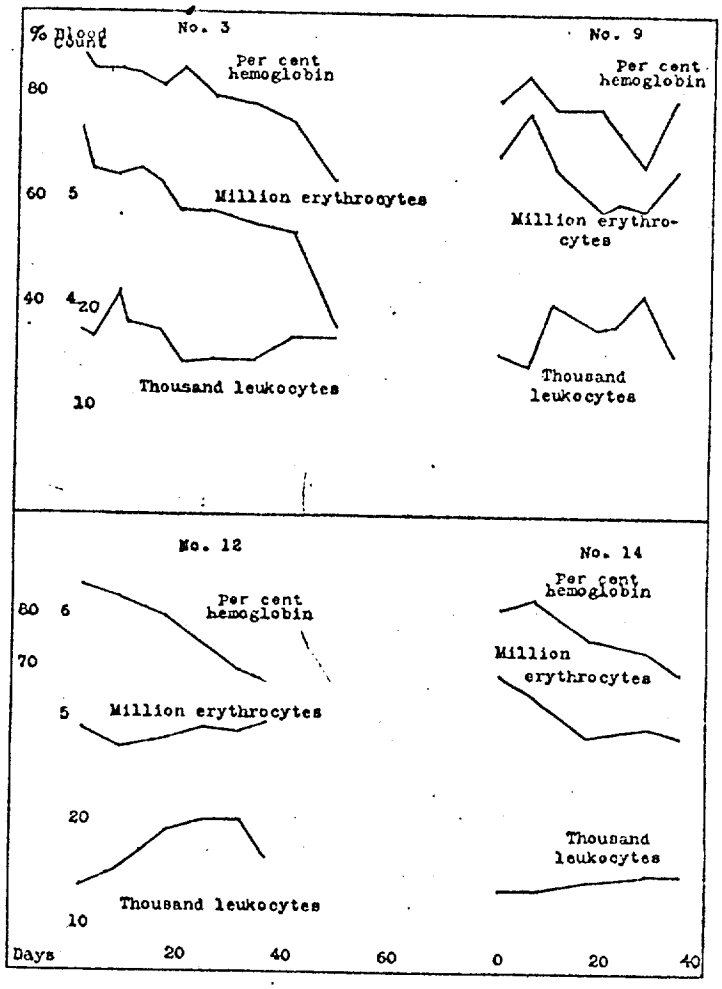

Chart 4.—The individual hemoglobin, erythrocyte and leukocyte curves of rabbits following daily intravenous injection of 0.01 Gm. each of aluminum chloride.

infection in the inner ear. In all four rabbits, swelling, edema, inflammation and necrosis of the ears appeared as soon and almost to the same extent as in rabbits treated with sodium aluminum sulphate. Nos. 3 and 12 in the later stages displayed swellings on the under side and

on the back of the neck. There was practically no loss of weight in any of the rabbits, but all became quiet and sickly.

Results of Control Injections of Acid Solutions.—Two rabbits, no. 5 (3.6 Kg.) and no. 11 (3 Kg.), for forty-five and thirty-six days, respectively, received daily intravenous injections of 5 cc. each of a sulphuric

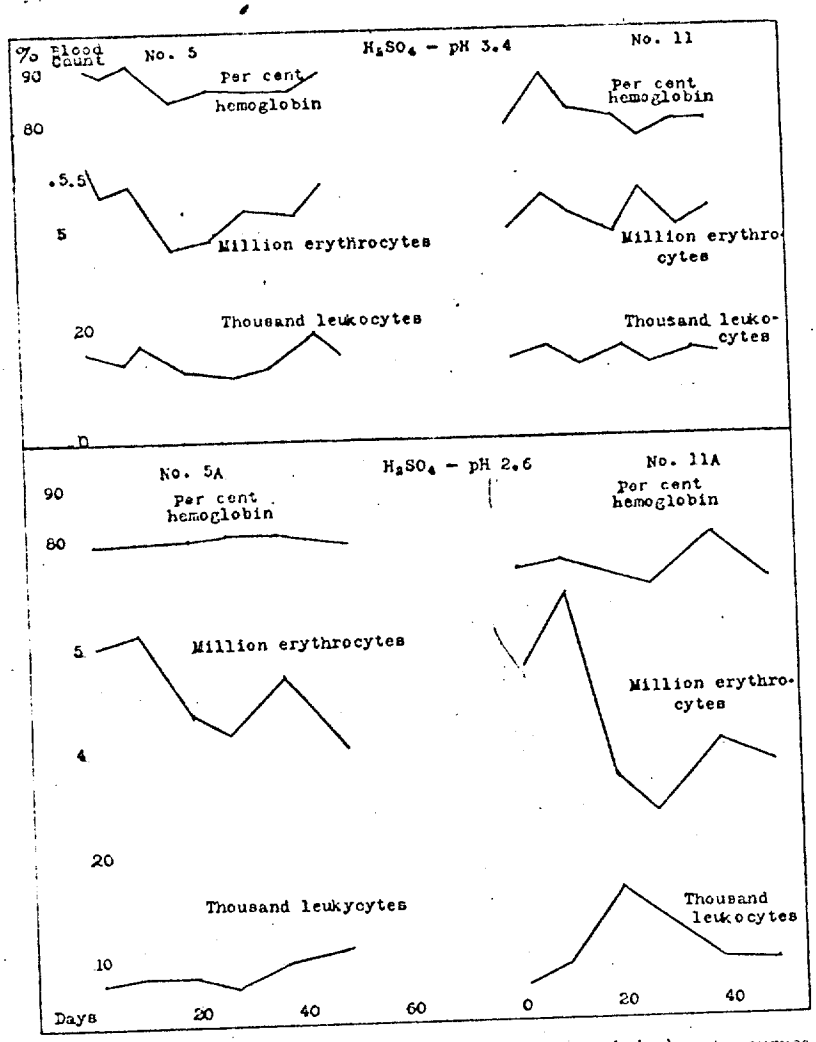

Chart 5.—The individual hemoglobin, erythrocyte and leukocyte curves of rabbits following daily intravenous injection of 5 cc. each of sulphuric acid.

acid solution of such strength that its p_H was 3.4. As indicated before, this was found to be the p_H of the 0.8 per cent solution of sodium aluminum sulphate injected (chart 5). At the end of and during these periods both rabbits appeared sleek and in perfect health and did not show any

of the marked symptoms or blood pictures described as occurring in the former groups of experiments.

Since more acid is liberated from sodium aluminum sulphate when it comes in contact with tissue than is free in the original solution, it is possible that some of the harmful effects reported following injections of the aluminum solution may be due to the acid liberated after the solution was injected. Therefore, sulphuric acid solution with a p_H of 2.6, which corresponds to the maximal amount of acid which can be liberated by the sodium aluminum sulphate, was injected intravenously daily into two rabbits, 5A (2.7 Kg.) and 11A (2.7 Kg.), for forty-nine days (chart 5). It is improbable, however, that this amount of acid would ever be free in the blood stream at any one time following injection of sodium aluminum sulphate solution because of the buffering capacity of the blood. There was no decrease in the percentage of hemoglobin, and there were no abnormal blood cells, but the total number of red cells did decrease. For a while during the experiment, the ear of one rabbit (11A) became swollen, owing to the irritant effects of the solution injected, and during this time the number of leukocytes rose. The other rabbit was in perfect health throughout the experiment and showed none of these symptoms.

THE LETHAL DOSE OF SODIUM ALUMINUM SULPHATE

Since the five rabbits that had received injections of sodium aluminum sulphate were able to tolerate for a considerable length of time daily doses of about 5 cc. containing 0.04 Gm. of the salt in 0.8 per cent concentration, it seemed of interest to determine what amount of the compound given in this way was lethal and in what concentration. Therefore, one rabbit (1.8 Kg.) was given an intravenous injection of 10 cc. of a solution containing 0.25 Gm. of sodium aluminum sulphate in 2.5 per cent concentration. Immediately, the rabbit's back bowed, and it cried, became limp, stopped breathing and died in spasms.

Another rabbit (1.8 Kg.) was then given an injection of the same total amount (0.25 Gm.) of sodium aluminum sulphate, but this time the salt was given in a large volume of solution or a weaker concentration, namely, 32.5 cc. of solution of 0.8 per cent strength. The rabbit trembled during the injection of the last 10 cc., but soon recovered completely. Three days later the rabbit was given 0.32 Gm. in 40 cc. in the same concentration over a period of two hours and the following day 0.16 Gm. in 20 cc. The rabbit did not collapse, but from then on it seemed to decline and became more quiet than normal, and its coat was roughened. Twenty-four days later it was found dead, having received a total of 0.73 Gm. in 72.5 cc. of a solution of a concentration of 0.8 per cent. At autopsy, petechiae were found in the mucosa of the stomach. The intestinal mucosa was red and microscopically showed

small areas of necrosis and mitosis in numerous epithelial cells. The spleen was small and fibrous, and microscopically it showed fibrosis and extensive necrosis of the pulp.

A third rabbit (1.6 Kg.) was given an intravenous injection of 10 cc. of 0.8 per cent solution (or 0.08 Gm.). Immediately, it showed slight symptoms of collapse. Eleven days later, it received the same dose and manifested no symptoms. Four days after this, it received five injections of 10 cc. each of a 1 per cent solution at hourly intervals and still showed no symptoms. The following morning, immediately after a single injection of 10 cc. of a 1 per cent solution, it died. This rabbit therefore died after receiving a total of 0.76 Gm. of the aluminum compound. At autopsy, extensive necrosis was found in the splenic pulp and some spontaneous nephritis, but no abnormalities of the alimentary tract.

Therefore, it can be stated approximately that a rabbit weighing 1.5 Kg. will survive an intravenous injection of 0.7 Gm. of sodium aluminum sulphate, if given in an 0.8 to 1 per cent concentration, but will be killed by any amount over this, given in a short period of time. And if the salt is more concentrated, the rabbit will not tolerate nearly so large an amount. However, small daily doses (5 cc.) of a solution of 0.8 per cent can be tolerated up to sixty days and longer, or until, as was the case in several of the rabbits receiving the injections, the rabbit is moribund and no longer offers a superficial vein for injection because of the necrotizing effect of the solution of aluminum on the surrounding tissues.

ANATOMIC STUDY OF THE RABBITS FED OR GIVEN INJECTIONS OF SMALL DOSES OF ALUMINUM

A careful study of the anatomic changes in the rabbits given injections of sodium aluminum sulphate and aluminum chloride, as compared with the control animals (those given injections of sulphuric acid solutions of corresponding acidity or fed lactose), revealed only one type of injury as certainly due primarily to the treatment, namely, pigmentation, thrombosis, necrosis and fibrosis in the spleen. On the whole, the spleens of the rabbits fed or given injections of the aluminum compounds were more heavily pigmented than were the spleens from the control rabbits given injections of sulphuric acid. However, later study of the spleens from rabbits fed only lactose and considered as normal revealed considerable pigmentation in three of four cases. It is true that necrosis of the splenic pulp cells was found only in the rabbits receiving injections of the solution of sodium aluminum sulphate, and this was extremely marked in several of these animals. The fact that there was such marked necrosis in the splenic pulp in the animals receiving large doses of alum, and that pigmentation and fibrosis were found so frequently

in the other animals, suggests the probability that the hemagglutinative effect of aluminum may be responsible for these changes in the spleen.

Some of the alimentary changes, such as petechiae and ulcers of the stomach and intestinal mucosa, probably had some relation to the treatments with alum, since they were found chiefly in those rabbits fed aluminum or given injections of it, even though they were not found constantly. This view is supported by the fact that similar intestinal lesions were found in mice fed alum by Schaeffer, Fontés LeBreton, Oberling and Thivolle.[5]

Focal necrosis of the liver cells seemed to be associated chiefly with infections, for example, bronchitis and pneumonia, and therefore it was found chiefly in the rabbits that had died of infections and cannot be attributed to the alum.

In summing up the microscopic study of the tissues of the twenty-six rabbits used in these experiments, it can be said that many of the pathologic lesions are not necessarily to be accredited to the treatment with aluminum compounds, since so many similar abnormalities were found in supposedly normal rabbits. The severe lesions in the spleens, however, for instance, the fibrosis and the necrosis, of even those rabbits receiving small daily doses of alum were probably due to the treatment with aluminum. The marked changes (to be described later) in the kidneys of the rabbits fed large doses of aluminum compounds and the extensive vacuolization and granular degeneration of renal epithelium in the rabbits fed small daily doses or given intravenous injections of sodium aluminum sulphate for a long period of time were not to be found in any of the control rabbits and were therefore, presumably, truly an aluminum effect. The agglutination thrombi found in the lungs, liver and kidneys of some of the animals were also never observed in the control rabbits.

EFFECTS OF FEEDING OF ALUMINUM

The experiments in this part deal with the effects following the feeding of small daily doses of sodium aluminum sulphate to rabbits over short periods (two months) and over long periods (up to one year); and the effects following the feeding of a single large dose or a few large doses of different aluminum compounds.

FEEDING OF SMALL DAILY AMOUNTS

Eight rabbits, nos. 8 (4.07 Kg), 16 (3.2 Kg.), 17 (2.5 Kg.), 35 (2.5 Kg.), 36 (2.5 Kg.), 37 (2.7 Kg.), 61 (2.8 Kg.) and 500 (4.07 Kg.), were each fed daily a gelatin capsule containing 0.1 Gm. of sodium aluminum sulphate ($Al_2[SO_4]_3.Na_2SO_4.24H_2O$) mixed with powdered lactose. This amount of the salt fed to a rabbit weighing 4 Kg. is comparable with the amount fed by Kahn to dogs. For example,

he fed 15 Gm. of aluminized biscuit per kilogram, and his analyses show that this amount of biscuit contains 2.4 mg. of Al_2O_3, which is equivalent to 21.53 mg. of sodium aluminum sulphate ($Al_2[SO_4]_3.NA_2SO_4.24H_2O$). In the case of four rabbits, the feeding was continued for only a short period, up to sixty days, and in the case of four others the feeding lasted from 150 to 350 days. In all except Nos. 35 and 37 (charts 6 and 7) the percentage of the hemoglobin

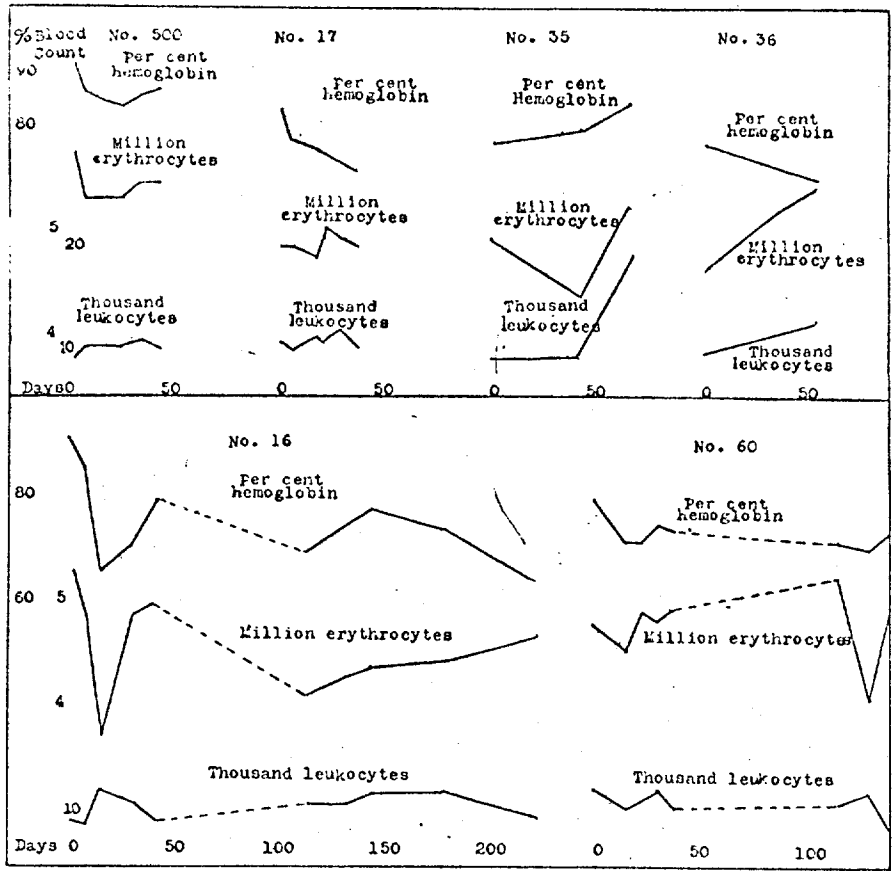

Chart 6.—The individual hemoglobin, erythrocyte and leukocyte curves of rabbits fed daily 0.1 Gm. each of sodium aluminum sulphate. The dotted line represents a period during which the feeding of the aluminum salt was suspended.

decreased, during the experiment, more than in the control rabbits (chart 8) fed only lactose. These two rabbits, nos. 35 and 37, as well as no. 36, showed a decided increase in the number of erythrocytes, comparbale to that in the control rabbits fed lactose. An explanation

of this phenomenon is wanting. It is noteworthy that in many of the alum-fed rabbits, especially rabbits 35, 16, 60 and 8, periods of marked depression of the number of erythrocytes are followed by periods of a rise in their number. There was practically no change in the total number of the leukocytes.

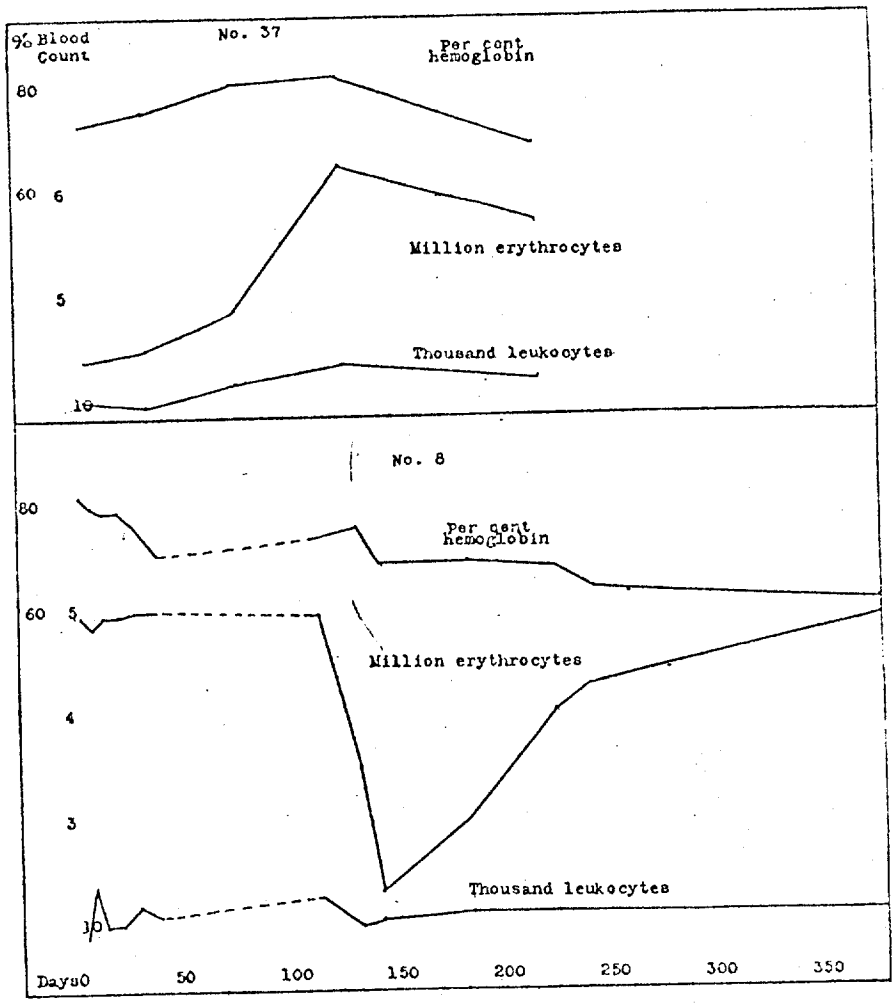

Chart 7.—The individual hemoglobin erythrocyte and leukocyte curves of rabbits fed daily 0.1 Gm. each of sodium aluminum sulphate. The dotted line represents a period during which the feeding of the aluminum salt was suspended.

Whether this somewhat consistent decrease in percentage of hemoglobin and the occasional marked depression in the number of erythrocytes is mere chance or whether it is significant may possibly be

decided by noting whether any considerable effects follow the feeding of large doses. If acute effects can be produced by the latter means, then are we not justified in suspecting that slight similar effects repeated over years may lead to chronic symptoms with a gradual failing of the hematopoietic system and the attending lowered resistance? It seemed worth while, therefore, to study the effects following the feeding of large doses of aluminum.

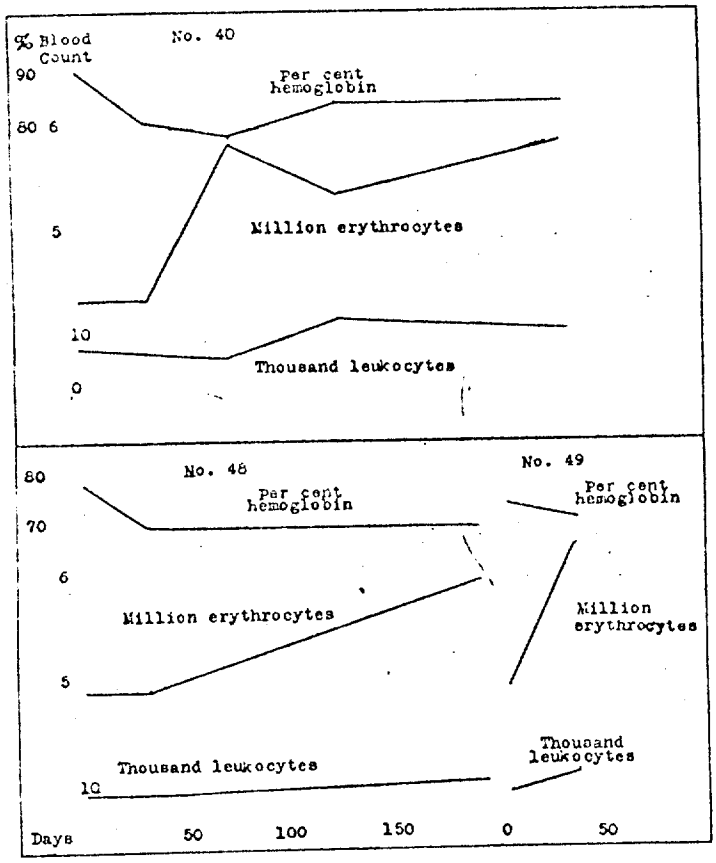

Chart 8.—The individual hemoglobin, erythrocyte and leukocyte curves of rabbits daily fed lactose only. These were controls on the rabbits fed sodium aluminum sulphate, which was mixed with lactose.

FEEDING OF A SINGLE LARGE DOSE OR A FEW LARGE DOSES OF ALUMINUM COMPOUNDS

Results with $AlCl_3.6H_2O$.—Rabbit 55 (1.5 Kg.) was fed 1.5 Gm. of aluminum chloride ($AlCl_36H_2O$) in gelatin capsules. In fifteen minutes, it began to breathe hard, displayed the motions of vomiting, stretched at length prostrate and jerked its head from side to side. It

then seemed to improve for a while, but after two hours died in convulsions. Autopsy revealed much liquid, containing aluminum, in the stomach, much mucous secretion and black hemorrhagic areas on the mucosa. The fluid in the stomach darkened blood when it came in contact with it, as does the aluminum ion.

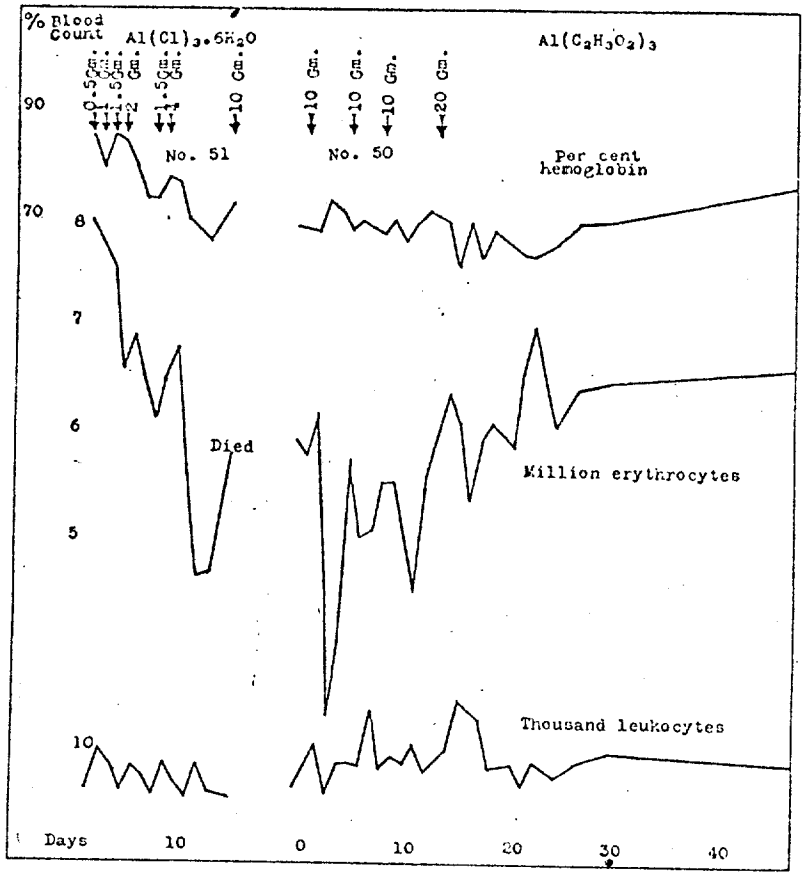

Chart 9.—The individual hemoglobin, erythrocyte and leukocyte curves of two rabbits following the feeding of single large doses of aluminum compounds over a period of fourteen days. An arrow indicates the time and the amount of each feeding.

Another rabbit, no. 51 (3 Kg.), was fed large single doses of aluminum chloride in gelatin capsules, ranging from 0.5 to 10 Gm. during a period of fourteen days. On chart 9, the arrows indicate the times of feeding and the amounts are mentioned. The total fall of over 3,000,000 in the number of erythrocytes and the fall of 18 per cent in hemoglobin, as well as the individual decreases after each dose, are striking. It is

noteworthy that the effect is registered soon after each feeding. This is indicative of a real toxicity of the aluminum, rather than of an impairment of digestive functions, which would lead to similar changes only later. There was no loss in weight. The rabbit finally died as a result of a feeding of 10 Gm.

The autopsy was performed within less than two hours after death. There were hemorrhages in the stomach, the mucosa of the whole small intestine was blanched, as if macerated, and there was a complete loss in tonus of the entire tract. The spleen was spongy, flabby and engorged; the kidney was small and soft and the cut surface was abnormal.

Microscopic examination showed marked changes in the kidney, which were essentially the effects produced by toxic agents. Changes suggesting the same condition were found in the spleen, and the lesions in the spinal cord were especially interesting.

The detailed protocols of the microscopic observations in rabbit 51 follow:

The liver was normal.

The spleen showed slight fibrosis of the pulp and slight diffuse pigmentation. In the pulp was blood pigment in phagocytes. Occasionally, large phagocytes contained both pigment and cells; also there were occasional areas of coagulated material not containing cells. There were numerous clumps of cells resembling somewhat cellular thrombi, although no distinct vessel wall could be seen about them, and there were also areas of hemorrhagic infiltration of the pulp.

The kidney showed swollen convoluted tubules, with, as a rule, no lumen remaining. There was practically no staining of nuclear material in the cells of the convoluted tubules. The epithelial lining appeared as solid coagulated masses, generally shrunken from the basement membrane. Loss of staining was so complete as to resemble an advanced postmortem autolysis, which can be excluded by the fact that the animal was sectioned two hours after death. Furthermore, the fusion of the renal epithelium into solid, structureless, granular masses, in places resembling granular casts, indicates that there had been a marked antemortem destruction of the swollen epithelium of the convoluted tubules in toto. Rarely, a cell was found in the convoluted tubular epithelium that retained nuclear stain. The nuclei of the basement membrane of these same tubules stained well, as did the nuclei of the loops of Henle, although these cells were swollen and often separated from one another. The collecting tubules showed swollen epithelium, the lumen rarely being visible, but the nuclei stained well. The glomeruli retained their nuclear staining. The tufts were generally swollen, but no hemorrhage or exudation was seen. The appearance indicated a selective toxic necrosis of the epithelium of the convoluted tubules.

The spinal cord showed marked destruction of the cytoplasm of many of the ganglion cells, some of which exhibited little besides the nucleus, containing many vacuoles and a granular detritus filling up the space formerly occupied by the ganglion cell. All the ganglion cells were more or less affected the same way. The white matter was also greatly vacuolated and granular.

The lung contained many small hemorrhages, slight edema and marked engorgement of the vessels. There was no inflammatory reaction. The red corpuscles seemed to be discrete, and hyaline thrombi were not recognized.

The stomach showed a diffuse loss of nuclear stain. There was no sharp necrosis, no inflammatory reaction. The vessels were engorged with blood.

The adrenal glands stained well and showed no recognizable alterations.

The duodenum showed a necrotic condition of the superficial portion extending about half through the mucosa, accompanied by a marked accumulation of stringy, blue-staining material, apparently mucin. There was no inflammatory reaction.

Lest the effects observed in these two experiments might be solely due to the acid liberated from the chloride compound, other aluminum compounds were similarly used.

Results with $Al(C_2H_3O_2)_3$.—Rabbit 50 (3.8 Kg.) was fed four times, at five day intervals, 10 Gm. of $Al(C_2H_3O_2)_3$ in approximately 100 cc. distilled water by stomach tube (chart 9). Before each feeding, the rabbit had been starved over night to insure a fairly empty stomach. In this experiment, the decrease in the number of erythrocytes after each feeding is striking, but not lasting, and in the final rebound the level is higher than it was originally. The percentage of hemoglobin is not seriously lowered, although there is evidence of a slight decrease after some of the feedings. Thirty-three days after the fourth feeding, this animal was killed by a blow on the back of the head and examined immediately. The histologic observations were as follows:

The lungs showed no change.

The liver showed slight fatty degeneration in the liver cells, nowhere marked, but diffuse through all parts of the lobule. There were vague masses staining red with scarlet R, some of which were in the blood vessels and some in the sinusoids. The nature of these is not clear, but their location raises the question whether they can be agglutination thrombi. A section of the same liver stained with hematoxylin and eosin showed in some of the vessels masses of fused red corpuscles corresponding in shape and size to the masses that stained red with fat stains. When the specimen stained with scarlet R was counterstained with hematoxylin, it was seen that some of the fat masses corresponded to large, fatty liver cells or to Kupffer's cells, but there were also masses staining like fat which plugged the sinuses.

The heart showed no change, and no fatty degeneration.

The duodenum showed diffuse loss of staining in its surface layers, occasional submucous hemorrhages. The excretory ducts of a few of the Brunner's glands were packed with leukocytes, although there were no changes in the adjacent tissues.

The adrenal glands revealed no change.

The spleen was generally normal in structure, except for occasional accumulations of brown pigment in masses, not generally in phagocytes.

The stomach was normal.

The kidney presented epithelium somewhat swollen and vacuolated. There was no fatty degeneration of the epithelium, but there were occasional irregular masses of fat stain, the location and nature of which could not be accurately determined.

Rabbit 730 (2 Kg.) was fed 30 Gm. of Al $(C_2H_3O_2)_3$ in a single dose in 100 cc. of water by stomach tube. Although it seemed to be all right following the feeding, on the third day it was found dead. At autopsy, the adrenal gland was normal. The spleen was small, thin and somewhat congested in areas. The stomach was filled with a brownish watery suspension, the muscles lacked tonus and the mucosa was completely coagulated into granular white folds. The small intestine seemed normal, but was filled with a jelly-like, yellowish-green substance. The heart was somewhat distended with venous blood, and showed profound fatty degeneration. In the lungs were many patches that looked extremely hemorrhagic and these were interspersed with pale areas. On cutting much dark blood oozed out. The microscopic observations were as follows:

In the lung, areas of edema were present without inflammatory change, and the vessels were engorged with blood. The testicle showed no spermatogenesis. There was no atrophy, but the spermatids and spermatozoa were entirely lacking. Many large multinucleated cells were present in the tubules. The epididymis contained no spermatozoa. The kidney showed profound fatty degeneration in all the straight tubules; the epithelium was much swollen, granular and finely vacuolated, occluding the lumen. Fat droplets were present in the convoluted tubules and glomeruli and also in the intima of the blood vessels. In the liver were branching streaks that took the fat stain, and the size and location of these streaks corresponded to those of the capillaries, but there was no fat in the liver cells. The fat masses were generally too large to correspond to the Kupffer cells. In the centers of some of the lobules, however, there was slight fatty degeneration in the liver cells. In some places, where the capillaries contained blood, the material that stained like fat could be seen definitely to be in the walls of the blood spaces. The sinuses of the spleen were much dilated and contained many granular masses and some resembling agglutinated red corpuscles as well as distintegrated red corpuscles. There were masses of mucus present on the surface of the stomach and extending slightly into the lumen of many of the glands. The glands near the surface were disorganized, but no inflammatory changes were seen.

Results with Metallic Aluminum.—Rabbit 53 (2.5 Kg.) was fed only two doses (3 and 4.5 Gm.) of powdered metallic aluminum in gelatin capsules (chart 10). The fall of 2,000,000 in erythrocytes and of 22 per cent in hemoglobin is good evidence that the aluminum goes into solution in the digestive juices and is absorbed. These decreases are further evidence that the changes in the blood noted in some of the previous experiments were due to the aluminum ion itself rather than to any acid radical accompanying it. Thirty-nine days after the last feeding, this rabbit was killed by a blow on the head and examined immediately. Few pathologic changes were noted. In the kidney, the renal epithelium was slightly swollen and granular, but the lumen usually persisted and in some of the tubules the epithelium was much vacuolated. There were no casts. Marked fatty changes were seen in the Kupffer cells, but no fat was seen in the liver cells.

Rabbit 771 (2 Kg.) was fed metallic aluminum in the following doses on four consecutive days: 2, 1.7, 2 and 2.1 Gm., respectively. Twenty days after the last feeding the rabbit was found dead, and the autopsy indicated that death was probably due to an infection of the lungs. The spleen was small and dark; the stomach and duodenal mucosa were blanched and the stomach was filled with gas.

Microscopically, the spleen was enormously engorged with blood, and the sinuses were greatly distended with blood, but without thrombosis or necrosis. The lungs showed acute suppurative pleuritis, which may account for the focal

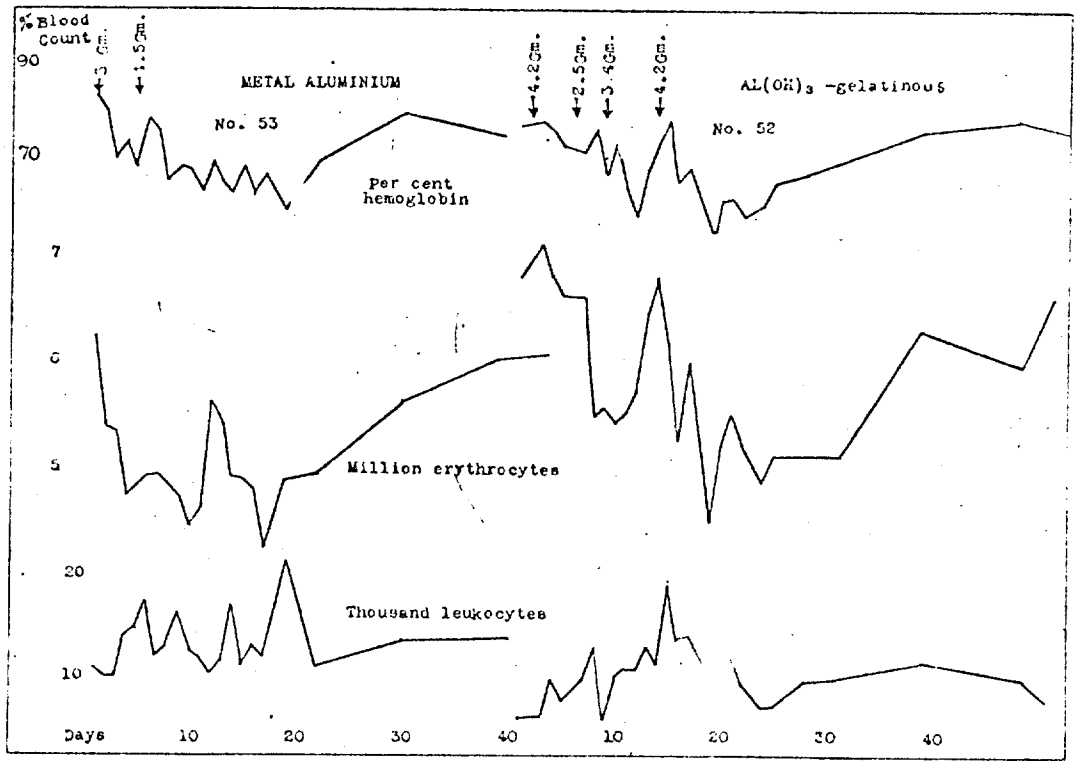

Chart 10.—The individual hemoglobin, erythrocyte and leukocyte curves of two rabbits fed aluminum compounds as follows: rabbit 53, metallic aluminum, and rabbit 52, $Al(OH)_3$ (gelatinous). An arrow indicates the time and the amount of each feeding.

necrosis in the liver. The tubular and cortical epithelium of the kidney was swollen and the glomeruli were much engorged with blood. In the liver, the centers of the lobules showed a decreased number of cells and loss of cord arrangement, and the liver cells here seemed somewhat coagulated, although they preserved their nuclei, which tended to be pyknotic. There were small areas of early focal necrosis and fatty changes in some of the Kupffer cells without fat in the liver cells.

Results with $Al(OH)_3$.—Since the aluminum ion, as it is usually ingested with foods, is chiefly in the form of $Al(OH)_3$, rabbit 52 (2.75Kg.) was fed with $Al(OH)_3$.[15] suspended in from 50 to 100 cc. of distilled water.

This suspension was fed by stomach tube to the fasting rabbit, over a period of from two to five hours, according to its ability to tolerate the aluminum. The accompanying curves of rabbit 52 (chart 10) show that this compound was apparently absorbed just as were the other aluminum compounds. The drop of 2,600,000 in erythrocytes and of 20 per cent in hemoglobin is evidence that the compound does not all pass on through the alimentary tract as inert material. When this rabbit was killed forty days after the last feeding no pathologic changes were found, gross or microscopic.

Rabbit 750 (2.5 Kg.) was fed, at one time, by stomach tube, 40 Gm. of $Al(OH)_3$ suspension prepared in the same manner as that described for the preceding experiment. At the end of three days, it was moribund. It was killed with ether and examined immediately. The stomach contained nothing except a watery mucous substance filled with dark brown blood clots, some of which were also on the mucosa. The same condition was observed in the duodenum. The lungs also contained hemorrhagic spots, and red and white patches. The blood was almost black, and ran freely. Otherwise, the tissues were normal.

The microscopic changes noted were chiefly those of fatty degeneration. In the kidney, the convoluted tubules and Henle's loops were chiefly involved, and the collecting tubules and the pyramids showed but slight, fatty changes. The blood capillaries were often filled with agglutinated and hemolyzed red corpuscles, forming thrombi. The glomeruli showed only occasional dustlike fat droplets. Homogeneous pink-staining material was found in the vessels of the heart and of the liver and marked fatty degeneration of the parenchyma cells was present. In the liver, the degeneration was least marked about the portal triads. In the lung, a few of the bronchioles contained leukocytes, but there were no pneumonic changes. A few leukocytes were found in the alveoli and alveolar walls. The blood in the small vessels had a brassy appearance, and in some it was hemolyzed. The testicle seemed normal. In the stomach was an area of superficial ulceration, in which were many polymorphonuclear leukocytes. These extended into many of the gastric glands, even those at considerable distance from the site of the ulcer. There were many mitotic figures in the glandular epithelium in this vicinity. The spleen was poor in lymphoid tissue and the pulp diffusely fibrotic. There was much brown pigment. There were many small masses of iron and extensive deposits in the capsule and trabecula, but only a faint iron stain in many of the phagocytes. The larger masses of dark brown material did not stain for iron.

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15. The $Al(OH)_3$ was prepared as follows: 50 Gm. $Al_2(SO_4).Na_2SO_4.24H_2O$ was dissolved in distilled water. Enough strong sodium hydroxide was added to neutralize the solution and to precipitate the aluminum as $Al(OH)_3$ in the form of a gelatinous mass. This precipitate was then filtered off and washed with hot water to free it from other soluble salts. The washed precipitate was then ground to a creamy paste in a mortar with about 100 cc. of distilled water.

Again 45 Gm. of a suspension of $Al(OH)_3$, made as previously described, was fed to rabbit 775 (2.3 Kg.) by stomach tube. The rabbit died suddenly four days later, probably from peritonitis caused by a perforation in the stomach, which was full of a black mash intermingled with some loose, white, mucous masses. There was considerable hemorrhage around this perforation. The lungs were full of raised white patches and dark red areas.

Microscopically, no changes were found in the liver, testicle, lungs and spleen (except postmortem changes). A few glands in the duodenum were dilated. The kidney showed marked postmortem changes. The peripheral portion was well fixed, but showed no definite changes, and there were also no fatty changes, as determined by special staining, except that the protoplasm of the epithelium of the convoluted tubules stained diffusely a little more than other elements.

$Al(OH)_3$ may exist in two forms—either as a gelatinous compound, which readily dissolves in the slight acidity or alkalinity of the digestive juices, or in a more granular form, which is supposed to be extremely insoluble. Two more experiments were therefore planned. For these, the solution containing 50 Gm. of sodium aluminum sulphate ($Al_2[SO_4]_3.Na_2SO_4.24H_2O$) was neutralized, changing it to $Al(OH)_3$, and washed, and the final suspension of gelatinous $Al(OH)_3$ was boiled two hours over a direct flame to change it to the granular, insoluble form. This process is comparable to what might happen to a greater or less extent in baking.

Rabbits 54 to 56 were fed by stomach tube with three doses each of this compound and chart 11 shows that even this presumably insoluble compound must have been absorbed, for the effects on the erythrocytes and the hemoglobin were similar to those obtained in the former experiments.

Rabbit 56 (2.7 Kg.) was fed its third dose of $Al(OH)_3$ at 4 p. m., and at 10 p. m. it seemed to be perfectly normal, with the exception of a slight diarrhea. (Rather severe diarrhea frequently followed some of the feedings.) The next morning, the rabbit was found dead. At autopsy, the stomach was found to be full of a brown mush mixed with a white, slimy precipitate, and the wall was thin and hyperemic. The small intestines were filled with a watery brown mixture, and the colon was distended with a thin, watery brown liquid.

Microscopically, the changes in the kidney resembled those found in another rabbit (51), which died as a direct effect of feeding aluminum chloride ($AlCl_3.6H_2O$). In the lungs, agglutination thrombosis was found, so extensive as to have been the probable cause of death. This is significant in connection with death from ingested aluminum, for it has been shown that aluminum is a strong agglutinating reagent with red corpuscles. The protocols of the histologic examinations follow:

The epithelium of the kidneys was swollen so that the lumen was often occluded. Occasionally, granular casts and loose cells were seen. Many of the cells of the convoluted tubules contained yellowish-brown material in granules, sometimes occupying vacuoles. It was not as dark brown as hemosiderin and had more the appearance of hemoglobin. A large proportion of the convoluted tubules showed this change. There were occasional hyaline casts and occasional tubules in which the epithelial cells were disintegrated and lay in loose masses filling up the tubules. There was no frank coagulation necrosis. The glomeruli generally seemed to be normal. The collecting tubules had swollen epithelium, but otherwise seemed normal.

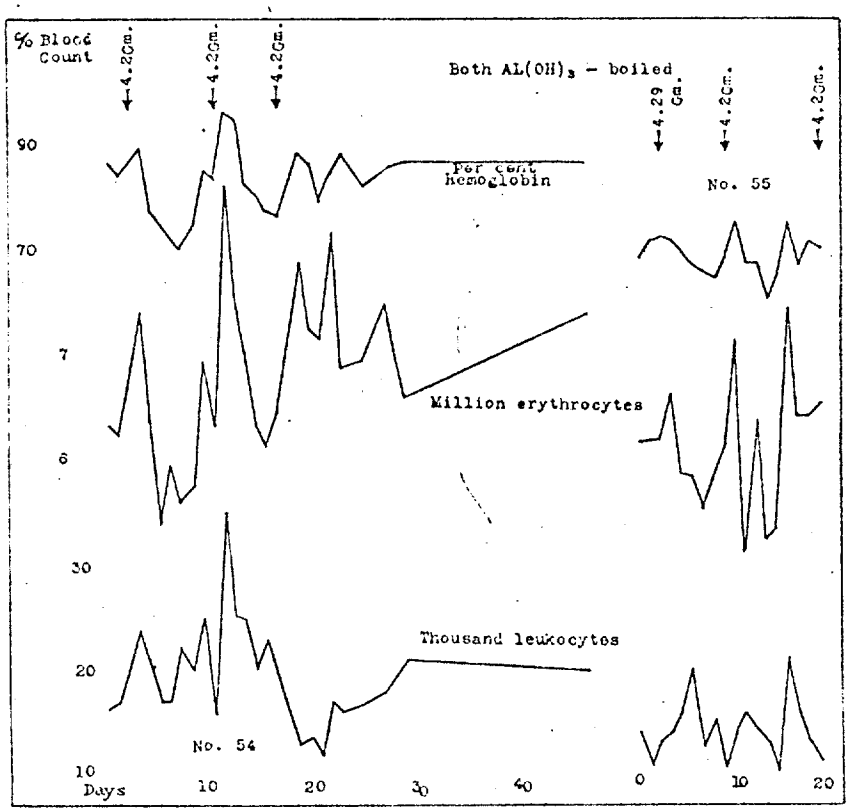

Chart 11.—The individual hemoglobin, erythrocyte and leukocyte curves of rabbits fed three large single doses each of a boiled aluminum compound $Al(OH)_3$ in the amounts and at the intervals indicated by the arrows.

The adrenal glands were normal.

The lungs showed marked engorgement of vessels with occasional small hemorrhages into the alveoli and edema involving perhaps one fourth of the alveoli. Some of this edematous fluid was also present in the bronchi. The capillaries were distended with masses of homogeneous structure, apparently thrombi from agglutination of red corpuscles. In the larger vessels, the red corpuscles were usually discrete. This condition of capillary agglutination thrombosis was extremely marked. There was no inflammatory process.

The myocardium was normal.

The stomach showed diffuse loss of stain on the surface, apparently postmortem.

The pancreas and the duodenum revealed no changes.

The spleen showed no pigmentation, hemorrhage or necrosis, except for an occasional phagocytic cell containing brown granules. The fibrous elements of the pulp were more conspicuous than usual.

The testicle showed spermatogenesis present but not abundant.

When the other rabbit, no. 54 (2 Kg.), which was fed boiled $Al(OH)_3$ also was killed, the tubular epithelium of the kidneys was generally normal, but in a few of the glomeruli, the capillaries were distended and contained homogeneous red cells, apparently thrombi from agglutination, but no hemorrhage or exudation. There was slight fatty degeneration in a few of the convoluted tubules and the endothelial cells. A few of the capillaries were plugged with masses staining for fat. The capillaries of the lungs were distended with homogeneous masses of red cells—agglutination thrombi—but there was no edema or hemorrhage. In sections of the liver stained for fat and counterstained with hematoxylin, there were many red masses corresponding in size and shape with single large liver cells, each with an intact nucleus surrounded by a solid mass of fat droplets, although the great majority of the liver cells contained no fat despite the fact that all the cells were deeply vacuolated. There were also smaller masses of stain that seemed to correspond to Kupffer's cells. Another section showed masses of fat granules, some corresponding to enlarged liver cells, others to Kupffer's cells, the latter being especially conspicuous in this liver. There were also masses of agglutination thrombi filling sinuses and small blood vessels.

The effects on the leukocytes in these rabbits fed large doses of aluminum compounds were plotted (chart 11) also, and occasionally a marked disturbance was noted.

A study of the blood pictures in these animals showed marked polychromasia, slight poikilocytosis and anisocytosis and an occasional normoblast and stippled cell.

EFFECT OF ALUM ON THE PRODUCTION OF COMPLEMENT AND ANTIBODIES

The experiments to determine the effects of aluminum on the production of complement and antibodies fall into four groups. Group 1 consisted of the control tests, in which one rabbit, no. 7 (2.7 Kg.), received a single injection of 5 cc. of a 50 per cent solution of washed sheep cells, and two rabbits, nos. 23 (2.9 Kg.) and 24 (2.8 Kg.), each received two injections of sheep cells seven days apart. Hemolysins and agglutinins were followed in each rabbit (chart 12). The development of agglutinin was followed by not adding complement to every other tube in the titration. A small amount of hemolysin was found following a single injection of sheep cells; but practically no agglutinin was demonstrable. There was a sudden rise, however, in both antibodies following a second injection of sheep cells. The chart shows, furthermore, an increase in native complement (fine dotted line in chart), following the second injection of sheep cells. The experiments are reported because

the contrast is so great between the results in groups 1 and 2 and those in groups 3 and 4 (with rabbits receiving considerable quantities of aluminum compounds) as to indicate strongly that decided effects result from injections of aluminum.

Group 2 consisted of three experiments in which rabbits 6, 21 and 25 (2.9 Kg. each) were each given an injection of sheep cells, followed

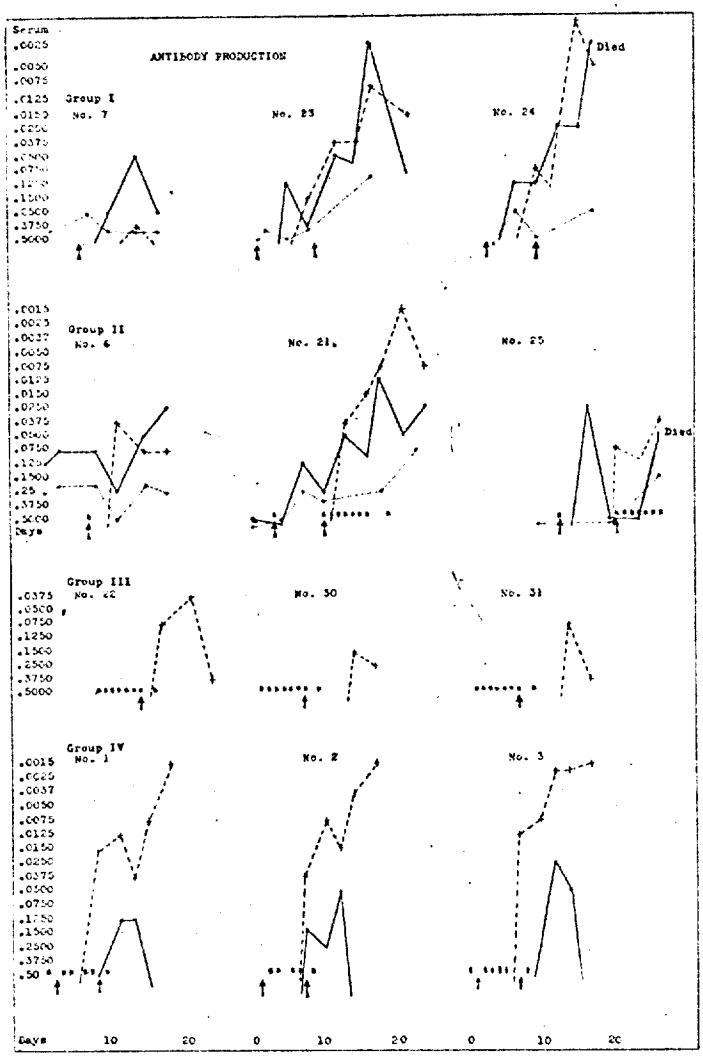

Chart 12.—The individual hemolysin, agglutinin and complement curves of rabbits treated with aluminum. The solid line represents hemolysin, the dash line agglutinin and the dotted line complement. An arrow indicates the day of injection of 5 cc. of a 50 per cent dilution of sheep cells. A star indicates the day of injection of $Al_2(SO_4)_3.Na_2SO_4.24H_2O$. A double-hilted dagger indicates the day of injection of $AlCl_3.6H_2O$.

by an injection of 0.04 Gm. of $Al_2(SO_4)_3.24H_2O$ in 5 cc. one hour afterward. Here, also, some hemolysin was produced, but little, and practically no agglutinin. Rabbits 21 and 25 were then each given a second injection of sheep cells seven days after the first injection, and then injections of alum for seven consecutive days. Rabbit 25 died immediately following the seventh injection of alum. In rabbit 21, the hemolysin rose following the second injection but never as high as in the controls; the agglutinin, however, rose high. In rabbit 25, little of either agglutinin or hemolysin was demonstrable. The remarks made concerning complement in group 1 are true also for this group.

Group 3 consisted of three experiments, in which the rabbits, no. 22 (2.7 Kg.), no. 30 (2.4 Kg.) and no. 31 (2.4 Kg.), were given injections of alum on seven consecutive days prior to the injection of the sheep cells. In all three cases, no hemolysin was demonstrable, and only a small amount of agglutinin. These results are therefore to be contrasted with the results in the control animals, in which after a single injection of sheep cells there was a small production of hemolysin, but practically no demonstrable agglutinin.

Group 4 consisted of three experiments in which rabbits 1, 2 and 3, which were showing a definite picture of anemia following extended injections of aluminum solutions (charts 1 and 4), were tested for production of antibodies. Rabbit 1, which had received forty-four previous injections of alum, was given an injection of sheep cells, then four more injections of alum, and a second injection of sheep cells six days after the first, as indicated on the chart. Rabbit 2, which had received thirty-two previous injections of alum, was treated similarly. Rabbit 3, which had received forty previous injections of $AlCl_3.6H_2O$, was also treated like nos. 1 and 2. All three showed marked production of agglutinin after the first injection of sheep cells, but no demonstrable hemolysin. Following the second injection of sheep cells, in all three rabbits, hemolysin appeared for only a short period, but the agglutinins were markedly increased. During the determinations of agglutinin, it was noted that there was a peculiar heavy precipitate in the tubes. This opens the question whether, in view of the lack of formation of hemolysin, the apparent increase in agglutinin might not really be added evidence concerning the poisoned condition of the blood, rather than a true formation of antibodies. It had been previously shown that during the poisoning of the blood with aluminum compounds the corpuscles became more resistant to hemolysis by saponin and hypotonic salt. Therefore, it seems possible that an increase in aluminum in the serum, due to injection of the aluminum compounds, prevents hemolysis of the sheep cells used in the test and at the same time causes marked agglutination of these same cells, thus giving the appearance of an increased formation of agglutinin.

COMMENT

From the results obtained in the experiments in which aluminum compounds were injected directly into the blood stream of rabbits, we conclude that the aluminum ion, introduced either combined as the sodium sulphate salt or as the chloride, produces a marked anemia, and when the dose is large enough, even causes death.

When daily intravenous injections of these two compounds were given, a definite secondary anemia was produced, i.e., there was a decrease in the percentage of the hemoglobin and in the number of the erythrocytes, an increase in the number of the leukocytes, chiefly the mononuclears, and an increased resistance of the corpuscles to hemolysis by hypotonic salt and saponin. Such increased resistance of the blood cells to hemolytic agents has been linked in lead poisoning with an increase in fragility of the corpuscles and denotes an injury to the cells. The final blood picture is also morphologically similar to that found in the later stages of lead poisoning, there being present normoblasts, poikilocytes, anisocytes, chromatophilic cells in considerable numbers and a few stippled cells.

Moreover, local irritant effects appear at the site of the injection—swelling, edema, inflammation and necrosis of the tissue and swelling of the neighboring lymph glands. All these symptoms were somewhat more marked in rabbits receiving injections of sodium aluminum sulphate than in those receiving injections of aluminum chloride.

These results are not wholly due to acid, since no similar effects were produced when a sulphuric acid solution of the same p_H as the solution of sodium aluminum sulphate was injected. A decrease in the number of erythrocytes, however, occurred when a sulphuric acid solution of p_H 2.6 was injected daily. This acidity corresponds to the maximal acidity that could be released from the alum compound, but it is improbable that a release of so much acid from alum would ever occur suddenly enough and at one time in the blood stream to give rise to the same effect as is produced by the injection of a solution of p_H 2.6.

A study of the minimal lethal dose emphasizes the toxicity of aluminum compounds. Rabbits seemed to be able to tolerate daily intravenous doses of from 0.011 to 0.017 Gm. of sodium aluminum sulphate in 0.8 per cent concentration, per kilogram, and from 0.003 to 0.004 Gm. of aluminum chloride, per kilogram, for as long as forty days, or a total of 1.5 Gm., or of from 0.416 to 0.625 Gm. per kilogram, of the former salt, before they showed visible signs of decline to such an extent that it seemed inadvisable to continue the injections without an interval of rest. However, as small a single dose as 0.25 Gm., when given in a solution of 2.5 per cent concentration, killed a rabbit weighing 1.8 Kg. immediately. This same amount (0.25 Gm.) was nonlethal when given at one time in a large volume of solution or in the concentra-

tion of 0.8 per cent to another rabbit of the same size. When 0.73 Gm. was given to this rabbit over a period of four days, death did not occur immediately, but the rabbit rapidly weakened and died twenty-four days later. A third rabbit of the same size died after receiving 0.76 Gm. over a period of seventeen days. Therefore, although a total as high as 1.5 Gm. or from 0.416 to 0.625 Gm. per kilogram of sodium aluminum sulphate could be tolerated, if given over a period of forty days, one-half that amount was lethal if given in a shorter time in the same concentration, and one sixth as much was lethal if given at one time in greater concentration.

A study of the tissues of all these rabbits revealed many lesions; namely, focal necrosis, infiltration with round cells and vacuolization of the liver cells; pigmentation, fibrosis, thrombosis and necrosis of the spleen; vacuolization of the renal epithelium and granular degeneration, necrosis and spontaneous nephritis in the kidney; lytic changes in the heart muscle cells; petechiae and ulcers in the stomach and intestines, etc. Except for the intestinal and splenic lesions, none of these lesions was found consistently in the alum-treated rabbits alone, and therefore they cannot be considered exclusively the result of treatment with aluminum compounds. It is significant that both rabbits killed by the intravenous injection of 0.73 and 0.76 Gm. of sodium aluminum sulphate over periods of from four to seventeen days showed extensive necrosis in the splenic pulp, while this never was seen in any of the control rabbits or in those that were fed alum. Similar lesions of varying degree were found in the spleens of several other animals that received aluminum, but not in the controls. Apparently, they resulted from injury to the blood, with formation of agglutination thrombi in the spleen sinuses.

These experiments leave no doubt that aluminum compounds are toxic and even lethal when put directly in contact with the blood and the tissues. There was still the question whether similar results can occur from feeding or whether the aluminum as usually ingested, i.e., in the residues of baking powder, may not be in such an insoluble form that it cannot be absorbed and therefore cannot produce these toxic symptoms.

Two sets of feeding experiments were undertaken in order to determine this point. In the first series, small daily doses of sodium aluminum sulphate (0.1 Gm. as $Al_2[SO_4]_3.Na_2SO_4.24H_2O$, which is the equivalent of from 0.0007 to 0.0011 Gm. aluminum per kilogram per day) were fed to rabbits weighing from 2.5 to 4 Kg. In these experiments, extending over varying periods of time up to one year, no outward clinical symptoms were detectable, but there was an undoubted moderate decrease in the percentage of hemoglobin in most of the rabbits. No such decrease occurred in the control rabbits. Moreover,

in contrast with the unexplainable increase in the number of erythrocytes in the control rabbits fed only lactose, there were marked decreases, at times, in the number of erythrocytes of the rabbits fed the aluminum compound.

That dramatic effects, such as follow intravenous administration, cannot be expected to result from feeding the same compound is illustrated by the experiments of Sollman[15] with $Pb(Co_3)_2$. Daily feeding of $Pb(CO_3)_2$ (0.15 Gm. per kilogram or 0.11 Gm. Pb per kilogram) to rats produced no effect in thirty-five weeks, except loss of appetite and checking of growth. And yet no one doubts the harmfulness of lead as an article of food.

Aluminum is particularly toxic to red corpuscles, and it may well be that, in analogy with chronic lead poisoning, years of slow absorption of minute amounts of aluminum are required to effect the same results that are accomplished in a short time by introducing the substance directly into the blood stream. That this is a possibility is suggested by the slight changes noted in the blood after prolonged feeding of small doses of an aluminum compound.

The strongest evidence, however, that the feeding of aluminum compounds, in any form, including that supposed to be so insoluble and inert ($Al[OH]_3$) may be harmful, lies in the results obtained in the second series of feeding experiments with large single doses of aluminum compounds. Quick responses, such as decreases of from 2,000,000 to 3,000,000 in the number of erythrocytes and of as much as 20 per cent in the hemoglobin were noted in a day or two following the ingestion of large doses of aluminum, in the form of $Al(Cl_3.6H_2O)$, $Al(C_2H_3O_2)_3$, metallic aluminum, $Al(OH)_3$ (soluble gelatinous form) and $Al(OH)_3$ (granular insoluble form).

Death followed immediately after the ingestion of $Al(Cl_3.6H_2O)$ in two rabbits (1.5 Gm. equaling 0.16 Gm. metallic aluminum, in a rabbit weighing 1.5 Kg. and 10 Gm., equaling 1.3 Gm. metallic aluminum, in a rabbit weighing 3 Kg.). One rabbit died during the night following a feeding of insoluble $Al(OH)_3$ (4.25 Gm., equaling 1.47 Gm. metallic aluminum in a rabbit weighing 2.7 Kg.). The other rabbits either died three, four or more days after the feedings or were killed at the end of a month.

At autopsy, these rabbits showed chiefly toxic effects in the kidneys, such as marked swelling of the tubular epithelial cells, in one with fusion of the cells into solid, structureless, granular masses resembling casts, with loss of nuclear staining. Thrombi from agglutination were especially prevalent in the lungs, liver and kidneys in some of the rabbits, and attention is called to the apparent relationship between this

15 Sollman, T.: J. Pharmacol. & Exper. Therap. **19**:375, 1922.

phenomenon and the hemagglutinative effect of aluminum. Fatty degeneration was found in the liver, kidneys and heart in most of them. Such changes were not seen in any of the control rabbits or in those fed smaller doses of aluminum.

That these lesions, as well as the marked and sudden although transient anemias, are due primarily to the toxicity of the absorbed aluminum ion, rather than to any acid radical attached, is evident from the fact that effects follow the ingestion of the neutral $Al(OH)_3$ compounds and the metallic aluminum itself similar to those that follow the intake of acid compounds in the form of the chloride and the sulphate. There would seem, therefore, to be no doubt concerning the possibility of the absorption of aluminum following the ingestion of aluminum compounds, even when in the form of the supposedly insoluble $Al(OH)_3$, since blood changes occur similar to those following the direct introduction of aluminum into the blood stream.

A brief preliminary study of the effect of aluminum on the immunologic defenses of the body indicated that in rabbits which were showing definite symptoms of anemia and intoxication, due to extensive intravenous injections of either sodium aluminum sulphate or aluminum chloride, two injections of sheep cells failed to increase the hemolysin, whereas the agglutinative power of the serum seemed to be greatly increased, in contrast with that of the controls. As mentioned previously, these effects may be added evidence of poisoning of the blood, rather than of a change in the production of antibodies. The experiments on this phase of the problem are scanty and are reported only because of the appearance of a distinct effect due to intoxication with aluminum and as a suggestion for further work.

SUMMARY

Daily intravenous injections into rabbits of from 0.011 to 0.017 Gm. sodium aluminum sulphate per kilogram in 0.8 per cent concentration, or of from 0.003 to 0.004 Gm. aluminum chloride per kilogram in 0.2 per cent concentration, caused a very definite secondary anemia, i.e., a decrease in the percentage of hemoglobin and in the number of erythrocytes, an increase in the number of leukocytes, chiefly the mononuclears, and an increased resistance of the corpuscles to hemolysis by hypotonic salt or saponin. The final blood picture showed normoblasts, poikilocytes, anisocytes, chromatophilic cells and stipple cells. Moreover, at the site of the injection, local irritant effects appeared, such as swelling, edema, inflammation and necrosis of the tissue and swelling of neighboring lymph nodes.

Daily intravenous injections of a sulphuric acid solution, the p_H of which was the same as that of the aluminum solution (3.4) produced no similar effects. An acid solution of an acidity equivalent to the

maximal acidity that could be, but probably would not be, released from the sodium aluminum sulphate solution at any one time (p_H 2.6) produced a decrease in the number of erythrocytes, and in one rabbit caused marked local irritation at the site of the injection. But a comparison of the results obtained with the aluminum solutions and the corresponding acid solutions makes it probable that the changes so marked in the rabbits treated with the former were due chiefly to the aluminum ion.

The lethal intravenous dose of sodium aluminum sulphate seemed to vary inversely with the period of time over which it was given and also with the concentration in which it was given. Rabbits were able to survive from 0.416 to 0.625 Gm. per kilogram in a 0.8 per cent concentration if given over a period of forty days, whereas one half of this amount was lethal, if given in a shorter period of time in the same concentration. One sixth of the amount killed instantly if given in a 2.5 per cent concentration.

The feeding of small daily doses over extended periods of time and the feeding of large doses of aluminum compounds produced changes in the blood and the tissues which indicate that aluminum is absorbed through the intestinal tract and then exerts the same toxic effects as follow intravenous injection.

Rabbits daily fed sodium aluminum sulphate, mixed with lactose, in amounts of from 0.0007 to 0.0011 Gm. per kilogram for periods varying from 60 to 350 days, showed a greater decrease in the percentage of hemoglobin, than did the control rabbits. The number of erythrocytes occasionally decreased considerably. In a few rabbits there was a slight increase, but less than in control rabbits fed only lactose for the same length of time.

Rabbits fed at varying intervals with large single doses of $AlCl_3$, $Al(C_2H_3O_2)_3$, metallic aluminum, $Al(OH)_3$ in soluble form and $Al(OH)_3$ in insoluble form all showed marked sudden drops in the number of erythrocytes (of 2,000,000 or 3,000,000) and in the percentage of hemoglobin (of as much as 20 per cent). The rebound, also, in most cases, was quick. Polychromasia and slight anisocytosis and poikilocytosis were found in some.

Two rabbits were killed by a feeding of $AlCl_3.6H_2O$ (1 and 3.3 Gm. per kilogram) and one rabbit by a feeding of the insoluble form of $Al(OH)_3$ (1.58 Gm. per kilogram), and the pathologic changes in the kidneys, spleen (in one case), lungs and spinal cord (one case) were marked. The lesions in the kidneys resembled those of a selective toxic necrosis and the agglutination thrombi in the lungs, liver and kidneys indicate the absorption into the blood stream of the hemagglutinative agent—aluminum. Profound fatty degeneration was found in the kidneys, liver, and heart in two rabbits fed large amounts of $Al(C_2H_3O_2)_3$ (30 Gm.) and $Al(OH)_3$ (4.05 Gm.), respectively.

In some of the rabbits treated intravenously and fed small doses of alum over extended periods, numerous pathologic lesions were found, but only the occasional lesions of the stomach or the marked changes in the spleen, namely, thrombosis, necrosis, pigmentation and fibrosis, can be considered as primarily due to the treatment with aluminum solutions, since the other lesions were often found in control rabbits, especially those with intercurrent infections. The changes in the spleen were marked and of a character indicating the presence of a poison causing damage to the red corpuscles with agglutination and hemolysis.

A few experiments on the effect of aluminum on immunologic defenses indicated that repeated intravenous injection of aluminum, to a degree leading to definite anemia, apparently reduced the capacity of the rabbits to produce hemolysin. However, the blood of these animals showed a heightened agglutinative effect, and this contradictory result raises the question whether both the heightened agglutination and the apparent lack of hemolysin may not have depended on the effect of the alum in the serum on the tested corpuscles.

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Phosphate Treatment of Alumina Gel Weakness in Young Chicks[1]

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ABSTRACT

STEINBORN, KURT, SIMON RODBARD AND CHRISTINE WILLIAMS. *Phosphate treatment of alumina gel weakness in young chicks.* J. Appl. Physiol. 11(3): 435–438. 1957.—Addition of large amounts of a reactive aluminum hydroxide gel (25 mg Al/gm of food) to the diet of young chicks produces a progressive syndrome of leg and wing weakness with retarded growth, leading to death within 10 days. Serum phosphorus levels were found to be one-third normal. This led to preventive experiments in which the response to daily intramuscular injections of phosphate salts in fat were tested in 402 chicks. The phosphate injections prevented the weakness and improved the growth and survival rate. When the phosphate treatment was begun after slight symptoms of weakness had developed, the symptoms did not progress; growth and survival rate improved. Injection of the fat vehicle alone had no effect. Since the syndrome was not completely reversed by the phosphate treatment, a multiple deficiency syndrome probably results from excessive intake of the gel; however, the weakness appears related directly to the phosphorus deficiency.

WE HAVE previously described a syndrome of progressive leg and wing weakness induced in newly hatched chicks by the addition of a reactive aluminum hydroxide gel[3] to the diet (1, 2). The syndrome led to death of all chicks within the first month. It could be reversed only if the animals were returned to a normal mash without the gel within 1 or 2 days after onset of weakness

Since this pattern differed significantly from other syndromes of weakness reported in young chicks, further studies were undertaken to determine its mechanism. The present study was based on a laboratory finding of abnormally low levels of inorganic phosphorus in the serum of chicks on the gel diet.

PREVENTIVE EXPERIMENTS

One hundred and thirty-five newly hatched male New Hampshire chicks were used in the first series. They were placed in a brooder and given a commercial mash which has proved adequate for normal growth and development. (This diet contains approximately 0.6% phosphorus.) At 11 days of age, these chicks were divided into three groups. The control group was continued on the same mash diet. The remaining chicks were fed a mixture of mash and aluminum hydroxide gel (Amphojel) which contained 25 mg of aluminum per gram of food. Half of these animals also received daily injections of a phosphate suspension.

The material for injection was prepared by suspending 30 gm of pulverized monosodium-phosphate ($NaH_2PO_4 \cdot H_2O$) in a menstruum of 20 ml of cottonseed oil and 40 gm of hydrogenated vegetable fat.[4] Homogenization

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[3] Amphojel, Wyeth, Inc., containing approximately 4% Al_2O_3.

[4] A commercial hydrogenated fat, labeled 'Covo' was used (Lever Brothers Co., New York).

was carried out in a blender. To prevent melting of the fat and sedimentation of the salt, the temperature was kept low during the blending process, and the suspension was then stored in a cool place. It had the consistency of a stiff cream and could be injected by a tuberculin syringe through a no. 18 or no. 20 needle. The phosphorus content was about 10%.

Injections into the leg muscles were given twice daily. The initial dose of 0.1 ml was increased by 0.025 ml daily to 0.2 ml per injection which was then maintained for about 2 weeks. The dosage was further increased as the animals gained weight, until a level of 0.5 ml per injection was being given by the end of the experimental period.

The small initial doses were necessary in the very young chicks since lethargy, collapse, and even death, occurred occasionally in the 1st hour following injection. This effect became less evident as the chicks grew older and developed an apparently improved tolerance to the phosphate suspension.

Blood samples drawn at intervals were usually pooled by groups for chemical analysis. Behavior, weakness and other symptoms, average weights and survival times were recorded. Autopsies were performed on all animals.

RESULTS

Food Intake, Growth, Stools, Feathers. Chicks on the gel diet ate significantly less than their controls, but some food was taken even after the weakness developed. Diarrheal stools containing mucus were observed occasionally. Feather development was retarded, the yellow pigmented down persisting abnormally. These findings were unaffected by the phosphate injections. Weight gain was considerably less than in the controls; however, the phosphate injected birds gained weight at a faster rate than did the untreated chicks. The average weight after 13 days (age 24 days) was 286 gm in the control group; the untreated animals on the gel diet weighed 104 gm and the phosphate-injected group averaged 136 gm.

Bones. Roentgen films taken at intervals showed slightly retarded bone development and poor mineralization in the groups on the gel diet; the phosphate-treated animals did not differ from the others.

Chemical Determinations.[5] The inorganic serum phosphorus of chicks on gel diet receiving no injections fell from a control of 7 mg% to 2 mg% in the 1st week. In the phosphate-injected group, blood samples drawn at least 12 hours after the injection also showed a similar fall. Later in the course of the experiments the phosphate-injected birds occasionally showed higher values in the range between 2 and 8 mg% on individual blood samples. This may have been due to an improved absorption from the augmented depots of phosphate salts.

The serum iron[6] was low in all groups on the gel diet. The control group had an average value of 140 γ%, whereas the gel-treated birds showed an average level of 70 γ% at the end of a 10-day period on the gel.

Serum calcium levels tended to be slightly higher in the groups on gel diet which received no phosphate injections, but the difference was not statistically significant from the other gel groups or the control group.

Weakness. The chicks on gel diet which did not receive the injections developed the typical weakness symptoms previously described (1, 2). However, the weakness did not appear in the birds injected with fat-phosphate suspension.

Mortality. The chicks receiving the phosphate injections for more than a few days had an improved survival rate. At the onset of the treatment period the mortality was higher in the phosphate group, but this may have been due to the trauma and the shock-like state which sometimes followed the injections. However, an improved tolerance became evident within a few days and the survival rate began to exceed that of the uninjected group. About half of the phosphate-injected group was still alive when all untreated birds on the gel diet had died (fig. 1).

A second series of 147 birds studied under slightly different conditions of housing and care 6 months later gave similar results: the phosphate-injected birds did not exhibit the weakness. The average weights after 11 days

[5] Serum phosphorus and calcium levels were determined by N. Hansl of the Chemistry Laboratory.

[6] Serum iron determinations were made available through the courtesy of Dr. Marvin Bloom, Buffalo General Hospital.

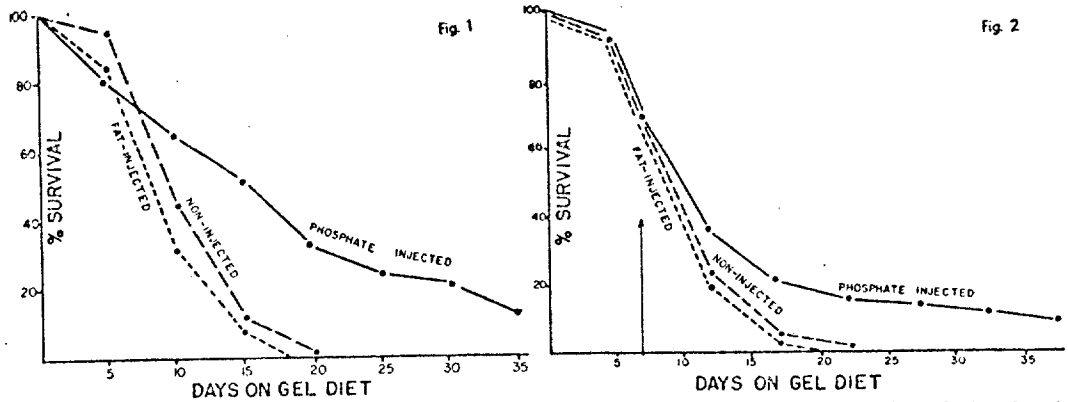

FIG. 1. Survival on alumina gel diet, as affected by daily injection of fat-phosphate or fat (282 chicks of *series 1* and *2*). Chicks not injected with phosphate developed weakness before death.

FIG. 2. Survival of chicks on alumina gel diet. Injection of phosphate-fat or fat was begun after 7 days on the gel diet, noted at *arrow* (120 chicks).

on the gel diet (age 22 days) were: controls 230 gm, phosphate group 158 gm, untreated group 143 gm. After all 30 of the untreated birds had died, 10 of the 48 phosphate-injected chicks were still alive (fig. 1). Experiments on another group of 49 chicks of this series demonstrated that injections of the fat vehicle, without the phosphate salt, neither prevented the weakness nor improved the survival rate. The survival of the fat-injected chicks was perhaps even poorer than that of the untreated birds (fig. 1). Their weight gain was as poor as that of the untreated birds on the gel diet (average of 143 gm after 11 days).

THERAPEUTIC EXPERIMENTS

A third series of experiments was conducted in which the gel diet was also begun on the 11th day after hatching, but injections were withheld for 7 days, at which time weakness had already become apparent. Of the 120 chicks originally placed in this series 37 had died. The remaining 83 chicks were then divided into three groups: one group of 27 birds was given phosphate in fat as in *experiment 1*; a second group of 26 chicks received the same amount of fat without the phosphate salt; the third group of 30 chicks was left untreated. These experiments showed that the phosphate injections, even when started after the onset of the weakness symptoms, improved the survival rate (fig. 2).

Autopsies. The viscera of birds on the gel diet were paler and contained less fat, and the livers were darker than in the birds on the normal diet. As previously noted, the spleens in most of the birds on the gel diet were much smaller and paler than those of the control chicks, while the gallbladders were relatively large and distended. Bile duct patency could be easily demonstrated. Hemorrhages were seen in the proventriculus, the ventriculus and the small intestine. Bones from the chicks on the gel diet did not appear to be softer than those of the controls. The injected regions of the leg muscles showed gross deposits consisting chiefly of fat with perhaps some local edema.

No notable difference in autopsy findings was seen between the untreated chicks and the phosphate-treated chicks.

DISCUSSION

The leg and wing weakness following excessive intake of a reactive aluminum hydroxide gel during the rapid growth phase seems clearly related to a critical phosphorus deficiency. This conclusion follows from the findings that the weakness was aborted or prevented by injection of phosphate salts even though an apparent phosphorus deficiency persisted, as indicated by low serum phosphorus levels. Rates of growth and survival which were partly improved by this treatment also appear to be related to the phosphorus depletion.

Excessive amounts of aluminum hydroxide gel as an intestinal adsorbing agent of high potency can be expected to disturb the absorption of vitamins and other substances to a

significant extent. The low carotene and vitamin A levels previously noted (1, 2) may depend on such an action. The low iron levels found in the serum of animals on the gel diet indicate that dietary iron is either bound by the gel or that the mechanism of enteric iron absorption is disturbed. The small spleens observed at autopsy may be associated with this iron depletion.

The diarrheal mucus-containing stools, and the intestinal hemorrhages found on autopsy, suggest that the excess gel or its salts modify enteric secretions and mucous membranes, disturbing normal intestinal function. The distended gallbladders may result from an increased tone of the biliary ductal sphincter, or possibly from an increased biliary secretory volume.

The weakness is of the flaccid type, apparently beginning in the distal muscle groups. Reactions to sensory stimuli seem to remain unaffected. The mechanism of the weakness remains obscure. It may be due to loss of essential contractile material, a neurological disorder or even pseudoparalysis caused by undefined modifications in bone structure.

'Weakness' is often present in florid rickets in man and has also been reported in phosphorus-deficiency rickets of other species. For example, a synthetic diet low in phosphorus has been reported to produce weakness in rats (3). Freeman and McLean (4) fed a diet deficient in phosphate to puppies and induced experimental rickets and a syndrome of weakness and unwillingness to stand and walk. Day and McCollum (5) produced "aphosphorosis" and a muscular weakness in rats, with an associated crippling gait and a progressive inability to walk, to stand and to breathe.

Parturient paresis (milk fever), which exhibits paralytic symptoms in dairy cattle at the onset of lactation (6), is usually considered to be caused by the low blood calcium found during this period of sudden mineral loss through colostrum secretion. However, phosphorus levels in cows with milk fever are also reported to be low, and acute phosphorus depletion may play a role in this syndrome.

A syndrome similar to that found in the chick has recently been reported during the period of rapid growth of pheasants on a commercial mash containing an adequate amount of phosphorus, most of which is bound as phytate and is therefore apparently of limited availability for absorption (7). Tibiotarsal and tarsometatarsal bone deformities were also noted.

The experiments cited, as well as our own studies, confirm the relation between phosphorus deficiency and certain weakness symptoms in several species, at least during the period of rapid growth. The possibility that the weakness is attendant upon a depletion of muscle phosphorus is under examination.

The present results demonstrate conclusively that the weakness syndrome is not due to "inanition," as suggested by Baeder, Beckfield and Seifter (8).

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THE INFLUENCE OF ALUMINUM SULFATE AND ALUMINUM HYDROXIDE UPON THE ABSORPTION OF DIETARY PHOSPHORUS BY THE RAT [1]

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The work of previous investigators has indicated that the feeding of sufficient amounts of soluble aluminum salts under experimental conditions renders much of the phosphorus of the ration non-assimilable. Thus, Cox, Dodds, Wigman and Murphy ('31) have reported that, if aluminum is added to the diets of rabbits or guinea pigs in quantities slightly in excess of that necessary to react with all the phosphorus contained in the ration (as $AlPO_4$), the absorption of phosphorus is practically nil. Deobald and Elvehjem ('35) have described similar results with chickens, using a natural grain ration to which various levels of aluminum salts were added. The addition of aluminum sulfate in amount equivalent to the phosphorus content of the ration resulted in severe symptoms of rickets, with low levels of blood inorganic phosphate. Likewise, Jones ('38) has described the production of marked rickets with low blood inorganic phosphate in rats fed a stock ration with high levels of aluminum sulfate or basic aluminum acetate.

The current use of aluminum hydroxide in the treatment of gastrointestinal disturbances such as peptic ulcer and

[1] This paper was presented in preliminary form at the meeting of the American Physiological Society in Chicago, April 16, 1941 (Street and Barlow).

J. Nutr. 24: 111-119 (1942)

gastritis has suggested a re-investigation of the effect of aluminum compounds upon phosphorus utilization.

Since this study was completed it has been reported by Fauley and co-workers ('41) that the administration of aluminum hydroxide gel to dogs in amounts equal to twice the phosphorus content of the diet reduced the urinary excretion of inorganic phosphate to approximately half its normal value.

The work reviewed above does not give precise quantitative data as to the effect of aluminum on phosphorus utilization since the degree of availability of the phosphorus in the cereal diets used is unknown (Jones, '39). The experiments described in this paper were designed to determine more quantitatively the influence of aluminum on phosphorus absorption.

METHOD

It was decided to study the utilization of phosphorus by means of the growth rate of young rats placed on a diet adequate except for phosphorus. The addition of sodium phosphate and aluminum compounds in various amounts and ratios to such a diet presumably should permit an accurate evaluation of the effect of aluminum on phosphorus utilization.

The low phosphorus diet of Schneider and Steenbock ('39), slightly modified, was chosen as the most suitable for the purposes desired. By adding phosphate to a diet of this type, which is composed of purified food materials, rations can be constructed in which the phosphorus is present chiefly in known chemical form in contra-distinction to the form and uncertain availability of the phosphorus in the cereal rations formerly used in studies of phosphorus utilization.

The percentage composition of the diet used was as follows: sucrose 44, starch 20, rice bran concentrate [2] 4, dried egg white 18, phosphorus-free salt mixture 4, and cottonseed oil 10. To this was added 0.3 cc. of a 0.3% solution of carotene in oil,

[2] Vitab rice bran concentrate, obtained from the National Oil Products Company, Inc., Harrison, New Jersey.

400 I.U. of vitamin D (viosterol), and 400 μg. of riboflavin per 100 gm. of ration.

The phosphorus content of this ration was reported by Schneider and Steenbock as 0.04%. Their data indicate that young rats placed on the basal ration alone grow very poorly, but the simple addition of sufficient sodium acid phosphate to bring the phosphorus content of the ration to 0.27% completely corrects the deficiency and results in satisfactory growth.

Various dietary formulae were developed from this basal ration by partially replacing the sucrose with sodium acid phosphate, or with both phosphate and aluminum sulfate or hydroxide in definite molecular proportions of aluminum to phosphorus. The phosphorus content of the basal ration was determined by the method of Fiske and Subbarow ('25) after digestion of the food by a wet ashing technique. Various lots of the basal diet were found to contain 0.035 to 0.042% phosphorus: the egg white used as the source of protein in the ration was found to have a phosphorus content of 0.08 to 0.10%. The drinking water used was a spring water of negligible phosphorus content.

The solubility of aluminum hydroxide in dilute acid and, therefore, in the stomach, depends upon its method of preparation. U. S. P. aluminum hydroxide is practically insoluble in tenth-normal hydrochloric acid. This material should be physiologically inert as a component of the diet, since it would not dissolve in the stomach. On the other hand, certain forms of commercial aluminum hydroxide used in clinical medicine are soluble in dilute hydrochloric acid.

The aluminum hydroxide used in this study was a powdered commercial tablet mixture.[3] Its solubility in acid was indicated by the fact that, when placed in tenth-normal hydrochloric acid, it was found to combine with and neutralize 75% of the theoretical amount of acid within a 20-minute period.

[3] Creamalin tablets, obtained from the Alba Pharmaceutical Company, Inc., Rensselaer, New York.

Young rats weighing 40 to 50 gm. were placed on experimental rations in groups of ten. The growth rates were determined at weekly intervals over a 6-week period, after which the animals were sacrificed and the blood inorganic phosphate determined individually by the method of Fiske and Subbarow ('25).

RESULTS

The massed results of feeding three series of rations are shown below in experiments I to III.

Experiment I. In this experiment different groups of animals were fed the basal low-phosphorus ration alone, or the basal ration plus sodium acid phosphate, or phosphate plus aluminum hydroxide, as indicated in table 1.

It will be seen that the animals on the basal ration grew at the rate of about 3 gm. per week, while the rats of groups 2 and 3, with phosphorus levels of 0.24% and 0.60%, respectively, gained approximately 17 gm. per week. With groups 4 and 5 fed a diet containing 0.6% and 3.0% $Al(OH)_3$, respectively, sufficient phosphate was added to the ration to make the value of the atomic ratio of aluminum to phosphorus equal to 1. In other words, the quantity of aluminum present was that calculated as just sufficient to precipitate all the phosphorus as $AlPO_4$, assuming complete interaction. On the basis of the growth curves the percentile formation of insoluble aluminum phosphate which actually occurred was much less than theory, since the animals of both groups 4 and 5 grew at approximately two-thirds the rate of those in groups 2 and 3 receiving optimum phosphorus.

It will be seen that the observed levels of the blood inorganic phosphate of the different groups were approximately proportional to the growth.

The somewhat subnormal growth of the positive controls in this experiment was due to the use of unheated egg white in the ration, thus resulting in early symptoms of egg white damage (Parsons and Kelly, '33). This artifact was proven by two following experiments in which the dietary egg white

was steamed, dried and ground, thus precluding the possibility of egg white damage.

It is clear that the effect of the aluminum feeding on phosphorus absorption in this experiment was much smaller than would be expected from the work of previous investigators. The two following experiments were carried out in an effort to explain this discrepancy.

TABLE 1

Summary of experiments I, II and III.

GROUP NO.	MATERIAL ADDED TO BASAL DIET	PHOSPHORUS CONTENT OF RATION	ATOMIC RATIO Al : P	AVERAGE GAIN IN WEIGHT IN 6 WEEKS	BLOOD INORGANIC PHOSPHORUS, AVER. VALUE
		%		gm.	mg./100 cc.
	Experiment I				
1	None	0.04		19.8	3.7
2	$NaH_2PO_4 \cdot H_2O$	0.24		105.6	8.2
3	$NaH_2PO_4 \cdot H_2O$	0.60		100.6	9.0
4	$NaH_2PO_4 \cdot H_2O + 0.6\%\ Al(OH)_3$	0.24	1:1	70.6	6.0
5	$NaH_2PO_4 \cdot H_2O + 3.0\%\ Al(OH)_3$	1.20	1:1	72.3	
	Experiment II				
1	None	0.04		11.1	3.54
2	$NaH_2PO_4 \cdot H_2O$	0.20		128.9	7.15
3	$NaH_2PO_4 \cdot H_2O$	0.60		145.2	8.37
4	$NaH_2PO_4 \cdot H_2O + 1\%\ Al(OH)_3$	0.20	2:1	43.3	3.19
5	$NaH_2PO_4 \cdot H_2O + 1\%\ Al(OH)_3$	0.60	2:3	137.7	7.87
6	$NaH_2PO_4 \cdot H_2O + 2.58\%\ Al_2(SO_4)_3 \cdot 18\ H_2O$	0.24	1:1	11.9	3.66
7	$NaH_2PO_4 \cdot H_2O + 2.58\%\ Al_2(SO_4)_3 \cdot 18\ H_2O$	0.60	2:5	142.4	9.00
	Experiment III				
1	None	0.04		4.7	2.40
2	$NaH_2PO_4 \cdot H_2O$	0.10		38.4	2.98
3	$NaH_2PO_4 \cdot H_2O$	0.15		95.9	4.46
4	$NaH_2PO_4 \cdot H_2O$	0.20		140.8	4.88
5	$NaH_2PO_4 \cdot H_2O + 1\%\ Al(OH)_3$	0.20	2:1	46.8	2.98
6	$NaH_2PO_4 \cdot H_2O + 1\%\ Al(OH)_3$	0.60	2:3	161.7	7.08
7	$NaH_2PO_4 \cdot H_2O + 0.5\%\ Al(OH)_3$	0.20	1:1	84.2	3.65
8	$NaH_2PO_4 \cdot H_2O + 2.58\%\ Al_2(SO_4)_3 \cdot 18\ H_2O$	0.24	1:1	17.7	2.27

Experiment II. In this experiment, summarized in table 1, the effect of aluminum sulfate on phosphorus utilization was compared with that of aluminum hydroxide. The growth of groups 2 and 3 indicates that the level of 0.20% phosphorus in the ration is slightly limiting, since a diet containing 0.60% phosphorus permitted a significantly greater growth. The incompleteness of combination of the ingested aluminum hydroxide with phosphate is indicated by the growth of group 4. Since the gain in weight of this group was one-third that of group 2, receiving the slightly limiting level of 0.20 gm. phosphorus per 100 gm. of ration, it may be assumed that the amount of phosphorus remaining available in ration 4 was approximately one-third that of group 2, or 0.067 gm. per 100 gm., indicating that 0.133 gm. of phosphorus per 100 gm. of ration combined with aluminum. Since sufficient aluminum was present to combine with 0.400 gm. phosphorus per 100 gm., the extent of the combination was only one-third of theory. The performance of group 5 indicates that this limiting effect of 1% aluminum hydroxide on growth can be completely eliminated by increasing the phosphorus level to 0.60%.

The data for group 6 show that when a soluble aluminum salt is fed in an amount just equal to the phosphorus content, there is almost complete failure of growth, indicating that most of the phosphorus in the ration has been made unavailable. Here again, the inhibiting effect of aluminum on growth can be prevented merely by increasing the phosphorus content of the ration, since group 7, receiving 0.60% phosphorus in addition to the aluminum sulfate, made optimum growth.

All three groups of rats showing maximal growth, that is, groups 3, 5 and 7, appeared in excellent condition, with smooth, sleek coats, despite the considerable amount of aluminum in the rations of groups 5 and 7. Thus, the only obviously harmful effect of aluminum feeding in these experiments was its interference with phosphorus utilization.

As in experiment I, the blood inorganic phosphate was low in the rats whose growth was seriously limited by the lack of sufficient available phosphorus, while the groups making normal growth exhibited normal levels of blood phosphate.

Experiment III. The results of feeding this series of rations are shown in table 1. This experiment was carried out in order to determine more quantitatively the effect of aluminum hydroxide on phosphorus utilization. From the data of the first four groups, a curve was constructed relating the per cent of available phosphorus in the ration to the growth obtained. Applying this curve to the performance of group 5, receiving 1% aluminum hydroxide, indicates that there was 0.108% available phosphorus in the ration, so that 0.092 gm. of phosphorus per 100 gm. of food, or 46% of the phosphorus present, was rendered unavailable. Since there was sufficient aluminum present to combine with twice the amount of phosphorus actually contained in the ration, it would appear that only 23% of the aluminum was changed to a soluble form so that it could combine with phosphate. Making the same calculation for group 7, the growth attained corresponds to 0.14% of available phosphorus, indicating that 0.06 gm. of phosphorus per 100 gm. of food was rendered unavailable. This is 30% of the phosphorus present in the ration. Since the amount of aluminum present was just sufficient to combine theoretically with all the phosphorus present, this indicates that 30% of the aluminum was converted to a reactive form.

The results obtained with the feeding of aluminum sulfate in this series were similar to those in experiment II. As in preceding experiments, the level of inorganic blood phosphate was approximately proportional to the growth obtained on the various rations.

DISCUSSION

These experiments indicate that when a soluble form of aluminum, as aluminum sulfate, is fed to young rats in amount equal to the phosphorus content of the ration, there is nearly complete precipitation of phosphorus in the intestinal tract.

This conclusion would appear justifiable, since the growth and levels of blood inorganic phosphate obtained under these circumstances are little, if any, greater than those on the basal low phosphorus ration alone. Since aluminum hydroxide is very insoluble in neutral solution, the ability of this form of aluminum to react with phosphate in the intestinal tract is undoubtedly due to its solution by the acid gastric contents, forming aluminum chloride. It follows, then, that the much smaller effect of the hydroxide on phosphorus absorption, as compared to the sulfate, indicates that only a portion of the aluminum hydroxide dissolves in the stomach.

SUMMARY

When aluminum sulfate was fed in amounts chemically equivalent to the phosphorus in the ration, essentially all of the phosphorus was rendered unavailable. On the other hand, when aluminum hydroxide was fed to young rats at levels of 0.5 and 1.0% of the diet about one-third to one-fourth of the aluminum was converted to a form reacting with phosphorus.[4]

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[4] Dick and Eisele ('42) have recently referred to our preliminary report (Street and Barlow, '41) as the basis in part for their statement, "The use of aluminum hydroxide and aluminum sulfate, which recently has become quite widespread, may have deleterious effects. Therapeutic doses may interfere with the absorption of inorganic phosphate from the intestinal tract, thereby deranging the calcium metabolism of the body." This statement is misleading. Aluminum sulfate is not, to our knowledge, used medicinally. The maximal therapeutic dose of aluminum hydroxide gel by normal oral administration recommended by the A.M.A. Council on Pharmacy and Chemistry ('41) would supply not more than 64 cc. in 24 hours. This amount of 5.5% gel would furnish 3.66 gm. of $Al(OH)_3$. If 30% of this were changed to soluble form as indicated by our experimental data it would combine with 0.44 gm. of P, since 1 gm. of aluminum hydroxide is equivalent to 0.4 gm. P. If a dietary contains 1.32 gm. of P, the level recommended by Sherman ('37), the precipitation of 0.44 gm. P leaves 0.88 gm. available, which is the figure given by Sherman as the minimal maintenance level for a 70 kg. adult. Thus, our data do not support the conclusions drawn from them by Dick and Eisele.

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STUDIES IN THE METABOLISM OF ALUMINIUM

III. Absorption and Excretion of Aluminium in Normal Man

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Aluminium compounds form a large part of the natural environment of plant and animal life. Escape from dust containing it is impossible. Significant amounts are found in many foods. In view of the fact that no substance can be considered absolutely insoluble in the laboratory of the animal body, it is to be expected that aluminium will find its way into the body in accordance with physico-chemical laws. An equilibrium must be established between the aluminium on what may be termed the outside of the body membrane, that is, the intestinal tract and the lung, and that on the inside—the tissues themselves. Considering therefore the aluminium which in one form or another must find its way into the body tube in food and into the respiratory tract in dust, it would be surprising indeed if it were not present in some parts of the body.

The results reported for the determination of aluminium in the blood of man are unconvincing (1). Our work (2) shows that aluminium is nearly always found in the blood of dogs, the amount increasing slightly after the feeding of aluminium-rich food. It seems probable that the negative results reported for man have been due to the shortcomings of the analytical methods used. The delicacy and reliability of our own method, outlined in the first paper of this series of studies in the metabolism of aluminium, prompted us to re-investigate the question of absorption of aluminium in man.

In this study it was desired to determine the following points:

1. Is aluminium present in the blood in normal man?
2. Is aluminium absorbed from food containing it?
3. Is aluminium excreted in the urine?

Two experiments were performed. In the first fifteen, in the second twelve subjects were used. These subjects were healthy young people, students in the Yale School of Medicine. In the first experiment two subjects, nos. 12 and 13, and in the second experiment, subject 7, were young women. To all we extend thanks for their valuable coöperation in this study.

Methods. *The determination of aluminium in human blood.* It is desirable, in working with human beings to make all analyses with the smallest possible amount of blood. In order to adapt the method previously reported (3) to the determination of aluminium in human blood the following procedure was used. Ten cubic centimeters of blood were drawn from an arm vein. Two 5 cc. portions were measured into silica crucibles, dried, ashed and dissolved as usual. Five cubic centimeters of the ash solution was shaken out with ether and ammonium thiocyanate. Then 5 cc. of the iron-free solution were transferred to a 10 cc. volumetric flask, and 2.5 cc. of the glycerine-citric acid mixture, and 1 drop of 0.25 per cent mono-sodium alizarine sulphonate added. The pH was adjusted as previously described. Then 0.5 cc. of 0.25 per cent alizarine solution and 0.2 cc. concentrated ammonium hydroxide were added, the neck of the flask washed, and the volume made to about 9 cc. Flasks stood, stoppered, for 24 hours, when 0.5 cc. of glacial acetic acid was added, the volume made to 10 cc., mixed and compared at once in the colorimeter. Standards and blanks were prepared simultaneously. Standards contained 0.00025, 0.0005, 0.00075, 0.001, 0.002 mgm. aluminium.

This manipulation must be executed with the utmost care as colors are very delicate, and color differences small. Any quantity of aluminium found in the blanks was substracted from that in the unknown. All determinations were done in duplicate.

Determination of aluminium in urine. In the first experiment 10 cc. portions, in the second 25 cc. portions of urine were dried, ashed and dissolved. Usually the determination was made directly on 5 cc. of the ash solution without previously shaking out with ammonium thiocyanate and ether. When the urine was found to contain enough iron to interfere with the color iron was removed as usual.

Preparation of the aluminium-containing biscuit. The aluminium containing biscuit used in these experiments was prepared with a well known brand of "alum-phosphate" baking powder, the composition of which is given in a previous paper (2). In the first experiment the biscuits were made according to the directions given on the can, and contained the following:

	grams
Flour	946
Baking powder	30
Salt	10
Lard	40
Water q.s.	

The biscuits in the second experiment were made according to a recipe in a widely used cook book (4) and contained:

	grams
Flour	4034
Baking powder	445
Salt	95
Lard	1000
Water q.s.	

When griddle cakes were made, the dry mixture used was the same, but more fluid was added.

The manipulation was carried out as is usual in household cooking.

The first experiment. Fifteen subjects reported at the laboratory at 9 a.m. after fasting since 7 p.m. of the previous evening. A blood sample, 10 cc., was drawn from the median basilic vein. A urine sample was collected in a paraffin coated bottle. These samples were considered normal with respect to contained aluminium. Ten subjects were given aluminium biscuit, five yeast bread.

The meal was given after all the samples had been collected. The meal consisted of bread or biscuit, butter, boiled ham, distilled water.

Each subject ate as much as he desired, the weight of bread or biscuit being recorded. Time was counted from the minute the meal was finished. The second blood samples were taken from each of three subjects at the end of the first, second, fourth, sixth and eighth hour. Urine samples were collected from all at the end of the second, fourth, sixth and eighth hour. No food was allowed during this period, but distilled water was allowed *ad libitum*.

The second experiment. Twelve subjects reported at the laboratory at 9 p.m., having fasted since noon. A blood and urine sample was collected from each. At 10 p.m. a meal consisting of roast beef, gravy, biscuit or bread, and butter was served. Each subject ate as much as he wished, weights of biscuit or bread being recorded.

The subjects reported at the laboratory at 8 a.m. the following morning, having collected the night urine in paraffined bottles. Breakfast, consisting of griddle cakes, sugar syrup, butter and coffee was served. Controls ate yeast bread or toast. Urine samples were collected during the day at 10 a.m., 2, 6 and 10 p.m. Two blood samples were taken from each subject at intervals of eight hours as is shown in table 3. Time was counted from the time of the meal the previous evening.

Lunch consisted of alum biscuit, or yeast bread, butter, boiled ham and coffee. Supper consisted of cold roast beef, boiled ham, alum biscuit or yeast bread, and butter. No other food was eaten during the experimental period. Cream and sugar were allowed in coffee. The coffee was made with distilled water and found to contain only a trace of aluminium.

RESULTS. *The blood.* The tables show the results of these experiments. It will be seen that aluminium often occurs in the blood of normal man. The amount present is small, the highest value found being 0.210 mgm. per 100 cc. (subject 1, first experiment). The amount is usually much less than this, the average being 0.060 mgm. for the first and 0.059 mgm. for the second experiment. When no value is given for the aluminium of the blood it means that the amount present is less than 0.001 mgm. per 100 cc. In the first experiment three subjects showed no detectable aluminium in the blood; in the second, two. Traces, that is amounts too small to estimate accurately, were shown by two in the first, by four in the second.

But comparison of tables 1 and 3 shows that the aluminium content of the normal blood of the same person may vary from a non-detectable to an appreciable amount. Below the results for the two experiments are tabulated.

SUBJECT	FIRST EXPERIMENT	SECOND EXPERIMENT
LaV.	0.124	Trace
E. T.	Trace	0.108
K.	0.000	0.104
P.	0.124	Trace
W.	0.096	0.000
R.	Trace	0.110
Subjects showing less change are:		
B.	0.210	0.167
J.	0.102	0.164
C.	0.016	Trace
G.	0.000	0.056
H.	0.053	0.000
R.T.	0.026	Trace

In the first experiment after the ingestion of aluminium-rich food the aluminium content of the blood was found to be definitely increased in two subjects, slightly in two others. In the first two the increase was over 100 per cent in each case. This increase has no apparent relation to the amount of aluminium-containing food ingested or the time after the meal.

In the second experiment definite increases in the blood are found in only two cases, the increase appearing 12 to 14 hours after the ingestion of large amounts of aluminium-containing food. One of these subjects (LaV.) did not show an increase in the first experiment. In all the others, including control subjects, the tendency was for any aluminium in the blood to decrease during the course of the experiment.

TABLE 1

The aluminium content of blood and urine after a single ingestion of aluminium containing food

HOUR	Al/100 cc. BLOOD	URINE VOLUME	Al/100 cc. URINE	TOTAL Al URINE	BISCUIT	Al/100 cc. BLOOD	URINE VOLUME	Al/100 cc. URINE	TOTAL Al URINE	BISCUIT	Al/100 cc. BLOOD	URINE VOLUME	Al/100 cc. URINE	TOTAL Al URINE	BREAD (YEAST)
	Subject 1 (B. wt. 160 pounds)					Subject 2 (LaV. wt. 155 pounds)					Subject 8 Control (E. T. wt. 146 pounds)				
		cc.		mgm.	grams		cc.		mgm.	grams		cc.		mgm.	grams
0	0.210	25	0.075	0.019	179.5	0.124	7.5	0.000	0.000	340	Trace	34	0.000	0.000	181
1	0.200					0.102					Trace				
2		37	0.000	0.000			47	0.000	0.000			65	0.000	0.000	
4		100	0.065	0.065			74	0.000	0.000			97	0.000	0.000	
6		67	0.072	0.048			170	0.000	0.000			170	0.000	0.000	
8		42	0.074	0.031			113	Trace	Trace			65	0.000	0.000	
				0.163					Trace					0.000	
	Subject 4 (K. wt. 155 pounds)					Subject 5 (W. wt. 145 pounds)					Subject 3 Control (J. wt. 138 pounds)				
0	0.000	37	0.098	0.036	213	0.096	55	0.050	0.025	141	0.102	15	0.103	0.015	170
2	0.000	41	0.000	0.000		0.204	59	0.049	0.029		0.094	24	0.096	0.023	
4		64	0.000	0.000			36	0.088	0.032			83	0.100	0.083	
6		62	0.102	0.063			63	0.080	0.050			87	0.051	0.045	
8		98	0.099	0.098			65	0.051	0.033			61	0.052	0.031	
				0.197					0.169					0.197	
	Subject 7 (C. wt. 135 pounds)					Subject 9 (R. T. wt. 147 pounds)					Subject 6 Control (H. wt. 199 pounds)				
0	0.016	16	0.000	0.000	235	0.026	148	0.078	0.038	137	0.053	19	0.053	0.010	227
2		45	Trace	Trace			190	0.078	0.148			47	0.053	0.025	
4	0.008	55	0.000	0.000		0.056	167	Trace	Trace		Trace	69	0.001	0.001	
6		60	0.098	0.059			108	Trace	0.000			77	0.000	0.000	
8		53	0.096	0.051			67	0.000	0.000			67	0.000	0.000	
				0.110					0.186					0.036	

	Subject 10 (N. wt. 215 pounds)					Subject 11 (G. wt. 138 pounds)					Subject 15 Control (R. wt. 137 pounds)				
0	0.032	170	0.000	0.000	255	0.000	67	0.000	0.000	205	Trace	25	0.024	0.006	152
2		100	0.000	0.000			48	0.088	0.043			70	0.016	0.011	
4		145	0.053	0.077			50	0.041	0.021			155	0.016	0.024	
6	0.026	160	0.000	0.000		Trace	156	0.049	0.077		0.000	135	0.016	0.022	
8		105	0.000	0.000			139	0.030	0.042			150	0.040	0.060	
				0.077					0.183					0.123	
	Subject 13 (P. wt. 110 pounds)					Subject 14 (M. wt. 150 pounds)					Subject 12 Control (L. wt. 119 pounds)				
0	0.124	158	0.021	0.030	166	0.120	187	0.050	0.094	128	0.000	185	0.048	0.089	
2		86	0.020	0.018			51	0.060	0.030			34	0.000	0.000	
4		150	0.020	0.030			52	0.053	0.028			102	0.000	0.000	
6		70	0.026	0.018			39	0.060	0.023			50	0.024	0.012	
8	0.095	42	0.019	0.008		0.140	24	0.047	0.011		0.000	45	0.032	0.011	
				0.104					0.186					0.112	

There is here some evidence that greatest absorption of aluminium occurs in the later hours after the ingestion of food to which aluminium has been added. Dogs (2) also show this delay. Does this mean that aluminium is not so easily absorbed from the upper part of the intestinal tract as from a lower part?

Where an increase of aluminium in the blood occurs, after the ingestion of aluminium-containing foods, we may be sure that this will be excreted later, in part at least, by the kidney.

The urine. The urine of normal subjects often contains aluminium. In the first experiment the normal urine of ten subjects showed aluminium,

TABLE 2

Summary—Averages—First experiment

TIME	BLOOD Al/100 cc.		URINE Al/100 cc.	
	Experimental subjects	Controls	Experimental subjects	Controls
Before meal	0.075	0.031	0.037	0.046
After meal	0.082	0.019		
2 hours			0.028	0.033
4 hours			0.033	0.023
6 hours			0.047	0.018
8 hours			0.042	0.025

	mgm.
Average for blood of all subjects before meal	0.060
Average for blood of all subjects after meal	0.062
Average for urine of all subjects, before meal	0.040
Average for total aluminium in urine, experimental subjects	0.137
Average for total aluminium in urine, control subjects	0.096

the average for all being 0.040 mgm. per 100 cc., a value two-thirds that of the blood value. After the ingestion of an aluminium-rich meal, the urine may be aluminium-free for a number of hours then aluminium may appear again, illustrated by subjects 1, 4, 7, 10 and 15. Or the rate of excretion of aluminium, as judged by the amount per 100 cc. for 2-hour periods may change very little (subjects 13 and 14), or the rate of excretion may steadily decrease (subject 9), or an initial rise may appear, followed by a progressive decrease (subject 11). The aluminium content of the urine of one control subject rose slightly (subject 15) but not above the preliminary level.

The difference in the amount of aluminium excreted by the experimental and control subjects in the first experiment is not great, as is shown in

table 2. The average total excretion for those ingesting aluminium is slightly higher than for the others.

In the second experiment the average aluminium content of normal urine was somewhat less than in the first, two subjects showing no aluminium, another only a trace.

The rate of excretion of aluminium was found to increase at some interval of the experimental period in subjects 1, 2, 4, 5, 7, 11 and 12, the latter a control subject. In subject 8, who showed a decided increase in the blood content, the amount of aluminium per 100 cc. of urine decreased during the experiment. Subject 10, after aluminium had been absent for a number of hours, showed a small amount. In subject 12 (control), the gain in the urine is partly accounted for by the loss from the blood. Subject 3 (control) showed no decrease in the amount of aluminium in the blood during the experimental period, and no aluminium appeared in the urine.

The total aluminium excretion for 24 hours was in no case over 0.5 mgm. and in one subject no aluminium, in another only a trace was excreted during the entire period. The total excretion of aluminium is decidedly higher for those who ingested aluminium-rich food than for corresponding controls.

From results for the urine we are led to believe that even though aluminium is often not detected in the blood it must at times be present since the urine shows definite amounts of aluminium. That the urine often shows a definite increase after the ingestion of aluminized foods points to absorption even though the increase in the blood is not always detected. While we know the condition of the blood only at the moment the sample is taken it is unlikely that large fluctuations will occur in the intervals.

The differences between the results for man and dog may be attributed to the differences in physico-chemical balance in the two different organisms, the human being a much more sensitively adjusted mechanism than the dog. It is to be inferred that the tendency of the body to keep a delicate and constant balance among the various inorganic ions cannot be easily disturbed by the ingestion of relatively large amounts of any one ion. If one may draw an analogy from experiments with the dog, one method of maintenance of this balance is by elimination of aluminium through the intestine (see paper IV).

TABLE 3

The aluminium content of blood and urine after the ingestion of aluminium containing foods

TIME	HOUR	Al/100 cc. BLOOD	URINE VOLUME	Al/100 cc. URINE	TOTAL Al URINE	BISCUIT	Al/100 cc. BLOOD	URINE VOLUME	Al/100 cc. URINE	TOTAL Al URINE	BISCUIT	Al/100 cc. BLOOD	URINE VOLUME	Al/100 cc. URINE	TOTAL Al URINE	BREAD (YEAST)
		Subject 1 (K. wt. 155 pounds)					Subject 2 (W. wt. 145 pounds)					Subject 3 Control (R. wt. 137 pounds)				
			cc.		mgm.	grams		cc.		mgm.	grams		cc.		mgm.	grams
Sat. 10 p.m.	0	0.104	240	0.020	0.028	162	0.000	143	0.011	0.016	62	0.110	80	0.000		70
Sun. 8 a.m.	10	0.105	210	0.069	0.153	260	0.000	195	0.021	0.051	260	0.111	135	0.000		106
10 a.m.	12		58	0.059	0.034			50	0.051	0.025			90	0.000		
12 m.	14					100					114		150	0.000		75
2 p.m.	16		190	0.044	0.084			140	0.042	0.058			50	0.000		
4 p.m.	18	0.096					0.000					0.111				
6 p.m.	20		220	0.059	0.130	65		100	0.042	0.042	42		375	0.000		62
8 p.m.	22												140	0.000		
10 p.m.	24		115	0.048	0.055			90	0.042	0.038			125	0.000		
					0.484	587				0.230	478			0.000		313
		Subject 4 (C. wt. 135 pounds)					Subject 5 (R. T. wt. 147 pounds)					Subject 6 Control (H. wt. 195 pounds)				
Sat. 10 p.m.	0	Trace	160	0.000	0.000	134	Trace	50	0.026	0.013	127	0.000	187	0.014	0.027	165
Sun. 8 a.m.	10		137	0.019	0.026	130		163	0.041	0.067	260		239	0.000	0.000	205
10 a.m.	12	Trace	58	0.019	0.011		Trace	70	0.032	0.022		0.000	66	0.000	0.000	
12 m.	14					150					96					113
2 p.m.	16		112	0.000	0.000			378	0.025	0.094			173	0.000	0.000	
4 p.m.	18															
6 p.m.	20	Trace	160	0.000	0.000	97	Trace	287	0.029	0.084	69	0.000	177	0.020	0.035	110
8 p.m.	22															
10 p.m.	24		95	0.000	0.000			113	0.025	0.028			131	0.000	0.000	
					0.037	511				0.308	552				0.062	593

		Subject 7 (P. wt. 110 pounds)					Subject 8 (G. wt. 138 pounds)					Subject 9 Control (E. T. wt. 146 pounds)				
Sat. 10 p.m.	0	Trace	39	0.019	0.007	119	0.056	338	0.029	0.099	176	0.108	95	Trace		116
Sun. 8 a.m.	10		265	0.030	0.079	152		75	0.030	0.022	400		199	Trace		108
10 a.m.	12		200	0.010	0.020			60	0.029	0.017			70	Trace		
12 m.	14	Trace				102	0.056				73	0.106				116
2 p.m.	16		652	0.024	0.152			175	0.009	0.016			257	Trace		
4 p.m.	18															
6 p.m.	20		572	0.029	0.168	71		223	0.010	0.022	106		210	Trace		70
8 p.m.	22	Trace					0.153					0.050				
10 p.m.	24		447	0.009	0.040			118	0.011	0.013			130	Trace		
					0.466	444				0.189	755				Trace	410
		Subject 10 (LaV. wt. 155 pounds)					Subject 11 (B. wt. 160 pounds)					Subject 12 Control (J. wt. 138 pounds)				
Sat. 10 p.m.	0	Trace	120	0.029	0.035	186	0.167	58	0.019	0.010	120	0.164	92	0.019	0.018	240
Sun. 8 a.m.	10		750	0.000	0.000	340		160	0.078	0.125	260		240	0.000	0.000	95
10 p.m.	12		41	0.000	0.000			60	0.009	0.006			61	0.020	0.012	
12 m.	14					183					143					108
2 p.m.	16	Trace	102	0.000	0.000		0.111	235	Trace	Trace		0.106	146	0.058	0.085	
4 p.m.	18															
6 p.m.	20		220	0.019	0.042	86		210	Trace	Trace	90		174	0.057	0.100	67
8 p.m.	22															
10 p.m.	24	0.086	165	0.020	0.032		0.108	165	0.020	0.033		0.109	102	0.057	0.058	
					0.109	795				0.174	613				0.273	510

TABLE 4

Summary—Averages—Second experiment

TIME	BLOOD Al 100 cc.		URINE Al 100 cc.	
	Experimental subjects	Controls	Experimental subjects	Controls
Normal	0.041	0.095	0.019	0.008
First blood sample	0.034	0.081		
Second blood sample	0.055	0.068		
10 hours			0.036	Trace
12 hours			0.026	0.005
16 hours			0.018	0.014
20 hours			0.025	0.019
24 hours			0.021	0.014

	mgm.
Average, blood of all subjects before experiment	0.059
Average, urine of all subjects before experiment	0.016
Average, total urine excretion, experimental subjects	0.250
Average, total urine excretion, control subjects	0.084
Average, 24 hour excretion, experimental subjects	0.024
Average, 24 hour excretion, control subjects	0.073

CONCLUSIONS

1. Aluminium may or may not occur in the blood of normal man. If it does occur the amount is not large, 0.21 mgm. per 100 cc. being the maximum found.

2. The aluminium content of the blood of the same normal person may vary from time to time between narrow limits.

3. The aluminium content of the blood may show definite increase after ingestion of aluminium-rich food. More often an increase is not detected.

4. The control subjects fed on food presumably poor in aluminium never showed an increased content of aluminium in the blood. On the contrary, the blood aluminium content usually decreased under these conditions.

5. The urine of normal man may or may not contain small amounts of aluminium.

6. The aluminium in the urine tends to increase after the ingestion of aluminium-rich foods.

7. Even after the ingestion of large amounts of aluminium-rich food the total urinary excretion for 24 hours does not exceed 0.5 mgm. aluminium.

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STUDIES IN THE METABOLISM OF ALUMINIUM

VIII. A Note on the Toxic Effects Produced by Subcutaneous Injection of Aluminium Salts[1]

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Pathological changes in animal tissues after the administration of large doses of aluminium salts have been described. Siem (1) using rabbits, cats and dogs, found the lethal dose of sodium aluminium tartrate to be 0.25 to 0.30 gram per kilo, expressed as aluminium oxide (equal to 1.72 to 2.07 grams of the salt). Even larger doses were given, death resulting in 7 to 31 days. The material was given subcutaneously in divided doses over a period of 2 to 4 weeks. How much the tartrate contributed to the effects observed we are unable to say, but it is now well known that the amount of tartaric acid present is sufficient to cause severe toxic symptoms (2) in the animals employed.

Döllken (3) described degenerative changes in the central nervous system after injections of the same material. Leary and Sheib (4) after feeding puppies 100 to 500 mgm. and rats 500 to 3000 mgm. of aluminium daily as aluminium hydroxide, found a slight disturbance of growth in the later periods for those receiving very large doses. Rats examined after 16 weeks showed no macroscopic lesions.

Roth and Voegtlin (5) fed aluminium tartrate and sodium aluminium lactate to rabbits, cats and dogs. Rabbits fed 20 mgm. of aluminium per day died in 2 months, cats fed 40 to 80 mgm. per day died in 3 to 17 days, dogs fed 20 mgm. per day died in 4 months. Symptoms noted were diarrhea (in cats, alternating with constipation), loss of appetite and weight, and albuminuria. Changes noted varied from congestion in the gastrointestinal tract to corrosion and hemorrhagic areas, as well as congestion of liver, kidney and spleen.

Seibert and Wells (6) found pathological changes in the blood elements after the intravenous injection of aluminium salts. The changes noted

[1] Constructed from a thesis presented by Anthony Sperandeo to the Yale School of Medicine in 1925 in partial fulfillment of the requirements for the degree of Doctor of Medicine.

resemble those found in lead poisoning. The spleen was also the site of pathological effects.

Since 1843 a half dozen cases of acute poisoning in man have been reported. All of these occurred after a large single dose of an aluminium salt, usually an alum. Symptoms described are those generally observed after large doses of metallic salts; i.e., frequent vomiting, acceleration of the pulse, tremor, followed by weakness, depression and sometimes coma and death.

The toxicology of aluminium in relation to plants does not concern us here. In general, small doses stimulate while large doses depress growth. The effect of aluminium salts on plants has been extensively studied by Stoklasa (7).

The present study was designed to determine the pathological effects of aluminium salts, uncomplicated by the influence of an organic radical. It is not proposed to detail the toxic symptoms which have been described sufficiently by others.

Rats, guinea pigs and rabbits were used. They were carefully selected from apparently healthy stock and given all care necessary to avoid factors which might complicate the result.

The chloride and sulfate of aluminium in 20 per cent solution in distilled water were used. Injections were made into the subcutaneous tissue of the thigh. Rats were given a single injection, while guinea pigs and rabbits were given divided doses beginning with 3 to 5 grams per kilo as the initial dose, following with 1 gram on alternating days. Doses are expressed as grams of the salt used.

When animals died autopsies were performed as soon as possible. Tissues to be examined were preserved in formaldehyde, and sections were stained with hemotoxylin and eosin. Tissues from 2 rats, 5 guinea pigs and 5 rabbits were examined microscopically.

Dr. H. Gideon Wells has examined the sections, and his description is included. We are greatly indebted to Doctor Wells for his interest and coöperation in this problem.

Results. The single lethal dose for rats is found to be 7 to 8 grams of aluminium chloride per kilo. This dose produces death in 1 to 3 days. The lethal dose for guinea pigs was 5 to 7 grams per kilo, when the initial dose was 3 grams followed by 1 gram on alternating days. This dose produces death in 3 to 7 days. The lethal dose of either the chloride or sulfate in rabbits is 7 to 8 grams per kilo, when the initial dose is 4 to 5 grams followed by 1 gram on alternating days. This dose produces death in 7 to 11 days (see table 1).

The symptoms produced in the three species were similar. When small doses were given, no symptoms were noticed. During the hours immediately following the injection of a large dose there was no deviation from

normal behavior. Loss of appetite was the first symptom noted. Animals soon showed inactivity and depression. They lay quietly in their cages, manifesting no interest in their surroundings. If forced to move they were slow and clumsy.

The gross post mortem examination revealed marked congestion in all the viscera, especially in the mesenteric vessels. The stomach was greatly distended, the mucosa somewhat swollen and containing a few pin point to pinhead sized hemorrhages. In two guinea pigs and one rabbit the intestinal mucosa contained a few small greyish superficial ulcers, but generally the intestinal tract was free of pathological changes. Intestinal contents were greenish, and contained no blood or mucus.

TABLE 1

Showing dosages and duration of life of animals receiving $AlCl_3$ and $Al_2(SO_4)_3$ subcutaneously

Doses as grams of salt per kilo body weight

ANIMAL	NUMBER	INITIAL DOSE		SUBSEQUENT DOSE	TOTAL	DEATH
		$AlCl_3$	$Al_2(SO_4)_3$			
		grams			*grams*	
Rat	1	7		None	7	3rd day
	2	8		None	8	2nd day
Guinea pig	1	3		1 gram alternate days	5	7th day
	2	3		1 gram alternate days	6	5th day
	3	3		1 gram alternate days	5	8th day
	4	5		1 gram alternate days	7	4th day
	5	3		1 gram alternate days	7	6th day
Rabbit	1	5		1 gram 8th and 10th day	7	10th day
	2	4		1 gram 3rd, 5th, and 8th day	7	8th day
	3		4	1 gram 3rd, 5th, 7th and 9th day	8	10th day
	4		4	1 gram 3rd, 5th, 7th day	7	8th day
	5		4	1 gram 3rd, 5th, 7th and 10th day	8	11th day

Livers of rats and guinea pigs were of a deep red color, soft and friable. Livers of rabbits showed extensive tissue change, consisting of congestion and greyish white, sharply defined areas of discoloration, involving the peripheral portion of the lobes and suggesting necrosis. The gall bladder, except for moderate distention, appeared normal.

The kidneys were pale and soft, but showed no gross evidence of parenchymatous change. The renal capsule could be stripped with ease. The lung and spleen shared in the general congestion. The brain showed no macroscopic change except for a pale color.

The microscopic examination by Doctor Wells is appended.

PATHOLOGICAL REPORT BY DOCTOR WELLS

Rat 1

Liver. Disintegrated along one side, appearance suggesting post mortem digestion from contact with stomach. Elsewhere liver shows numerous foci of necrosis, mostly central. A large vein contains many leucocytes, resembling an infected thrombus, but there is much post mortem change.

Kidney. Much disintegration of the convoluted epithelium, but of so diffuse a character as to suggest that it is at least partly post mortem.

Heart. Shows no definite changes, although there are alterations suggestive of post mortem change.

Brain. No definite changes recognized in the brain.

Rat 2

Liver. Well preserved. Shows no definitely abnormal conditions.

Kidney. Epithelium of convoluted tubules shows numerous areas of necrosis, much vacuolization, many granular casts, and on the whole, marked evidence of acute toxic injury. No changes in glomerules.

Spleen. Contains much blood, but not an abnormal amount. No definitely pathological changes seen.

Lung. Larger bronchi show extensive round cell infiltration of a chronic bronchitis, but the smaller bronchi are free from this change.

Heart. No recognizable changes.

Stomach. Seems normal.

Guinea pig 2

Liver. No distinct focal necrosis, but a few small groups of cells stain intensely with eosin, and there is a great variability in the staining of the liver cells throughout the section.

Kidney. Epithelium of convoluted tubules is generally much swollen. In some there is necrosis and large hyaline casts are numerous, more so than in any of the rabbit kidneys.

Stomach. No changes.

Intestine. No changes.

Guinea pig 3

Liver. Numerous vacuoles suggesting fatty infiltration to a moderate degree. Epithelium swollen, but no definite necrosis.

Kidney. Epithelium of convoluted tubules shows much vacuolization, with occasional granular casts, and occasional areas of calcification in the tubules.

Spleen. Small Malpighian bodies, atrophic pulp, distinctly fibrotic, containing many cells full of brown pigment.

Stomach. Mucous membrane shows numerous areas of necrosis which are sharply limited, and stain brownish as if with blood pigment. There is no inflammatory reaction about them.

Guinea pig 4

Liver. No noticeable changes.

Kidney. Considerable necrosis of the epithelium. Numerous mitotic figures are seen in the renal epithelium. There are occasional granular, but few hyaline casts.

Spleen. Engorged with blood but not abnormally so. No definite pathological changes.

Stomach. No changes.

Guinea pig 5

Liver. Post mortem digestion on one side. No recognizable changes in other portions.

Kidney. No definite changes.

Spleen. Atrophic; pulp, slightly fibrotic, but without pigmentation.

Intestine. Post mortem changes only.

Stomach. Changes seen, apparently post mortem only.

Pancreas. Normal.

Guinea pig 6

Liver. Slight vacuolization and swelling of epithelium but no other changes except post mortem digestion on one side.

Kidney. No distinct pathological changes except slight spontaneous nephritis.

Spleen. No change.

Intestine. Post mortem changes only.

Rabbit 1

Liver. Extensive typical focal necrosis, chiefly in centers of lobules, not all of which are involved. One large hepatic vein surrounded by necrotic tissue contains a thrombus, which does not fill it completely. No inflammatory reaction.

Kidney. Epithelium of some of convoluted tubules is vacuolated; the lumen of nearly all the tubules is obliterated by swelling of the epithelium. There are distinct areas of necrosis in the renal epithelium, but no casts. The glomerular tufts are swollen, engorged, and contain what resemble hyaline thrombi.

Skin. Shows large area of necrosis with edema, without inflammatory reaction. One section shows a necrotic border, beneath which is a growth of young connective tissue. There are also areas of necrosis in the deeper tissue involving especially the muscles.

Stomach. No changes.

Intestine. No changes.

Rabbit 2

Liver. Shows several large areas of focal necrosis, distinctly central, but the greater part of the liver is free from change.

Kidney. Much less change than no. 1, although there is some swelling and vacuolization in the epithelium of the convoluted tubules. The glomerular tufts are not so much swollen but in some places they show a protein exudate between the tuft and the capsule. Many of the glomerular capillaries contain what seem to be hyaline thrombi.

A section of the alimentary tract which is somewhat disintegrated and cut tangentially, so that it cannot be identified, seems to be highly edematous.

Rabbit 3

Liver. No section present.

Kidney. Epithelium of convoluted tubules badly injured; in some tubules there is severe coagulation necrosis, with and without vacuolization of the cells. Many tubules have lost the epithelium completely, and none of the tubules of any kind are normal; even the collecting tubules show casts and free epithelial cells in their lumen. The glomerules are not swollen as in rabbits no. 1 and no. 2, and some of them show shrinkage of the tufts. Neither are hyaline thrombi present. There are no interstitial changes. The necrosis resembles in severity that seen in tartrate kidney.

Stomach. Mucous membrane seems to be somewhat edematous but unless this was removed from the animal immediately after death the change may be artefact. However there is distinct edema between the muscularis mucosa and the circular muscle.

Intestine. Duodenum shows no changes that can be recognized as ante-mortem.

Pancreas. Same as intestine, as to changes.

Rabbit 4

Liver. No necrosis, but the cells in the periphery of each lobule are much larger and paler than those in the center. Can this represent a stage of regeneration after central necrosis? One section shows a coccidia abscess. Fat stain; a few lobules show a small collection of fat in small granules, partly, apparently in the liver cells, and partly in the Kupffer cells.

Kidney. The changes here are less marked than in the three preceding, the alterations being limited to a slight amount of protein precipitate in some of the tubules; swelling with and without vacuolization in part of the convoluted tubules; a few small areas of epithelial necrosis and an occasional small scar of a hemorrhagic nephritis.

Rabbit 5

Kidney. The convoluted tubules show some necrosis; many contain low flattened epithelium, and some are distended with coagulated protein, which in the collecting tubules takes the form of casts. The appearances here suggest a somewhat older process than in no. 1 and no. 2. The glomerules seem to be little affected.

Discussion. The lethal dose of aluminium chloride and aluminium sulfate for rats, guinea pigs and rabbits, varied from 5 to 8 grams per kilo. This dose, given subcutaneously, produced death in 2 to 11 days.

In the pathological results the outstanding features are injury to the liver and kidney, although probably the spleen shares in this, since the spleens in the rats showed congestion with paucity of lymphoid tissue and increased pigmentation. That liver and kidney damage is often excessive is shown by the description of the sections. The principal site of damage in the kidney seems to be the convoluted tubule, although the glomerular tufts are sometimes swollen. In the liver central necrosis and sometimes fatty infiltration are noted.

The findings described here correspond closely with those of Siem (1). He found a slight congestion in the gastro-intestinal tract as well as a finely granular fatty degeneration in the liver. The kidneys showed

congestion, fatty degeneration and sometimes blood extravasations. There were well filled vessels in the glomeruli and degeneration of the epithelium of the descending ends of the convoluted tubules. The lumens were filled with hyaline casts.

SUMMARY

The pathological results of the subcutaneous injection of lethal doses of aluminium chloride and aluminium sulfate, in rats, guinea pigs and rabbits, are described.

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STUDIES IN THE METABOLISM OF ALUMINIUM

VII. The Aluminium Content of Some Fresh Foods

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The aluminium content of foods has received attention from a number of investigators. Langworthy and Austin (1) published extensive analyses of foodstuffs and natural waters. Kratzman (2) found aluminium distributed in many plants. The work of Stoklasa (3) and his associates is summarized in a recent book. They found aluminium in many plants, but they were more interested in its relation to botanical grouping and function of the parts analyzed than in the amount in the edible portions. Myers and Voegtlin (4) reported that a large percentage of the aluminium found in grains and a few root vegetables was water soluble. Gonnerman (5) reports analyses of various breads and brans. Wolff et al. (6) report a few analyses of eggs.

Knowing that small amounts of aluminium may escape detection by the usual methods of analysis we wished to determine the aluminium content of fresh foods by means of the very sensitive method already described (7).

The materials were purchased in various local markets. They were of the best quality obtainable at the particular season. Usually vegetables and fruits were quite fresh and crisp. They were carefully cleaned, peeled when necessary, dried at 105° and ashed as previously described. The edible portion only was used. Meats were fresh.

Because of the possibility of wide variation in moisture content four analyses were usually carried out on each lot of material. The average figures are given. Variation was usually not large. Results are reported for aluminium in milligrams per 100 grams of fresh edible material.

Results. The tables show the results obtained. It is found that of all the materials analyzed cherries and onions contain by far the largest amount of aluminium. Further, both the Connecticut sour and California sweet cherries contain a large amount of aluminium. The three different lots of onions showed a high aluminium content.

The foods containing between 1 and 2 mgm. of aluminium per 100 grams are lettuce, milk, flour and liver. It will be noted that liver from the calf

TABLE 1

The aluminium content of fresh foods

MATERIAL	DESCRIPTION	NUMBER OF SOURCES	Al/100 GRAMS FRESH	AVERAGE	REMARKS
Beans, string	Varying degrees of ripeness	3	0.36 0.61 0.91	0.63	
Beets	Peeled	3	0.34 0.48 0.61	0.48	
Corn	Sweet Cut from cob	2	0.25 0.26	0.26	
Lettuce	Head	3	2.50 0.58 0.46	1.18	1st sample very young
Onions	Peeled	3	4.60 3.76 4.59	4.31	
Peas	Shelled	3	0.28 0.32 0.33	0.31	
Potatoes	Peeled	3	0.38 0.32 2.20	0.97	1st sample Florida 2nd sample Connecticut 3rd sample unknown
Apples	Variety unknown Duchess	1 1	0.015 0.08	0.047	
Cantaloupe	Edible portion	2	0.40 1.14	0.77	
Cherries	Pitted	2	3.18 3.80	3.49	Native sour California sweet
Oranges	Peeled	1	0.088	0.088	California seedless
Peaches	Peeled and stoned	2	0.93 0.83	0.88	Free stone Cling stone
Watermelon	Ripe heart	2	0.027 0.028	0.028	

TABLE 1—*Concluded*

MATERIAL	DESCRIPTION	NUMBER OF SOURCES	Al/100 GRAMS FRESH	AVERAGE	REMARKS
Eggs	Without shell	2	0.015		White shelled
			0.019	0.017	Brown shelled
Flour		3	2.30		"Gold Medal"
			1.62		"Pastry"
			1.16	1.69	"Mother's Best"
Liver	Calf	2	1.78		
			1.69	1.73	
Liver	Pig	3	2.07		
			1.70		
			1.54	1.77	
Beef		1	0.50		
Pork		1	0.44		
Mutton		1	0.43		
Milk	Whole	3	1.39		
			1.53		
			1.71	1.54	

TABLE 2

Summary—average aluminium content of foods

Al as milligrams per 100 grams

SUBSTANCE	Al/100 GRAMS	SUBSTANCE	Al/100 GRAMS
Apples	0.047	Liver—pork	1.770
Beans—string	0.630	Mutton	0.430
Beef	0.500	Milk	1.540
Beets	0.480	Onions	4.310
Cantaloupe	0.770	Oranges	0.088
Cherries	3.490	Peaches	0.880
Corn—sweet	0.260	Peas	0.310
Eggs	0.017	Pork	0.440
Flour	1.690	Potatoes	0.970
Lettuce—head	1.180	Watermelon	0.028
Liver—calf	1.730		

and from the pig have practically the same aluminium content. The materials having less than 1 mgm. but more than 0.2 per 100 grams are

string beans, beets, cantaloupe, sweet corn, peaches, pears, potatoes and meat (beef, pork, mutton). The lean meat from three animal sources has very nearly the same aluminium content. The lowest aluminium content was found in apples, eggs, oranges and watermelon, which had less than 0.1 mgm. per 100 grams.

In these plant products we can find no correlation between aluminium content and botanical group. If many more materials had been analyzed this might have been apparent since Stoklasa has found more aluminium in hydrophytic than mesophytic or xerophytic plants.

It is apparent that a diet containing liberal amounts of fresh fruits and vegetables may contain a significant quantity of aluminium. This would perhaps account for the aluminium found at times in the blood of normal man (8).

SUMMARY

Analyses showing the aluminium content of various fresh foods have been presented.

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THE EFFECTS OF PERORAL APPLICATION OF COMPOUNDS OF ALUMINUM ON THE METABOLISM OF GLYCIDES

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The observed unfavorable action of the increased intake of various derivatives of aluminum on the metabolism of phosphorus (Vozar, 1957; Krizek, 1957; Truffert, 1950) gave impetus to further studies especially to investigation of the possible effect of compounds of aluminum on glycide metabolism. In addition to this fact, the finding regarding the disruptive action of aluminum salt in relation to the absorption of glucose in the small intestine (Gisselbrecht et al.,1957) also led us to such a study. The mentioned studies also led us to the fact that we were unable to find in available literature data on the effect of aluminum on the metabolism of glycides.

Because of the amphoteric nature of aluminum we chose aluminum chloride and aluminum oxide for the experiments. This was done so that the total connection between the increased intake of aluminum and the metabolism of glycides would be in the forefront as much as possible. In this report we will assemble all the results obtained after investigating the reaction of application by mouth all at once of aluminum chloride and aluminum oxide on glycemia and the level of glycogen in the liver and skeletal musculature.

Method

The experiments were made in two stages on male rats of the

Wistar line. In the first stage we investigated the glycogen (liver, skeletal musculature) and glycemia in four groups of rats after 5 and 10 days of regular feeding of $AlCl_3.6\ H_2O$ in a daily dose of 15mg Al/100g of the total weight of the rats. In the second stage we investigated just like in the first stage the levels of the mentioned components of glycide metabolism. The difference was based only on this that a suspension of Al_2O_3 in a daily dose of 15 mg Al/100g of the weight of the rats was given to the experimental animals in the second stage. The aluminum compounds were given in a probe in a 1.5 ml of physiological solution. The animals in the control groups received an equivalent amount of the physiological solution.

There were 12 rats in each group. In the beginning of the experiment their weight fluctuated from 125 to 175 g. A total of 96 rats were used for the experiments.The rats were fed daily with Larzen's diet (Fabry, 1955) and as much drinking water as desired. Before the experiments were begun the animals were adapted to the experimental conditions. After the expiration of the appropriate length of the experimental period the rats were decapitated. Glycemia was determined in the blood from the cervical arteries and veins with the aid of a color reaction with an arsenomolybdenum reagent (Horejsi et al., 1957). Determination of glycogen in the liver and skeletal musculature was done by the antron method (Niederland et al., 1956).

Results

After application of aluminum chloride in the amount of 15mg Al/100g of the weight of the rats there was a definite rise in the level of glycogen in the liver and the skeletal musculature.

From the results listed in Table 1,it is evident that after 5 days of regular daily application of aluminum chloride the level of glycogen in the liver rises by 132.0% (P=0.001). In the same time segments a 5.2% (P=0.05) rise in the level of glycogen was also verified in the skeletal musculature. After a regular 10-day application of aluminum chloride a definite rise in the level of glycogen was verified only in the liver. In the experimental rats in comparison to the control rats the level of glycogen increased by 132.1% (P=0.001). After application of aluminum chloride in a time segment of 5 and 10 days no changes in the values of glycemia occurred.

After application of aluminum oxide in the amount of 15 mg Al/100g of the weight of the rats the recorded changes in the glycides had a different character than after application of aluminum chloride. From Table 2, it is evident that feeding a suspension of aluminum oxide in the course of 5 days will cause a decline in the values of glycogen and glycemia. Glycogen in the liver will decline by 82.1% (P=0.001), in the skeletal musculature by 61.3% (P=0.001) and the values of glycemia will decrease by 11.4% (P=0.05). Similar changes, even as to glycemia, were noted after 10 days of feeding aluminum oxide. After this time segment glycogen in the liver decreased by 63.9% (P=0.001) and in the skeletal musculature by 52.2% (P=0.001).

It is therefore evident from the results obtained that application by mouth of derivatives of aluminum is reflected in the metabolism of glycides. Aluminum chloride causes an accumulation of glycogen in the cellular tissues and aluminum oxide removal of

of glycogen from the cellular tissues. A more substantial significance cannot be attributed to the changes in glycemia because they fluctuate within the limits of the physiological norm. Changes in glycogen are conclusive because they are very easily identified.

Discussion

Examination of some of the components of glycide metabolism after application by mouth of aluminum compounds disclosed that aluminum compounds impair the sequence of biochemical processes in the metabolism of glycides. This is seen in the changes noted in the glycide reserves of the organism. It is noteworthy that the effect of the action in an aqueous medium of completely diluted aluminum chloride is different than the effect of the action of aluminum oxide undiluted in water. We see in these diverse properties of the aluminum compounds the source of the changes relating to the reserved glycides. We presume that the observed changes in the glycide metabolism have one common denominator -- a malfunction in the metabolism of forms of phosphorus. The mechanism of such action of aluminum compounds is very complex and very many specific studies will have to be made regarding its accountability. In agreement with research data (Gisselbrecht et al.,1957) we presuppose that aluminum chloride and aluminum oxide impair the metabolism of glycides by a blockade of the phosphorus, that is, phosphorylation processes. It can possibly be expected that because of the diverse character of aluminum chloride on the one side and aluminum oxide on the other the resulting effect, that is, changes in the reserved glycides will also be different. The results of our work clearly point out this fact. A study of

further parameters, especially of the metabolism of the labile enzymatic forms of phosphorus, will certainly provide valuable data to account for the mechanism of the action of aluminum compounds on the metabolism of glycides.

Conclusion

In the experiments on rats it was determined that application by mouth of aluminum compounds causes changes in the glycide reserves of the organism. After 5 and 10 days of regular application of aluminum chloride in the amount of 15mg Al/100g of the weight of the rats resulted in a rapid increase in the level of glycogen in the liver and skeletal musculature. After similar application of aluminum oxide the opposite effect of the action was recorded -- a decrease in the level of glycogen in the liver and the skeletal musculature. The values of glycemia did not leave the physiological area in a single case. Observed changes are presented in relation to the blockade of phosphorus, that is, phosphorylation processes, through the reaction between the applied aluminum and the phosphorus of the organism.

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Table 1

Level of glycogen and glycemia in rats of the control group and rats that were regularly fed $AlCl_3.H_2O$ in the amount of 15 mg Al/100 g of weight during a period of 5 and 10 days.

Period of feeding (days)	Group of rats	Of the number of rats	Glycogen liver g%	standard error	skeletal musculature g%	standard error	Glycemia mg%	standard error
1	2	3	4	5	6	7	8	9
5	control	12	1.306	0.137	0.413	0.063	110.07	5.43
	experimental	12	3.683	0.211	0.440	0.049	103.32	3.86
10	control	12	0.958	0.108	0.481	0.061	96.13	2.69
	experimental	12	2,224	0.405	0.583	0.032	98.26	3.72

Table 2

Level of glycogen and glycemia in rats of the control group and rats that were regularly fed Al_2O_3 in the amount of 15 mg Al/100 g of weight during a period of 5 and 10 days.

Period of feeding (days)	Group of rats	Of the number of rats	Glycogen				Glycemia	
			liver g%	standard error	skeletal muscula-ture	standard error	mg%	standard error
1	2	3	4	5	6	7	8	9
5	control	12	1.317	0.131	0.320	0.037	103.08	4.40
	exper-imental	12	0.237	0.023	0.124	0.017	91.43	1.61
10	control	12	0.975	0.086	0.251	0.019	101.07	3.31
	exper-imental	12	0.304	0.028	0.120	0.012	102.32	3.77

VPLYV PERORÁLNEJ APLIKÁCIE ZLÚČENÍN HLINÍKA NA METABOLIZMUS GLYCIDOV

Libor Vozár

Ústav pre výskum výživy ľudu v Bratislave

Pozorovaný nepriaznivý účinok zvýšeného príjmu rôznych derivátov hliníka na metabolizmus fosforu (Vozár, 1957; Křížek, 1957; Truffert, 1950) dal podnet pre ďalšie štúdiá, najmä pre výšetrovanie možného vplyvu zlúčenín hliníka na glycidový metabolizmus. Vedľa tejto skutočnosti nás k takémuto štúdiu viedol aj poznatok o brzdiacom účinku soli hliníka vo vzťahu k strebávaniu glukózy v tenkom čreve (Gisselbrecht a kol., 1957). Spomínané štúdie si vynútilo aj to, že sa nám v dostupnej literatúre nepodarilo nájsť údaje o vplyve hliníka na metabolizmus glycidov.

V dôsledku amfotérnej povahy hliníka sme volili pre experimenty chlorid a kysličník hlinitý. Bolo to preto, aby celková súvislosť medzi zvýšeným príjmom hliníka

a metabolizmom glycidov čo najviac vystúpila do popredia. V tomto oznámení zhrniene výsledky dosiahnuté pri vyšetrovaní odrazu per os aplikácie chloridu a kysličníka hlinitého na glykémiu a hladinu glykogénu v pečeni a kosternom svalstve.

Metodika

Experimenty sa robili v dvoch etapách na krysách samcoch z línie Wistar. V prvej etape sa vyšetroval na štyroch skupinách krýs glykogén (pečeň, kosterný sval) a glykémia po 5 a 10 dňoch pravidelného podávania $AlCl_3 . 6 H_2O$ v dennej dávke 15 mg Al/100 g celkovej váhy krýs. V druhej etape sme vyšetrovali práve tak ako v prvej etape hladiny spomínaných zložiek glycidového metabolizmu. Rozdiel spočíval len v tom, že pokusným zvieratám druhej etapy bola podávaná suspenzia Al_2O_3 v dennej dávke 15 mg Al/100 g váhy krýs. Zlúčeniny hliníka sa podávali sondou v 1,5 ml fyziologického roztoku. Zvieratá kontrolných skupín dostávali ekvivalentné množstvá fyziologického roztoku.

V každej skupine bolo 12 krýs, ktorých váha na začiatku experimentu kolísala od 125 do 175 g. Celkove sa pre pokusy použilo 96 krýs. Zvieratám sa denne podávala Larzenova diéta (Fábry, 1955) a pitná voda ad libitum. Pred zahájením experimentov sa zvieratá adaptovali na experimentálne podmienky. Po uplynutí príslušnej dĺžky experimentálneho obdobia boli krysy dekapitované. Glykémia sa stanovovala v krvi z krčných tepien a žíl pomocou farebnej reakcie s arzenomolybdénovým reagensom (Hořejší a kol., 1957). Stanovenie glykogénu v pečeni a kosternom svalstve sa robilo antrónovou metódou (Niederland a kol., 1956).

Výsledky

Po aplikácii chloridu hlinitého v množstve 15 mg Al/100 g váhy krýs dochádza k výraznému vzostupu hladiny glykogénu v pečeni a kosternom svalstve. Z výsledkov uvedených v tab. 1 vyplýva, že po 5 dňoch pravidelnej dennej aplikácie chloridu hli-

Tabuľka 1

Hladina glykogénu a glykémie u krýs kontrolnej skupiny a krýs, ktorým sa v období 5 a 10 dní pravidelne podával $AlCl_3 . 6 H_2O$ v množstve 15 mg Al/100 g váhy

Čas podávania (dní)	Skupina krýs	Z počtu krýs	Glykogén				Glykémia	
			pečeň g %	stredná chyba	kosterný sval g %	stredná chyba	mg %	stredná chyba
1	2	3	4	5	6	7	8	9
5	kontrolná	12	1,306	0,137	0,418	0,068	110,07	5,43
	pokusná	12	3,683	0,211	0,440	0,049	103,32	3,86
10	kontrolná	12	0,958	0,108	0,481	0,061	96,18	2,69
	pokusná	12	2,224	0,405	0,583	0,032	98,26	3,72

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ntého sa zvýši hladina glykogénu v pečeni o 182,0 % (P = 0,001). V tom istom časovom úseku sa zaznačilo aj 5,2 % (P = 0,05) zvýšenie hladiny glykogénu v kosternom svalstve. Po pravidelnej 10 dňovej aplikácii chloridu hlinitého bol výrazný vzostup hladiny glykogénu zaznačený len v pečeni. U pokusných krýs v porovnaní s kontrolnými sa hladina glykogénu zvýšila o 132,1 % (P = 0,001). Po aplikácii chloridu hlinitého v časovom úseku 5 a 10 dní nedošlo k zmenám v hodnotách glykémie.

Po aplikácii kysličníka hlinitého v množstve 15 mg Al/100 g váhy krýs mali zaznačené zmeny v glycidoch iný charakter ako po aplikácii chloridu hlinitého. Z tab. 2

Tabuľka 2

Hladina glykogénu a glykémie u krýs kontrolnej skupiny a krýs, ktorým sa v období 5 a 10 dní pravidelne podával Al_2O_3 v množstve 15 mg Al/100 g váhy

Čas podávania (dní)	Skupina krýs	Z počtu krýs	Glykogén: pečeň g %	stredná chyba	kosterný sval g %	stredná chyba	Glykémia: mg %	stredná chyba
1	2	3	4	5	6	7	8	9
5	kontrolná	12	1,317	0,181	0,320	0,037	103,08	4,40
	pokusná	12	0,237	0,023	0,124	0,017	91,43	1,61
10	kontrolná	12	0,975	0,086	0,251	0,019	101,07	3,31
	pokusná	12	0,304	0,028	0,120	0,012	102,32	3,77

vyplýva, že podávanie suspenzie kysličníka hlinitého v priebehu 5 dní spôsobí pokles hodnôt glykogénu a glykémie. Glykogén v pečeni poklesne o 82,1 % (P = 0,001), v kosternom svalstve o 61,3 % (P = 0,001) a hodnoty glykémie sa znížia o 11,4 % (P = 0,05). Obdobné zmeny, až na glykémiu, boli zaznačené po 10 dňoch podávania kysličníka hlinitého. Po tomto časovom úseku poklesol glykogén v pečeni o 68,9 % (P = 0,001) a v kosternom svalstve o 52,2 % (P = 0,001).

Z dosiahnutých výsledkov teda vyplýva, že per os aplikácia derivátov hliníka sa odráža na metabolizme glycidov. Chlorid hlinitý vyvoláva hromadenie glykogénu v tkanivách a kysličník hlinitý odbúravanie glykogénu z tkanív. Zmenám v glykémii nemožno pripísať podstatnejší význam, pretože kolíšu v hraniciach fyziologickej normy. Zmeny v glykogéne sú presvedčivé pre vysokú preukaznosť.

Diskusia

Vyšetrovanie niektorých zložiek glycidového metabolizmu po per os aplikácii zlúčenín hliníka odhalilo, že zlúčeniny hliníka narušujú v metabolizme glycidov sled biochemických procesov. Vidieť to zo zaznačených zmien v glycidových rezervách organizmu. Je pozoruhodné, že efekt účinku vo vodnom prostredí dobre rozpustného chloridu hlinitého je iný ako efekt účinku vo vode nerozpustného kysličníka hlinitého. V týchto rozdielnych vlastnostiach zlúčenín hliníka vidíme zdroj zmien dotýkajúcich sa rezervných glycidov. Domnievame sa, že pozorované zmeny v glycidovom metabolizme majú jediného spoločného menovateľa — poruchy v metabolizme foriem fosforu. Mechanizmus takéhoto účinku zlúčenín hliníka je veľmi zložitý a na jeho zodpovedanie bude treba urobiť veľké množstvo špecifických štúdií. V zhode s literárnymi údajmi (Gisselbrecht a kol., 1957) predpokladáme, že chlorid aj kysličník hlinitý blokádou fosforu, resp. fosforylačných procesov narušia metabolizmus glycidov. Dá sa čakať, že pre rozdielnu povahu chloridu hlinitého na jednej strane a kysličníka hlinitého na strane druhej bude aj výsledný efekt, teda zmeny v rezervných glycidoch, iný. Výsledky z našej práce na túto skutočnosť jednoznačne poukazujú. Štúdium ďalších parametrov, najmä metabolizmu labilných enzymatických foriem fosforu, iste poskytne cenné údaje pre zodpovedanie mechanizmu účinku zlúčenín hliníka na metabolizmus glycidov.

Záver

V experimentoch na krysách sa zistilo, že per os aplikácia zlúčenín hliníka spôsobuje zmeny v glycidových rezervách organizmu. Po 5 až 10 dňoch pravidelnej aplikácie chloridu hlinitého v množstve 15 mg Al/100 g váhy krýs došlo k rapídnemu vzostupu hladiny glykogénu v pečeni a kosternom svalstve. Po obdobnej aplikácii kysličníka hlinitého bol zaznačený opačný efekt účinku — pokles hladiny glykogénu v pečeni a kosternom svalstve. Hodnoty glykémie neopustili ani v jednom prípade fyziologickú oblasť. Pozorované zmeny sa dávajú do súvisu s blokádou fosforu, resp. fosforylačných procesov, prostredníctvom reakcií medzi aplikovaným hliníkom a fosforom organizmu.

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ВЛИЯНИЕ ПЕРОРАЛЬНОЙ ПОДАЧИ СОЕДИНЕНИЙ АЛЮМИНИЯ НА МЕТАБОЛИЗМ ГЛИЦИДОВ

Либор Возар

Резюме

При экспериментах на крысах было установлено, что подача соединений алюминия per os вызывает изменения в резервах глицидов в организме. После 5 и 10 дней регулярного введения хлорида алюминия в количестве 15 мг Al/100 г веса крысы на-

[illegible] быстрое повышение уровня гликогена в печени и скелетных мускулах. Посл[illegible] [illegible]логичного применения [illegible] алюминия наблюдался противоположный эффект — [illegible]нижение уровня гликогена в печени и скелетных мускулах. Уровень сахара в кров[illegible] [illegible] случае не понижался под физиологический. При вышеописанных процесс[illegible] [illegible] также блокада фосфора или фосфорилирующих процессов, что проце[illegible] [illegible] посредством реакции между введенным алюминиумом и фосфором организм[illegible]

EINFLUSS EINER PERORALEN APPLIKATION VON ALUMINIUMVERBINDUNGEN AUF DEN METABOLISMUS DER GLYCIDE

Libor Vozár

Zusammenfassung

In Experimenten auf Ratten wurde festgestellt, dass eine per os Applikation von Aluminiumverbindungen Änderungen in den Glycidreserven des Organismus bewirkt. Nach 5 und 10 Tagen regelmässiger Applikation von Aluminiumchlorid in einer Menge von 15 mg Al/100 g Rattengewicht kam es zu einem rapiden Anstieg des Glykogenspiegels in der Leber und in der Skelettmuskulatur. Nach einer ähnlichen Applikation von Aluminiumoxyd wurde ein umgekehrter Wirkungseffekt verzeichnet — ein Sinken des Glykogenspiegels in der Leber und der Skelettmuskulatur. Die Werte der Glykämie verliessen in keinem Falle den physiologischen Bereich. Die beobachteten Änderungen lassen sich mit der Blokade des Phosphors bzw. der Phosphorylierungsprozesse, durch Vermittlung der Reaktionen zwischen dem applizierten Aluminium und dem Phosphor des Organismus erklären.

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THE EFFECT OF THE INGESTION OF ALUMINUM HYDROXIDE ON THE SERUM SALICYLATE LEVEL*

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THE oral administration of aspirin or sodium salicylate frequently results in gastrointestinal upsets, especially pyrosis, diarrhea, nausea, and vomiting, and it is customary to administer with aspirin or salicylate an equal dose of sodium bicarbonate to relieve such symptoms. However, if it is desired to maintain the serum salicylate concentration at a certain level, the administration of sodium bicarbonate is contraindicated, since it has been shown by Smull, Wégria, and Leland that such doses of sodium bicarbonate considerably lower the serum salicylate level.[1] Since the main mechanism responsible for such lowering of the serum salicylate level is the increase in the renal excretion of salicylate or its derivatives produced by sodium bicarbonate, it was thought of interest to study the effect on the serum salicylate level of a drug such as aluminum hydroxide, which has been suggested to relieve the gastrointestinal symptoms produced by aspirin or salicylate, presumably by alkalinizing the contents of the gastrointestinal tract and yet is not absorbed.[2,3]

Method and Results

One normal person (subject 3, Table 1) and four patients with active rheumatic fever (subjects 1, 2, 4, and 5, Table 1) were given from 0.9 to 1.2 Gm. of aspirin every four hours, day and night. Their serum salicylate level was determined by the method of Brodie, Udenfriend, and Coburn on the serum of a sample of venous blood drawn every day just before the administration of a new dose of aspirin.[4] When a stable serum salicylate level was established, the subjects took, with each dose of aspirin, 5 cc. of commercial aluminum hydroxide. After a few days, the aluminum hydroxide was discontinued, and the subjects, taking the same dose of aspirin as before, were followed for a variable number of days. In subject 2, the effect of sodium bicarbonate on the serum salicylate level was also studied by giving the patient 2.4 Gm. sodium bicarbonate with each dose of aspirin. The results observed are summarized in Table 1.

In subjects 1, 3, 4, and 5, the administration of aluminum hydroxide for three to seven days did not modify the serum salicylate level. In subject 2, there was a definite rise of the serum salicylate level during the period of aluminum hydroxide administration, but this increase in the salicylate level persisted after the aluminum hydroxide was discontinued. It seems, therefore, that aluminum hydroxide was not responsible for the rise in the serum salicylate level. Indeed we have observed such rise in some patients with active rheumatic fever on a constant intake of aspirin or salicylate, probably around the time at which the rheumatic activity subsides. It can also be seen that a dose of 2.4 Gm. of sodium bicarbonate taken with each dose of aspirin resulted (subject 2), within twenty-four hours, in a fall in the serum salicylate level which rose again when the sodium bicarbonate was discontinued.

TABLE 1.—Successive Daily Salicylate Levels (Micrograms per Cubic Centimeter of Serum)

	Subjects				
	1	2	3	4	5
Control period—aspirin alone	360, 400, 403, 404	328, 319, 317, 335	332, 326, 328	480, 475, 479, 478, 493, 492	215, 211, 210, 214, 214
Aspirin (control dose) and 5 cc. aluminum hydroxide	374, 392, 400, 390, 408	351, 362, 368, 365, 412, 417, 426, 448	328, 319, 329	414, 493, 489, 477, 506	231, 191, 210, 227, 238, 220, 219
Aspirin (control dose) continued—aluminum hydroxide withdrawn	390	486, 484, 444, 480	326	477, 488, 451	239, 216, 232
Aspirin (control dose) and 2.4 Gm. sodium bicarbonate		337, 242			
Aspirin (control dose) continued—sodium bicarbonate withdrawn		244, 354			

Conclusion

Doses of 5 cc. of commercial aluminum hydroxide every four hours day and night do not modify the serum salicylate level.

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Weakness in Young Chicks on a Diet Supplemented with Aluminum Hydroxide Gel

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IN THE course of experiments on atherosclerosis in young chicks (Rodbard *et al.*, 1950, 1951a, 1952) we observed a syndrome of weakness which followed the ingestion of a diet to which a reactive aluminum hydroxide gel had been added.

METHODS

A total of 7 experiments was performed using newly hatched commercial incross-bred (Hyline) or New Hampshire chicks placed in a brooder and given a commercial starter mash[1] which has consistently been adequate for normal growth and development. The experimental animals were given the same mash mixed with a specially prepared gel containing aluminum hydroxide.[2] The resulting mash contained 25 mg. of aluminum per gram of food (experiments 1 to 7). Lower concentrations of the gel were also tested (experiments 2, 3). Fresh batches were prepared at intervals of three days. The ration tended to cake and it was necessary to break up the feed at least three times daily so that it could be eaten by the young chicks.

RESULTS

Weight and feed intake. Chicks on the aluminum hydroxide mash gained weight at a slower rate than did the chicks on the same diet without the gel. These animals ate less than their controls even though the mash was broken up at frequent intervals (Table 1). With lower dosages of the gel (3 or 8 mg. of aluminum) (experiments 2, 3) feed intake and weights approached control values.

Mortality. The incidence of mortality, which usually occurred about 4 days after onset of weakness, is given in Table 2. The primary cause of death was probably not inanition, since other chicks of the same age have been fasted for periods up to 5 days without showing similar signs of the weakness (Rodbard *et al.*, 1951b). The high incidence of mortality was seen only at the higher dosage levels.

Weakness. The weakness syndrome was observed in all the series of experiments in which the highest concentration of the gel was given. With continuous feeding

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[1] The chick starter mash used in these experiments has the following composition: 40% corn, 5% oats, 5% wheat bran and 10.5% wheat standard middlings, 5% dehydrated alfalfa meal, 3.75% meat scraps, 1% dried buttermilk, 22% soybean oil meal, 1% bone meal, 1.5% of a salt mixture containing calcium carbonate, manganese sulfate and potassium iodide, 2.5% of a APF premix and 2.5% of a vitamin premix. Based on average analysis this formula contains the following nutrients: 3.09% fat, 1.044% calcium, 0.62% phosphorus, 0.5% salt, 0.015% potassium iodide and 0.75% manganese sulfate. For each 100 pounds of feed there are 500,000 I.U. of vitamin A (based on the alfalfa meal and the starter premix only); 27,000 A.O.A.C. units vitamin D (based on the starter premix only); 324 mg. riboflavin; 70,000 mg. choline; 550 mg. pantothenic acid; 156 μmg. of vitamin B_{12}.

[2] USP aluminum hydroxide gel (amphogel), courtesy of Drs. George Farrar and F. B. Cooper, Wyeth Laboratories, Philadelphia, Pa. This gel binds the HCl in the stomach.

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TABLE 1.—*Feed intake and weight gains of chicks on diet supplemented with aluminum hydroxide gel*[1]

Diet	Weeks on diet					
	1		2		3	
	Feed[2]	Wt.[3]	Feed	Wt.	Feed	Wt.
Aluminum-gel experimental group	51	62	102	63	85	62
Control group	62	67	137	79	155	102

[1] Experiment 1.
[2] Grams, chick/day.
[3] Average of 10 chicks expressed in grams.

of the gel, the weakness was apparent as early as 10 days after hatching, being seen most frequently in the 3rd and 4th weeks of life. During the course of development of the syndrome, the chicks gradually showed an inability to stand erect. They tended to remain in a squatting position with head and wings dropped and with eyelids closed. The leg movements appeared uncoordinated with a resulting unsteady gait. No convulsive movements or twitchings were observed in any of the birds. The normal yellowish coloration of the shanks and beaks was replaced by a pale "washed out" appearance of these parts in the incrossbred chick but appeared normal in the New Hampshire. Only one possible case of xerophthalmia was seen. The early feathers appeared ruffled and poorly developed. In over 80% of these birds the combs had a bluish cast. X-rays showed a retarded development of skeletal structure, but no specific malformation. The droppings showed a change in character as the weakness progressed, caking readily on the hind quarters and being difficult to remove. Diarrhea was not noted.

TABLE 2.—*Survival of newly-hatched chicks on diet supplemented with aluminum hydroxide gel*[1]

Diet \ Age	% surviving			
	7 days	14 days	21 days	28 days
Control group (110 chicks)	100%	98%	98%	98%
Aluminum-gel experimental group (35 chicks)	100%	100%	80%	43%

[1] Experiment 4.

Chemical determinations. It was noted that plasmas of blood drawn by heart puncture from the aluminum-fed animals frequently appeared more hemolyzed than control plasmas. There were no differences in the hematocrits or in the saline fragilities of the erythrocytes in the two groups. The animals receiving the gel had consistently lower plasma levels of potassium, carotene and vitamin A[3] (Table 3) than those on control diet (Bessey *et al.*, 1946).

On regular mash diets, with or without gel, the plasma cholesterol levels were normal, ranging around 100 mg.%. However, with the concurrent feeding of cholesterol diets to produce hypercholesteremia, the aluminum-treated birds showed lower cholesterol levels (Schoenheimer and Sperry, 1934) than did other animals of the hatch on the same diet without the gel.

Autopsy findings. Fat deposits were notably absent in the organs of the gel-fed chicks. In general, the livers appeared darker red than the controls. The gastrointestinal tract generally held little food;

TABLE 3.—*Chemical determinations of plasma of chicks on diet supplemented with aluminum hydroxide gel*[1]

	Control group	Aluminum gel experimental group
Carotene, μg. %	482	302
Vitamin A, μg. %	47	0.7
Cholesterol,[2] mg. %	460	182
Potassium, meq./liter	6.1	5.1

[1] Experiment 5.
[2] On 2% cholesterol plus 5% cottonseed oil diet.

[3] We wish to thank Dr. Elizabeth Kaiser of the Department of Pediatrics, Michael Reese Hospital, Chicago, Illinois, for her assistance in the determination of carotene and vitamin A contents.

the proventriculus and the ventriculus often contained a thick brownish fluid. Occasional hemorrhages were seen in the proventriculus, ventriculus or small intestines. About 80% of the birds showed abnormally small spleens, in some instances the spleen being only about one-fifth of the size seen in control animals. Urate crystals were occasionally noted in the renal calyces and in the ureters. No specific gross or microscopic pathology which could account for the muscular weakness could be demonstrated in the brain, spinal cord or muscular system.

Reversibility. Several attempts were made to treat the weakness syndrome and prevent death of the animals. Seventy chicks were placed on the aluminum gel at the time of hatching (experiment 4). For the first two weeks of life, the percentages of deaths in the control and in the experimental groups were comparable. During the third and 4th weeks, when weakness became apparent in the animals on the gel diet, the remainder of the group was subdivided; 15 of the animals were continued on the experimental gel diet, while 35 were transferred to regular chick starter mash without gel. During the 4th and 5th weeks, all the animals on the gel diet died, while only three died in the group transferred to the normal mash diet. The weakness syndrome regressed and the animals appeared normal. The condition is therefore reversible, provided a normal diet is substituted shortly after the onset of symptoms. In other series it was found that when the chicks were continued on the gel diet for two days after weakness had become marked, replacement of the gel with a normal diet was of no avail.

Toxicity. An attempt was made to determine the dose toxicity by feeding the aluminum hydroxide gel in various concentrations (experiment 2, 3). Separate groups of chicks received 25, 8 or 3 mg. of aluminum (as aluminum hydroxide) per gram of food. Animals receiving the 8 or 3 mg. dosages showed no weakness or death (Table 4). The 25 mg. concentrations resulted in weakness and death of most of the animals during the third and fourth week as noted.

TABLE 4.—*Effect on survival of various dosages of gel diet*[1]

Aluminum equivalent in 1 gm. feed	No. of chicks	Percentage of chicks surviving				
		Age				
		7 days	14 days	21 days	28 days	35 days
25 mg.	45	96%	20%	20%	2%	0%
8 mg.	30	100%	100%	100%	97%	97%
3 mg.	25	96%	96%	96%	92%	92%

[1] Experiment 2.

Grit (experiment 5) was added to the gel diet in one group as a means of increasing the fragmentation of the clumped particles. An aggravation of all symptoms was noted. In particular there was a very marked decrease of the plasma vitamin A and carotene contents. The tendency to weakness and death was unaffected.

A non-reactive aluminum hydroxide gel[4] (25 mg. of aluminum equivalent per gram of food) (experiment 4) was tested for its possible effects in 60 chicks. Only an occasional chick showed a transient weakness which gradually disappeared even though the diet was continued.

Aluminum phosphate gel (experiment 7), given in concentrations so that the aluminum intake would be the same as on the hydroxide gel, produced only occasional signs of weakness. In 15 chicks there were 4 deaths in the first two weeks of the experiments. At the end of the second week the chicks appeared weak and listless, but the syndrome was not progressive. Two weeks later the weakness had disappeared and there were no deaths during the remaining two weeks that the

[4] B-gel, Wyeth Laboratories, a non-reactive gel, is prepared so that it does not bind the HCl in the stomach.

animals were kept on the special diet.

Neuromuscular studies. Since the neuromuscular system appeared to be involved, studies were conducted on the sensitivity of the chicks to d-tubocurarine and decamethonium[5] (experiment 6). The gel-treated chicks showed their first symptoms in the leg. By contrast, chicks paralyzed with d-tubocurarine showed the earliest and most marked weakness in the neck muscles with inability to raise the head. Neostigmine injections (50 to 1,000 μg./kg.) had no effect on the muscular weakness, further suggesting that the weakness was unlike that of myasthenia gravis.

Although facilities for assaying vitamin deficiencies were not available, preliminary attempts at dietary or parenteral treatment with vitamin supplements were undertaken. While on the gel diet, daily prophylactic dosages of oral and intramuscular vitamin E (100 mg.), vitamin A (10,000 units) and a polyvitamin mixture[6] were given without affecting the weakness syndrome or the mortality rate.

The weakness was unaffected by dietary supplements of cholesterol (2%) and cottonseed oil (5%) or by injections of estradiol benzoate (1 mg./chick/day) which were given as a part of other experimental work (Rodbard *et al.*, 1950, 1953).

DISCUSSION

Muscular weakness may occur in a wide variety of conditions in the chick (Biester and Schwarte, 1952). However, none of the recorded patterns of syndromes or pathology are identical with the weakness observed in the chicks fed with a mash mixed with the reactive aluminum hydroxide gel.

The possibility of a vitamin deficiency was suggested on the basis of the known adsorptive qualities of the aluminum hydroxide gel (Dasler, 1948). For example, in other experiments the gel adsorbed dietary cholesterol (Rodbard and Bolene-Williams, 1952). The syndrome closely resembles those states reported for deficiences of vitamins A, D, E and thiamine However, treatment of the chicks with these vitamins or with polyvitamin mixtures was without success in changing the course of the weakness and mortality patterns.[7] Aluminum toxicity was eliminated as a cause of the syndrome by the comparable feeding of a non-reactive aluminum hydroxide gel or an aluminum phosphate gel.

It is of interest to note that this condition was seen only in very young chicks. Older animals fed the same diet showed normal weight gain, growth, and development, and no signs of the muscular weakness. The special nature of the weakness syndrome would appear to deserve further investigation.[8]

[5] These data were obtained through the courtesy of Dr. Cedric M. Smith and Dr. Klaus Unna of the Department of Pharmacology, University of Illinois, School of Medicine, Chicago, Illinois.

[6] Poly-vi-sol. The dosage given contained 5,000 units of vitamin A, 10,000 units of D, 50 mg. of ascorbic acid, 1 mg. of thiamine, 0.8 mg. riboflavin and 5 mg. niacinamide.

[7] In a recent series of experiments, birds with the weakness syndrome have been found to have decreased plasma uric acid. This is considered as further evidence that a vitamin A deficiency is not the responsible factor for production of the weakness pattern.

[8] The syndrome of muscular weakness has been confirmed by Baeder *et al.* (Baeder, D. H., W. J. Beckfield, and J. Seifter, 1954. Proc. Soc. Exp. Biol. and Med. 86: 326.) These workers feel that the syndrome is due to general inanition. However, previous experiments in our department show that chicks starved for periods of 5 days or more do not develop the weakness syndrome. It must therefore be ascribed to other more specific causes. Further, it has been clearly shown that inanition, while the animals are on a restricted high cholesterol diet, results in a tendency to hypercholesteremia rather than a lowered level as reported by Baeder *et al.* The effect must be attributed to the effects of the aluminum gel rather than to inanition *per se*.

SUMMARY

A syndrome of weakness leading to death within a week after onset was produced by feeding young chicks a diet containing high concentrations of a reactive aluminum hydroxide gel. Lower concentrations did not produce the weakness, nor did non-reactive aluminum hydroxide gel or aluminum phosphate gel in comparable amounts. The weakness syndrome was associated with a reduction in the plasma carotene, vitamin A and cholesterol values. The syndrome was reversible if a regular mash diet replaced the gel diet within two days after onset of weakness. Replacement with vitamin supplements did not reverse the weakness syndrome. The syndrome was unlike the weakness due to decamethonium and it did not respond to neostigmine.

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