Monograph on **Gelatin** #95 8/17/73

GELATIN #95

M O N O G R A P H

O N

G E L A T I N

TR-72-1552-35

Submitted Under:
Contract No. FDA 72-104

August 17, 1973

INFORMATICS INC.
6000 Executive Boulevard
Rockville, Maryland 20852

GELATIN

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GELATIN

Summary

An excellent introduction to a monograph on gelatin is presented by Glicksman (209); the following three paragraphs are taken directly from his work.

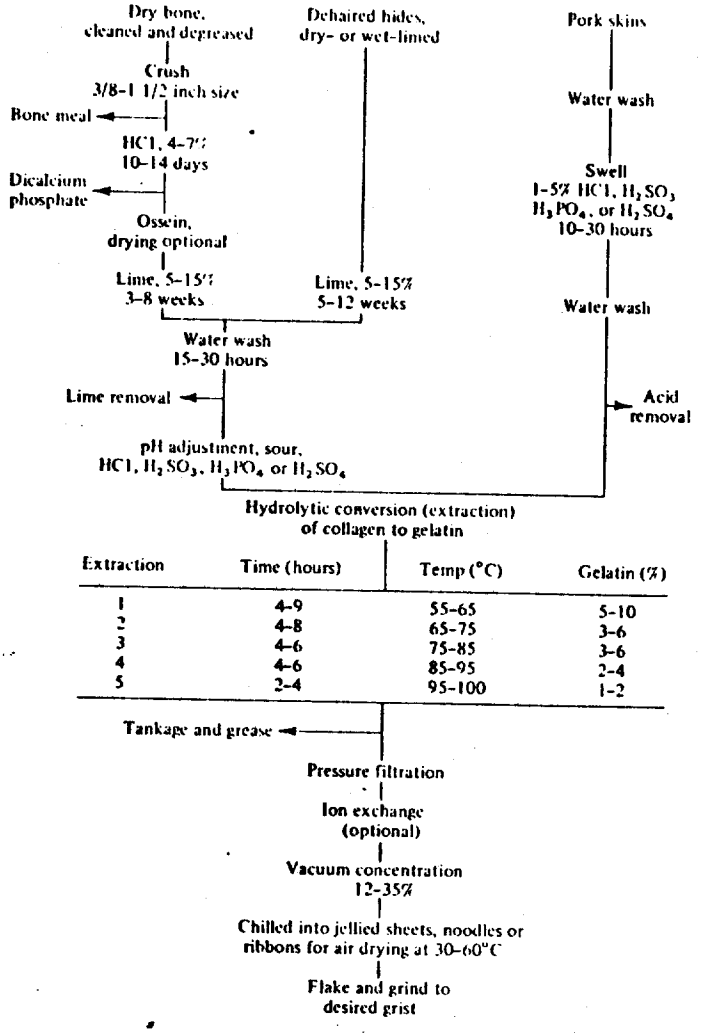
"Gelatin," derived from the Latin verb gelare meaning "to congeal," has been known for thousands of years. The extraction of glue by cooking hides dates back at least to the time of the ancient Pharaohs of Egypt. Bogue (1922) cites a 3000-year-old stone carving, found in the ancient city of Thebes and belonging to the period of Thotines III, which describes the gluing of a thin piece of rare red wood veneer to a yellow plank of sycamore.

Later, in the Roman era, Pliny, Lucretius, and others referred to the manufacture of glue. Pliny wrote, "Glue is cooked from the hides of bulls." Elsewhere in his writings he referred to glue, which had been mixed with gums, milk, eggs, and wax as a vehicle for paints, used by the ancient Egyptians. Much later, in the Elizabethan period (ca. 1600 A.D.), Shakespeare and Bacon made frequent references to glue in their writings. Indeed, a commercial glue industry appears to have been established in England about 1700. A similar industry developed in the United States within the following century.

During the early years of the Napoleonic era, gelatin was manufactured on a large scale in an attempt to alleviate the food shortages resulting from the English Naval blockade of Europe. The first manufacture of edible gelatin is credited to Arney, who was granted a patent in 1846 for the preparation of a powdered gelatin "for forming compositions from which may be prepared jellies and blanc manges; also, when mixed with farina, or starch, or starchy vegetable flour, for thickening soups, gravies, etc.". At present, gelatin is manufactured in the United States by at least eleven companies in the amount of about 60-70 million pounds annually.

Gelatin does not occur in nature as such, but is derived by hydrolysis from collagen, which is the chief protein component in connective tissues; these tissues, themselves may be regarded in a practical sense as "gluing" the body-parts of mammals and fish together. Quantitatively, collagen is concentrated in skin, bone of the skeletal system, and tendons relating muscles to skeleton; it is found, however, throughout the tissues and organs of the animal to a lesser degree. Chemically, collagen and gelatin are virtually indistinguishable, but the processing of collagen results in altering the fibrous, water insoluble, highly organized, crystal-like macromolecules (tropocollagens) irreversibly into gelatin with dissimilar physical characteristics. Other proteins, and proteins complexed with micropolysaccharides; keratin and aldehydes are also present in the dermal skin but are mostly removed from the gelatin produced during treatment of the collagen. The variations in gelatin properties due to source and treatment make it a highly diverse and heterogeneous substance, particularly with regard to molecular weights, etc. Cattle hides and bone, and pork skins are the major sources of the collagen, with a limited amount of fish skin;

the resulting gelatin is of two types: Type A and Type B, depending upon which of two processes are used to convert the collagen to gelatin. (More details will be found in the monograph, Chemical Information section). A flow diagram of the manufacture of gelatin is shown below (Glicksman, 209):



Extraction	Time (hours)	Temp (°C)	Gelatin (%)
1	4-9	55-65	5-10
2	4-8	65-75	3-6
3	4-6	75-85	3-6
4	4-6	85-95	2-4
5	2-4	95-100	1-2

FIG. 3. Flow diagram for gelatin manufacture.

In view of the enormous quantities of gelatin consumed worldwide for more than a century (Cooper, in 1845 was granted a U.S. patent for "portable gelatin" for use in desserts: Glicksman (209) it is not surprising to discover that no reports were encountered in the literature

indicating acute or chronic toxicity of gelatin. Gelatin is, itself - a protein food, and lacks only three amino acids (tryptophan, an "essential" amino acid; cystine and cysteine) which disqualify gelatin as a "complete protein" in man's diet. Many of the papers reviewed for this monograph were nutrition studies which demonstrate that gelatin, in the absence of other amino acid sources does not meet the protein requirements of test subjects, but can be an important dietary supplement.

Jackson et al. (298) in an early study (1928) of the nutritional value of gelatin fed to rats in various diets showed, however, that diets of gelatin supplemented with tyrosine, cystine and tryptophan resulted in half the animals dying of nutritional failure within 9 to 48 days. The deaths were attributed to renal failure rather than directly to nutritive failure, but the gelatin appeared to be the causitive factor. A number of other diets were tested in which gelatin comprised 35% of the diet for the most part, in combination with various amino acids; casein plus gelatin; and in one test, casein plus glycine and alanine, two prominent amino acids of gelatin. In the last two diets, early weight losses in the animals were followed by recovery and rapid growth, indicating that neither gelatin nor a mixture of two of its component amino acids added to an otherwise normal diet of casein permanently inhibit growth. High concentrations of gelatin in the diet did retard growth, and frequently produced fatalities, apparently due to renal failure.

Robscheit-Robbins et al. (591) performed twelve separate experiments to determine the qualities of gelatin as a plasma substitute, using doubly depleted dogs (anemic and hypoproteinemic). The gelatin, administered intravenously as 6% solutions in 0.9% NaCl was of bone collagen origin for the most part, some was of unknown origin. Urinary nitrogen, hemoglobin levels and plasma protein were determined. The tolerance of the animals for dosages of 1 to 3 g/kg body weight over a period of several days was variable; a total dosage of 2-3 g/kg produced severe intoxication in some dogs, while others tolerated a dosage of 7 g/kg without significant disturbance. Gelatin given over 1 to 2 weeks duration for totals of 3-17 g/kg produced serious disturbances: inhibition of blood protein production, intoxication, weight loss, and even fatalities. The doubly-depleted animals were very sensitive "testing machines" for evaluating plasma substitutes, and by inference, the variable results found in their reactions to gelatin suggested careful use of gelatin as a plasma substitute in human cases.

Some studies (see monograph section, Biological Data: Special Studies) have indicated that subcutaneous injections of aqueous gelatin in mice have produced a small incidence of lung adenomas, hepatomas, and thymus lymphomas over periods of about a year following treatment.

Questions have been raised (1950's) regarding the antigenicity of gelatin: Maurer (433) injected human volunteers with oxypolygelatin and got results showing antibody production even in preimmunization studies; Ratner and Crawford (575) performed anaphylactic studies in guinea pigs using a variety of gelatins and concluded that gelatin was

non-anaphylactogenic. Maurer includes in his report a lengthy discussion suggesting that some antibody molecules serve functions other than protective, and discards that notion that the antibodies he found in the plasma of the human subjects were due to impurities in the gelatin used; Ratner and Crawford attributed to impurities any allergenicity of gelatin.

Several cases in which allergic symptoms were observed in humans involved in beef-sensitivity were reported by Randolph (571); ingestion of Knox gelatin by beef-sensitive patients produced severe reactions among 3 of 4 tested; one showed no reaction, as did 4 patients not beef-sensitive. The ingested gelatin produced in the beef-sensitive cases nasal stuffness, nausea, diarrhea, tinnitus and cramps. The same beef-sensitive patients showed no reactions when tested with commercial porcine gelatin.

Mendez and Hughes (445) described a case of a young woman who suffered from urticaria and angioneurotic edema after ingesting boiled and stewed meats or gravy made from meat stock; she was also allergic to ice cream and jellies. Extracts of gelatin and foods containing gelatin all produced positive allergic reactions; hyposensitization with gelatin caused the clinical allergic symptoms to disappear. Skin-sensitizing antibodies in the patient's serum were shown by passive transfer tests. A thousand allergic cases tested indicated positive skin reactions to gelatin in about 1 in 150 and a clinical sensitivity in 1 of 500. The authors concluded that gelatin was a rare allergen in man.

Gelatin solutions were proposed more than half a century ago as plasma substitutes in medical practice, or surgery involving loss of blood, and were extensively used for that purpose during and after World War II with considerable success. Several reports dealing with the application of various gelatins and modified gelatin solutions as plasma substitutes are included in this monograph. Vander Brook et al. (715) performed studies with dogs, maintained on a high protein diet, bled of about 29-54% of their estimated blood volume at weekly intervals, and having 80% of the blood removed replaced by a 5% gelatin solution. After periods of 2 to 13 weeks, the animals were sacrificed and autopsied. No gross pathology attributed to the gelatin infusion was observed; histological evidence of some liver and renal damage was shown in some instances.

During the same year that Vander Brook's paper appeared, (1947), Skinsnes (641) reported the results of gelatin given to 23 hospital patients who eventually expired from medical or neoplastic diseases, or from therapeutic surgical measures. Postmortem examinations were performed, usually within 2-10 hours of death; renal lesions were observed, to which the author attributed an etiological role of gelatin. Gelatin nephrosis was shown to be a reversible phenomenon, appearing within half an hour after intravenous injection of the gelatin, and disappearing within 120 hours. It was suggested that caution be exercised in giving gelatin to patients with a history of renal impairment; several cases were cited in which gelatin was a suspected factor in coronary occlusion and vascular thrombosis.

Znamenskaya (765) in a recent (1968) report surveyed the literature related to the use of gelatin plasma substitutes. Some forms of gelatin solutions described were: a 4-8% osseous gelatin in isotonic NaCl solution (extensively used during WW II); oxypolygelatin (OPG) developed in 1951 by treating gelatin with glyoxal; a modified fluid gelatin (MFG) produced by treating gelatin with decarbonized acids or their anhydrides and, first appearing in the mid-1950's; "Blutersatz G" comprised of 6% gelatin, methyl-glucosamine and 2% fructose; "Gemaktsel", a synthetic polymer of three similar polypeptides (molecular weight of 12,000) combined into a lattice structure of molecular weight 35,000 - the first homogeneous gelatin plasma substitute, appearing in 1962. Several other varieties of substitutes were described: "ferrofusine", a Ringer-Lokk salt solution with colloidal iron and 1% gelatin; and "gelatinol", 8% partially hydrolyzed edible gelatin, with 0.9% NaCl, which appeared in 1960. The plasma substitutes were tested in treatment of acute radiation sickness induced in dogs by exposure to 600 r of radiation over a period of several days. The gelatin was injected as 100 ml/kg body weight, together with bone marrow transplants; a significant reduction of the radiation effects was observed following this therapy. The same radiation dose of 600 r in controls without therapy results in an LD_{100}.

Nixon and Mawer (511) recently reported the results of experiments in which protein test meals were given 6 human volunteers and subsequent determinations from intestinal contents at various levels along the intestinal tract were made of the distribution of the protein components or amino acids. Arginine, lysine, tyrosine, valine, phenylalanine, methionine and leucine appeared to be absorbed in the free form; glycine, threonine, serine, the imino acids, and dicarboxylic amino acids were not indicated as being absorbed in the free form.

Numerous metabolism (nutrition) reports are found in the literature of gelatin over the past half-century attesting generally to the inadequacies of gelatin as a nutritional protein source in the diet. A wide variety of diets have been studied: gelatin alone; gelatin plus amino acid supplements; gelatin plus soy - protein, fish protein; casein, barley, bread or zein. Ashley and Fisher (26) in a recent study (1966) fed 5 day-old chicks a variety of diets with results that showed gelatin (10%) plus an amino acid mix of some 15 amino acids gave satisfactory results as measured by food utilization and growth. The key amino acids supplementing the gelatin diet were lysine and threonine, previously underestimated quantitatively in gelatin, and methionine, due to the "stress" created by the high glycine and arginine content of the gelatin-favoring creatine synthesis.

Nutritional studies with gelatin in rats were reported by Nilson and Lemon (508). Diets containing 5 to 30% gelatin produced stunting of growth, most marked at 30% - less at 20%, and minor retardation at 10% gelatin.

Casein and gelatin diets in comparative nutritional studies were performed by Ramakrishnan (569). Rats fed 20% casein thrived and grew normally; rats fed 16.5% gelatin lost weight and suffered from inanition. Liver xanthine oxidase determinations in sacrificed animals showed 70% less oxidase activity in the animals fed gelatin than in those fed

casein. Methionine supplements in the gelatin diet doubled the oxidase activity but did not improve the growth.

Downey (142) nearly half a century ago (1925) made detailed studies using rats fed various diets to test the nutritional value of gelatin. Eight experimental diets were tested in pair groups for comparisons, from which Downey concluded that gelatin is not nutritionally useful as a major source of protein; but has value as a supplement to various natural foods, particularly in dairy products (milk and ice cream).

Pagé and Gingras (532) studied nutritional inanition in hospital patients unable to eat normally; physiological glucose administered parenterally has not satisfied the nutritional requirements of the patient. Experiments with rats, fed several diets, were performed to determine the best mixture of nutrient elements that could be given parenterally: casein and gelatin were compared, with casein plus vitamin supplements producing the longest survival time; casein and gelatin, each with sucrose added, showed little difference, with the casein plus sucrose slightly better; an optimum mix of 50-80% casein with 50 or 20 parts of sugar gave maximum survival in the rats. Gelatin was declared unsatisfactory in overcoming inanition or in promoting growth.

It has been observed that diets deficient in one or more essential amino acids result in a lowered allantoin excretion, regardless of which amino acid(s) is missing. The purine metabolism of rats given diets deficient in essential amino acids was studied by Ricceri (586). The animals were pair-fed a variety of diets, consisting of casein with various supplementary amino acids; the casein was replaced by gelatin, and comparisons drawn by an analysis of the urine for allantoin; uric acid, total nitrogen, and creatinine. Four diet-stages were observed: casein, alone; gelatin, alone; gelatin plus cystine and tryptophan; and casein, alone. All four urine analytical results from the four stages followed similar patterns: normal value, a marked decrease in values, a slow increase in values and a return to normal.

Gelatin, wheat gliadin, and casein as supplements in the diet of rats were compared in experiments by Ellis and Rose (153). Each protein was fed as 5% of the diet together with a mixture of amino acids. The gelatin; gliadin and casein promoted growth in that order from least to most effective.

Mason and Palmer (429) experimented with rats fed diets of gelatin, casein or zein to establish relative nutritional values. A preliminary, basic, "nitrogen free" diet was followed by the addition of one of the three proteins added in the amount of about 0.4 g; feces and urine were collected and analyzed for excreted nitrogen. Additional amounts of the protein were added to the diet in quantities required for a nitrogen balance; after several days, the animals were returned to the "nitrogen free" diet and excreted nitrogen again determined. The gelatin and casein were well digested, but the zein was irregularly or poorly digested. The gelatin was poorly utilized, compared to both casein and zein utilization.

Intravenous injections of daily doses of 150 ml of 12% gelatin solutions were given to dogs depleted of protein stores in studies by Brunschwig (73) to determine the nutritional value of gelatin administered in this manner. Total nitrogen was determined for each gelatin dose, and for nitrogen loss in feces and urine. In 5 of 6 experiments with 4 dogs, a positive nitrogen balance or an equilibrium was established; it was assumed that at least some of the injected gelatin was metabolized.

The urinary excretion of intravenous doses of 1000 ml, 5% gelatin, given to 42 normal human subjects was studied by Hoffman and Kozoll (277). Three gelatins of molecular weights averaging 37,000; 47,000; and 58,000 were used; at 6 hrs. after injection, the gelatin excreted was quantitatively inversely related to the molecular weight. After 72 hrs., 80% of all gelatins had been excreted; some excretion was still observable after 4-5 days. The authors stated that little gelatin appeared to be catabolized.

Dill and Horvath (140) studied the influence of a high gelatin diet on the excretion of nitrogen, creatinine and creatine in healthy young men. Four subjects were given 60 g gelatin for 40-50 days; creatine excretion increased in two, but the average creatinine excretion was not changed. Both creatine and creatinine excretion did increase after the gelatin diet was terminated, indicating possible storage of the materials during the experimental diet.

Gelatin solutions have been used as plasma expanders for many years, and various modifications of gelatin to minimize the gelling at room temperatures have been devised. Two such modified gelatins, introduced in the 1950's, one a condensation of gelatin with glyoxal and H_2O_2 has been called oxypolygelatin (OPG), and a second form from gelatin treated with dicarboxylic acid anhydride, called "fluid gelatin", have been tested in 12 hospital patients by Siiteri and Highland (639). The modified gelatins were administered intravenously over an hour's time as 500 ml to begin the experiments, and given as 250 ml at half-hour intervals for 6 hours. Blood samples and urine were collected at intervals; urine analyses showed the OPG and "fluid gelatin" were mostly excreted during the first 6 hours, with OPG excretion more rapid than that of "fluid gelatin". The analyses of blood samples for gelatin, plasma protein, albumin-globulin ratios and hematocrit showed no significant differences in the two gelatins beyond the retention of "fluid gelatin" in the blood for longer periods of time than for OPG.

Scott and Ryan (627) performed experiments with 10% gelatin solutions given intravenously to 24 hospital patients as a single injection of 500 ml. Blood and urine sample analyses over a period of 48 hours showed no significant results; amounts of only about 0.1 g/100 ml blood gelatin were observed after 48 hours. The authors suggested that further, similar studies should be performed under various clinical conditions in order to better understand the metabolism of gelatin. (The paper appeared in 1951).

A recent series of experiments (1970) were performed by Hueckel and Rogers (287) in five different animal species fed diets of gelatin

or amino acid supplements and mixtures. Urine analyses for free and peptide-bound amino acids were made. Human subjects given orally 30 g of casein or gelatin showed significant increases in urinary peptide-bound proline and hydroxyproline, and free hydroxylysine; no change in free protein or hydroxyproline was observed. Rats fed a diet of 20% gelatin plus 8% essential amino acids, or diets of 28% free amino acids, etc., excreted large quantities of free proline and hydroxyproline; a 50% gelatin diet yielded greatly increased urinary peptide-bound proline, hydroxyproline, glycine and aspartic acid. Hamsters fed 23-40% gelatin showed great increases of urinary peptide-bound proline and hydroxyproline. Dogs on diets of a 50% amino acid mixture or of 5% gelatin showed no change in urinary amino acids, free or bound. Monkeys, given diets similar to those of the dogs, also showed no significant urinary amino acid excretion; one monkey did show a five-fold increase in free, urinary hydroxylysine on the day it was fed the gelatin diet.

A modified fluid gelatin was transfused into 17 healthy dogs, replacing about 50% of the blood which was withdrawn, in experiments reported recently by Habif et al. (236). Over a period of 1-4 days, blood samples were analyzed for acid-base equilibrium, ionic and albumin concentrations, plasma gelatin, and rate of urinary excretion. Observations of the animals were continued for several weeks after the experiment. At 24 hours after transfusion, plasma volume was normal, plasma total protein and albumin decreases seen during transfusion rapidly returned to about 80% of normal values; about 10% gelatin remained in the plasma. After 4 days, total plasma protein and albumin values were about 90% of normal. The half-life of the gelatin in the plasma was 150 minutes. Coagulation studies, <u>in vitro</u> and <u>in vivo</u>, using albumin tagged with I^{131} were made with human blood samples, and indicated that the modified fluid gelatin did not alter coagulation time and was an efficient and safe plasma substitute.

MacKay and MacKay (418) in studies of the relationship of protein intake and the kidney weights of male albino rats fed various proteins, demonstrated that gelatin-fed animals grew poorly but showed significant increases in kidney size. Histological evidence from kidneys appeared normal, but the authors suggested that the kidney enlargement represented an early stage of renal damage due to gelatin.

The relationships of urinary excretion and tissue levels of ascorbic acid, and gelatin or gelatin plus essential amino acids fed to rats was the subject of experiments performed by Chandrasekhara et al. (92). Diets of 10 or 20% gelatin, or of 10% gelatin plus the amino acid equivalents of 10% casein, given rats for several weeks resulted in both urinary and liver ascorbic acid levels nearly as low as rats fed on a protein-free diet in those diets of 10-20% gelatin; the 10% gelatin plus amino acids, however, increased the ascorbic levels nearly two-fold. When only methionine and tryptophan were added to a gelatin diet, ascorbic acid levels increased; the increase was even greater when all missing (from gelatin) amino acids were added to the diet. Tryptophan increased urinary glucuronic acid levels; glucuronic acid is the first essential precursor in the synthesis of ascorbic acid.

An early study by Pryor and Knapp (563) demonstrated that gelatin diets given 70 college women, one ounce gelatin daily for several weeks and half an ounce for several more weeks, resulted in very small gains in weight and strength; strength and endurance were not, however, significantly affected by the gelatin diet. Ray et al. (579) had previously reported in a similar study, that women subjects on gelatin diets appeared to have their endurance unaffected by gelatin, but that men increased the amount of work done before fatigue occurred by 37-240%. Men presumably store creatine in the muscle (due to a creatinogenic action of the glycine). The doses of gelatin in this experiment were much larger than in that reported by Pryor and Knapp: men were given 60 g daily; the women, 45-67.5 g daily. Maison (425) refuted the results reported by Ray et al. from experiments using measurements of fatigue on a single muscle only, the extensor digitorum communis of the second and third fingers. Six male subjects were given intensive training for five weeks, after which they received daily doses of 60 g of unflavored gelatin. After a month on the gelatin diet, the muscular ability of the men continued to increase; during the next four weeks, without gelatin in the diet, the muscular abilities still continued to increase. No measurable influence of gelatin on improvement of muscular ability was seen, nor did the ability decrease when the gelatin was no longer added to the diet.

The Council on Foods report in a summary of 1936 (772) concluded that gelatin was a wholesome food when used as a supplement, particularly in soups or desserts, and was well tolerated in the diet; that it is an aid to the digestion of milk, has not been established, nor has gelatin value in treatment of some myopathies. Gelatin has no special significance as a source of amino acids, but rather is "notoriously deficient" in certain essential amino acids.

Eight years before the Council on Foods report appeared, Perlman (551) published the results of feeding gelatinized milk to a number of infants of from under a year of age to two years of age. The results indicated that gelatinized milk produced less vomiting and greater gains in weight than did cow's milk formulas. Perlman cited the ease of preparation of gelatinized milk, its palatability, inexpensiveness and easy retention as factors encouraging its use, particularly in difficult feeding cases among infants.

The early popularity of gelatin given orally as an hemostatic in preoperative applications where excessive bleeding could be anticipated, such as tonsillectomies, dental extractions, or surgery, was questioned by Tainter et al. in 1939 (679). Six healthy human subjects were given 15 g gelatin 3 times daily for 10 days to 2 weeks, and blood coagulation times were determined. The data from controls and experimentals were virtually indistinguishable; the authors concluded that gelatin had little or no therapeutic value as an hemostatic. (Reference to Dorland's Medical Dictionary, 17 Edition, 1937 - lists "Wright's hemostatic, 22 cc. of solution of formaldehyde and 40 cc of gelatin: used as a dental hemostatic").

GELATIN

Chemical Information

I. Nomenclature

A. Common Name

Gelatin

B. Chemical Names

1. Gelatin
2. Gelatin Sponge, Absorbable
3. Gelatin, Succinylated

C. Trade Names

Puragel
Burtonite Edible Gelatin (Burtonite)
Velvetex (Swift)

D. Chemical Abstracts Services Unique Registry Number

1. PM9000-70-8
2. 977005-08-5
3. 977050-78-4

II. Empirical Formula

Approx. $C_{105}H_{151}O_{39}N_{31}$

(From NAS/NRC information: "Burtonite Technical Data, 1970")

The following information is taken from Glicksman (209):

A. Amino Acids

Gelatin consists of 19 amino acids joined by peptide linkages to form long polymer chains. It gives a typical protein reaction and may be hydrolyzed by any of the proteolytic enzymes to yield its constituent amino acid or peptide components. Gelatin is not a nutritionally complete protein in that it is lacking in the essential amino acid, tryptophan. However, it contains a small amount of the rare amino acid hydroxylysine. Gelatins from different sources may exhibit small variations in amino acid composition, as shown in Table II.

The variations in chemical structure between the Type A and Type B gelatins produce differences in the physical properties, including the isoelectric and isoionic point.

TABLE II

AMINO ACID COMPOSITION OF DIFFERENT GELATINS

(GRAMS/100 GRAMS GELATIN)

Amino acid	Type A[a] (%)	Type B (skin)[b] (%)	Type B (bone)[c] (%)
Alanine	8.6 -10.7	9.3 -11.0	11.3
Arginine	8.3 - 9.1	8.55- 8.8	9.0
Aspartic acid	6.2 - 9.7	6.6 - 6.9	6.7
Cystine	0.1	None-Trace	Trace
Glycine	26.4 -30.5	26.9 -27.5	27.2
Glutamic acid	11.3 -11.7	11.1 -11.4	11.6
Histidine	0.85-. 1.0	0.74- 0.78	0.70
Hydroxylysine	1.04	0.91- 1.2	0.76
Hydroxyproline	13.5	14.0 -14.5	13.3
Isoleucine	1.36	1.7 - 1.8	1.54
Leucine	3.1 - 3.34	3.1 - 3.4	3.45
Lysine	4.1 - 5.2	4.5 - 4.6	4.36
Methionine	0.80- 0.92	0.80- 0.90	0.63
Phenylalanine	2.1 - 2.56	2.2 - 2.5	2.49
Proline	16.2 -18.0	14.8 -16.35	15.5
Serine	2.9 - 4.13	3.2 - 4.2	3.73
Threonine	2.2	2.2	2.36
Tyrosine	0.44- 0.91	0.2 - 1.0	0.23
Valine	2.5 - 2.8	2.6 - 3.4	2.77

[a] Neumann (1949), Graham *et al.* (1949), and Eastoe (1955).
[b] Tristram (1953) and Eastoe (1955).
[c] Eastoe (1955).

III. Structural Formula

Not Applicable

IV. Molecular Weight

Glicksman (209) states that the best values of collagen monomer molecular weight are in excess of 300,000. Since each monomer yields 3

gelatin chains or molecules, the molecular weight of gelatin should be greater than 100,000. The following diagram from Glicksman shows the possible paths of collagen conversion to gelatin:

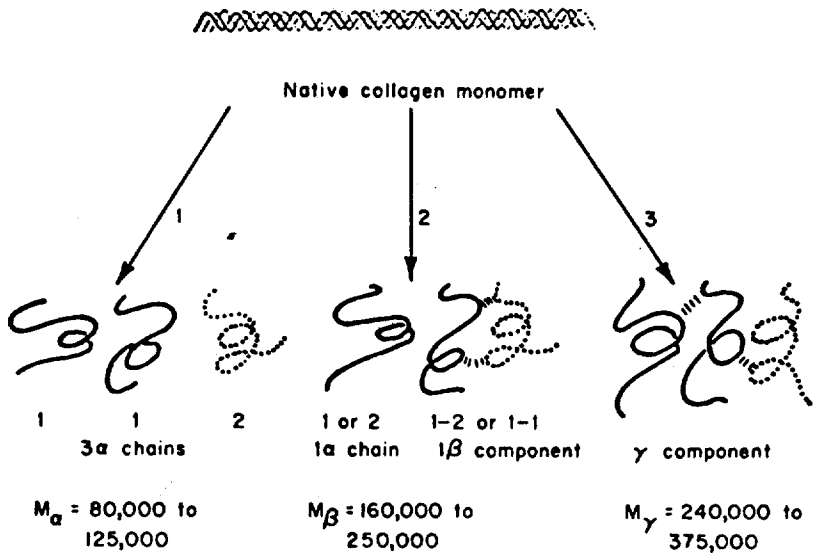


FIG. 2. Possible paths of collagen coversion to gelatin (Veis, 1964).

The molecular weights for gelatins given in the experimental studies reviewed in this monograph vary considerably, but were generally much less than the theoretical value noted above. Glicksman attributes peptide bond hydrolysis as an explanation for the lower molecular weights reported.

V. Specifications

From NAS/NRC: Gelatin, Grocery Type 1. Dried protein material obtained by water extraction from partially hydrolyzed collagen derived from animal tissues; conforms with the provisions of the Federal Food, Drug and Cosmetic Act; yellow-amber to slight gray powder free from lumps which do not break up readily under slight pressure; free from foreign flavors, off odors, and visible evidence of contamination.

Analytical requirements:

Property	Limits
Bloom	180-225 g
Viscosity	32-48 mp
Load weight	27.7 ± 2.0 lbs.
pH	4.3-5.9
Solubility	
Solution time	3 min. max.
Insolubles	Equal to or better than min. standard
Clarity	Equal to or better than min. standard Lot No. 03005
Flavor and odor	Equal to or better than min. standard Lot No. 6733003
Moisture	8-12%
Granulation U.S. 50 mesh	
On U.S. Screen No. 40	0.1% max.
On U.S. Screen No. 50	2.0% max.
On U.S. Screen No. 60	15.0% max.
Thru U.S. Screen No. 200	10.0% max.
Heavy metals	50 ppm max.
Arsenic	1 ppm max.

From NAS/NRC: Gelatin For Use In Frozen Cooked Beef Products.
Standard of quality:

Ash	2% max.
Nitrogen	15% min.
Insoluble solids in water	Absent
As	1.4 ppm max.
Cu	100 ppm max.
Pb	5 ppm max.
Zn	Absent
SO_2	250 ppm max.
Moisture	12-15%
pH	5.5-7
Bloom	125-175
Protein	82.5%
Total bacteria count	5000 per gram max.
Coliforms	negative in 10 g

VI. Description

A. General Characteristics

Colorless or slightly yellow, transparent, brittle, practically odorless, tasteless sheets, flakes, or coarse powder.

B. Physical Properties

Swells up and absorbs 5-10 times its weight of cold water; soluble in hot water, glycerol, acetic acid; insoluble in organic solvents. Glicksman notes two types of gelatin: type A, derived primarily from pork skin, by an acid processing; and type B, derived from primarily cattle hides and bone (ossein), by an alkaline or lime processing. Some of the physical properties are shown below:

TABLE I
CHARACTERISTICS OF GELATIN

Characteristic	Type A (pork skin, acid treated)	Type B (ossein or calfskin, lime treated)
Moisture	8-12%	8-12%
pH	3.8-5.5	5.0-7.5
Isoelectric point (pH)	7.0-9.0	4.7-5.1
Gel strength (Bloom)	50-300 g	50-275 g
Viscosity	20-70 mps	20-75 mps
Ash	0.3% (mainly Na^+, Cl^-, SO_4^{2-})	0.5-2.0% (mainly Ca^{2+}, PO_4^{3-}, Cl^-)

Details of the physical properties of gelatin can be found in Glicksman, Chapter 11, pages 370-373, particularly with regard to the determination of gel strength or Bloom; the protective colloid action of gelatin which is most effective; and the ability of gelatin to stabilize emulsions.

C. Stability

From NAS/NRC information:

The product shall be conveyed (50 lb.) in 3 ply kraft paper bags. The bags are to be sealed top and bottom with hot melt adhesive or sewn full width at both ends such as to protect the contents from extraneous solids and from loss of the product under normal conditions of handling, shipment, and storage.

Gelatin from protective inventory (1 year or more) shall be conveyed in 300 lb. fiber drums containing 4 mil polyethylene liners. Each liner shall be tied and each drum shall be well closed and properly sealed.

Packaging materials shall not transmit contaminants or objectionable substances to the product and shall conform to all applicable food additive regulations.

Storage

The product shall be stored at room temperature carefully protected from moisture, off odors and other contaminants.

VII. Analytical Methods:

A qualitative test may be found in Methods of Analysis, AOAC (774) section 15.042.

VIII. Occurrence

The following information is taken from Advances in Food Research, Vol. VII, 1957 (775):

a. Raw Materials. The essential raw material for gelatin is collagen, the principal protein of connective tissue. The major supply of collagen is procured from hides and bones. Muscle tissue, horns, and hoofs are of no value. Hide is, of course, so valuable to the tanner that only those parts which cannot be used find their way to the gelatin manufacturer. The actual materials consist of the leavings of tanneries and slaughter houses, i.e., trimmings, pates, fleshing, cheek-pieces, etc. The stock may be either "dry" or wet and is sometimes degreased and dehaired. The hides are composed primarily of splits and calfskin. When hides are removed from cattle they are run through a splitting machine. The outside of the hide, which is marked with the characteristic grain, is used for the manufacture of leather. This is the so-called top-grain product. The inside portion of the hide does not contain the grain and this is known as a split, and may be obtained in the form of fresh splits, dried splits, and cured or pickled splits. In the United States, a large amount of fresh pigskin is processed which arrives at the gelatin plant in a frozen state. In addition to the species, the age and condition of the animal are of importance.

If green or wet stock is not going to be used within a relatively short time it must be preserved to prevent putrefaction. Salt, lime, and desiccation are most commonly used. However, most stock is immediately "conditioned" by either an alkaline or acid process, after a preliminary water wash to remove dirt and blood, which may interfere with the color of the finished product. The bulk of gelatins are "limed" or treated with a suspension of calcium hydroxide.

Most of the bone is furnished by South America and India; the hide pieces, by the United States, South America, and England; the frozen porkskin is used only in the United States. In Europe, 20% to 35% more gelatin is produced from ossein than from hide pieces. The production of ossein in the United States is small.

Glicksman observes that in 1965, about 40-45 million pounds of edible gelatin were sold in the United States for use in foods; some estimates have even been as high as 50 million pounds used in desserts, confections, meat products, ice cream, and other foodstuffs. It has been further estimated that the foregoing amounts of gelatin comprise about two-thirds of the total production, with the remaining gelatin being used in pharmaceutical applications (10%), photographic uses (20%), and about 5% in specialized industrial application.

The pharmaceuticals using gelatin include tablets, pill-coatings, capsules, suppositories and numerous preparations utilizing the protective-colloid characteristic of gelatin. Details of gelatin use in foodstuffs may be found in Glicksman under headings: gelatin desserts, confections (as marshmallows, etc.), ice cream, dairy products, meat products, bakery goods, food coatings, flavor fixation and miscellaneous.

Biological Data

I. Acute Toxicity

None

II. Short-Term Studies

Rat

Jackson et al. (298) made a comprehensive study of the nutritional value of gelatin following earlier evidence that the simple addition of the three conspicuously absent amino acids, cystine, tryptophan and tyrosine did not serve to render gelatin an adequate source of dietary nitrogen. Not only the conspicuous amino acid deficiencies were compensated for in the experimental diets, but other factors as well were incorporated in the studies. Comparative amino acid composition of casein and of gelatin suggested the addition of other amino acids to the gelatin diets: valine, hydroxyglutamic acid, and isoleucine. Glycine and alanine which abound in gelatin were added to casein diets. The influence of the metabolism of gelatin on appetite was tested by feeding gelatin as a third of the total diet which already included an abundance of nitrogen. The effect of a complete hydrolysis of gelatin before including it in dietary studies was undertaken to demonstrate a possible existence of amino acid linkages in gelatin that were not amenable to cleavage by digestive enzymes.

White rats of at least 100 g weight were first tested on a casein diet for growth behavior and then transferred to various diets of compositions shown in table on the following page.

TABLE I.

Composition of Diets.

Diet	A.	B.	C.	D and E.*	F.	G.	H and I.*
	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Casein	18.0	18.0	18.0				
Gelatin			35.0	35.00	30.00	25.00	15.00
Dextrin	34.0	22.0		15.30	16.30	20.90	24.00
Sucrose	15.0	15.0	14.0	15.00	15.00	15.00	15.00
Salt mixture	4.0	4.0	4.0	4.00	4.00	4.00	4.00
Agar	2.0	2.0	2.0	2.00	2.00	2.00	2.00
Cod liver oil	5.0	5.0	5.0	5.00	5.00	5.00	5.00
Lard	22.0	22.0	22.0	22.00	22.00	22.00	22.00
Tyrosine (*l*)				0.90	0.90	0.90	0.90
Cystine (*l*)				0.50	0.50	0.50	0.50
Tryptophane (*l*)				0.35	0.35	0.35	0.30
Histidine monochloride (*l*)					0.26	0.32	0.40
Valine (*dl*)					2.70	2.70	4.00
Phenylalanine (*dl*)					0.60	0.80	2.00
Glutamic acid (*d*)					2.10	2.40	4.00
Aspartic " (*l*)							1.00
Leucine (*dl*)							1.33
Isoleucine (*dl*)							1.33
Norleucine (*dl*)							1.33
Glycine		9.0					
Alanine (*dl*)		6.0					

* Diets E and I contained hydrolyzed gelatin but in other respects were exactly like Diets D and H respectively. With the exception of Diets E and I, all of the gelatin diets contained the protein in an unhydrolyzed form.

Vitamin B was given daily to promote growth. The gelatin was a special product of the Wilson Laboratories, Chicago and was added to the diets either unhydrolyzed or hydrolyzed (Diets E and I), at a 35% level in most experiments.

Of 26 rats given the Diet D which was a gelatin diet supplemental with tyrosine, cystine and tryptophan , 13 died at intervals of 9 to 48 days from nutritive failure; autopsies showed badly deteriorated kidneys. Renal failure was probably responsible for early deaths, rather than nutritive failure, directly; gelatin appeared to be the causitive factor. The 13 animals which survived Diet D usually lost weight rapidly but subsequently improved, though probably suffering kidney injury.

Diets F and G containing 30 and 25% gelatin plus the three amino acids of Diet D and four more, histidine, valine, phenylalanine and glutamic acid were given to four rats each. The resulting growth curves were no better than those of Diet D animals; 2 died within 16 days, 3 lost weight so rapidly that they were taken off the diet, and the remaining 3 animals slowly recovered. One of

the latter and 3 others from a casein diet were transferred to Diet H, gelatin 15% and 11 amino acids (leucine, isoleucine norleucine, and aspartic acid added to the previous 7). These animals made slow but consistent weight gains. Efforts to improve the quality of the diets by increasing the gelatin concentration up to 55% resulted in 2 of 3 rats tested promptly dying and the third becoming moribund. The experiment was terminated.

Diets B (casein, glycine and alanine) and C (casein and 35% gelatin) given to 2 and 3 rats respectively, resulted in weight losses followed by recovery and rapid growth, demonstrating that neither gelatin nor a mixture of two of its component amino acids added to an otherwise normal diet permanently prevents growth. Temporary inhibition of growth may result from gelatin as a large proportion of the diet, however, proving detrimental as well as inadequate as a protein.

Diets E and I, with hydrolyzed gelatin at 35 and 15% levels proved very little better in growth promotion than did the corresponding diets D and H with unhydrolyzed gelatin. This would tend to deny the proposition that amino acid linkages, not subject to digestive cleavage, exist in gelatin and serve to explain the nutritive inadequacy of the protein.

There appeared to be no clear indication in the experiments that the amount of food consumed determined weight charges. Appetite failed after, not before - failure in growth. Renal involvement and not amino acid deficiency was suggested by the authors as the immediate cause of death in many of the foregoing experiments; it appeared that some deleterious action is exerted by gelatin, particularly at a 35% level in the diet.

Dog

The impairment of blood-protein production in dogs given gelatin intravenously was reported by Robscheit-Robbins et al. (591) in experiments designed to demonstrate the good and bad qualities of gelatin as a plasma protein substitute. Most of the dogs used experimentally were from an anemia colony of long standing, representing a white bull terrier and coach strain; other animals were mongrels. All animals were maintained on a very low protein diet, and before gelatin treatment were doubly depleted (anemic and hypoproteinemic) by blood removal, a non protein diet and abundant iron intake. The gelatin used was bone collagen, given variously as about 6% solutions in 0.9% NaCl; as degraded gelatin; or in a dry, granular form. Some gelatin of unknown origin, a commercial brand, was also employed. Vitamin additions, amino acid preparations and a casein digest were also variously included in the diets.

The gelatin solutions were injected by the drip method in about an hour's time; in some cases, the amino acids given were dissolved in saline and administered subcutaneously. Urinary nitrogen studies were made in many experiments; hemoglobin levels and plasma protein levels were also determined.

Twelve separate experiments were described in considerable detail as may be seen by reference to the document included in this monograph. A summary of the toxic effects of intravenous gelatin is presented in the following table:

Toxicity of Gelatin by Vein

Dog No.	Table No.	Gelatin total intake	Weight	Gelatin intake per kilo	Type of gelatin molecular size	Toxicity
		gm.	kilo	gm.		
43-24	1	108	8.7	12.4	Large	Death
40-34	2	99	15.5	6.4	Large	+
41-50	3	118	17.6	6.7,	Large	0
37-82	3	49	15.0	3.3	Large	++
40-32	8	20.6	17.9	1.2	Large	0
40-32	8	24.2	18.1	1.3	Large	0
40-32	4	272	16.2	16.8	Large	++
37-87	5	147	16.0	9.2	Small	Death
37-85	6	144	14.1	10.2	Small	+
41-53	9	33.4	12.4	2.7	Small	+
43-250	9	36.3	15.1	2.4	Large	0
41-50	8	36.3	19.1	1.9	Large	0

The summary of the significant overall findings from the studies follows:

Gelatin given by vein to doubly depleted dogs (anemic and hypoproteinemic) gives no immediate toxic response, no anaphylactoid reactions, and may contribute something to the building of new hemoglobin and plasma protein.

Gelatin given by vein during 1 to 2 weeks (total 3 to 17 gm. per kilo) usually causes serious disturbances--inhibition of blood protein production, signs of intoxication, much weight loss, and even death.

Gelatin given by vein for 2 to 3 days (total 1 to 3 gm. per kilo) may not cause any recognizable abnormalities, but dogs vary greatly in their response to gelatin by vein. Some dogs may tolerate a total of 7 gm. per kilo without significant disturbance and other dogs may be seriously intoxicated by 2 to 3 gm. per kilo. No one can predict which animal will be least tolerant.

Some experiments with gelatin by vein for 2 to 3 days (total gelatin 1 to 2 gm. per kilo) given with and followed by amino acids or casein digests do show absence of intoxication and ample production of new hemoblobin and plasma protein during the weeks following the

injection of gelatin. This may suggest possible usefulness of gelatin with amino acids or casein digests in acute emergencies (shock, hemorrhage).

These doubly depleted dogs are very susceptible to various injurious agents as compared to normal dogs. They may serve as sensitive testing machines for evaluating plasma substitutes.

Where the gelatin by vein inflicts its damage is not clear and there is little if any significant histological evidence but the disturbance of blood protein production implicates the liver.

Gelatin of smaller molecular weight (degraded by autoclaving) is no less toxic than the standard gelatin.

Gelatin by mouth may contribute to but cannot alone support the production of new hemoglobin and plasma protein.

Gelatin by vein has definite limitations in dogs and, by implication, when used in human cases the amount given should be very carefully watched.

III. Long-Term Studies

None

IV. Special Studies

Carcinogenicity

The following data are taken directly from "Survey of Compounds Which Have Been Tested For Carcinogenic Activity", HEW Public Health Service Publication No. 149, 1969: 1968-1969 Volume and Supplements 1 and 2:

829. GELATINE, AQUEOUS

REFERENCE	ANIMAL	STRAIN OR TYPE AND SEX	PREPARATION AND DOSE	ROUTE AND SITE	PATH EXAM LEVEL	ANIMALS WITH TUMORS								DETAILED INFORMATION	SURVIVAL	DURATION OF EXPERIMENT
						TOTAL T.B.A.	HEMAT.	SKIN	S.C.	MAMM.	RESP.	G.I.	LIVER	OTHER			
Ball, J.K., 1968.	66 mice	CFW Swiss, newborn.	1%, vol n.s., 1 x.	S.C. Inter-scap	3	1.5%	*								1.5% thym lymph at 160 d.		160 d.
	94 mice	CFW Swiss, newborn.	As above, plus bone marrow cells 8-10 x 10^6 (from DMBA ttd donors).	S.C. Inter-scap	3	0									0 thym lymph.		
Gorrod, J.W., et al., 1968.	100 mice	Swiss (Porton), M, F, newborn.	0.02 ml 1/d x 3.	S.C. Inter-scap	3		*				*		*		1/88 thym lymph. 1/88 lung aden. 5/41 (M), 2/47 (F) hep.	41 (M), 46 (F) at 48 wk, sacr 48-52 wk.	52 wk.
Adrian, R.W. and John, U., 1969.	78 mice		0.05 ml 1% 1 x.	S.C.	3	7					*				7 lung aden.	38 at 1 yr.	1 yr.

Antigenicity

In response to questions regarding the antigenicity of gelatin, which is generally regarded in its edible form as nonantigenic, Ratner and Crawford (575) conducted a series of experiments in which anaphylaxis induced in guinea pigs, and the Schultz-Dale technique on isolated tissue, were used to test both the precursors of gelatin and various grades of gelatin itself.

Normal guinea pigs, weighing about 250 g; having never been fed beef or gelatin, were used as test animals. Sensitization and shock procedures for anaphylaxis testing employed a comminuted ossein prepared from bovine bone; crude gelatin extracted from the ossein; food-grade (Knox Sparkling) gelatin; and highly purified intravenous-grade gelatin. Bovine plasma was variously used as either the sensitizing or shock injection alternately with one of the ossein or gelatin preparations; usually, sensitization was done intraperitoneally and shock, intravenously. Six anaphylaxis experiments in all were performed and the author's summary is presented in the following tables:

I. (See Table VII.) Comminuted ossein was found to sensitize against bovine plasma in fourteen of twenty-five animals (56 per cent). Food-grade gelatin, refined from comminuted ossein, sensitized only three of forty-five animals (6.6 per cent) when challenged with bovine plasma. In none of thirty-two animals did intravenous-grade gelatin sensitize against bovine plasma.

TABLE VII. COMPARATIVE REACTIVITY OF CONTAMINATING BOVINE BLOOD ELEMENTS IN CRUDE AND REFINED GELATINE WHEN USED AS A SENSITIZING AGENT

NUMBER OF ANIMALS	SENSITIZATION	SHOCK	RESULTS		
			POSITIVE	NEGATIVE	PER CENT POSITIVE
25	Comminuted ossein	Bovine plasma	14	11	56.0
45	Food-grade gelatin	Bovine plasma	3	42	6.6
32	Iv.-grade gelatin	Bovine plasma	0	32	0.0

II. (See Table VIII.) In none of 141 animals sensitized with bovine plasma, comminuted ossein, crude gelatin, food-grade gelatin, and intravenous-grade gelatin was there any reaction when the animals were challenged with any of the forms of unrefined or refined gelatin.

TABLE VIII. NONREACTIVITY OF CRUDE AND REFINED GELATIN WHEN USED AS ANAPHYLACTIC SHOCK AGENTS

NUMBER OF ANIMALS	SENSITIZATION	SHOCK	RESULT
14	Comminuted ossein	Iv.-grade gelatin	0
9	Crude gelatin	Crude gelatin	0
19	Bovine plasma	Crude gelatin	0
19	Food-grade gelatin	Food-grade gelatin	0
22	Bovine plasma	Food-grade gelatin	0
30	Bovine plasma	Iv.-grade gelatin	0
10	Iv.-grade gelatin	Iv.-grade gelatin	0
18	Bovine plasma	Comminuted ossein	0

III. (See Table IX.) Whether the intravenous-grade gelatin was used as a sensitizing injection or as a shock injection, in no instance in fifty-eight animals was there any sensitization or shock manifested, even when they were cross-tested with bovine plasma.

TABLE IX. ABSENCE OF ANY ANAPHYLACTOGENIC REACTIVITY OF INTRAVENOUS-GRADE GELATIN

NUMBER OF ANIMALS	SENSITIZATION	SHOCK	ANAPHYLAXIS
14	Comminuted ossein	Iv.-grade gelatin	0
24	Bovine plasma	Iv.-grade gelatin	0
10	Iv.-grade gelatin	Bovine plasma	0
10	Iv.-grade gelatin	Iv.-grade gelatin	0

Schultz-Dale experiments were conducted using the uterus taken from virgin female guinea pigs previously sensitized to comminuted ossein. The uterine muscle, immersed in oxygenated Tyrode's solution, when challenged by food-grade gelatin showed no evidence of antigenicity; challenged by bovine plasma, however, the muscle contracted vigorously, supporting the findings of the anaphylactic experiments. Ratner and Crawford concluded that, within the limits of their experiments, gelatin is non-anaphylactogenic; any allergenicity attributed to gelatin in man is due to contamination by blood elements of the species from which the gelatin is derived. The authors disagreed with the statements made by Maurer that antibodies found in man by that investigator demonstrate the antigenicity of gelatin. (A review of Maurer's paper is presented next.)

A highly purified form of gelatin that has been modified to remain liquid at room temperature, to serve as a plasma expander, is called oxypolygelatin (OPG). Maurer (433) studied the antigenic properties of clinical OPG (molecular weight about 27,000), a fractionated, high molecular weight OPG (molecular weight, 36,000) and a gelatin from which OPG was prepared (mol. wt. 50,000 - 75,000). The initial experiment with clinical OPG used 20 healthy medical volunteers in four groups of five each. A preimmunization sample of 50 ml of blood was first taken and each subject then received two intracutaneous injections of about 0.01-0.02 ml of the OPG preparation; skin reactions were read 15 minutes later. Each group received nine daily injections of clinical OPG containing 2, 5, 10 or 25 mg protein per ml. of solution. A second 50 ml sample of blood was taken 3 weeks after the last injection, and intracutaneous tests performed; 500 ml samples of blood were obtained from the test subjects who showed good antibody responses. The pre- and postimmunization blood sera were assayed for antibody nitrogen by a quantitative precipitin method. The results are shown in the following table:

TABLE I

Response of Humans to Injections of Clinical Oxypolygelatin S 230 X

Subject	No.	OPG solution injected (9 injections)	Preinjection		3 weeks post injection	
			μg. antibody N precipitated per ml. of serum from 3 ml. serum treated with			
			3 μg. OPG N	9 μg. OPG N	3 μg. OPG N	9 μg. OPG N
		mg./ml.				
DAV	1	2	0.0	0.5	0.4	4.4
DJF	2	2	1.0	2.0	—	12.2
CAB	3	2	0.2	1.0	1.5	4.6
JZ	4	2	1.3	2.7	2.0	6.0
JAC	5	2	0.3	0.8	2.2	3.9
DGC	6	5	1.8	3.3	3.3	7.5
JHA	7	5	0.8	3.4	0.8	2.9
NS	8	5	0.7	3.1	1.9	7.5
HJ	9	5	1.1	6.7	1.9	4.7
PHM	10	5	0.0	0.0	2.2	3.6
LH	11	10	0.1	1.3	1.5	2.7
TCH	12	10	0.8	3.1	2.3	4.3
WJH	13	10	0.6	6.4	—	10.3
RNL	14	10	0.0	3.4	0.8	3.3
CHL	15	10	0.6	3.6	1.4	—
SW	16	25	0.8	2.0	0.0	—
RAP	17	25	0.8	3.1	0.1	3.7
TBL	18	25	0.5	—	3.6	—
RRB	19	25	1.1	4.0	3.5	9.4
JRL	20	25	0.2	0.2	—	—

A similar experiment was conducted; repeating the clinical OPG immunizations and performing immunizations with high molecular weight OPG, and gelatin. In general, gelatin precipitated much more antibody than clinical OPG, and surprisingly, antibody was found in pre-immunization sera. The effectiveness of the three antigens in producing antibodies ranked gelatin first, fractionated OPG second and clinical OPG last. The report concludes with a lengthy discussion by Maurer in which, among other points, the author discards the explanation of impurities in the gelatins as being responsible for the antibodies found, and suggests two possibilities for the pre-immunization antibodies: the diet, which frequently contains gelatin; or enzymatic action on the collagen of the body's connective tissue. Since no skin reactions (wheal or erythema) were observed after injection of the antigens (gelatin and derivatives), the possibility that some antibody molecules do not function in the protective mechanism, but rather in a metabolic system, was noted.

Allergology

T. G. Randolph (571) reported briefly on gelatin as an allergen in several cases involving beef sensitivity in patients who suffered acute allergic symptoms during food tests with beef. In view of the common use of beef gelatin solutions as plasma substitutes, Randolph subjected four beef-sensitive patients to experimental ingestion of Knox Gelatin solutions. In one test, 2 doses of 1/4 oz. gelatin taken an hour apart produced severe reactions in 3 of the 4 patients, including nasal stuffiness, cramps, tinnitus, nausea, diarrhea, etc. One case showed no reaction, as did four patients known not to be beef-sensitive. Gelatin, prepared for intravenous use, was given as 150 ml orally, followed an hour later by 75 ml; the reactions in three of the four beef-sensitive test patients were the same as before; the patient who had reacted so violently to the first test was not tested in the second experiment. Tests on the beef-sensitive patients with commercial porcine gelatin showed no symptoms; pork-sensitivity did not exist.

Mendez and Hughes (445) reported three cases illustrating different types of sensitivity to meat and derivatives, including a case of sensitization to gelatin. A young woman of 22 years suffered from urticaria and angioneurotic edema associated with meals of boiled and stewed meats, soups and gravies made from meat stock. Ice cream and jellies also caused the allergic symptoms. A series of extracts of gelatin and various gelatin-containing foods all gave positive allergic reactions; hyposensitization with gelatin caused reduction of the skin reaction and disappearance of clinical symptoms. Passive transfer tests were performed with serum from a blood sample of the young lady and demonstrated the presence of circulating skin-sensitizing antibodies. Further tests in 1000 allergic cases showed only about 1 in 150 with positive skin reaction to gelatin and clinical sensitivity of 1 in 500. Gelatin would appear to be a rare allergen in many, and has been shown in other studies unable to give rise to anaphylaxis in animals.

Plasma Substitutes

Vander Brook et al. (715) experimented with dogs to observe the toxic effects of gelatin solutions proposed as plasma substitutes for use in transfusions.

Nine mongrel dogs, supplied with a high protein diet supplemented with raw liver were bled of 29 to 54% of their estimated blood volume at weekly intervals for periods of 2 to 13 weeks (average 7 weeks). The blood was replaced at the same time by infusion of 5% gelatin solution in amounts equal to 80% of the blood removed. Control animals consisted of 2 dogs bled as severely as the experimentals, but without parenteral fluid replacement, and 20 apparently healthy dogs with no treatment. Observations at autopsy revealed no gross pathology, except for scar tissue and thrombi in some instances of

the sites of bleeding and gelatin infusion. Histological examination of the kidneys of gelatin-infused dogs showed, in some instances, slight departures from normal; the epithelial cells of the tubules of the proximal convolutions were sometimes swollen and occasionally vaucolated. An increase in sudanophil droplets in Kupffer's cells was noted. Gelatin did not appear to be stored in liver or kidneys and was excreted by the kidneys.

The use of gelatin as a blood substitute in medical practice and the possible pathological changes produced were the subject of a report by Skinsnes (641).

Gelatin was given to 23 humans who finally expired from medical or neoplastic disease or from complications of therapeutic surgical measures. Gelatin was given intravenously in quantities ranging from 100 to 17600 cc with widely varying intervals between the final injection and death of the patient. Postmortem examinations were performed 17 1/2 hours after death and in the majority of cases the postmortem interval was 2 to 10 hours. A study was made to evaluate the role of gelatin in the pathogenesis of renal lesions, the morphological nature of these changes and to appraise their functional significance. The author considers that the renal hydropic changes observed in these patients demonstrate an etiologic role of gelatin. These changes are morphologically similar to those produced by the intravenous injection of sucrose. Gelatin nephrosis is shown to be a reversible change occurring within 1/2 hours after intravenous injection of gelatin and disappearing within 120 hours following the last injection. Caution is suggested in the use of gelatin in patients with previous renal impairment. Three cases are reported as suggesting that gelatin was a potential precipitating factor in coronary occlusion and vascular thrombosis in the presence of circulatory disturbances and vascular disease.

Znamenskaya (765) published in 1968 a literature review and chronology of the status of gelatin plasma substitutes during the period from the earliest use in 1915, throughout the following half century. An autoclaved, partially hydrolyzed form of 4-8% osseous gelatin, in isotonic NaCl solution, was extensively used during the WW II period as a blood plasma substitute with considerable success. In 1951, an improved form of gelatin with glyoxal (oxypolygelatin, or OPG) was introduced. In the mid-1950's, a modified fluid gelatin (MFG) was developed by the treatment of gelatin with decarbonized acids or their anhydrides and the plasma substitute soon appeared under various international trade names; particular note is made of a Merck preparation, "Blutersatz G", prepared from 6% gelatin, methylglucosamine and 2% fructose. Up to this point, the gelatin plasma substitutes were heterogeneous in structure, consisting of peptide chains ranging in molecular weight from 10,000 to 100,000. In 1962, the creation of an homogeneous gelatin plasma substitute was reported, comprised of only peptides with a molecular weight of 12,000, synthesized

into a polymer of three polypeptides making up a lattice structure of a molecular weight of 35,000. The Russian term for this plasma substitute is "gemaktsel"; it appeared in Europe under a number of trade names, and has been indicated as being absolutely harmless in many applications.

A Ringer-Lokk salt solution containing colloidal iron and 1% gelatin was described, "ferrofusine" - which under clinical conditions has proved useful. A Russian preparation, "gelatinol", composed of 8% partially hydrolyzed edible gelatin with 0.9% NaCl, and of heterogeneous molecular weights, was described in considerable detail regarding applications; this plasma substitute was created in 1960 by the Leningrad Institute of Hematology and Blood Transfusion, with whom the author, Znamenskaya, was associated.

It is of some interest to note that the new gelatin plasma substitutes were tested for medical effectiveness in experiments in complex treatment of acute radiation sickness in dogs given doses of 600 roentgen over several days' time. Gelatin solution injected as 100 ml/kg body weight, together with bone marrow transplants, reduced significantly the acute radiation effects; controls without treatment yielded an LD_{100} at the dose of 600 r.

Biochemical Aspects

I. Breakdown

None

II. Absorption - Distribution

Nixon and Mawer (511) reported the results of studies with six human experimental subjects to determine the rate of release of free amino acids in the intestinal contents following a protein test meal and whether the amino acids leave the intestinal lumen in the free form or not. Three different test meals were used: milk-protein; gelatin; and low-protein. Samples of the intestinal contents were taken at various levels along the tract and at various intervals following ingestion of each diet. Centrifugation and gel filtration were employed to fractionate the intestinal samples into four fractions. The large molecules of the protein in the test meals were rapidly broken down within the intestinal lumen; by 15 minutes after ingestion of the meal the distribution of the various components within the intestinal samples was similar to that found in samples taken 2 hours later. Arginine, lysine, tyrosine, valine, phenylalamine, methionine and leucine were presumably absorbed in the free form; glycine, threonine, serine, the amino acids and dicarboxylic amino acids were not indicated as being absorbed in the free form. The authors suggested that experiments in which amino acid mixtures, simulating a dietary protein; are fed to experimental animals to determine the rates of amino acid absorption, do not present a true picture of the events in the small intestine following the ingestion of a protein meal.

III. Metabolism and Excretion

Nutrition

The poor performance of amino acid-supplemental gelatin diets was suggested by Ashley and Fisher (26) to be due to peculiarities in the amino acid balance and/or differences in composition from reported values. Experiments using 5 day-old chicks and carefully calculated diets were conducted; body weights and feed utilization were measured over 2 or 3-week growth periods. The amino acid composition of gelatin as analyzed by Ashley and Fisher is shown in the following table compared with the data from other studies:

TABLE 1.—*Amino acid composition of gelatin*

	Present Analysis gm./16 gm. N	Block and Weiss (1956) gm./16 gm. N
Arginine	6.81	7.7
Glycine	24.66	21.9
Histidine	0.56	0.8
Isoleucine	1.41	1.6
Leucine	2.85	3.2
Lysine	3.18	4.0
Methionine	0.75	0.9
Cystine	0.11	0.2
Phenylalanine	0.79	2.2
Tyrosine	0.52	0.9
Threonine	1.76	2.6
Valine	2.35	2.8
Glutamic acid	10.24	10.1
Proline	12.41	12.8
Hydroxy proline	10.84	12.4
Serine	3.01	4.0
Aspartic acid	5.71	5.7
Alanine	9.22	9.1

Five separate experiments, with a variety of diets and combinations of gelatin, amino acid supplements, soy-protein, casein and fish protein, gave results which indicated that a 10% gelatin plus an amino acid mix of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, aspartic acid, cystine, glutamic acid, serine and tyrosine plus additional amounts of lysine, threonine and methionine gave a balanced protein diet that was quite satisfactory. The key amino acids required in a gelatin diet as supplements were lysine and threonine (in lower amounts in gelatin than previously calculated), and methionine which has a higher requirement due to the high arginine and glycine content of gelatin favoring creatine synthesis, creating a 'stress' for additional methionine.

Nilson and Lemon performed nutrition studies with algin and gelatin in rats (508); the gelatin studies are reported here.

Male albino rats with an average initial weight of 53 to 54 g were fed diets containing 5, 10, 20 or 30% gelatin for 10 weeks. Each experimental group and the control contained 6 animals. The average daily gain in weight varied from 0.99 g for the rats on 30% gelatin to 3.99 g for the control rats. The stunting of growth was marked at the 30% gelatin diet, slight less so at the 20%, and only minor at the 10 and 5% levels. All animals survived the experimental period.

Downey (142) presented a very detailed study of the nutritive value of gelatin in a paper published nearly fifty years ago. Numerous investigations on the direct utilization of gelatin in tissue building and conservation performed during the first twenty years of the 1900's were noted by Downey, as were the colloid-chemical aspects of gelatin. His own experiments were of two types: investigations of the supplementary relations between gelatin and other protein foodstuffs; and studies of the influence of the colloidal property of gelatin on the utilization of some dairy products. A total of 8 feeding experiments with white rats were performed, each experiment consisting of 2 groups of several animals each given various test diets. In the first 4 experiments, the basic diet was properly constituted for promoting well-being and growth except for protein which was regulated at too low a level to promote normal growth; in the last 4 experiments small amounts of gelatin were added to various dairy products. Each experimental diet and results are summarized:

No. 1, group 1, gelatin plus tryptophan , cystine and tyrosine; group 2, gelatin only. Group 1 growth better, but subnormal; one rat from group 2 died.

No. 2, group 1, pearled barley plus gelatin; group 2, barley only. Group 1 growth nearly normal; group 2 growth very poor.

No. 3, group 1, white wheat bread 97.5% of diet; group 2, bread 92.5% plus gelatin 5%. Group 1 growth subnormal; group 2 growth normal.

No. 4, group 1, rolled oats 60% plus gelatin 5%; group 2, rolled oats 60%. Group 1 growth normal; group 2 growth subnormal.

No. 5, group 1, cow's whole milk 100%; group 2, milk 99% plus gelatin 1%. Addition of 1% gelatin to diet increased availability of nutrients from milk to a marked degree.

No. 6, group 1, cow's whole milk 95%, egg 5%; group 2, milk 94%, egg 5%, gelatin 1%. Group 1 growth subnormal, females sterile; group 2 growth and reproduction normal.

No. 7, group 1, ice cream formula without stabilizer; group 2, same formula plus 1% gelatin. Group 1 growth slightly below normal, females sterile; group 2 growth and reproduction normal.

No. 8, a variation of No. 7, group 1, 0.5% gelatin added to ice cream mix; group 2, 0.75% gelatin plus ice cream mix. Group 1 growth normal but female reproduction below normal; group 2 both growth and reproduction normal. The gelatin given group 2 was a low jelly-strength, degreased green bone gelatin. The author suggested that skin-stock gelatin (used in all other diets) had improved nutrition, but that green-bone gelatin contained a substance that favorably improved reproduction.

There follows a lengthy discussion by Downey of experimental results and he concludes that gelatin lacks properties necessary in a

major source of protein, but as a supplement with various natural foods it has value, particularly in dairy products.

One measure of the metabolism of the purine bases of nucleic acids is in the quantity of allantoin eliminated; diets deficient in one or more essential amino acids result in a lowered allantoin excretion, regardless of which essential amino acid is missing. Ricceri (586) performed studies on the purine metabolism of rats fed diets deficient in essential amino acids: zein, a protein from maize is deficient in lysine and tryptophan, and argine and histidine are present only slightly; gelatin from animal hides and bones is deficient in tryptophan and cystine; with methionine, histidine, tyrosine and isoleucine present in small amounts. For the purpose of this monograph, only the gelatin studies are described.

Male adult albino rats weighing 200-350 g were used in 2 experiments lasting 62 days each; 2 rats having the same body weights were used in each experiment. The diet to begin and end with contained casein as the protein which was replaced by gelatin during the deficiency studies. Each experiment consisted of 4 stages: the 1st and 4th were controls; the 2nd, the deficiency study; and the 3rd stage was supplemented by various amino acids, - tryptophan, cystine, leucine, isoleucine, methionine and tyrosine, each to make up 5% of the protein in the diet. The urine was collected and analyzed for allantoin, uric acid, total nitrogen and creatinine. During the 2nd stage, with gelatin only, allantoin decreased, slowly rising when both cystine and tryptophan were added as supplements during the 3rd stage; allantoin excreted during the casein, 4th stage returned to normal amounts. Uric acid and creatinine, as well as the total nitrogen excreted followed much the same sequence; the charts on the following page show part of the results for two of the rats from each experiment:

The author presents a view of the biochemical mechanism in explanation of the effects of a diet deficient in essential amino acids and the subsequent slowdown of the nucleic acid metabolism. It is not due to a decreased synthesis of the enzyme systems related to the metabolism of nucleic acids, but rather to the fact that the absence of a single amino acid reduced the synthesis of the entire nucleoprotein molecule, slowing down the anabolism and successive catabolism of nucleic acids.

The supplementary effect of proteins in the diet of rats was studied by Ellis and Rose (153). Gelatin, gliadin and casein were added to the diet at a 5% level together with a mixture of amino acids; 3 rats were used in each diet for about 36 to 45 days. All animals showed weight losses during the first four days, after which slow growth was observed. The gelatin-fed animals showed the least growth, gliadin slightly better, and casein-fed, the most growth. An

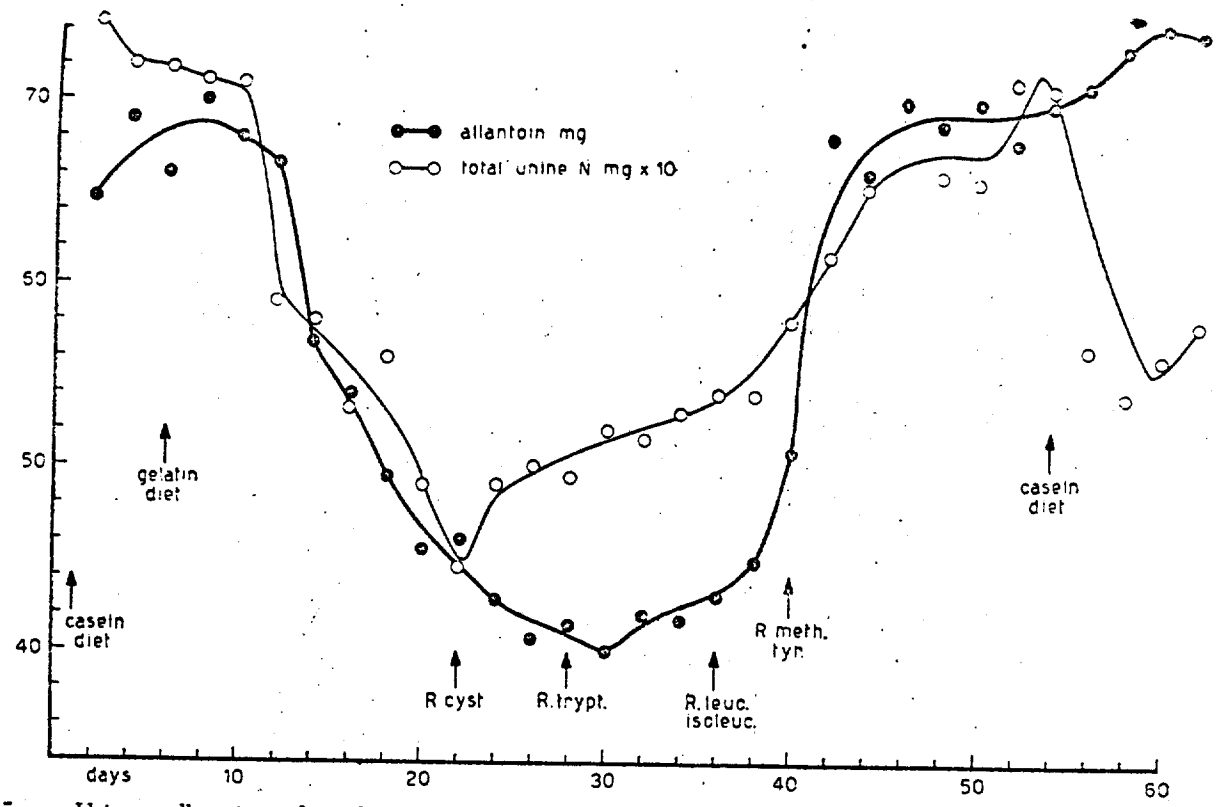


Fig. 5. — Urinary allantoin and total nitrogen eliminated per day by rats fed a diet containing gelatin as the only sour of amino acids and then supplemented with amino acids (rats E an F). aprot. = protein-free diet. R = diet supplement with amino acids in the following order: cystine, tryptophan, leucine and isoleucine, methionine and tyrosine.

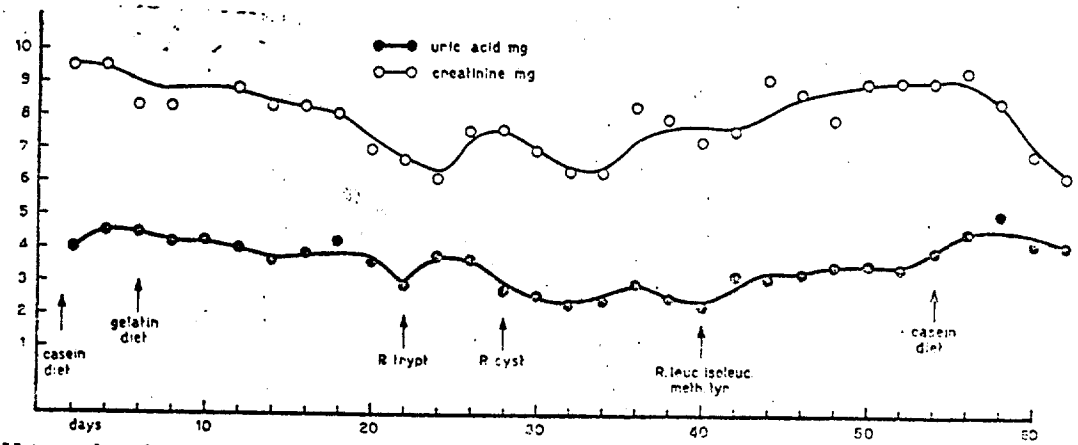


Fig. 8. — Uric acid and creatinine eliminated per day by rats fed a diet containing gelatin as the only source of amino acids and then supplemented with amino acids (rats G and H). For further details see the legend of fig. 5.

unknown growth essential was postulated in proteins to explain the stimulation of growth, especially through the addition of casein.

Protein metabolism studies using gelatin, casein and zein fed to rats were performed by Mason and Palmer (429). Similar studies during the 1920's and 30's concerning the relative nutritional value of various protein sources had produced widely varying results.

Particular attention was given to a basic 'nitrogen free' diet that was palatable; specially prepared casein and zein were selected, and a commercial gelatin used. All three proteins were analyzed for total nitrogen content as were fecal and urine samples taken during the experiment. Adult male rats, 350-500 g body weight, of various strains, were kept in individual metabolism cages from which urine and feces could be collected. A 3-day preliminary period of basal diet was observed followed by addition of about 0.4 g of the protein to be studied. Analysis of the nitrogen excreted determined the amount of protein then added daily to the diet for the next 3 days; or just enough to balance the daily nitrogen loss. After the 3-day protein supplemented diet, the animals were returned to the basal 'nitrogen free' diet and further nitrogen excretion measured. Difficulties in rendering the 3 protein palatable were described. The number of nitrogen balance experiments performed were: 20 with casein; 18 with gelatin; and 20 with zein. The relative utilization of the 3 proteins are shown in the following table:

TABLE 3

Percentage retention of casein, zein and gelatin calculated by McCollum's method

RAT NO.	CASEIN	ZEIN	GELATIN
11		60.7	20.1
13			23.5
14			23.1
16	76.0		22.9
17	78.8	69.0	31.8
17		74.3	23.3
20	67.6	51.5	32.8
27	84.6		35.5
27			16.7
29	73.9	50.9	
30			17.8
33	76.3	47.3	17.1
34			17.0
35	75.0	50.0	
36	82.7		
37	70.7		40.4
38			30.3
39	76.3	49.2	22.7
41	77.7		15.1
42	74.5	58.5	14.9
45	78.2		
50	80.3		
51		72.8	
52	77.0	51.9	
52		72.9	
55	64.0		
56	72.5	59.3	
59	71.7	64.0	
60	70.0	58.9	
61	60.1	47.8	16.1
61		57.1	
63		44.1	
64		46.6	
67		59.9	
Mean	74.4	57.3	23.4
Standard deviation	5.95	9.41	7.69

Statistical analysis of the data showed a highly significant correlation between the animal's weight and its endogenous urinary nitrogen. Weight losses during the experiment were attributed to a decrease in the alimentary tract contents and in loss of fat. Experimental difficulties were discussed in some detail, and several supportive experiments were performed in response to various interpretations of the experimental results. The gelatin and casein were well digested and the percentage retention correlated closely to the biological values calculated; zein was badly or irregularly digested but presented a significant correlation between exogenous fecal nitrogen/nitrogen ingested, and exogenous urinary nitrogen/nitrogen resorbed.

Brunschwig et al. (73) reported some observations on the intravenous injection of gelatin for nutritional purposes, noting that studies of this type "apparently have not been hitherto recorded". Aqueous solutions of gelatin, about 12%, were given as 150 ml daily doses intravenously to dogs depleted of protein stores by maintenance on a very low nitrogen diet (1.23 g N/505 g of diet). Total nitrogens were determined for each gelatin dose employed and used to calculate nitrogen intake. Urine and feces were collected every 24 hours and the daily nitrogen loss determined. In 5 of 6 experiments with 4 dogs, a positive nitrogen balance or a nitrogen equilibrium was obtained. A rapid and appreciable rise in plasma protein was seen, due in large part to the presence of circulating gelatin. In general, the dogs tolerated the injections quite well; occasional emesis was observed and one animal developed marked erythema on the head and extremities. The authors concluded that at least a portion of the injected gelatin appeared to be metabolized since there was a substantial increase in non-protein nitrogen excretion following the injections.

Studies of nutritional inanition, particularly among hospitalized patients who are unable to consume a normal amount of food, led to experiments with rats by Pagé and Gingras (532). Physiological glucose solutions given parenterally have not satisfied the nutritional requirements in the past, and amino acid solutions or protein hydrolysates have been proposed, which together with glucose should prevent inanition. The problem, stated by Pagé and Gingras, is to determine the best mixture of nutritive elements that can be administered parenterally: hydrolyzed proteins, glucose, vitamins and mineral elements.

Young adult rats were fed various diets limited to about half the caloric level for the basal metabolism, assuring that the animals would consume the entire ration; this corresponded to roughly the calories given sick persons intravenously, or 3 liters of 5% glucose solution equal to 600 calories. The criteria used for judging the results of the various diets were length of survival of the animals, and the degree of emaciation at the time of death.

The first experiment compared casein and gelatin, with and without vitamin supplements. The results showed that casein with vitamins produced a greater length of survival than gelatin, with or without vitamins; the 80% mortality rate was reached in 12 or 13 days on gelatin, but casein extended survival to 17 or 20 days. A second experiment with sucrose, gelatin and casein, conducted at a lower ambient temperature, gave results which scarcely distinguished one diet from another, except that the animals on the casein-sucrose diet survived two days longer than the others. The influence of temperature on the length of survival was assumed to be related to the gradual slowing down of the metabolism.

A third experiment established that an optimum mix of 50-80% casein with 50-20 parts of sugar assured a maximum length of survival. Gelatin was shown in the first two experiments to be as unsatisfactory in overcoming inanition as in promoting growth. In prolonged inanition, vitamin supplements were shown to be advantageous, and mixtures of protein and sugar are superior to either given alone.

The 'protein quality' of gelatin, or its ability to promote synthesis of tissue proteins and enzymes, was determined by Ramakrishnan (569), based on determination of the liver xanthine oxidase in rats fed diets containing gelatin, and for comparison, casein. Two groups of 4 albino rats, weighing 80 ± 10 g, were fed different diets, one containing 20% casein and the other, 16.5% gelatin. After 4 weeks, the animals on the gelatin diet had lost considerable weight and were very weak; those fed the casein diet had grown steadily and appeared healthy. All rats were sacrificed and the liver xanthine oxidase determined; the gelatin-fed animals showed 70% less oxidase activity than did the casein-fed. The experiment was repeated, but with 532 mg % of methionine added to the gelatin diet to compensate for the absence of that amino acid in gelatin. No improvement in growth was observed, but the liver xanthine oxidase level in the gelatin-fed group was doubled by the addition of methionine. The 'protein quality' of gelatin appeared very poor compared to casein in synthesizing an enzyme protein like xanthine oxidase.

Excretion

Human

Quantities of free and peptide-bound amino acids were determined in the urine of man, rat, dog, hamster and monkey fed normal and gelatin-containing diets by Hueckel and Rogers (287). Four human subjects given 30 g of casein of 30 g of gelatin showed a significant increase in the urinary excretion of peptide-bound proline and hydroxyproline and of free hydroxylysine. The ingestion of gelatin had no effect on the amount of free proline or hydroxyproline excreted. The amounts of bound and free amino acids are shown below:

TABLE 1—URINARY EXCRETION OF AMINO ACIDS AFTER THE INGESTION OF EITHER GELATIN OR CASEIN BY MAN

	μmoles/5½ hr			
	Free form		Bound form	
Amino acid	Control	Gelatin	Control	Gelatin
Hydroxyproline	<2*	10*	38 ± 13†	794 ± 126
Aspartic acid	23‡	22‡	160‡	310‡
Glutamic acid	5 ± 1	5 ± 2	600 ± 189	892 ± 295
Proline	3 ± 1	2 ± 1	108 ± 38	649 ± 114
Glycine	307*	1310*	734*	1391*
Alanine	95 ± 44	163 ± 55	33 ± 5	112 ± 45
Lysine	101*	107*	57*	66*
Histidine	337*	349*	136*	145*
Hydroxylysine	2 ± 1	39 ± 8	4 ± 2	17 ± 7

* Average of three values.
† ± Standard error of mean of four values.
‡ Average of two values.

Rat

In a study with rats an experimental diet contained 20% gelatin which was balanced by adding 8% essential amino acids. The control diet contained 28% free amino acids in the same proportion as the experimental diet. Four rats were given an amino acid diet, 4 rats were given 20% gelatin diet and 4 rats were given the casein diet. In a separate experiment 4 rats were given a 50% gelatin diet in the same manner. The rats excreted large quantities of free proline and hydroxyproline after eating diets high in proline or hydroxyproline. The peptide-bound hydroxyproline, proline, glycine and aspartic acid were greatly increased by dietary gelatin as shown in the following figures:

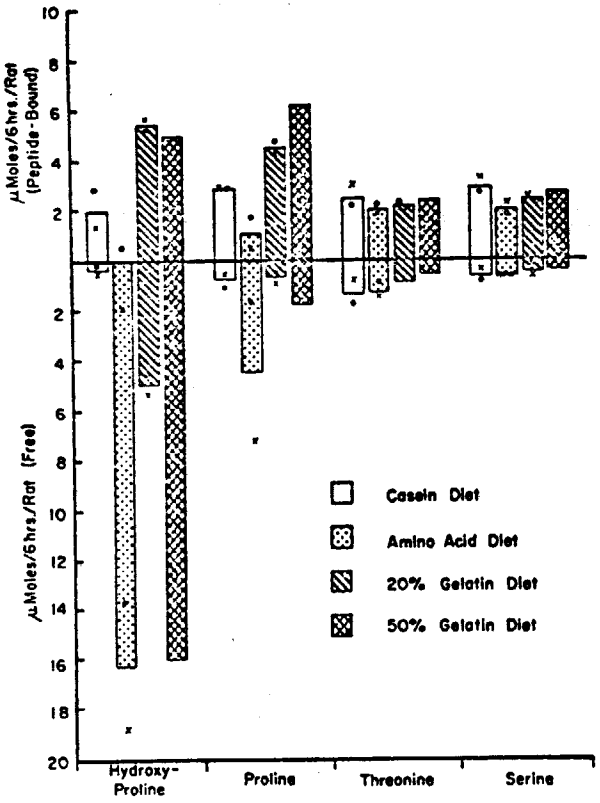


FIG. 1A

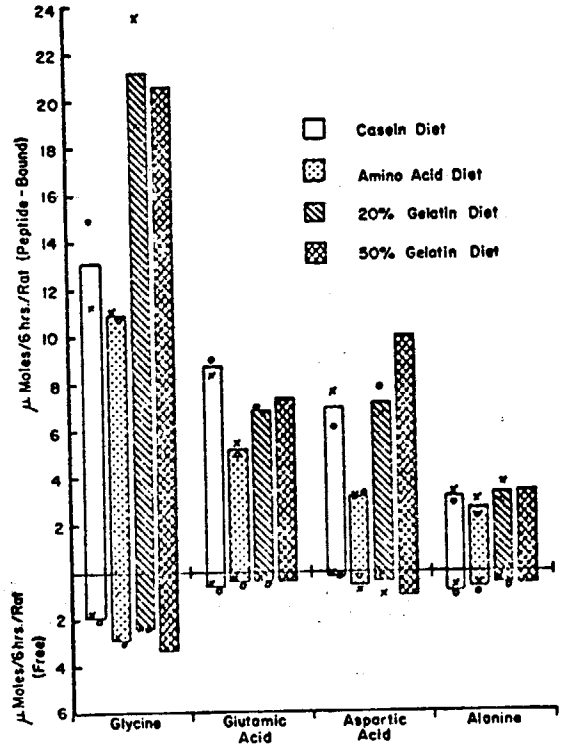


FIG. 1B

FIG. 1A, B. Micromoles of free and bound amino acids from urine of rats. O represents the pooled urine of four rats in a 6-hr period after feeding on the first day of collection. × represents the pooled urine of four rats in a 6-hr period after feeding on the fourth day of collection. For the casein, amino acid and 20 per cent gelatin diets, each bar represents the average of O and × values. For the 50 per cent gelatin diet each bar represents the pooled urine of ten rats in a 6-hr period after feeding. Values below the zero line are the amino acids found free in the urine; values above the zero line are peptide-bound amino acids.

Hamsters

In a study with hamsters fed 23 or 40% gelatin diets there was an increase of fourfold in bound hydroxyproline and a twofold increase in bound proline in urine of hamsters fed the 50% gelatin diet. A twofold increase in bound hydroxyproline and a 1.5 fold increase bound proline occurred in the urine of hamsters fed the 23% gelatin diet, as shown below:

TABLE 2—MOLAR RATIOS OF BOUND AMINO ACIDS TO THAT OF BOUND ALANINE IN URINE OF HAMSTERS FED GELATIN DIETS

	Diets					
	First experiment				Second experiment	
Amino acid	Control*	40% Gelatin*	40% Gelatin*	Control*	Casein†	23% Gelatin†
Hydroxyproline	0·16	0·75	0·68	0·18	0·26	0·56
Aspartic acid	0·76	1·65	1·32	1·00	—	—
Threonine	1·55	1·44	1·46	1·29	—	—
Serine	1·40	1·04	0·82	1·36	—	—
Glutamic acid	1·73	2·18	2·28	1·51	1·79	2·28
Proline	0·75	1·53	1·32	0·70	0·94	1·52
Glycine	6·05	6·50	5·55	7·70	3·80	5·05
Alanine	1·00	1·00	1·00	1·00	1·00	1·00

* Pooled urine of two hamsters.
† Urine of one hamster.

Dog

Four dogs were fed a basal diet for 2 days. On the third and fourth day they were given the basal diet containing 5% of an amino acid mixture resembling gelatin, and on the fifth day they were given the basal diet containing 5% gelatin. The results were expressed in terms of the ratio of the various amino acids in the urine to that of alanine. There was no change in the amount of free or bound amino acids with either the amino acid mixture or the gelatin diet. The results are shown on the following page:

TABLE 3—MOLAR RATIOS OF BOUND AMINO ACIDS TO THAT OF BOUND ALANINE IN URINE OF BEAGLES FED GELATIN

Amino acid	Control diet*	Amino acid diet†	Gelatin diet*
		μmoles/μmole of Ala	
Hydroxyproline	0·12 ± 0·01	0·29 ± 0·06	0·29 ± 0·05
Aspartic acid	1·96 ± 0·09	1·90 ± 0·08	2·39 ± 0·13
Glutamic acid	1·31 ± 0·10	1·18 ± 0·06	1·28 ± 0·04
Proline	0·87 ± 0·09	1·01 ± 0·07	0·99 ± 0·04
Glycine	3·33 ± 0·15	3·54 ± 0·21	3·67 ± 0·05
Alanine	1·00	1·00	1·00
Hydroxylysine	0·10‡	0·16 ± 0·02	0·12 ± 0·01
		μmole/ml of urine	
Alanine	2·28 ± 0·24	1·35 ± 0·29	1·27 ± 0·36

* Average of three values.
† Average of four values.
‡ Average of two values.

Monkey

Two monkeys were fed either an amino acid mixture resembling gelatin or gelatin in their diets on different days. There was no change in the amount of free or bound urinary hydroxyproline as a response to dietary gelatin. The only positive response to the dietary gelatin was a fivefold increase of free hydroxylysine in one monkey on the day gelatin was fed. The urinary excretion of the amino acids is shown by the following table:

TABLE 4—URINARY EXCRETION OF FREE AND BOUND AMINO ACIDS BY CAPUCHIN MONKEY AFTER FEEDING GELATIN

	μmoles/day					
	Control		Amino acids		Gelatin	
Amino acid	Free	Bound	Free	Bound	Free	Bound
HO proline	0	24·2	6·9	45·2	27·8	65·1
Aspartic acid	0	*	0	74·2	1·2	58·8
Threonine	0·5	32·2	9·7	33·4	30·8	32·1
Serine	0·4	33·0	1·0	34·8	54·2	8·3
Glutamic acid	0	190·0	0·3	179·5	11·8	122·8
Proline	0	62·0	0	80·2	1·6	65·6
Glycine	0·3	441·6	111·5	603·0	306·9	490·5
Alanine	0	75·2	2·4	76·7	21·6	45·0
Cystine	4·9	1·5	4·4	5·1	3·8	1·6
Valine	0·3	20·5	0·5	22·3	4·6	14·9
Methionine	0·3	10·2	0·5	11·0	1·9	4·8
Isoleucine	0·3	12·5	0·2	12·9	6·8	9·4
Leucine	1·3	19·8	2·2	21·9	6·8	9·4
Tyrosine	2·2	6·8	2·4	7·4	5·2	1·5
Phenylalanine	0·9	8·9	1·0	14·3	3·0	7·8
HO lysine	0·8	13·4	0·5	15·7	1·5	6·0
Lysine	1·2	39·4	*	*	6·4	22·3
Histidine	10·0	6·1	13·1	3·5	30·0	10·4

* Not determined.

Scott and Ryan (627) reported the results of administering 500 ml of a 10% gelatin solution intravenously to 24 hospital patients with non-surgical conditions, malnutrition, liver dysfunction, edema, etc. Blood and urine samples were collected over a 48-hour period and subjected to various tests; no changes of significance were observed. The gelatin disappeared rapidly from the blood following injection, only small amounts (0.1 g/100 ml, or less) remaining after 48 hours; similarly, no measurable gelatin was detected in the urine of most patients 48 hours after injection. The average total excretion in the urine during the first 24 hours was 14.4 g for 23 of the patients; individual amounts varied from 7.6 to 20.5 g. The blood volume was observed to increase immediately and the hematocrit decreased; urine volume increased 24 to 48 hours after injection. The principal conclusion reached by the authors was that additional studies of gelatin metabolism in patients with various clinical conditions were needed.

Gelatin has been used as a plasma expander in the treatment of shock in human beings for more than half a century. Ordinary solutions of gelatin, however, have the disadvantage of gelling at room temperatures and several methods have been devised to modify the solutions to prevent this obvious shortcoming. Siiteri and Highland (639) have studied the retention and excretion of two modified gelatin solutions in normal men: a condensation of native gelatin with glyoxal, further oxidized by H_2O_2, is liquid at room temperature in 5% concentration and has been named oxypolygelatin (OPG); Knox P-20 gelatin, treated with dicarboxylic acid anhydride at specified pH and temperature, has been called 'fluid gelatin' and is liquid at room temperature in 3% concentration.

Twelve hospital patients convalescing from minor burns were divided into two groups: 8 men received infusion of OPG, and 4 received fluid gelatin. Two patients from the OPG group were equipped with indwelling catheters for urine collection; voluntary voiding served in all other collections, and all but the two catheterized patients received orally, 500 ml H_2O at the beginning of each experiment, and 250 ml each half hour for the next 6 hours.

Blood samples were taken immediately after the 1-hour perfusion of 500 ml gelatin solutions and at 3, 5 and 20 hours thereafter. Gelatin, plasma protein concentrations, albumin-globulin ratios and hematocrit were determined. The results summarized for each of the two gelatin solutions are shown in the following tables:

TABLE 3. FLUID GELATIN. SUMMARY OF DATA OBTAINED ON 4 PATIENTS INFUSED WITH 500 ML FLUID GELATIN

Patient	Time Postinfusion	Plasma Vol.	Plasma Protein Conc.	Hct.	Gelatin Conc.	CO_2
		ml	*gm %*		*gm %*	*mEq/l*
1	Preinfusion	2480	8.02	46.5	0	27.6
	Immediately posinfusion	3035	7.05	41.5	0.301	23.5
	3 hr.	2912	6.71	42.5	0.180	
	5 hr.	2685	7.65	44.5	0.190	
	20 hr.	2748	7.95	44.0	0.085	
2	Preinfusion	3090	7.85	45.5	0	21.6
	Immediately postinfusion	3640	6.85	41.5	0.268	30.2
	3 hr.	2970	8.16	46.5	0.185	
	5 hr.	2910	7.93	47.0	0.149	
	20 hr.	3420	7.41	43.0	0.050	
3	Preinfusion	2650	7.67	51.0	0	26.0
	Immediately postinfusion	3610	6.20	43.5	0.320	25.4
	3 hr.	3262	6.79	46.0	0.208	
	5 hr.	3010	6.95	47.5	0.180	
	20 hr.	2620	6.93	51.5	0.068	
4	Preinfusion	3695	6.97	46.5	0	29.1
	Immediately postinfusion	4170	6.33	43.5	0.230	24.5
	3 hr.		6.43		0.138	
	5 hr.	4080	6.51	44.5	0.116	
	20 hr.	3840	6.13	45.5	0.060	

TABLE 2. OXYPOLYGELATIN. SUMMARY OF DATA OBTAINED ON 8 PATIENTS INFUSED WITH 500 ML OPG

Patient	Time Postinfusion	Plasma Vol.	Plasma Protein Conc.	Hct.	Gelatin Conc.	CO_2
		ml	*gm %*		*gm %*	*mEq/l*
1	Preinfusion	2806	6.98	50.0	0	29.2
	Immediately postinfusion	3228	5.78	46.5	0.425	28.9
	5 hr.	2806	6.50	50.0	0.170	
	20 hr.	2861	6.73	49.5	0.060	
2	Preinfusion	3382	6.12	45.5	0	29.0
	Immediately postinfusion	3900	5.33	42.0	0.350	27.9
	5 hr.	3585	5.53	44.5	0.188	
	20 hr.	3452	5.78	45.0	0.060	
3	Preinfusion	2921	6.84	45.5	0	29.1
	Immediately postinfusion	3438	5.57	41.5	0.400	29.7
	5 hr.	2805	6.26	46.5	0.210	
	20 hr.	2980	6.75	45.0	0.050	
4	Preinfusion	2301	6.67	51.0	0	31.0
	Immediately postinfusion	2650	5.28	47.5	0.500	30.8
	5 hr.	2255 (2430)*	6.19	51.5	0.250	
	20 hr.	2492	6.53	49.0	0.095	
5	Prefusion	3056	7.34	48.5	0	30.5
	Immediately postinfusion	3380	5.96	46.0	0.434	29.9
	5 hr.	3182	6.89	47.5	0.195	
	20 hr.	2940	7.43	49.5	0.078	
6	Preinfusion	2639	7.65	50.0	0	30.2
	Immediately postinfusion	3035	6.48	46.5	0.453	27.7
	5 hr.	3160 (2900)*	6.71	45.5	0.195	
	20 hr.	2860	7.09	48.0	0.072	
7	Preinfusion	2797	7.43	51.5	0	29.2
	Immediately postinfusion	3080	6.80	49.0	0.398	25.6
	5 hr.	3215 (2812)*	7.43	48.0	0.192	
	20 hr.	2478 (2600)*	7.55	54.5	0.0	
8	Preinfusion	2973	6.60	45.0	0	28.1
	Immediately postinfusion	3362	6.18	42.0	0.558	28.3
	5 hr.	2973	6.88	45.0	0.152	
	20 hr.	2865	6.94	46.0	0.0	

* T-1824 blood volume.

Both forms of gelatin were excreted mostly during the first 6 hours; fluid gelatin was retained to a greater degree than OPG with significantly smaller urinary excretion.

Dill and Horvath (140) studied the influence of a high gelatin diet on the excretion of nitrogen, creatinine and creatine in healthy young men.

Sixty grams of gelatin was added to the diet of 4 young men. The feeding of gelatin continued for 41 days for 3 subjects and for 51 days for the fourth. The addition of gelatin was accompanied by a measured excretion of creatine in 2 of the subjects. The average excretion of creatinine was not increased by the high protein intake. There was, however, measured excretion of both creatinine and creatine in all subjects in cessation of gelatin ingestion indicating a possible storage during the previous period of gelatin feeding. All subjects normally had some creatine in the urine and also showed considerable variation in the 24-hour excretions of creatinine.

The fate of intravenously injected gelatin was studied in 42 normal human subjects by Hoffman and Kozoll (277). Each individual was given 1000 ml of 5% gelatin. Three types of gelatin were used, namely heavy, intermediate and light with molecular weights of the order of 58,000, 47,000 and 37,000 respectively.

The urinary excretion of gelatin was variable, however, a pattern of excretion was discernible. The heavy gelatin excretion in 6 hours averaged 14.7 g while the light was 22.7 g. The intermediate gelatin values were between these. By 72 hours 80% of all types of gelatin was excreted. There was some excretion on the 4th and 5th days. The author stated that it appeared unlikely that any considerable quantity of gelatin was catabolized.

IV. Effects on Enzymes and Other Biochemical Parameters

The relationship of protein intake and kidney weight in male albino rats was investigated by MacKay and MacKay (418). Four proteins were used; casein, egg albumin, blood albumin and gelatin. The gelatin and casein were fed together in the diet of about 20 rats of 26 to 70 days of age. The gelatin-fed animals showed the poorest growth increases, but kidney weights in sacrificed rats were notably larger than in the other diets. Histological evidence was not abnormal, and the authors suggested that the kidney enlargement was due to early stages of renal damage due to the gelatin.

The influence of the quality of protein on urinary excretion and tissue levels of ascorbic acid was studied by Chandrasekhara

et al. (92) using gelatin and supplementary essential amino acids missing in gelatin. Male adult albino Wistar rats, weighing 140-170 g, were fed a diet containing 10 and 20% gelatin or 10% gelatin plus amino acids sufficient to equal a 10% casein. After 2 weeks on the experimental diet, urine was collected for 3 days for analysis of ascorbic acid and glucuronic acid. A week later, the animals were sacrificed and the livers removed and prepared for analyses of the ascorbic and glucuronic acids.

The animals on a 10% gelatin diet showed both urinary and liver ascorbic acid levels nearly as low as those rats fed a protein-free diet; the 20% gelatin lowered the levels even further. The 10% gelatin plus amino acids increased the ascorbic acid levels almost two-fold. Only a combined methionine-tryptophan supplementation substantially raised ascorbic acid levels when given as the sole amino acids added; when all missing amino acids were used, the highest urinary ascorbic acid excretion was obtained. Only tryptophan added to a gelatin diet increased urinary glucuronic acid which is the first essential precursor for the biosynthesis of ascorbic acid.

An important, nonnutritional application of gelatin lies in its use as a plasma expander in the treatment of massive hemorrhage. Habif et al. (236) reported several experiments using a modified fluid gelatin to replace, in dogs, volume for volume, blood loss of about 50%, and to observe the effect of the gelatin on the coagulation of blood samples from healthy human donors.

Seventeen healthy dogs of either sex, weighing 15-23 kg, 1 to 4 years of age were anesthetized after 12 hours of fasting and prepared by catheter inserted in femoral vessels for continuous measurement of blood loss. A 3% modified fluid gelatin suspended in a balanced solution was transfused in amounts equal to the blood lost. For a period of from 1 to 4 days, the influence of the gelatin solution on the acid-base equilibrium and ionic and albumin concentration of plasma was observed as were the plasma gelatin concentration and rate of urinary excretion. The results are shown in the following table: (the animals were observed for two weeks following the experiment).

TABLE 2. *Effects of Blood Volume Replacement with BFG 3% (Plasmagel-F) Following Hemorrhage in Dogs*

Bleeding Time (min.)	Transf. Time (min.)	Blood Volume (ml.)	Blood Loss (%)	Blood Pressure (mm. Hg)			Heart Rate (beats/min.)			Number of Survivors
				Control	p Hem.	1 h p Transf.	Control	p Hem.	1 h p Transf.	
3.7 ±0.6 (17)	3.4 ±0.4 (17)	1880 ±88 (17)	46.9 ±1.1 (17)	150.0 ±3.2 (17)	34.6 ±5.5 (17)	115.2 ±5.0 (17)	168 ±8 (17)	142 ±10 (17)	171 ±8 (17)	16

Arterial pH			Arterial Pco_2 (mm. Hg)			Bicarbonate (mEq./l.)		
Control	p Hem.	1 h p Transf.	Control	p Hem.	1 h p Transf.	Control	p Hem.	1 h p Transf.
7.39 ±0.02 (10)	7.32 ±0.02 (11)	7.42 ±0.03 (11)	38.5 ±2.2 (10)	39.0 ±2.1 (11)	36.5 ±2.1 (11)	22.6 ±0.6 (10)	19.3 ±0.9 (11)	23.0 ±0.7 (11)

Plasma Volume (ml)		Hematocrit (%)	
Control	15 h p Transf.	Control	15 h p Transf.
1201 ±88 (9)	1240 ±82 (9)	39.3 ±1.2 (9)	22.4 ±1.0 (9)

Numbers in parentheses indicate number of animals.
Values are means ± S.E.

Plasma volume, 24 hours after transfusion, was normal. Total protein and plasma albumin concentrations decreased in proportion to blood loss, but increased rapidly to 82% and 78% of control values at 24 hours and 90 and 89% after 4 days. Urinary elimination of the gelatin showed about 10% remaining in the plasma after 24 hours; the half-life of the gelatin in the plasma was 150 minutes.

Forty-eight samples of human blood were studied in vitro using albumin tagged with radioactive I131 for coagulation time; the gelatin solution, diluted 1 to 5 did not significantly alter plasma or platelet factors of coagulation. Use of this same balanced fluid gelatin in surgical procedures performed in man and reported by other investigators did not alter coagulation factors in vivo. The modified balanced fluid gelatin appears to be an efficient and safe

plasma substitute.

Seventy college women on definite exercise schedules took gelatin for 28 days in a study by Pryor and Knapp (563). Each girl took one ounce of gelatin daily for the first 2 weeks and 1/2 ounce daily for the second 2 weeks. A similar group of 70 women on the same schedule served as controls. Weight and strength were measured and recorded before and after the test period for both the experimental and control groups. Very small gains in weight and strength were made by the gelatin group. No similar gains were made by the controls. There were no significant changes in strength or endurance as a result of the gelatin feeding.

Ray et al. (579) tested the effect of gelatin on muscular fatigue in 6 men and 4 women; other studies had indicated that glycine delayed fatigue in normal individuals, particularly in men, presumably from the creatinogenic action of glycine and the ability of males to store this substance in muscle. Large amounts of glycine produce some discomfort, however, and gelatin was selected in this study due to its large glycine content (25%) and easy digestibility. A bicycle-generator device was employed to measure work output. After a training period to establish constancy of daily work, the subjects were placed on a gelatin diet: all men received 60 g daily; 3 women took 45 g and one, 67.5 g of gelatin daily. The gelatin was mixed with orange and lemon juice. The results of 3 representative experiments are shown in the following figure:

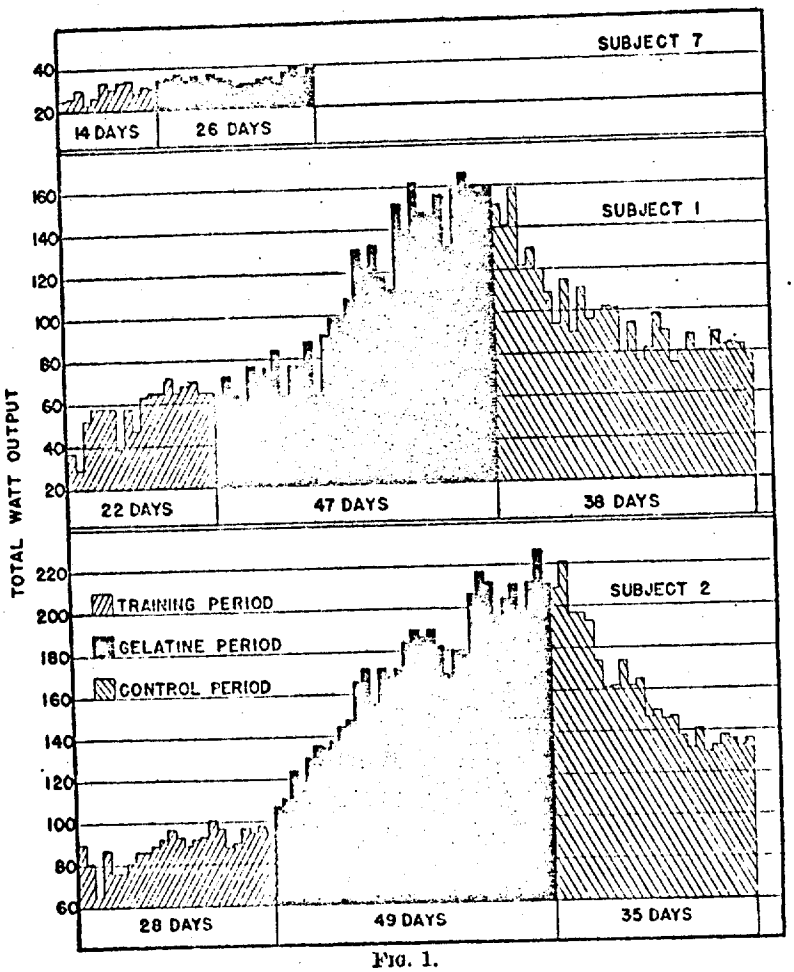


FIG. 1.

Subject 7, a woman, showed a suggestion of an increase (3%) during the gelatin diet (45 g/day); Subject 1, a 25-year-old man, received 60 g gelatin daily, as did Subject 2, age 33.

Men, when given an adequate amount of gelatin, invariably increased the amount of work done before fatigue set in - with results ranging from 37 to 240% increases above the training level. No appreciably effect was observed in the women subjects, again suggestive that the gelatin action was due to the creatinogenic property of its amino acids, particularly glycine.

A report by Maison (425) which appeared shortly after that by Ray et al, refuted the experimental results indicating that gelatin

or glycine influenced muscular fatigue. Maison conducted several experiments, one of which repeated in effect that of Ray et al, but measuring the work done and fatigue of the single muscle, the extensor digitorium communis, by a weight ergograph designed especially for the extensor of the fingers. Six male subjects ranging in age from 23 to 45 years were employed in the training period of five weeks; in order to distinguish between the neuromuscular abilities from combined neuromuscular-circulatory systems, the blood supply to the right arm was interrupted during the work periods by a sphygmomanometer cuff. After 5 weeks the muscles were still increasing work ability, and each subject was given 60 g of unflavored gelatin daily in two doses. Gastrointestinal intolerance caused two of the subjects to discontinue the gelatin after 10 days; the remaining four continued the program for 21-31 days. During the gelatin tests, muscle ability continued to increase, as it did for four weeks after the gelatin was stopped. No measurable effect of gelatin was observed throughout the experiment in either improvement of muscle ability or decreased ability from omission of gelatin from the daily routine.

Two of the subjects continued the daily work for a full year. After three months, 15 g of glycine were ingested daily for 30 days with no improvement observed, nor did ability decline upon discontinuing the glycine. After several more months, the subjects were given 60 g gelatin daily for 42 days, a total of about 5 pounds of gelatin. Neither improvement or decreased ability in the muscle ability was observed. Maison concluded his report with critical comment on the Ray et al. study.

The Council on Foods (772) reported in 1936 in the J.A.M.A., "The Nutritional Significance of Gelatin" comprising a general review of the knowledge to that time. After discussing the various claims for gelatin, the Council concluded with the following:

Allowable Claims for Gelatin - In view of the available evidence the Council believes that gelatin properly made is a wholesome food, that it has special usefulness when one desires to add variety to the diet by incorporating gelatin in nutritious soups or pleasant desserts which appeal to the appetite of many persons, and that for these reasons gelatin is often a useful food for inclusion in the diet of healthy persons or of sick or convalescent patients. Gelatin appears to be well tolerated. The claim that gelatin is an aid in the digestion of milk, however, is in the opinion of the Council not established. The claim that gelatin is of value as a source of aminoacetic acid in the treatment of some of the myopathies cannot be recognized; in the light of present evidence, gelatin has no special significance as a source of amino acids in the diet. Indeed, it is notoriously deficient in certain essential amino acids.

This report is of some interest in view of the following paper by Perlman which appeared in 1928, eight years before the Council on Foods report.

Half a century ago, gelatin enjoyed widespread publicity and popularity as an infant food in gelatinized milk, particularly in difficult feeding cases involving low weight gains or regurgitation. Perlman (551), a former Medical Director of the Ocean City Seashore Home for Babies in New Jersey, reported research into the value of gelatin in infant feeding. The Home dealt principally with the care of malnourished infants. Thirty-six infants (18 controls, 18 tested) selected for the gelatin study were divided into two groups: under one year of age, and between one and two years old. Powdered gelatin was added to the daily milk as a half teaspoonful for infants under 6 months and a teaspoonful for those over 6 months of age. Of the tested, gelatin-fed infants, 8 were under one year of age, and 10 were between 1 and 2 years; the controls, receiving simply milk dilutions, were 17 under one year and 1 sixteen months of age.

The results were summarized by the author as follows:

1. Gelatinized fed babies fare far better than infants fed upon simple dilutions of cow's milk.

2. In the series of infants studied, those fed upon cow's milk formulas vomited more frequently in contrast to those fed upon gelatinized milk.

3. Weekly gains in weight per capita were greater in the gelatinized fed babies than in those fed upon simple dilutions of whole milk.

4. Of eight infants between the ages of six months and one year fed upon gelatinized milk, six gained more than 2.7 ounces weekly, one gained less than this amount weekly, while one infant lost weight. Contrasted with the series of 17 controls of the same age, ten gained above 2.7 ounces weekly, seven less than 2.7 ounces weekly.

5. Of the ten infants from one to two years who received gelatinized milk, all gained over 1.8 ounces weekly. Contrasted with the only control baby of similar age, the latter gained 7.2 ounces weekly.

6. The average weekly gain per capita in infants during the first year fed by means of "gelatin milk" was 6.1 ounces while in the controls, 4.2 ounces. The average weekly gain in infants during the second year upon "gelatin milk" was 4.4 ounces as compared to the single case in the control series of 7.2 ounces.

7. Gelatin milk is superior to whole milk dilutions as a means of increasing avoirdupois in artificial malnourished infants.

8. The preparation of gelatinized milk is comparatively simple, palatable, inexpensive, easily retained by the stomach, and its use encouraged in difficult feeding cases.

A popular but questionable use for gelatin as an hemostatic for preoperative use in tonsillectomies, extractions (dental) and other instances where excessive bleeding might be anticipated, was the subject for discussion and experimentation by Tainter et al. (679). Since the gelatin is given orally, the authors found it difficult to understand how it could serve as an hemostatic. Six healthy human subjects took 15 g of gelatin 3 times daily for from 10 days to 2 weeks, observing the blood coagulation times; control times for coagulation were established during several weeks before and following medication. The control average for the six subjects was 196 seconds; the average time for coagulation during gelatin medication was 194 seconds. The authors concluded that there was little or no therapeutic value in gelatin as an hemostatic; similar studies were made on coagulen, fibrogen and histidine with similar results. "The dental practioner is advised to direct his efforts at controlling hemorrhage to local application of demonstrably effective agents rather than to try to speed up the coagulation process by systemic medication."

V. Drug Interaction

None

VI. Consumer Exposure Information

The hydrocolloidal properties of gelatin, together with its food value as a supplementary protein source, have resulted in very large quantities of gelatin being used in desserts, confection, marshmallows, consommes, ice cream, dairy products, baked goods and jellied meats. So ubiquitous is gelatin, that it is difficult to imagine a typical diet free from gelatin.

Exact estimates of the amount of edible grade gelatin used in the United States alone are not available, but Glicksman (209) indicates that an annual sales of gelatin (edible) may well be in the neighborhood of 50,000,000 pounds, distributed as follows:

Dessert products	37,000,000 lbs.
Confections (primarly marshmallows, toppings, fillings)	8,000,000
Meat products and ice cream	4,000,000
Other	1,000,000

For further details relative to the applications of gelatin in foodstuffs, refer to Glicksman (209) in the documents appended to this monograph.

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Poultry Sci. 1966 45(3): 541-546

The Amino-Acid Deficiencies of Gelatin for the Growing Chicken[1]

JOHN H. ASHLEY AND HANS FISHER
Nutrition Laboratories, Department of Animal Sciences, Rutgers-The State University, New Brunswick, New Jersey

(Received for publication October 18, 1965)

EARLY attempts by Jackson *et al.* (1928) and Kruse *et al.* (1934) to promote optimal growth in rats fed gelatin-containing diets supplemented with the amino acids then known (1928–34) to be deficient failed. At that time, however, threonine had not been identified as an essential amino acid. Even in the light of newer knowledge a decade later, Hier *et al.* (1944) were still unsuccessful in obtaining good growth in rats fed amino acid-supplemented gelatin diets. These workers reported slower growth on the amino acid-sup-

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, New Brunswick. Supported in part by grants in aid from the U. S. Public Health Service A-4904 and The National Science Foundation GB 2007.

plemented gelatin diets than for the amino acids fed separately. They concluded that the difficulty with gelatin might be due to an inhibitory effect caused by the unusually high content of glycine and proline. Fourteen years later Summers and Fisher (1962) again tried, unsuccessfully, to simulate the amino acid pattern and the growth response of isolated soy-protein in chicks fed amino acid-supplemented gelatin diets. In these studies no inhibitory effects could be demonstrated when an isolated soy-protein diet was supplemented with amounts of glycine, proline, and hydroxyproline present in equivalent levels of gelatin.

Since an earlier study (Jackson *et al.*, 1928) as well as unpublished results from this laboratory had established that the digestibility of gelatin for rats was high (90%) and therefore not the answer to the problem, it apppeared that the poor performance of amino acid-supplemented gelatin diets had to be resolved in terms of peculiarities in amino acid balance and/or differences in composition from reported values (Block and Weiss, 1956).

EXPERIMENTAL PROCEDURE

Duplicate lots of five day-old chicks (Columbian ♀ × New Hampshire ♂) were allotted to each treatment (except in Experiment 5, where several triplicate lots of 5 were used). Body weights and feed utilization were measured for 2- or 3-week experimental periods. Statistical comparisons were based upon variance analyses and 't' tests (Snedecor, 1956).

The experimental diets were calculated, based on our nitrogen analyses of the protein sources, to supply 13 or 16% crude protein (N × 6.25). The standard reference diet used contained 13% isolated soy-protein supplemented with 0.2% DL-methionine. The basal diet, exclusive of the nitrogen source, contained (in percent): corn starch, 26.0; dextrin, 5.0; non-nutritive fiber, 5.0; mineral mix,[2] 4.9; corn oil, 3.0; choline chloride (70%), 0.3; vitamin mix,[3] 0.2; and glucose monohydrate to 100.

TABLE 1.—*Amino acid composition of gelatin*

	Present Analysis gm./16 gm. N	Block and Weiss (1956) gm./16 gm. N
Arginine	6.81	7.7
Glycine	24.66	21.9
Histidine	0.56	0.8
Isoleucine	1.41	1.6
Leucine	2.85	3.2
Lysine	3.18	4.0
Methionine	0.75	0.9
Cystine	0.11	0.2
Phenylalanine	0.79	2.2
Tyrosine	0.52	0.9
Threonine	1.76	2.6
Valine	2.35	2.8
Glutamic acid	10.24	10.1
Proline	12.41	12.8
Hydroxy proline	10.84	12.4
Serine	3.01	4.0
Aspartic acid	5.71	5.7
Alanine	9.22	9.1

The average amino acid composition of gelatin, as given by Block and Weiss (1956), was used in calculating the amino acid supplements. These values are compared in Table 1 with others determined by us during these studies.[4] For these determinations, the gelatin sample was hydrolyzed with concentrated HCl at 110°C. for 11 or 22 hours (there was no significant difference between the two times of hydrolysis) in an evacuated, sealed tube. The

[2] As percentage of total mineral mix: $Ca_3(PO_4)_2$—17.2; KH_2PO_4—21.3; NaCl—16.2; $CaCO_3$—38.5; Fe gluconate—1.05; $MgSO_4$—5.06; $MnSO_4 \cdot H_2O$—0.4; KI—0.02; $CuSO_4$ ·anhyd.—0.025, $ZnCO_3$—0.4; $Na_2MO_4 \cdot 2H_2O$—0.02.

[3] In mg./kg. of diet: thiamine ·HCl—25; riboflavin—16; Ca pantothenate—20; vitamin B_{12}—0.02; pyridoxine·HCl—6; biotin—0.6; folic acid—4; 2-methyl naphthoquinone—5; ascorbic acid—250; niacin—150; per kg diet: vitamin A, 10,000 I.U.; vitamin D_3, 3,600 I.U.; and a-tocopheryl succinate, 5 I.U.

[4] The authors wish to express their gratitude to Drs. R. W. Wannemacher, Jr. and J. P. H. Wessels who carried out these analyses.

amino acids were then determined with a Spinco Model 150 Analyzer according to the method of Spackman *et al.* (1960). For the isolated soy-protein, the amino acid values given by the manufacturer (Archer-Daniels-Midland Co., Cincinnati, Ohio) were used, while the values of Block and Weiss (1956) were used for the amino acid content of casein and fish meal.

RESULTS AND DISCUSSION

Following our earlier (Summers and Fisher, 1962) unsuccessful attempts to obtain reasonably good growth on amino acid-supplemented gelatin diets, we based our first renewed attempt to do so on the observations of Longenecker and Hause (1959). By measuring the changes in plasma amino acid levels in dogs, following the feeding of a gelatin test meal, they found the order of limiting amino acids in gelatin to be tryptophan, methionine, isoleucine and histidine. Based largely on their results, we conducted a series of unsuccessful experiments in which these amino acids were preferentially added to gelatin in various combinations. Next we returned to the 10% gelatin protein diet supplemented with amino acids at a level equal to that supplied by 13% isolated soy-protein with added DL-methionine. It should be noted that the gelatin diets supplied certain amino acids in excess of those present in 13% isolated soy-protein (arginine and glycine among others). The composition of this amino acid supplement, referred to as amino acid mix 1, is shown in Table 2, footnote 'd'. In Experiment 1 (Table 2), 3% additional protein in the form of isolated soy-protein, fish meal, or casein was added to a diet containing 10% gelatin protein plus amino acid mix 1.

The 2-week body weights, together with the gain-to-feed ratios resulting from feeding these nitrogen supplements, are presented in Table 2. In comparison with the body weights of chicks fed either an isonitrogenous gelatin diet (16% protein) supplemented only with L-tryptophan, or 13% gelatin protein plus 3% casein protein plus additional tryptophan, the body weights of chicks fed gelatin supplemented with amino acid mix 1 increased significantly ($P < 0.01$). When either 3% fish, casein, or isolated soy-protein was also added to the gelatin diet already supplemented with amino acid mix 1, a further highly significant increase ($P < 0.01$) in body weight was obtained. The increased growth response was greater for fish and casein protein than for the isolated soy-protein. The growth obtained with the combination of 10% gelatin, amino acid mix 1, and 3% fish or casein protein was essentially equal to that observed on the positive control diet containing 13% isolated soy-protein with added methionine. That the gelatin was contributing to the growth response was apparent from the inferior growth on the

TABLE 2.—*Body weights and feed utilization of chickens fed gelatin with different amino acid and/or protein supplements*

Nitrogen Supplements	Two-Week[a] Body Weight gm.	Two-Week[a] Gain-to-Feed Ratio
16% gelatin protein[c]+0.21% L-tryptophan	44±1[b]	0.01
13% gelatin protein+3% casein protein+0.11% L-tryptophan	49+2	0.12
10% gelatin protein+amino acid mix 1[d]	89±3	0.41
+3% isolated soy-protein[e]	116±9	0.54
+3% fish protein[f]	134±5	0.55
+3% casein protein [g]	133±7	0.54
amino acid mix 1+3% casein protein	65±4	0.26
+3% isolated soy-protein	81±6	0.30
13% isolated soy-protein+0.2% DL-methionine	139±5	0.51

[a] Average day-old starting weight, 41 gm.
[b] Mean ± SE for duplicate lots of 5 chicks per treatment.
[c] Edible gelatin, Duche Uni-Gum Division, Clifton, New Jersey, provided 91% crude protein (N×6.25).
[d] Based on the amino acid composition of gelatin by Block and Weiss (1956) (See Table 1) this mix was designed to meet the amino acid levels supplied by 13% isolated soy-protein +DL-methionine (in percent of diet): L-arginine HCl 0.41; L-histidine HCl·H_2O 0.33; L-isoleucine 0.88; L-leucine 0.88; L-lysine HCl 0.64; DL-methionine 0.22; L-phenylalanine 0.50; L-threonine 0.22; L-tryptophan 0.16; L-valine 0.61; L-aspartic acid 0.36; L-cystine 0.08, L-glutamic acid 1.20; L-serine 0.76; and L-tyrosine 0.48.
[e] Assay Protein C-1, Archer-Daniels-Midland Co., Cincinnati, Ohio, provided 82% crude protein (N×6.25).
[f] Viobin Corp., Monticello, Illinois, provided 77% crude protein (N×6.25).
[g] Borden Chem. Co., New York, provided 82% crude protein (N×6.25).

TABLE 3.—*The effects on body weight and feed utilization of feeding chickens gelatin supplemented with amino acid mixtures simulating isolated soy- and casein proteins*

Nitrogen Supplements	Two-Week[a]	
	Body Weight gm.	Gain-to-Feed Ratio
16% gelatin protein+0.2% L-tryptophan	44±1[b]	0.03
10% gelatin protein+amino acid mix 1[c]	81±4	0.37
+3% casein protein	129±6	0.54
+amino acid mix 2[d]	142±5	0.64

[a] Average day-old starting weight, 43 gm.
[b] Mean±SE for duplicate lots of 5 chicks per treatment.
[c] For composition see Table 2, footnote "d".
[d] Amino acids simulating 3% casein protein based on the composition by Block and Weiss (1956) (in percent of diet) L-arginine HCl 0.198; L-histidine HCl·H_2O 0.146; L-isoleucine 0.311; L-leucine 0.615; L-lysine HCl 0.35; DL-methionine 0.358; L-phenylalanine 0.242; L-threonine 0.179; L-tryptophan 0.092; L-valine 0.297; L-aspartic acid 0.326; L-cystine 0.026; L-glutamic acid 1.014; L-serine 0.275; L-tyrosine 0.329; glycine 0.077; L-alanine 0.135; and L-proline 0.512.

two diets which supplied only amino acid mix 1 and either 3% casein or isolated soy-protein. While both of these diets were significantly better ($P<0.01$) than the negative control gelatin diets, they were inferior to their counterparts—which also contained 10% gelatin protein.

As a result of the success obtained by adding either 3% casein or fish protein to the 10% gelatin diet simulating 13% isolated soy-protein, all further studies were carried out with 3% casein protein or its amino acid components. In Experiment 2 an amino acid mixture simulating 3% casein protein (amino acid mix 2, Table 3, footnote 'd') was compared to the intact casein protein as a supplement to the diet containing 10% gelatin protein plus amino acid mix 1. The results (given in Table 3) show the simulated casein amino acid mix to promote growth even better than that supported by intact casein.

Table 4 gives the results of adding amino acid mix 2 (simulating casein) to the diet containing 10% gelatin protein and amino acid mix 1 at levels corresponding to 1, 2, or 3 percent casein protein. The effect of omitting the nonessential amino acids from amino acid mix 2 was also studied (amino acid mix 3, Table 4, footnote 'e'). The best growth was obtained with the level of amino acid mix 2, which corresponded to 3% casein protein. The omission, however, of the nonessential amino acids from mix 2 had no deleterious consequences. Attention is again called to the similar weights and gain-to-feed ratios for the gelatin diet supplemented with amino acid mixes 1 and 2, and that of the control diet containing isolated soy-protein with added methionine.

It seemed reasonable to assume that the

TABLE 4.—*Body weights and feed utilization of chickens fed gelatin with additional amino acids*

Nitrogen Supplement	Two-Week[a]	
	Body Weight gm.	Gain-to-Feed Ratio
13% isolated soy-protein+0.2% DL-methionine	150±5[b]	0.50
10% gelatin protein+amino acid mix 1[c] +amino acid mix 2[d] simulating 1% casein protein	98±4	0.46
2% casein protein	108±5	0.53
3% casein protein	134±7	0.58
10% gelatin protein+amino acid mix +amino acid mix 3[e]	140±6	0.58

[a] Average day-old starting weight, 42 gm.
[b] Mean±SE for duplicate lots of 5 chicks per treatment.
[c] For composition see Table 2, footnote "d".
[d] For composition see Table 3, footnote "d"; the amino acids simulating one and two percent casein protein were scaled down 2/3 and 1/3 of the amounts shown.
[e] Essential amino acids simulating those provided by 3% casein protein. See Table 3, footnote "d", omitting the additions of L-aspartic acid, L-cystine, L-glutamic acid, L-serine, and L-tyrosine.

TABLE 5.—*Body weights and feed utilization of chickens fed gelatin and amino acids simulating 3% casein protein from which one essential amino acid at a time was omitted*

Nitrogen Supplements	Two-Week[a]	
	Body Weight gm.	Gain-to-Feed Ratio
13% isolated soy-protein+0.2% DL-methionine	152±7[b]	0.52
10% gelatin protein+amino acid mix 1[c]	130±7	0.60
+amino acid mix 2[d]	141±6	0.62
+complete amino acid mix 3[e]	143±7	0.62
−L-arginine	141±6	0.61
−L-histidine	145±4	0.59
−L-isoleucine	140±7	0.62
−L-leucine	129±7	0.57
−L-lysine	80±5	0.38
−DL-methionine	140±8	0.57
−L-phenylalanine	77±3	0.35
−L-threonine	130±7	0.61
−L-tryptophan	143±4	0.61
−L-valine		

[a] Average day-old starting weight, 41 gm.
[b] Mean±SE for duplicate lots of 5 chicks per treatment.
[c] For composition see Table 2, Footnote "d".
[d] For composition See Table 3, Footnote "d".
[e] For composition See Table 4, Footnote "e".

TABLE 6.—*Two- and three-week body weights and feed utilization of chickens fed amino acid-supplemented gelatin diets or* DL-*methionine-supplemented isolated soy-protein diets*

Nitrogen Supplements	Two-Week[a]	
	Body Weight gm.	Gain-to-Feed Ratio
13% isolated soy-protein+0.17% DL-methionine	128±4[b] (165±7)[c]	0.49 (0.40)[c]
+0.27% DL-methionine	128±5	0.49
+0.37% DL-methionine	118±4	0.51
10% gelatin protein+amino acid mix 1[e]+amino acid mix 3[f]	134±6[d] (185±6)[c,d]	0.63 (0.40)[c,d]
+0.351% L-lysine HCl+0.179% L-threonine		
+0.17% DL-methionine	120±2	0.55
+0.27% DL-methionine	132±4	0.58
+0.37% DL-methionine	134±4[d] (193±6)[c,d]	0.61 (0.54)[c,d]

[a] Average day-old starting weight, 41 gm.
[b] Mean±SE for duplicate lots of 5 chicks per treatment.
[c] Bracketed data are results at 3-weeks of age.
[d] Mean±SE for triplicate groups of 5 chicks per treatment.
[e] For composition See Table 2, footnote "d".
[f] For composition See Table 4, footnote "e".

efficacy of 3% casein protein in providing good growth on the gelatin-amino acid diet was due to one or more of the essential amino acids it supplied. In Experiment 4, therefore, the essential amino acids simulating 3% casein protein (mix 3) were studied as supplements in successive diets from which one amino acid at a time was omitted. The results (shown in Table 5) confirmed that amino acid mix 3 (nonessential amino acids omitted) was as effective as was mix 2 (nonessential amino acids included) in the previous trial (Table 4). Omission from mix 3 of the amino acids methionine and threonine significantly ($P < 0.01$) reduced the growth rate of chicks to the level previously observed (Table 2) when the gelatin was only supplemented with amino acid mix 1. Omission of lysine resulted in slightly (not significantly) lower body weights and gain-to-feed ratios.

From this experiment it appeared that either greater amounts of methionine and threonine were required on a diet containing gelatin, or that the gelatin sample used in these studies had a composition which differed in the amounts of these amino acids from that given by Block and Weiss (1956), which had been used in the calculation of the amino acid supplements. The amino acid analysis for the specific gelatin sample used in these studies (Table 1) was markedly lower in phenylalanine, and moderately lower in threonine and lysine, but not appreciably lower in cystine and methionine. Based on the new gelatin analysis, amino acid mix 1, which was designed to supplement 10% gelatin protein to the amino acid levels of 13% isolated soy-protein, contained 0.09% less L-lysine and 0.08% less L-threonine than isolated soy-protein. The methionine and cystine levels were lower by a negligible amount only (0.01%).

The last trial was designed to answer two questions: (A) would the addition of only the three amino acids—lysine, threonine and methionine—to a diet containing 10% gelatin protein plus amino acid mix 1 support growth of the same order as that obtained with the 13% isolated soy-protein diet plus methionine—and equivalent to that obtained with the complete amino acid mix 3 (simulating the essential amino acids of 3% casein protein); and (B) was there a higher methionine requirement on the gelatin diet than on the isolated soy-protein diet.

The results in Table 6 suggested affirmative answers to both questions. Over a

3-week growth period the diet containing 10% gelatin protein, amino acid mix 1, and the three amino acids (lysine, threonine and methionine) supported growth which was significantly ($P<0.05$) better than that of the isolated soy-protein diet. There was a suggestion of a growth depression with the highest methionine addition to the isolated soy-protein diet, but chicks on the gelatin diet showed improved growth and gain-to-feed ratios from such supplementation. It has been shown that muscle creatine continues to increase with increased levels of dietary arginine beyond the point where dietary arginine affects growth rate (Fisher *et al.*, 1956). Since methionine is also necessary for creatine biosynthesis, a higher methionine requirement may arise from the relatively large amount of arginine and glycine contained in gelatin.

SUMMARY

A series of studies were conducted with chicks in an effort to solve the problem of the poor growth response obtained with gelatin as the protein source even when supplemented with amino acids to the level of isolated soy-protein plus added methionine. By adding small amounts of various intact proteins it was found that 3% casein would improve a 10% gelatin protein-amino acid mixture to the level obtained with isolated soy-protein. The same response was also obtained by the amino acids contained in 3% casein, and the solution to the problem was finally traced to the amino acids lysine, threonine, and methionine. Analysis of the gelatin used in these studies showed it to contain less lysine and threonine than suggested by Block and Weiss (1956), whose values were used in the calculation. For methionine there appeared to be a higher requirement with the gelatin diet than observed with the isolated soy-protein diet. It was postulated that the high arginine and glycine content of gelatin favors creatine synthesis, creating thereby a 'stress' for additional methionine.

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Proc. Soc. Exper. Biol. & Med. 1943, 52:46-48

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Observations on the Intravenous Injection of Gelatin for Nutritional Purposes.*

A. Brunschwig, V. B. Scott, N. Corbin and R. Moe.

From the Departments of Surgery and Medicine, The University of Chicago.

In view of the recent interest in parenteral injection of nitrogenous nutriment, the question of nutritive value of gelatin injected intravenously is being investigated. Observations of this type apparently have not been hitherto recorded. Such a study is complicated by the heterogeneous nature of gelatin. The sample employed in this study was designated as 3 Cu, and consisted of a light brown moderately coarse powder. Aqueous solutions were prepared by adding 120 to 125 g to 1000 cc of hot water. This was autoclaved at 15 pounds pressure and 250°F for 45 min prior to injections in animals. Total nitrogen determinations were made on each sample and this data employed to calculate nitrogen intake. Non-protein nitrogen varied widely in the several liter samples prepared, the maximum in one sample being 570 mg % but in most others it was under 100 mg %. This gelatin was found to contain no cystein or tryptophane and .8% tyrosin (analyses by Mr. Carter Johnston, Department of Biochemistry).

Dogs depleted of protein stores by maintenance on a Weech diet[1] for about one month received 150 cc of the gelatin solution (which was approximately 12% by weight) a day intravenously as the sole source of protein. During the injection period Weech diet was afforded *ad libitum* but the appetite varied widely. Urine (and feces) was collected every 24 hr and the daily loss of

* This work was conducted under a grant from the Edible Gelatin Manufacturers' Research Society.

1 Weech, A., *et al., J. Exp. Med.*, 1935, **61**, 299. (505 g of diet contains 1.23 g N.)

TABLE I.
Nitrogen Balance in Dogs Receiving Intravenous Injections of Gelatin.

	No inj.			Inj. period*				
Dog	No. days	N loss, g	Avg daily N loss, g	No. days	Total N inj., g	Total N loss, g	Avg daily N bal., g	N balance total period, g
352 (4.3 kg)				7	18.5	10.17	+1.2	+ 8.33
354 (6.4 kg)								
Exp. I	8	4.58	.57	8	27.9	14.32	+1.69	+13.58
Exp. II	5	2.92	.58	7	18.5	17.25	+ .18	+ 1.25
357 (5.6 kg)	7	4.77	.68	10	28.00	30.58	— .26	— 2.58
358 (5.6 kg)								
Exp. I	5	3.38	.68	15	41.70	41.30	+ .03	+ .40
Exp. II	5	1.28	.26	7	18.30	11.02	+1.04	+ 7.28

* Each dog received 150 cc of 12% gelatin solution daily.

nitrogen determined. This was compared with nitrogen intake as represented by the gelatin injections. Results are summarized in Table I. In 5 of the 6 experiments a positive nitrogen balance or a nitrogen equilibrium was obtained; in one instance there was a negative balance. In the latter (Dog 357) however, the daily net loss of nitrogen was less than in the period during which no injections of gelatin were received.

The fact that a positive nitrogen balance or equilibrium was obtained, does not indicate necessarily a utilization of gelatin for body or plasma protein synthesis or for energy. At least some gelatin remains unchanged and circulates in the plasma since samples of blood withdrawn 24 hr after an injection gel when placed in the ice box, and after several daily injections, some samples of urine also geled at 8°C. On the other hand it would appear that some of the gelatin is broken down by the organism since, as shown in Tables I and III, after daily injections were begun the nitrogen output exhibited a substantial increase and this was for the most part in the form of non-protein nitrogen.

When 2, 2.5 and 5% aqueous solutions of gelatin were analyzed as "blood plasma" for plasma proteins by the semi-micro Kjeldahl

TABLE II.
Plasma Protein Changes Resulting from Intravenous Injection of Gelatin.

Dog		Hematocrit, %	N. P. N., mg %	Plasma prot., g %	A/G
352	Before injection	44.5	18.2	4.52	2.09/2.43
	After 6 injections*	32	50	6.54	2.25/4.29
	4 days after last injection	38	28	5.54	2.03/3.51
	8 days after last injection	32	22	4.93	2.56/2.33
354	Before injection	37	17.5	4.73	2.65/2.08
	After 9 injections	30	26	6.10	1.52/4.58
	7 days after last injection	32	17	5.35	1.68/3.67
358	Before injection	48	23	4.22	2.41/1.81
	After 14 injections	23	—	5.79	2.65/3.14
	7 days after last injection	30	34	5.18	2.23/2.95
	14 days after last injection	28	26	4.30	2.22/2.08
356	Before injection	40	21	3.87	1.56/2.31
	After 5 injections	25	32	4.88	1.84/3.04
353	Before injection	49	17	3.90	2.15/1.75
	After 3 injections	38	322‡	8.63	2.71/5.92

*Daily injection of 150 cc of 12% gelatin solution.
‡Dog was uremic, died 24 hours after last injection.

TABLE III.
Showing Increased Urinary N Output During Period of Intravenous Injection with Gelatin. Dog 358. On Weech Diet. (Partial data.)

	No injections				Daily inj. 150 cc gelatin solution 6th to 20th day				No injection after 20th day			
Days	1	2	3	4	7	8	9	10	21	22	23	24
Urine vol., cc	280	240	250	160	240	380	400	300	200	100	140	120
Total N excreted, g	.47	.61	.56	.44	2.90	3.71	3.98	2.03	1.81	.76	1.06	.75
Total N.P.N. excreted, g					2.88	3.61	3.80	1.93	1.66	.68	.96	.65

method for total nitrogen, and precipitation with 21% sodium sulphite carried out as for separation of albumin from globulin, it was found that most of the gelatin precipitated as "globulin." For example, a 2.1% aqueous solution of gelatin yielded .68% "albumin" and 1.41% "globulin." A 5.33% solution of gelatin yielded 1.72% "albumin" and 3.61% "globulin." In another series of experiments gelatin was added to dog plasma low in proteins and the greater part of the gelatin was precipitated with the globulin fraction.

In the protein depleted dogs, the injections of gelatin resulted in a rather rapid and appreciable rise in plasma protein, this being in a large measure due to the presence of circulating gelatin. From the above mentioned observations it would therefore appear that the increase in "plasma proteins" thus brought about would constitute essentially an increase in the "globulins." Some of the data illustrating this is shown in Table II. Following the cessation of injections the nitrogen output fell appreciably and there was a gradual fall in the plasma protein level, the decrease being again principally the globulin fraction with relatively less fluctuation in the albumin fraction (with exception of Dog 354).

The animals tolerated the injections quite well. Occasionally there was emesis; in one animal marked erythema about the head and over the extremities developed after each injection. Severe reactions were not observed Elevations in temperature were likewise no observed. Since the animals were depleted of proteins, being on a very low protein diet, they were not in good condition at the onset of the experiments. Throughout the injection period this diet was available in unlimited quantities but as stated they ate poorly and, although maintained for periods in positive nitrogen balance or in nitrogen equilibrium, they continued to lose weigh and their condition deteriorated. The latter would be expected from maintenance on an incomplete diet.

Summary. 1. A positive nitrogen balance or nitrogen equilibrium may be maintained in protein-depleted dogs with a gelatin solution injected intravenously as practically the sole source of nitrogen.

2. Elevation of depressed blood plasma protein levels may obtain from intravenously administered gelatin as practically the sole source of nitrogen. The increase is apparently in the globulin fraction and represents to a large extent circulating gelatin. It was found that most of the gelatin used above in aqueous solution, or if added to plasma *in vitro,* precipitates with 21% sodium sulphite (thus behaving like a "globulin").

3. At least a portion of intravenously injected gelatin appears to be metabolized since there is a substantial increase in non-protein nitrogen excretion following injections.

Indian J. Biochem. 2:44-46, Mar. 1965

Ascorbic Acid Status of the Rat on Gelatin Diets*

N. CHANDRASEKHARA, M. V. L. RAO & M. SRINIVASAN
Central Food Technological Research Institute, Mysore 2

Manuscript received 9 December 1964

In adult male albino rats fed diets containing gelatin as the sole protein source, urinary excretion and liver levels of ascorbic acid are very low. Supplementation of 10 per cent gelatin diet with all the essential amino acids in which gelatin is deficient, increases urinary excretion of ascorbic acid nearly two-fold; the liver levels of the vitamin are also enhanced substantially. Of the various combinations of the amino acids employed, only methionine and tryptophane together bring about a similar increase. It is concluded that the amino acid composition of the dietary protein is an important factor determining the ascorbic acid status of the rat.

THAT dietary protein levels directly influence the ascorbic acid status of the rat[1] and that high levels of protein in the diet counteract the adverse influence of sulpha drugs[2] have been previously reported. In seeking the mechanism through which dietary protein exerts these effects, the influence of the quality of protein on urinary excretion and tissue levels of ascorbic acid has been studied. The effects of employing gelatin as the basal protein in the diet and of supplementing essential amino acids in which gelatin is deficient have been investigated. The results are presented in this paper.

Materials and Methods

Animals — Adult male albino rats (Wistar strain), weighing 140-170 g., were grouped according to the randomized block design and fed the experimental diets *ad lib.*

Composition of the basal diet — 'Difco' gelatin, 10 per cent; peanut oil, 10 per cent; sucrose, 10 per cent; salt mixture (Hubbel, Mendel and Wakeman) containing 40 mg. zinc carbonate/kg. mix, 2 per cent; vitaminized starch, 1 per cent; and corn starch was added to make up to 100 per cent (ref. 2). Vitamins A, D and E (20, 10 and 1 IU respectively) were separately provided in peanut oil.

Amino acid supplements — From the published data on the amino acid composition of gelatin (Difco)[3] and casein[4], the quantities of the essential amino acids to be added to a 10 per cent gelatin diet to make it equivalent to that of casein were calculated. They were dissolved in slightly acidulated water and required aliquots were added to the diet cups just before feeding. The following were the doses of the amino acid supplements (g./100 g. diet): L-lysine monohydrochloride, 0·55; DL-tryptophane, 0·50; DL-phenylalanine, 0·83; DL-methionine, 0·40; DL-threonine, 0·58; L-leucine, 1·40; DL-isoleucine, 1·40; and DL-valine, 1·06.

Collection of urine and liver tissue — After two weeks on the experimental diets, the rats were transferred to individual metabolism cages. Urine was collected in trichloracetic acid (final concentration, 5 per cent) for 3 days[1]. A week later, the animals were sacrificed under ether anaesthesia. Livers were excised, washed with cold isotonic KCl, wiped with filter paper, homogenized with distilled water in a Waring blender and made up to volume. An aliquot was deproteinized with trichloracetic acid (5 per cent) and the filtrate used for total ascorbic acid and glucuronic acid estimations.

Analytical methods — Total ascorbic acid in urine and tissues was estimated according to the method of Roe and Kuether[5] and glucuronic acid by Dische's procedure[6].

Results

In the first experiment, urinary excretion and liver levels of ascorbic acid were determined in rats fed diets containing 10 and 20 per cent gelatin and a 10 per cent gelatin diet supplemented

*Preliminary results obtained in these studies have been reported in the *Proceedings of symposium on vitamin metabolism*, Bulletin No. 25, National Institute of Sciences of India, New Delhi, 1962, 66.

with a mixture of amino acids in which gelatin is deficient to make it equivalent to a 10 per cent casein diet (Table 1). On a 10 per cent gelatin diet, excretion of ascorbic acid in the urine was nearly the same as that on a protein-free diet[1]. The liver level of the vitamin was also quite low. Increasing the gelatin content of the diet to 20 per cent lowered both urinary excretion and liver levels of ascorbic acid further. However, supplementation of the 10 per cent gelatin diet with the needed amino acids increased the urinary excretion and the liver levels of ascorbic acid almost two-fold.

In the second experiment, the effect of supplementing the basal 10 per cent gelatin diet with the essential amino acids in three different combinations was examined (Table 2). In the third experiment, the effect of individual supplements of methionine and tryptophane was ascertained (Table 3). It was observed that the excretion and the liver level of the vitamin were increased very significantly only when the diet was supplemented with tryptophane and methionine together.

Discussion

The results presented in Tables 1-3 show that in rats fed diets containing 10 per cent gelatin as the sole source of protein, both urinary excretion and liver ascorbic acid levels are very much lower as

TABLE 1 — URINARY EXCRETION AND LIVER LEVELS OF ASCORBIC ACID IN RATS FED GELATIN DIETS WITH AND WITHOUT SUPPLEMENTS OF ESSENTIAL AMINO ACIDS

(Adult male rats distributed into groups of 6 each according to the randomized block design)

Group	Diet	Urinary ascorbic acid* mg./day	Liver ascorbic acid†	
			Whole liver mg.	Per g. fresh liver mg.
I	10% gelatin	0·68 ± 0·11‡ (14 df)	1·61 1·09 2·30 ±0·33¶ (13 df)	0·37 0·22 0·45 ±0·06¶ (13 df)
II	20% gelatin	0·33 1·22 ±0·099‡ (14 df)		
III	10% gelatin + all deficient essential amino acids			

STATISTICAL ANALYSIS§

Group III ~ group I	Significant at 1% level	Significant at 1% level	Significant at 5% level
Group II ~ group I	Significant at 5% level	Significant at 5% level	Significant at 0·1% level

*One sample value in group I was missing. This was estimated by the missing plot technique.
†Two sample values, one in group I and the other in group III, were missing. They were estimated by the missing plot technique. The averages include the estimated values.
‡Standard error of the mean.
¶Standard deviation per rat.
§One-tailed test.

TABLE 2 — INFLUENCE OF SUPPLEMENTS OF AMINO ACID COMBINATIONS TO GELATIN DIET ON ASCORBIC ACID EXCRETION AND RELATED PARAMETERS

(Adult male rats distributed into groups of 6 each according to the randomized block design)

Group	Diet	Urinary ascorbic acid mg./day	Urinary glucuronic acid mg./day	Liver ascorbic acid	
				Whole liver mg.	Per g. fresh liver mg.
I	Basal 10% gelatin diet	0·30	4·88	0·83	0·19
II	Basal diet + methionine + tryptophane	0·63 ±0·06 (15 df)	5·49 ±0·38 (15 df)	1·66 ±0·10 (15 df)	0·31 ±0·02 (15 df)
III	Basal diet + threonine + lysine + phenylalanine	0·46	5·69	1·17	0·24
IV	Basal diet + leucine + isoleucine + valine	0·32	5·20	1·17	0·25

STATISTICAL ANALYSIS*

Group II ~ group I	Significant at 0·1% level	Not significant	Significant at 0·1% level	Significant at 0·1% level
Group III ~ group I	Significant at 5% level	do	Significant at 5% level	Not significant
Group IV ~ group I	Not significant	Significant at 5% level	do	Significant at 5% level

*According to one-sided *t* test.

TABLE 3 — EFFECT OF SUPPLEMENTS OF METHIONINE AND TRYPTOPHANE TO GELATIN ON URINARY ASCORBIC ACID AND RELATED PARAMETERS

(Adult male rats distributed into groups of 6 each according to the randomized block design)

Group	Diet	Urinary ascorbic acid mg./day	Urinary glucuronic acid mg./day	Liver ascorbic acid: Whole liver mg.	Liver ascorbic acid: Per g. fresh liver mg.
I	10% gelatin diet	0·34	5·25	1·12	0·23
II	10% gelatin diet + methionine	0·52	5·62	1·23	0·26
III	10% gelatin diet + tryptophane	0·34	8·05	1·14	0·23
		± 0·03 (10 df)	± 0·63 (10 df)	± 0·10 (10 df)	± 0·02 (10 df)
	STATISTICAL ANALYSIS				
Group I ~ group II		Significant at 0·1% level	Not significant	Not significant	Not significant
Group I ~ group III		Not significant	Significant at 1% level	do	do

compared to animals maintained on the corresponding level of casein[1]. Only supplementation with the two most limiting essential amino acids—methionine and tryptophane—serves to raise substantially the urinary excretion and tissue levels of the vitamin. Methionine alone causes a small but significant increase in the urinary excretion only. Tryptophane by itself has no influence on either of the parameters. Supplementation with all the amino acids in which gelatin is comparatively deficient gives the highest urinary ascorbic acid excretion.

In the first experiment, the average daily food intake of rats receiving the amino acid supplements was 6·5 g. per rat, as compared to 6 g. per rat in the group fed the basal gelatin diets. There were similar differences in other experiments. These variations are too small to account for the observed changes in urinary ascorbic acid excretion and other parameters which have, therefore, to be directly ascribed to the amino acids provided.

In their studies on the phenobarbital stimulated excretion of vitamin C in the rat, Roberts and Spiegel[7] also observed that supplementation of a low casein (5 per cent) diet with cystine and methionine considerably augmented the urinary excretion.

The amino acid supplements in the present study, excepting tryptophane in the last experiment (Table 3), have not been found to affect the urinary glucuronic acid, unlike the specific effects of cysteine and tyrosine reported by Martin and Stenzel[8]. This precludes any possible interpretation of the observed increases in the ascorbic acid excretion through increased production of glucuronic acid which is the first essential precursor for the biosynthesis of the vitamin.

The findings reported in this paper point to the amino acid make-up of the protein as an important factor determining the urinary excretion and liver levels of ascorbic acid in the rat. Since the pattern of changes in the urinary excretion and liver ascorbic acid levels are the same, these changes may be interpreted as reflecting the biosynthesis of the vitamin. It is known that all the enzymes involved in ascorbic acid biogenesis in the rat are located in the liver. The liver levels of several important enzymes are also known to be markedly influenced by the plane of protein nutrition[9]. It would, therefore, appear reasonable to infer that some of the enzymes concerned with the biosynthesis of ascorbic acid may also be influenced in a similar manner. This aspect is now under study.

Acknowledgement

The authors' thanks are due to Dr H. A. B. Parpia, Director of the Institute, for his kind interest in the work and to Shri A. N. Sankaran for the statistical analysis of the data.

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Amer. Jour. Physiol. 133(3):520-524, 1941

THE INFLUENCE OF GELATIN INGESTION UPON THE CREATININE-CREATINE EXCRETION OF NORMAL MEN

D. B. DILL AND S. M. HORVATH

With the technical assistance of F. CONSOLAZIO

From the Fatigue Laboratory, Harvard University, Boston, Mass.

Accepted for publication April 4, 1941

It is generally taught that creatine is not present in the urine of adult men apart from such pathological conditions as complete starvation, Graves' disease, and progressive muscle dystrophy. Attempts by various workers to bring about a creatinuria by feeding high protein diets have given conflicting results (Denis and Minot, 1917; Rose et al., 1918; Lewis and Doisy, 1918; Bollman, 1929–30). Following the demonstration by Brand et al. (1929) that feeding glycine to persons with progressive muscle dystrophy increased their output of creatine, several investigators fed glycine to normal individuals (rats and man) (Bodansky, 1935–36; Beard et al., 1931–32, 1939; Borst and Möbius, 1936). Contradictory results were again obtained, and the substitution of gelatin, which is 25 per cent glycine, gave no better agreement (Denis and Minot, 1917).

In connection with other studies on the influence of a high gelatin diet, we followed the excretion of nitrogen, creatinine and creatine in four healthy young men (20–29 years old) who were engaged only in laboratory work during the course of the investigation. Twenty-four hour urines were collected for three consecutive days while on their usual diet. This diet was then supplemented with 60 grams of ossein gelatin daily, 30 in the morning and 30 in the evening. No precise control was exercised over the diet during the gelatin feeding; each man ate as he chose.

Feeding of gelatin continued for 41 days in the case of 3 subjects and for 51 days with the fourth subject, B. C., a colored laboratory helper. Twenty-four hour urines were collected for the first 3 days of each week and excretion studies were continued for at least 7 days following cessation of gelatin feeding. Analyses were performed immediately. Nitrogen was determined by the Kjeldahl method and preformed creatinine by adding alkaline picrate to the fresh urine as described by Folin (1904). For total creatinine the sample was autoclaved with HCl at a temperature of 120° for 30 minutes, and to an aliquot alkaline picrate was also added. Creatine values were obtained by difference. All color comparisons were made in

an Evelyn photocolorimeter. The final values for the creatinine equivalents were obtained from a calibration curve prepared from analyses of pure creatinine solutions.

RESULTS. For convenience, the data are presented graphically (figs. 1 and 2). The added gelatin was equivalent to the daily feeding of 8.8 grams of nitrogen and if its glycine nitrogen were completely converted to creatinine and creatine nitrogen the yield would be about 6 grams (or according to the theory of Beard (1939) the yield would be about 48 grams). The nitrogen excretion of all subjects increased greatly, although in two of the subjects (B. C. and S. M. H.) the extra nitrogen output was less than in the control period when the gelatin nitrogen intake was deducted. Since the subjects were not on a constant diet, we do not know whether nitrogen was retained or the intake of non-gelatin nitrogen was decreased. The excretion of nitrogen in one subject (F. C.) reached 33.5 grams in 24 hours. The nitrogen excretion of three subjects had returned to pre-gelatin values

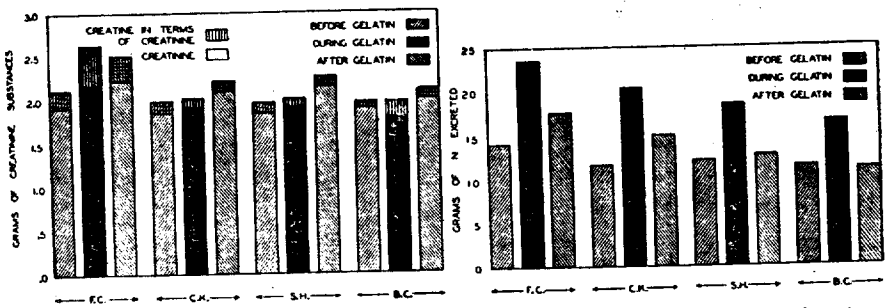

Fig. 1. The average 24-hour excretions of nitrogen, creatinine, and creatine (expressed as creatinine) for four normal men before, during and after a period of gelatin ingestion.

about four days after discontinuing gelatin and in the fourth subject shortly thereafter. (The average values of nitrogen excretion for each subject before, during and after the period of gelatin feeding are shown in fig. 1.)

Although Light and Warren (1934) found no creatine in the urine of males above the age of 19, each of our subjects normally had a measurable creatine excretion even up to 0.27 gram (expressed as creatinine). This is in agreement with Hobson (1939), who recorded creatine excretion in 96 of 97 males. The influence of extra gelatin on the creatine excretion is inconclusive. Two subjects had increases. This was particularly apparent in F. C., who in one 24-hour period excreted over 0.8 gram of creatine (about one-third as much as his creatinine excretion) (fig. 2). In these subjects the increases are unmistakable: in one only 2 of 19 and in the other 2 of 13 urines contained less creatine than in the control periods. However, the other two subjects had extremely variable excretions, and

their average creatine output for the entire period of gelatin ingestion is lower than in control periods. After discontinuing gelatin, the average creatine excretion was slightly above previous control levels. The probability that some creatine had been stored and was being slowly excreted (fig. 2) is indicated by the return of the daily excretions of creatine to pre-

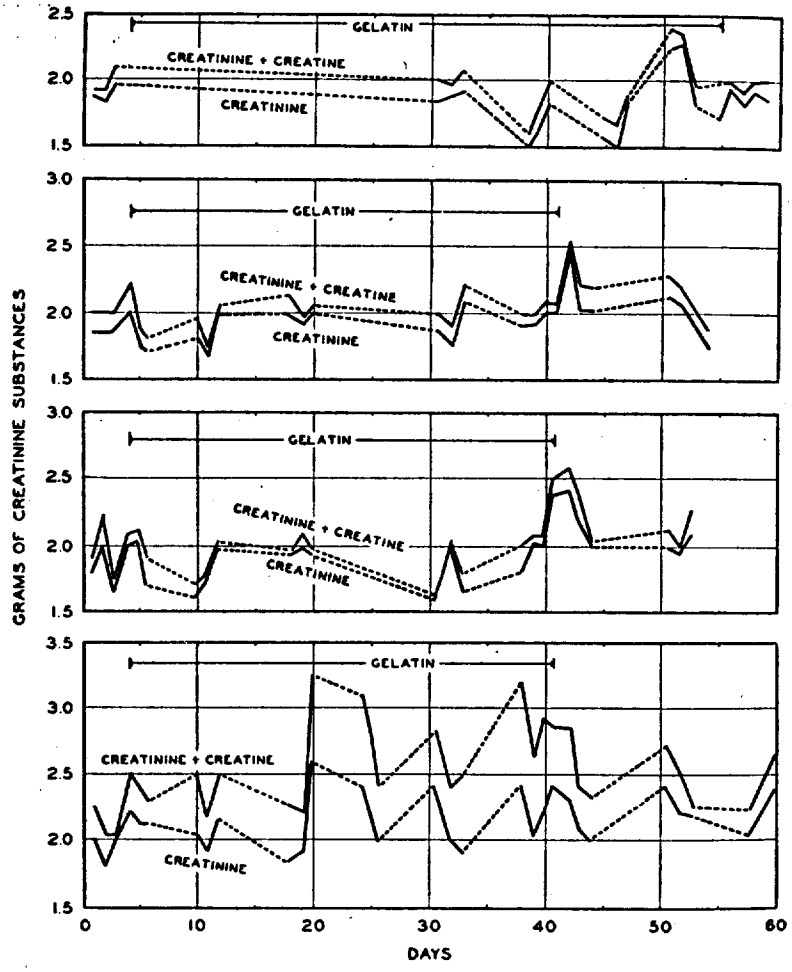

Fig. 2. The daily variations in the excretion of creatinine and creatine of four men before, during, and following the period of gelatin ingestion. (The solid lines indicate days during which the determinations were made.)

gelatin values toward the end of this period. Denis and Minot (1917) were unable to obtain creatinuria in two normal males on a high protein diet which included 50 grams of gelatin. Lewis and Doisy (1918) and Rose et al. (1918) also found no excretion during high protein diets.

As shown by Folin in 1905 and by other workers of that period, the creatinine output in 24 hours is nearly constant for a healthy person and

is uninfluenced by such factors as a high protein, meat free diet. Hobson (1939) states that he was unable to find any significant variation in creatinine excretion of subjects when changed from an adequate but high carbohydrate diet to a low carbohydrate, high protein diet. His mean values for creatinine outputs in 11 subjects are 2.139, 2.140 and 2.107 grams while on a high carbohydrate, and the first and fourth days of a low carbohydrate, high protein diet, respectively. The data presented by us show less regularity in creatinine excretion. The values of creatinine excretion for the four men show an extreme range from minimum to maximum of 0.85 (1.78–2.63); 0.87 (1.57–2.44); 0.82 (1.68–2.50); 0.85 (1.53–2.38) during the entire period of the investigation.

Utilizing average values for creatinine (fig. 1) there is little change in 2 of 4 subjects in its excretion during the period of gelatin ingestion. One of the 4 subjects had a lower average excretion, while another had a much higher excretion. Following the discontinuance of gelatin, the excretion of creatinine was higher in all subjects than in the control periods. Since this was also observed in three subjects during the period of gelatin feeding, the possibility of excretion of exogenous creatinine from stored creatine cannot be entirely ignored. However, the amount actually excreted is small compared to the amount theoretically possible.

Since one-fourth of gelatin is glycine, it is possible that any physiological effects produced by feeding glycine might also be obtained by feeding proportionally larger amounts of gelatin. In treatment of muscle dystrophies gelatin has been found satisfactory by some investigators and unsatisfactory by others. However, gelatin can be effectually substituted for glycine as a detoxifying agent (Griffith, 1934). Denis and Minot (1917) did not observe creatinuria in two males fed 50 grams of gelatin daily. Our data show that creatinuria is possible when subjects ingest 60 grams of gelatin, although it may not necessarily occur to any great extent. This lack of uniform effect is also evident in the feeding of glycine. Borst and Möbius (1936) have failed to observe any influence on the urinary creatine and creatinine of normal adults given 50 grams of glycine. Zwarenstein (1928) also noted no influence (10 grams), while Beard et al. (1939) were able to recover slightly more than the theoretical amount of creatine and creatinine (16.2 grams) formed from the feeding of 5 grams of glycine. Bodansky (1935–36) noted only an effect on creatine output.

We were able to verify Hobson's observation of the presence of creatine excretion in normal adult males. These observations are contrary to general opinion (Folin, etc.). Furthermore, the daily excretion of creatinine is not as consistent as many investigators have reported, although such constancy may be observed in some individuals. As a result, we do not believe in the validity of the assumption that creatinine excretion can be used as a test of the completeness of a 24-hour urinary output (Folin, 1905).

SUMMARY

The addition of 60 grams of gelatin daily to the diet of four males was accompanied by an increased excretion of creatine in two of the subjects. The average excretion of creatinine was not markedly increased by the high protein intake. There was, however, increased excretion of both creatinine and creatine in all subjects on cessation of gelatin ingestion indicating a possible storage during the previous period of gelatin feeding.

In contrast to most previous reports all the subjects normally had some creatine present in their urine and also showed considerable variation in the 24-hour excretions of creatinine.

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J. Metab. Research 5:145-168, April-June 1924

A STUDY OF THE NUTRITIVE VALUE OF GELATIN*

By THOMAS B. DOWNEY** †

INTRODUCTION

The investigation of gelatin reported in this paper has had for its purpose the extension of the knowledge of the supplementary relations existing between gelatin and other proteins, and the determination of the influence of the colloidal properties of gelatin upon the absorption of nutrients when ingested with other food products.

According to the classification of proteins adopted by the American Society of Biological Chemists and by the American Physiological Society, gelatin belongs to the sub-group of simple proteins known as albuminoids. The English classification of gelatin as a sclero-protein is descriptive of its parent substance, collagen, which is present in skin and bone. It was the first protein to be separated in quantity in an approximate state of purity.

Gelatin was introduced as an economy food during the period of the French Revolution. In view of the status of the knowledge of nutrition of that day, it is not surprising that there arose exaggerated opinions, both pro and con, regarding the actual food value of the product.*** It is only in quite recent times that the results of earlier investigations on gelatin as a food can be interpreted with accuracy.

* A paper presented before the Division of Biological Chemistry of the American Chemical Society, Ithaca, N. Y., September 11, 1924.

** Senior Industrial Fellow, Mellon Institute of Industrial Research, University of Pittsburgh, Pa.

*** Much of this early work, particularly the activities of the three French Commissions, is summarized in the Catalogue of the Surgeon General's Library, (1) 5, 824; (2) 6, 116.

† Received for publication January 11, 1925.

DIRECT UTILIZATION OF GELATIN IN TISSUE BUILDING AND CONSERVATION

The research of Voit,[1] who attempted to substitute with gelatin a part or all of the meat proteins in his diets with the dog and found that gelatin as the sole source of protein did not prevent loss of body nitrogen, gave impetus to further studies of this odd behavior. Orum,[2] Munk,[3] and Bischoff and Voit[4] continued this work with similar observations, while Kirschmann[5] and Krummacher[6] determined the extent to which gelatin reduces the waste of body protein in metabolism (23 to 37.5 per cent., according to the level of total heat requirements at which it is fed). It was thus apparent that gelatin must differ from the protein mixtures with which it had been compared, although it contained about the same amount of nitrogen, possessed a similar calorific value and yielded in the diabetic the same amount of sugar.[7]

In 1905, Kaufmann[8] demonstrated that the inability of gelatin to function as the sole source of protein was due to a shortage of certain essential amino-acids in its cleavage products. In his experiments, dogs whose diets contained gelatin supplemented with tyrosine, cystine and tryptophane to the extent of 4.2 and 1 per cent., respectively, of the total nitrogen in the diets, replaced completely the body protein lost through metabolism. Kaufmann also confirmed these results by experimentation upon himself. Abderhalden[9] has corroborated these findings by similar experiments. McCollum[10] has pointed out that Kaufmann's experiments were of such short duration that the omission of certain dietary factors, apparently, did not interfere with the outcome of the study. As one would expect, the attempts of Rona and Muller[11] to bring dogs into nitrogenous equilibrium by feeding gelatin with tyrosine and tryptophane (but not cystine) met with failure. This experimental work will be discussed subsequently. Sure[12] found that gelatin, fed at 12 per cent. planes of intake in the presence of 0.4 per cent. each of cystine and tryptophane and 0.6 per cent. of tyrosine, supports maintenance for several weeks, followed by decline.

An extensive investigation of the substitution of gelatin for other proteins in mixed diets, with maintenance of nitrogen equilibrium at the fasting level, has been carried out by Murlin.[13] He showed that as much as two-thirds of the total protein nitrogen in certain combinations could be satisfactorily replaced by gelatin nitrogen, and also that the utilization of gelatin was not due to the 50 per cent. (65 per cent., according to Janney) of glucose which arose from it in metabolism, but to the specific chemical nature of gelatin.

McCollum[14] found that pigs, brought into a condition where they exhibited only the endogenous type of metabolism, excreted in the urine only a part of the nitrogen ingested by them in the form of the incomplete proteins, zein and gelatin. 40 per cent. of the gelatin nitrogen and 73 per cent. of the zein nitrogen were "retained for some purpose in the tissues, presumably for a partial repair of tissue wastes".

It is evident from the foregoing researches that the value (for maintenance and repair) of incomplete proteins, of which gelatin, zein and hordein

are excellent examples, is small where they are fed to the total exclusion of others. This conclusion however does not indicate that these proteins may not be of decided value where taken in appropriate combinations.

The most complete analysis of the amino-acid radicles of the gelatin molecule was made by Dakin,[15] who has accounted for 91.31 per cent. of its cleavage products. Tryptophane and cystine are absent and tyrosine is present in very small amount. Glycine, an amino-acid capable of synthesis in the animal body, is present in surprisingly large amounts (25.5 per cent.). Of particular significance in supplementary relations is the relatively large quantities of lysine (5.9 per cent.) as well as of arginine (8.2 per cent.), proline and hydroxyproline (9.5 and 14.1 per cent., respectively). Sure[16] has found that the addition of 0.4 per cent. of proline to a diet containing 6 per cent. of edestin and 0.4 per cent. each of cystine and lysine accelerated growth, whereas other additions of amino acids did not, and concludes that "proline is an essential amino-acid for growth."

An extensive investigation of the supplementary relations between gelatin and the cereal grains has been made by McCollum and his coworkers.[17] They found that gelatin is a remarkably efficient supplementary protein where fed with proteins of wheat and oats, presumably through the high lysine content of gelatin. Maize, however, is not improved in biological value, as the limiting amino-acids in zein, the chief protein of corn, are cystine and tryptophane. The following pertinent statements are quoted from this paper: "So small an addition as 3.5 per cent. of gelatin, because of its high lysine content, greatly increases the utilization of the wheat proteins. Without this addition growth takes place at a rate approximating half the normal expectation. With gelatin addition growth proceeds at the normal rate". With respect to oats: "Our standard food mixture (Chart 5), with its 9 per cent. of oat protein, becomes adequate to support growth at the normal rate to the full adult size when 10 per cent. of gelatin is added. This serves as a remarkable illustration of the need of an understanding of the degree to which the proteins of one natural foodstuff make good the supply of the cleavage products of another which are present in smallest amount. Gelatin as a source of protein is wasted when combined with maize proteins, but its nitrogen is of value when combined with oat or wheat proteins". McCollum concludes that these results show that "oat proteins are improved by the addition of 3.5 per cent. of gelatin." The remarkably efficient supplementary value of gelatin upon oats is confirmed by Osborne and Mendel,[18] whose food consumption records are particularly striking. They report that a rat fed on 45 per cent. oat concentrate (2.7 per cent. nitrogen) in an otherwise adequate diet over a 2-week period gained 10 gms. in weight, consuming 92 gms. of the food mixture. During the succeeding 2 weeks, this animal was fed on the same diet, except that ¼ of the oat nitrogen was replaced with gelatin nitrogen, and the rat gained 23 gms. in weight, consuming 87 gms. of the food mixture. There is a wide difference between 9.2 gms. and 3.8 gms. of food consumed on the respective diets for each gm. gained in weight. It is difficult to understand why Mitchell[19] failed to obtain evidence of supplementation by his special biological method.

McCollum[20] has found that gelatin, like lactalbumin, is unsuited for making good the deficiencies of pea proteins. Sure has recorded similar observations with the Georgia velvet bean[21] and arachin,[22] the globulin from the peanut. When the velvet bean was supplemented with cystine, only slow growths were obtained upon the addition of gliadin or zein; with gelatin addition, however, a remarkable and continuous response in growth resulted. According to Hogan,[23] kafirin (an alcohol-soluble protein extracted from kafir) is only partially supplemented by gelatin, as lysine is the first limiting factor and cystine the second. Sure[24] has found that edestin at 6 per cent. intake is inadequate, but supports fair growth upon the addition of 6 per cent. of gelatin; this growth is markedly increased by the further addition of 0.4 per cent. cystine. In subsequent experiments, Sure[25] reports that proline is a third limiting amino-acid in edestin. As noted previously, gelatin contains considerable proline as well as hydroxyproline.

COLLOID-CHEMICAL ASPECTS

A property of gelatin that has been of particular interest to the colloid chemist, is the influence of protective colloids, such as gelatin, gum arabic and starchy decoctions (barley water cococtions) upon the digestibility of cow's milk. Indeed, the use of these protective agents is common practice in infant feeding as well as for sick children, where difficult feeders are unable to digest and assimilate their foods. Alexander and also Alexander and Bullowa[42] have concluded that the casein of milk, an irreversible colloid, is protected from precipitation by the reversible colloid lactalbumin. They have shown that the digestibility of milks from various animals are in the order of the relative proportions of casein and lactalbumin contained in them. For example, cow's milk contains about 6 times as much casein as lactalbumin, is the least protected of the milks examined and is the most difficult of digestion; in striking contrast is the milk of the ass, which is highly protected and exceedingly easy of digestion. The addition of small amounts of gelatin or of gum arabic to cow's milk results in such protective action that its behavior towards acid and rennin coagulation is similar to the deportment of milks that are highly protected by lactalbumin and are known to be digested readily (human milk). Alexander consequently advocates the use of gelatin in infant feeding. Moore and Krombholz[26] from their studies of emulsions conclude that the use of emulsifying agents, such as gelatin and albumin, are of significance in maintaining fats in a finely divided emulsified condition during digestive processes (see also Clayton[26]).

Gelatin is of particular interest to biochemists because it is unexcelled as a protective colloid, according to its gold number by the method of Zsigmondy,[27] and because it is an excellent emulsifying agent. Tadokoro and Sato[28] have shown that gelatin in 0.5 per cent. solutions is more efficient against coagulation of milk by acids and rennin than dextrin and various starches, although it is excelled by sodium nucleate and glutinate. The pH of these alkaline salts may have had a significant influence in these determinations.

The gastric digestion of milk in the presence of gelatin has been investigated by Hawk,[29] who found the addition of 1 per cent. of gelatin to cow's milk reduces the gastric digestion period in man; curd formations are modified in size and texture, the flow of gastric juice is greater as measured by titrations of total acidity, but free acid is about the same as found after the ingestion of plain milk. Doubtless this difference in acidity is taken care of by the amphoteric nature of gelatin. Hawk reported that sick children and hospital patients were improved by the addition of gelatin to their dietaries.

The value of gelatinated milk in infant feeding has been endorsed by medical specialists.[30]

EXPERIMENTAL PART

By means of feeding experiments with the white rat, the food value of gelatin has been investigated from two angles: first, the supplementary relations existing between gelatin and the proteins of other food products; and second, the influence of the colloidal properties of gelatin upon the utilization of certain dairy products.

In the study of the supplementary value of gelatin, the technic employed was essentially that used by McCollum and coworkers.[72] A ration consisting of the particular food under investigation was supplemented with inorganic salts, butter fat or cod-liver oil, and alcoholic extract of ether-extracted wheat embryo so as to be properly constituted for promoting growth and prolonged well being, except that the protein was regulated, by the addition of starch in certain cases, at too low a level to promote growth at the normal rate. The comparative ration was made up in the same way, except that a definite amount of gelatin replaced starch. The bread rations (see Chart III) are exceptions, as starch was not added to them. The data are given in Charts I-IV and their accompanying explanatory notes.

In Charts V-VIII are shown the results of feeding relatively small amounts of gelatin in various dairy products. In each of these experiments the following plan was observed: shortly after weaning one or more litters of young were divided into two groups, one group was fed entirely upon the dairy product, the other upon the dairy product in which was dissolved a definite proportion of gelatin. The rats, 2 or 3 to the cage, were fed daily, *ad libitum*, in agate cups. Records of the food consumption were kept for 6 weeks or longer. The design of the cups prevented appreciable losses of the food mixtures. A small error, consistent

in each experiment, was introduced by loss of moisture through evaporation.

Chart V, groups 1 and 2, presents the observations with diets of pasteurized whole cow's milk (3.7 per cent. of fat; 3.4 per cent. of protein) and of pasteurized whole cow's milk containing 1 per cent. of gelatin.* The gelatin was dissolved in the milk by soaking for 5-10 minutes at room temperature, followed by heating in a water-bath at 55-60°C for about 10 minutes. The whole milk was also subjected to this heat treatment. Diets were made up at 3-day intervals. While Chart V shows that the value of milk for the promotion of growth and continued well being is increased by the addition of gelatin, of further significance is the comparison of food consumption as given in the following table.

TABLE I.

Period 1 of 3 weeks Ration	Animals	Total gains in body weight in gm.	Total food intakes in gm. (wet basis).	Food intake in gm. for gm. gain in body weight (wet basis).
Pasteurized whole milk (See Chart V, Group I)	145, 146 130, 131	213	3324	15.6
Pasteurized whole milk containing 1 per cent. of gelatin	147, 149 132, 133	221	2628	11.8
Period 2 of 3 weeks				
Pasteurized whole milk	145, 146 130, 131	149	4520	30.3
Pasteurized whole milk containing 1 per cent. of gelatin	147, 149 132	127	3258	25.6

During the second period of 3 weeks, female 133 was not included in the tests (see note under Chart V). By the addition of 1 per cent. of gelatin to the whole milk, the total solids were increased from 12.6 to 13.4 per cent., and the protein from 3.4 to 4.3 per cent. During the entire six-week period the average consumptions of foods for gm. gains in body weight were 21.7 gm. of milk and 16.9 gm. of gelatin-milk, corresponding

* Reference is had here to gelatin of the quality known as "edible", which possessed the following characteristics: low ash and compliance with governmental legislation in sulfur dioxide and heavy metal content, pleasing bouquet and absence of pathogenic bacteria.

to food intakes of 2.7 gm. of milk solids and 2.2 gm. of milk solids and gelatin for the respective rations. If this result is expressed as percentage of whole milk successfully replaced by gelatin, the saving is 28 per cent., or, considering gelatin as part of the solids in the gelatinated milk, the saving of solids is 23 per cent. On the basis of actual protein consumed per gm. gain in body weight, it is found that 0.73 gm. of proteins was ingested in each diet.

This experiment was later confirmed on a different strain of rats.* Eight males and 2 females, selected from 2 litters of young, 25-28 days old, were placed on the respective diets. Each diet group consisted of young from both litters for the males, while the females were selected from the same litter. The results of the test over a period of 6 weeks, starting 1 week after the animals were first placed on the diets, are given in the following table.

TABLE II.

Period 1 of 3 weeks Ration	Animals	Total gains in body weight in gm.	Total food intakes in gm. (wet basis).	Food intake in gm. for gm. gain in body weight (wet basis).
Pasteurized whole milk	4 males 1 female	227	4999	22
Pasteurized whole milk 1 per cent of gelatine	4 males 1 female	255	4516	17.7
Period 2 of 3 weeks				
Pasteurized whole milk	4 males 1 female	118	5138	43.9
Pasteurized whole milk 1 per cent of gelatine	4 males 1 female	163	5409	33.2

During the entire period of 6 weeks, 4 males and 1 female fed on milk gained 345 gm. in weight, consumed an average of 29.5 gm. of milk (3.7 gm. of milk solids) per gm. gain in body weight, while their 4 brothers and 1 sister, fed on milk and gelatin, gained 413 gm. in weight and consumed 23.7 gm. of milk-gelatin (3.2 of milk solids and gelatin) per gm. gain in body weight. It follows that there were savings of 24 and 16 per cent., as calculated above. The proteins ingested were 1.00 and 1.03 for the respective diets. It will be observed that the food consumptions of these animals were greater than those previously recorded. This result was doubtless due to the larger sizes of the animals when first placed on the diets and their sub-

* These animals were provided by Mr. K. K. Jones, nutritionist of Swift and Company, Chicago, Illinois, to whom the author wishes to express his thanks.

sequent growth during the period of one week before the record of food consumption was started.

Chart VI, group 1 and 2, shows the growth of rats on milk containing 5 per cent. of whole egg (moist basis) and upon this combination with the further addition of 1 per cent. of gelatin. The raw egg was incorporated with the milk by beating and the gelatin was dissolved in a portion of this mixture by soaking and warming at 45-50°C. for about 10 minutes, both diets receiving the same heat treatment. It was found that the addition of egg improved the growth value of milk to a marked degree, which was further increased by the addition of 1 per cent. of gelatin. In this experiment, the utilization of milk-egg-gelatin combination was better than milk-egg to the extent of about 14 per cent., based on food intake for equivalent gain in weight.

Charts VII and VIII are illustrative of the development of rats fed on the dairy products, plain ice cream plus 0.5, 0.75 and 1 per cent. of gelatin. In these charts the compositions of the diets are expressed in percentages of solids, a system customarily used in the industry. The ration of group 1, Chart VII, plain ice cream (the other three diets were prepared in identically the same manner with the addition of the designated percentages of gelatin) was made up as follows: raw milk, unsalted butter or 30 per cent. cream, skim milk powder, and cane sugar were mixed in the proper proportions to give a total solid content of 36 per cent., 11 per cent of which was butter fat, 14 per cent. cane sugar, and 11 per cent. milk solids not fat. The mixture was pasteurized at 60°C. for from 25 to 30 minutes, passed through a viscolizer* at 2000 lb. pressure, cooled and ripened for about 24 hours at 4°C., then frozen, weighed into small glass jars, and held at about —18°C.** A jar of each diet was removed daily and the animals fed liberally. Sufficient rations were usually made up for 3 weeks feeding.

The rats used in this series were taken from 4 litters of young at 25-28 days age and apportioned to the four comparative diets. After having been on the respective diets for 1 week, a record of their food consumption for 56 days was kept. The pertinent data are given in the following table.

* The machine used was generously loaned by Cherry-Bassett-Winner Company, Philadelphia, Pa.

** The author acknowledges his gratitude to the C. Nelson Manufacturing Company, St. Louis, Mo., for the use of a mechanically refrigerated ice cream cabinet.

TABLE III

	Period of test in days	Rats on test	Total gains in body weight in gm.	Total food intake in gm. (wet basis).	Food intake in gm. for gm. gain in body weight (wet basis).	Food intake in gm. for gm. gain in body weight (dry basis).
Plain ice cream formula (See Chart VII, group 1).	56	♂281, ♂282, ♀203, ♀204, ♀205	454	6024	13.3	4.79
Composition same as above, except 0.5 per cent. gelatin added (See Chart VIII, group 1).	56	♂285, ♂286, ♀209, ♀210, ♀211	481	5465	11.4	4.16
Composition same as above, except 0.75 per cent. gelatin added (See Chart VIII, group 2).	56	♂287, ♂288, ♀212, ♀213, ♀215	509	6227	12.3	4.52
Composition same as above, except 1.0 per cent. gelatin added (See Chart VII, group 2).	56	♂283, ♂284, ♀206, ♀207, ♀208	581	6486	11.2	4.14

In the diets containing 1 and 0.5 per cent. of gelatin, respectively, the same gelatin sample was used, namely, a calf stock gelatin, Bloom gelometer reading 116 gm., viscosity 25 millipose, pH 6.0, ash 1.8 per cent.; while the 0.75 per cent. gelatin was made from green bone, Bloom gelometer reading 69 gm., viscosity 22 millipose, pH 6.2, ash 2.0 per cent. These differences in part account for the lower efficiency of food utilization on the 0.75 per cent. gelatin diet. Chart VIII shows however that the second gelatin has had a marked effect on growth and a profound influence on the ability of animals to reproduce and rear their young.

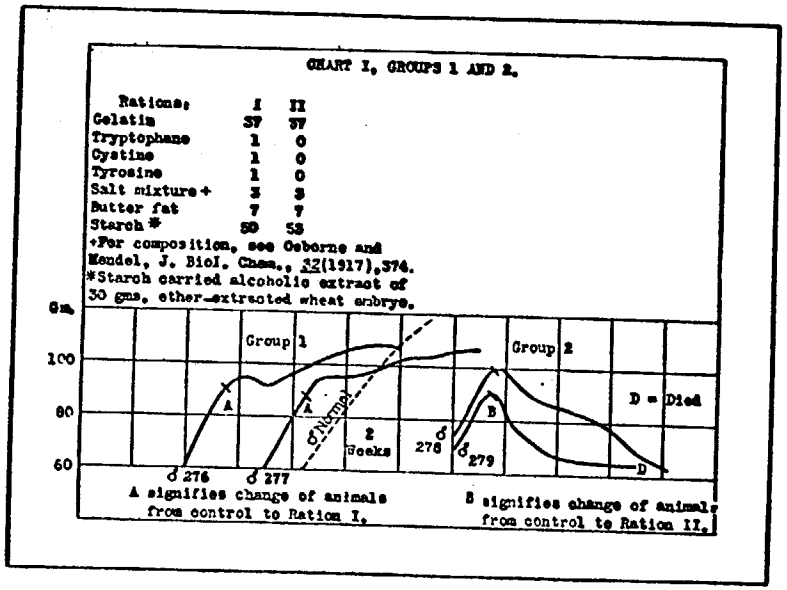


Chart I, group 1, shows that the deficiencies of gelatin as the sole source of protein in an otherwise adequate diet, illustrated by Group 2, ration II, are supplemented by the addition of the amino-acids tryptophane, cystine and tyrosine. The transfers of group 1 and group 2 animals from the control diet to rations I and II, respectively, are indicated by intersecting lines and letters A and B. The rations were fed in the form of still jellies (50 per cent. of water), because previous experience had shown that foods containing large proportions of gelatin are not freely consumed when dry. Even with this modification the food consumption after the first week was considerably less and variable. This result leads to the belief that ration I with normal food intake would show a greater repair value than that registered by group 1 rats.

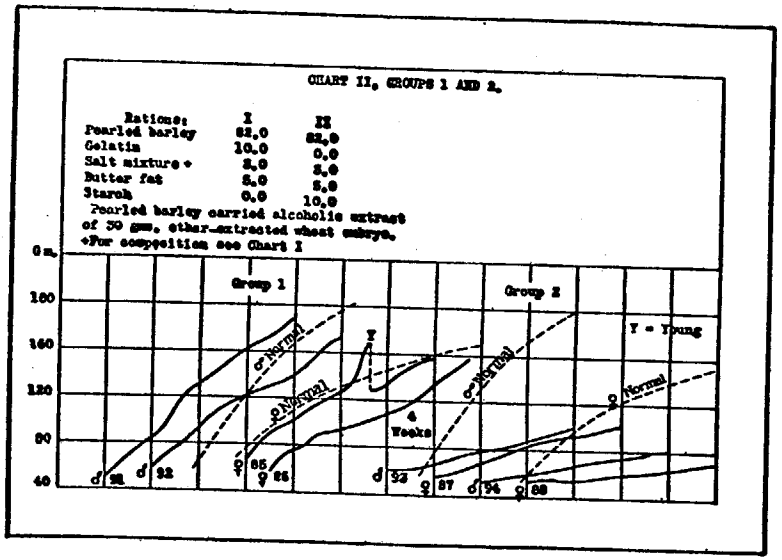


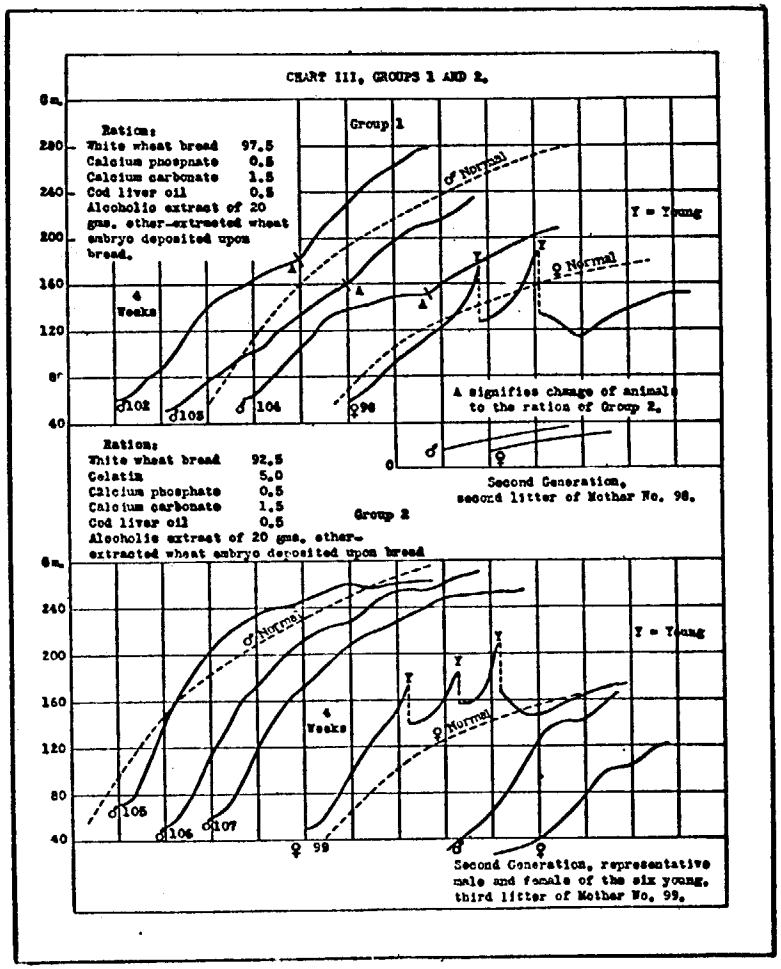
Chart II, group 1 and 2, with rations I and II, respectively. — This chart demonstrates that the protein deficiencies of pearled barley, where fed at a level of 7.2 per cent. in an otherwise adequate ration, are efficiently supplemented* by the addition of 10 per cent. of gelatin. The rats used in this experiment were taken from the same litter. While female 85, group 1, gave birth to young, she did not raise them.

In a second experiment, terminated after 2 months, it was found that a food mixture of 91.5 per cent. of pearled barley (approximately 8 per cent. of barley proteins), 3 per cent. of salt mixture, 5 per cent. of starch, and 0.5 per cent. of cod-liver oil supported growth at about one-half normal expectation. Where the starch was replaced with 5 per cent. of gelatin, a normal growth of all rats was obtained.

In a third test it was observed that sifted, ground whole barley, where fed as the sole source of protein (9 per cent.) in an otherwise adequate diet, was efficiently supplemented by the addition of 5 per cent. of gelatin.

Chart III demonstrates that the proteins (approximately 12.0 per cent.) of commercial white wheat bread are improved in biological value by the inclusion of 5 per cent. of gelatin. The rations were prepared by passing moistened bread and other designated constituents of the diets through a food chopper. As these food mixtures had been made adequate in salt and

* In a restricted sense, the results of these tests would be described in terms of additive value rather than of "supplementary value."



vitamine contents, the development of the rats was controlled by the quality of bread proteins for group 1 and by bread proteins plus 5 per cent. of gelatin for group 2. The results on group 1 show that the proteins of white wheat bread do not support normal growth. The growth of female 98 was comparatively rapid. She bore 2 litters of young and was able to rear 2 of the 7 born in the second litter. These young were exceedingly scrawny,

weighing only 36 and 31 gm. at the age of 4 months. After 4 months on the ration of group 1, the 3 males were changed to group 2 ration and a pronounced increase of their growth rate was observed, particularly in the case of male 102.

The findings with group 2 show that the addition of 5 per cent. of gelatin to bread proteins promotes growth at the normal expectation. Female 99 on this diet bore three litters of young and reared six of the seven born in the third litter. The second generation consisting of 4 males and 2 females was continued on group 2 ration for 7 months. The female whose curve is shown above bore a litter after six months, but was unable to raise them. Apparently the other second generation female was sterile.

This experiment confirms the observations of others, that the proteins of wheat are supplemented in a satisfactory manner where fed with relatively small percentages of gelatin.

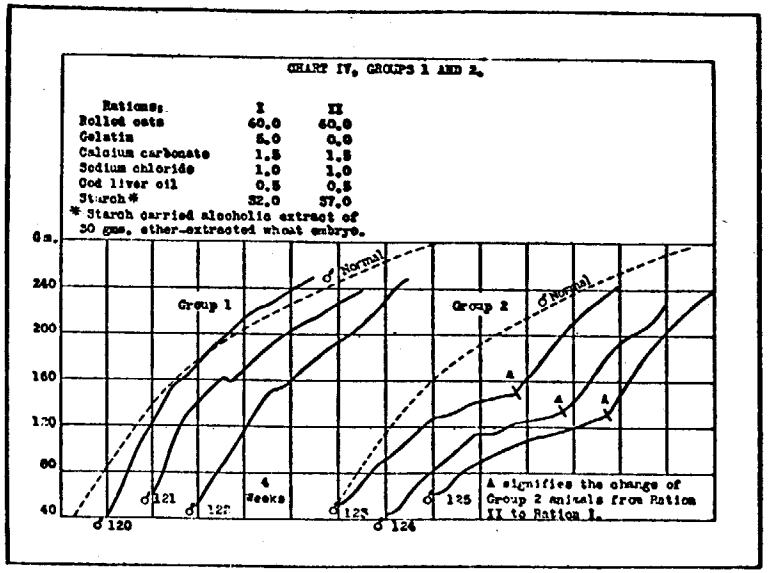


Chart IV shows that rolled oat proteins, fed at approximately 9 per cent. level in an otherwise adequate ration, do not support normal growth, but become adequate in this respect where fed with the addition of 5 per cent. of gelatin. Group 1 rats were fed on ration I without change during the entire period of the test, except that 2 per cent. of agar-agar was added for 2 weeks time (5 weeks after start of tests) to overcome the difficulties of the elimination of feces, a disturbance that is reflected by the break in growth curves. The addition of agar-agar at frequent intervals was neces-

sary and fairly effective for group 2 rats on ration II. Apparently the gelatin in ration I alleviated to a considerable extent the difficult elimination of the characteristically pasty feces resulting from oats. As indicated by the intersecting lines and letter A, group 2 animals were changed after 15 weeks to ration I, and their recovery in appearance and growth rate was immediately apparent.

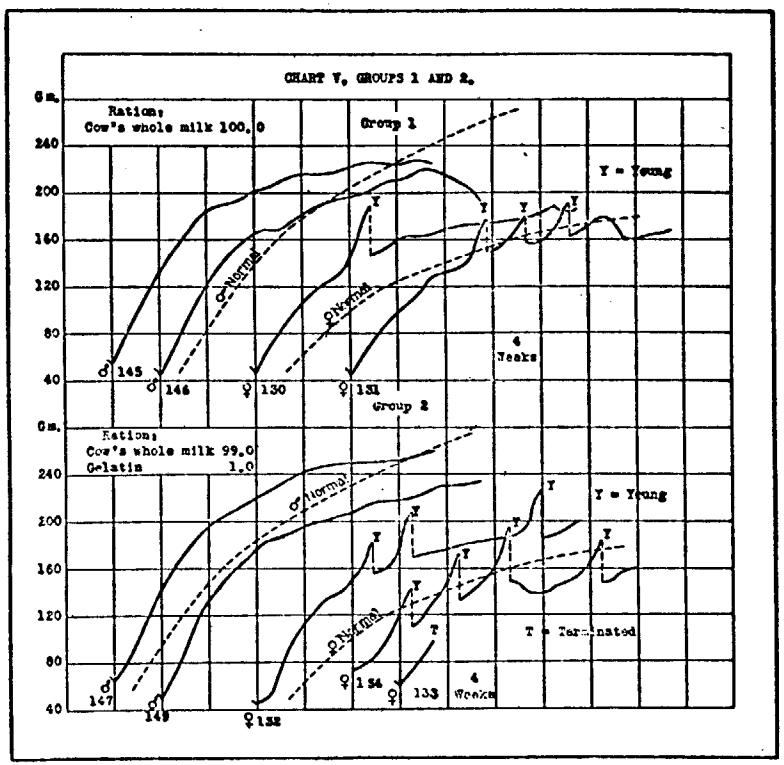


Chart V. — In this experiment the rats with the exception of female 134 were taken from the same litter of young. Female 134 replaced 133, which escaped. A comparison of the curves of the males of groups 1 and 2 reveals that the addition of 1 per cent. of gelatin to milk has promoted growth rate and continued well being. Males fed on milk alone showed a tendency to decline after 24 weeks on the diet. While females 130 and 131 on the milk diet gave birth to 4 litters of young, lactation did not follow, and the young were usually disposed of during the first day. On the other hand, females

132 and 134 on the gelatin-milk diet gave birth to 7 litters of young with lactation for 5-10 days. The third litter of female 134 was an interesting exception, as 4 of the 10 young (1 died and 5 were removed) were reared in excellent condition to an age of 19 days, when the mother ceased to lactate and disposed of them.

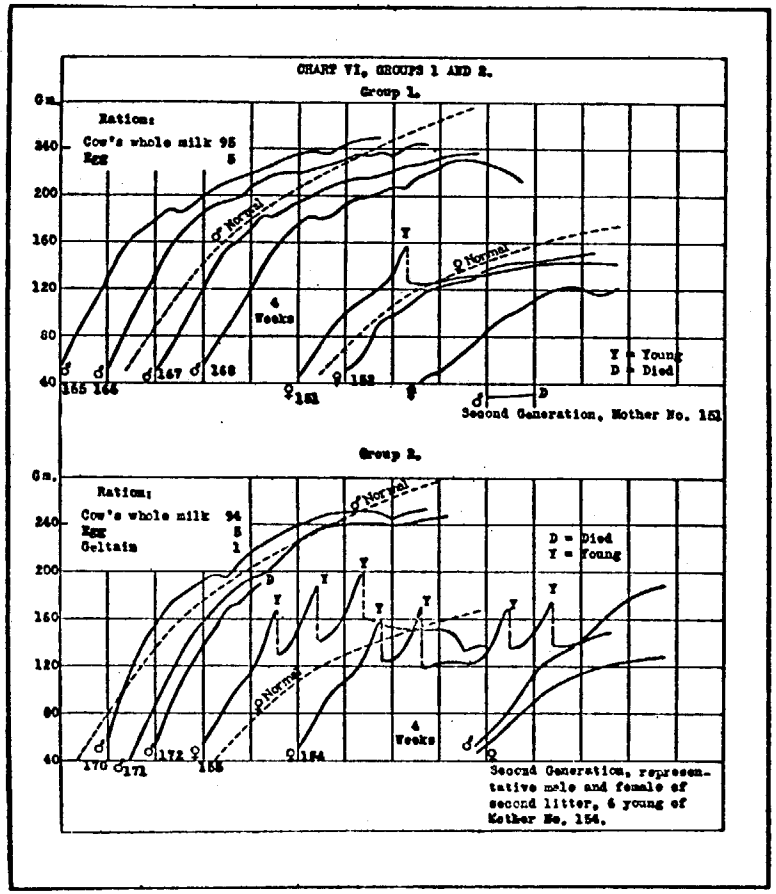
Of particular significance in this experiment are the amounts of milk solids consumed per gm. gains in body weight. This record is given in Table I and shows that the addition of 1 per cent. of gelatin to milk has increased the availability of nutrients to a marked degree. See also Table II, which confirms these observations with a larger number of rats from a different strain.

Chart VI. — The observations on group 1 show that the growth value of milk is markedly improved by the inclusion of 5 per cent. of whole egg. The poor reproductive ability of females 151 and 152 is surprising and does not conform with the results on group 1 in Chart V, where females on a plain milk diet gave birth to 4 litters, although they were unable to rear them. On the milk-egg mixture female 151 gave birth to 1 litter of four young and reared two of these animals to the weaning period. Both of these young,however, were abnormal: the male was small and was killed by becoming fast in the meshes of the cage bottom, and the female grew at less than normal expectation and showed poor skeletal development, her teeth growing as long tusks curving over the lip or into the nostril. It is believed that these observations on reproduction are not truly representative of the value of this diet.

In striking contrast is the reproduction shown by females 154 and 155 of group 2, which gave birth to 4 and 3 litters, respectively. When afforded the opportunity, both females successfully reared their young, although at the expense of considerable body weight. The curves for representative second generation rats (4 males and 2 females of the second litter by mother No. 154) are shown. These second-generation rats were continued on the gelatin-milk diet for 6 months, but they did not reproduce.

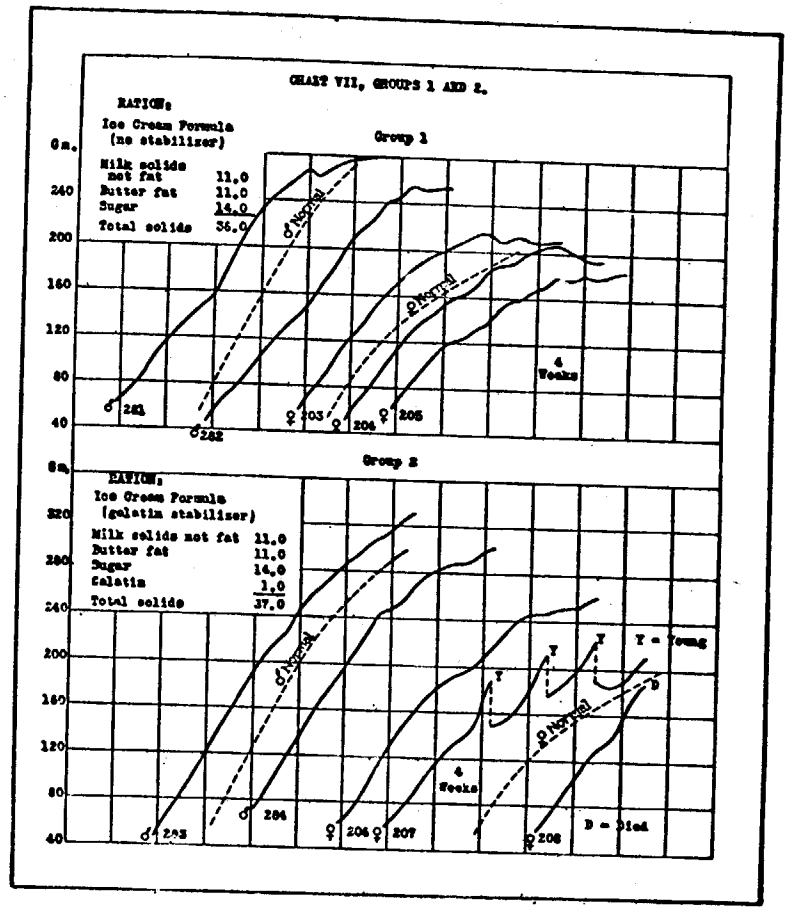
There is little difference in the growth rate of the males of groups 1 and 2. However, the record of food consumptions for all animals shows that the utilization of gelatin-milk-egg mixture for equivalent gains in body weight was the better to the extent of about 14 per cent. During the first six weeks of the test, several of the group 1 rats were subject to diarrhea. The feces of these animals during the entire test were poorly formed, drying down on the cage in hard masses, characteristic of undigested egg white. Group 2 rats were not troubled by diarrhea and their feces were well formed.

Chart VII confirms the observation given in Chart V. This experiment and its continuation in Chart VIII were designed to determine the effects of gelatin additions upon the food value of ice cream of average commercial composition. Group 1 ration constituted the basis of all ice-cream diets. On the dry basis it contained about 12.0 per cent. of protein, 30.5 per cent. of butter fat, 38.8 per cent. of sucrose, 2.7 per cent. of ash, and lactose as the other principal constituent.



The results with group 1 show that upon the plain dairy product growth is slightly below normal expectation for the first 24 weeks and is followed by a decidedly slow growth with bare maintenance in most cases. While the growth of females was good, all of them were apparently sterile.

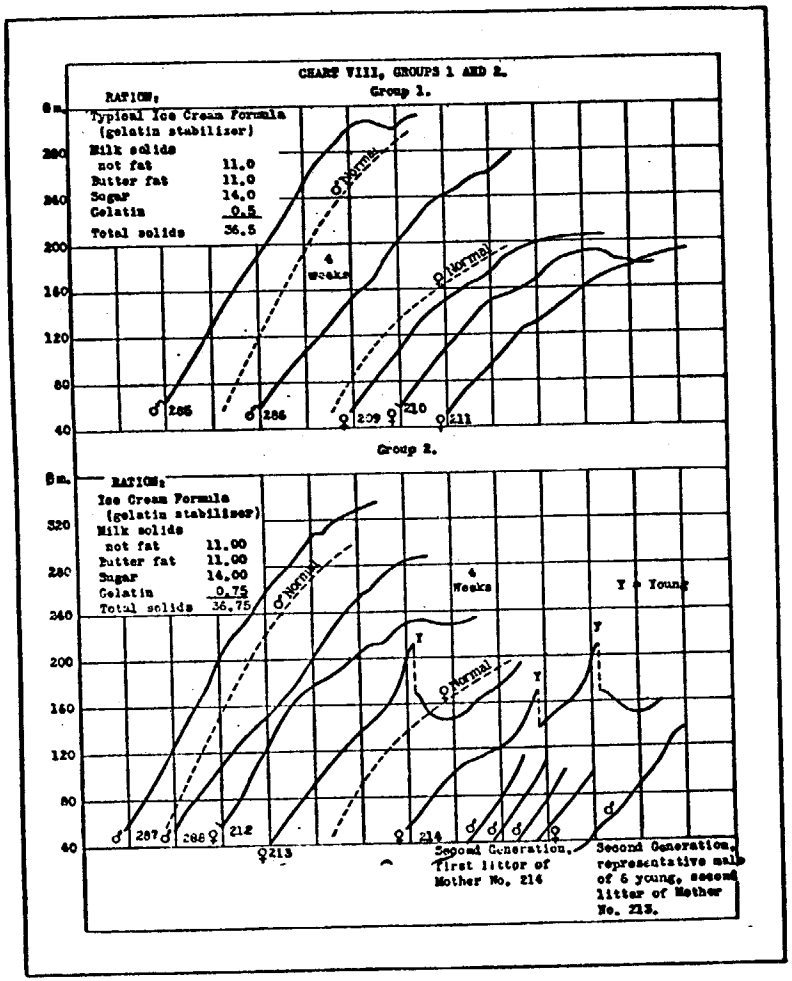
In group 2 the addition of 1 per cent. of gelatin to the above diet promoted normal growth of all animals and reproduction in the case of female 207 (female 208 died during parturition). The young were not reared. The size and appearance of these group 2 rats were greatly superior at the



conclusion of the tests to those of group 1. See Table III for a record of food intakes by groups 1 and 2.

On the completion of the feeding, the femurs of all animals were removed, dried and ashed. The weights of these ashed bones showed that the skeletal development of group 2 animals was greater than that of the rats of group 1.

Chart VIII indicates that so small additions as 0.5 and 0.75 per cent. of gelatin to ice cream (see Chart VII, group 1) increase its biological value.



The rats of group 1 (ration contained 0.5 per cent. of the same gelatin used in Chart VII, group 2) grew at approximately normal expectation, although reproduction was not shown by the females. The development of group 1 was better than in the case of animals fed on plain ice cream. The record of food consumption for equivalent gains in weight (see Table III) shows that ice cream containing 0.5 per cent. of gelatin is a decidedly more

economical food than plain ice cream and is almost equal to ice cream + 1 per cent. gelatin.

The results with group 2 show that the addition of 0.75 per cent. of a low jelly-strength gelatin made from degreased green bone had a remarkable influence on the biological value of ice cream. A comparison of this group with group 1 of Chart VII shows that the growth of animals as well as ability to reproduce have been increased. Of the 3 females in group 2, two females gave birth to young and successfully reared them. The curves for certain of these scond generation rats are given. The young of mother 214 were superior to those of mother 213. The severe strain of lactation is reflected in the curves of both mothers. These feeding tests showed that reproduction did not result on a plain ice cream diet, that reproduction without lactation occurred on the addition of skin-stock gelatin, and that reproduction and successful rearing of young ensued when 0.75 per cent. of green-bone gelatin was added to the feed. It is plausible therefore that the use of skin-stock gelatin improved nutrition, whereas green-bone gelatin contains a substance that has a favorable influence on reproduction. This explanation seems reasonable in view of the convincing character of the experimental results.

DISCUSSION OF RESULTS

That gelatin as the sole source of protein in an otherwise satisfactory diet becomes adequate for maintenance and repair when the amino-acids crystine, tryptophane and tyrosine are added thereto is shown in Chart I. These experimental results confirm the findings of Kaufmann[8] and Abderhalden[9] on man and dog, which were based on observations of work carried on for only a short period of time without attention to certain dietary factors now known to be essential. With this point well established and with the results of Dakin's[15] analysis of the cleavage products of gelatin, it is possible to interpret and predict with a fair degree of assurance the value of gelatin as a protein where fed in combination with many other food products in which the amino acid composition of the proteins is known.

The cereal grains are relatively low in lysine, and McCollum[17] has interpreted the efficient supplementation of wheat and oat proteins by gelatin on the basis of lysine content. The results given in Chart III support McCollum's conclusions and also embrace white wheat bread with its content of 12.0 per cent. of protein. Chart IV confirms the findings of McCollum[17] and of

* Of the young, second generation, two were females and one of these has successfully reared a litter of 6 young to the age of 10 days, which are in excellent condition and of fair size and show all indications of being rearable to weaning age.

Osborne and Mendel[18] with oats and shows the remarkable recovery of animals when 5 per cent. of gelatin is added to the 9 per cent. of oat protein. Chart II shows that barley proteins are improved in biological value by the addition of 10 per cent. of gelatin. The author has subsequently found that 5 per cent. of gelatin with 8 per cent. of pearled barley proteins supports normal growth. He has also noticed that the protein mixture of sifted whole barley is improved to a marked degree by the addition of gelatin. Animals fed with rye proteins have shown a less but still decided improvement when fed with gelatin additions. The results of Sure[16] with edestin and gelatin are of significance, because they reveal that in certain combinations the proline content of gelatin is also a deciding factor for growth.

Several investigators[24 25] have expressed the opinion that protective colloids of the emulsoid type are of great significance in the utilization of nutrients of milk by the animal organism, and the validity of this view has been repeatedly demonstrated by clinical experience in the feeding of bottle babies and children. To the knowledge of the writer, however, animal experimentation has not been previously resorted to as a means of determining the extent of this action. The rations represented in Charts V-VII were selected to test this effect with gelatin as the protective agent where fed with certain dairy products in liquid form. In each comparative experiment gelatin was added to the dairy product and resulted in an increase in protein content and a small decrease in calories per gm. of food mixture. It is the belief of the author that the resultant improvements of the dairy products are not to be ascribed entirely to gelatin as a protein, but principally to its protective and emulsifying effects and possibly peptogenic activity.* The levels of milk protein intakes were relatively high in these experiments, and it is also a well established fact that cystine, an amino-acid absent in the cleavage products of gelatin, is the limiting protein factor in

* In a preliminary test with a small number of rats, the author has failed to observe any noteworthy differences in growths or food intakes for growth where 1 per cent. of low jelly strength gelatin is incorporated with sweetened condensed milk diluted with an equal volume of water. This result indicates that the improvements in the nutritive values observed in other dairy products are chiefly of a colloidal nature. Hawk (N. Y. Med. J., 117, 1923, 609) reports that the curds of condensed milk are smaller and of looser texture. The more stable emulsion of fats (the fat particles are extremely finely divided) may also be a determining factor. "Homogenized milk and cream are more easily digested than the usual products, owing to the fine subdivision of the fat globules" (Clayton, "The Theory of Emulsions and Emulsification," 1923, 123).

both casein and lactalbumin. While Sure[21] has found that the addition of 8 per cent. of gelatin to a ration containing 50 per cent. of skim milk powder, cystine, agar-agar, salt and vitamines promoted reproduction, his curves do not show growths superior to the results obtained on the same diet without gelatin.

In the first test of this type, Chart V, it is observed that the addition of 1 per cent. of gelatin to whole milk produces marked improvements in growth rate, prolonged well being and reproduction. The records of food intakes for these rats during 6 weeks of the test are of particular significance (see Table I as well as Table II). They show that the utilization of solids for growth has been increased from 16 to 23 per cent. by the addition of gelatin. If the comparison is extended to the utilization of proteins, it is found that practically the same amount was ingested in the comparative rations for equivalent gains in body weight. In these tests, as with other dairy products fed in liquid form, it was observed that the feces were better formed where gelatin was present, an indication of increased absorption.

When egg was fed with milk (Chart VI) the feces were liquid, amounting to diarrhea in several cases. Chart VI shows the improved growth of animals where fed with milk and egg and the further improvement as shown by the reproduction of females where 1 per cent. of gelatin was added. While egg protein is an efficient protective colloid, its value in this respect in these diets was doubtless seriously interfered with by diarrhea. Where gelatin was included in the milk-egg mixture (ration of group 2), there was little disturbance of this nature. This observation is in accord with clinical experience of physicians who have used gelatin for combatting diarrhea in infants and young children.

The experimental results on the use of a fair average of commercial cream (Charts VII and VIII) support the observations with whole milk. The results of feeding the combination of milk, butter fat and sugar (12 per cent. of milk proteins and 30.3 per cent. of fat on the dry basis) are compared with the results obtained where 0.5, 0.75 and 1.0 per cent. of gelatin are added thereto. By the addition of 1 per cent. of gelatin, the

protein content is increased 2.7 per cent., i. e., from 12 to 14.7 per cent. It will be observed from these charts that these additions of gelatin have increased the growth rate and prolonged growth beyond the period where rats on the plain ice cream barely sustain maintenance. The improvements in both respects are roughly proportional to the gelatin content. The record of food intakes (Table III) shows that the 5 rats on each of the respective diets, plain dairy product and this combination plus 0.5, 0.75 and 1.0 per cent. of gelatin, during a period of 8 weeks gained 454, 481, 509 and 581* gm. respectively in body weight, consuming an average of 4.79, 4.16, 4.52 and 4.14 gm. of the respective food mixtures (dry basis) per gm. gains in body weight. These results demonstrate an increased utilization of solids for growth in the gelatin diets of 15.0, 6.0 and 15.5 per cent., respectively. On these diets the respective ingestions of proteins per gm. gains in weight were 0.57 gm. (milk proteins, plain ice cream) and 0.54, 0.61 and 0.58 gm. (milk proteins plus gelatin, i. e., gelatin ice cream).

It is observed that 0.75 per cent. of a particular gelatin (greenbone gelatin of low jelly strength) did not promote as efficent utilization of solids for growth (food intakes per equivalent gains in body weight) as the 0.5 and 1.0 per cent. of ordinary gelatin (higher jelly strength made from calf skin), although the green-bone gelatin appeared, in this limited group of animals, to influence reproduction as shown in Chart VIII, group 2. The first observation supports the contention that the colloidal effects produced by gelatin are of significance, for the low jelly strength is the result of hydrolysis of the gelatin-complex to proteoses, peptones and amino-acids, with corresponding decrease in viscosity and protective colloid action. The second result (reproduction) may possibly be accounted for by the source of this gelatin; there may be present in it certain extractives from the green bone and its marrow which influence favorably the ability of animals to reproduce and rear their young.* It should be noted that although young were born by female 207 on the ration with 1 per cent. of gelatin, the mother did not care for them.

The femurs of all animals were well shaped, and the weights

* This figure is high, as it takes in a short period of pregnancy of one female.

of the dried and ashed bones showed that increased body weights attained by the animals whose foods contained gelatin were attended with correspondingly greater bone development.

CONCLUSIONS

Gelatin is a protein whose chemical composition and properties preclude its use as the sole or major source of protein in the dietary.

Where fed in combination with proteins of certain natural food products, such as oats, wheat and barley, it possesses a decided supplementary value, presumably through its high proportion of the amino acid lysine; where fed with corn, peas and beans, its cleavage products are of little value.

The value of pasteurized whole milk and of ice cream (average of commercial product) for growth and continued health of animals is improved to a marked extent by the inclusion of gelatin. Better nutritional status of females fed on the gelatin-containing diets was reflected in increased reproduction in the rats studied, although successful rearing of young was obtained on only one specific diet. The data indicate that gelatin is well utilized as a protein when fed in conjunction with milk proteins and further that the effects of gelatin as a colloid of the emulsoid type exert a significant influence upon digestibility and absorption where ingested with these dairy products with the exclusion of other foods. In these particular diets, the colloidal effects of gelatin may be equal in importance to, if not indeed greater than, its value as a protein.

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30. See, for example, Jacobi, "*Intestinal Diseases of Infancy and Childhood*", 1887, p. 79; Starr and Westcott, "*Diseases of Children*", 1900, p. 23; Griffth, "*The Care of the Baby*", 1908, p. 386; and Friedenwald and Ruhrah, "*Diet in Health and Disease*", 1923, pp. 295, 466. On the utility of gelatin in chronic intestinal infection, see Herter, "*Infantilism from Chronic Intestinal Infection*", 1908, p. 101.
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Journ. Biol. Chemistry 94: 167-171, 1931

FEEDING EXPERIMENTS WITH MIXTURES OF HIGHLY PURIFIED AMINO ACIDS

II. THE SUPPLEMENTING EFFECT OF PROTEINS*

BY RUTH H. ELLIS† AND WILLIAM C. ROSE

(From the Laboratory of Physiological Chemistry, University of Illinois, Urbana)

(Received for publication, August 14, 1931)

In the preceding paper the conclusion was reached that growth-promoting proteins probably carry at least one indispensable dietary component other than the twenty generally accepted amino acids, and that the inadequacy of diets containing synthetic mixtures of the known amino acids is due to the absence of this hitherto unrecognized essential. If this conception is correct one should be able to demonstrate at least a moderate improvement in the quality of the food as a result of replacing a portion of the amino acid mixture with an equivalent quantity of protein. In order to test the validity of this hypothesis the following experiments were conducted in which the supplementing action of gelatin, gliadin, and casein was determined.

EXPERIMENTAL

Three diets were formulated, each of which contained 5 per cent of one of the proteins indicated above in place of a like amount of "effective" amino acids. The amino acid mixture was identical in composition with Mixture I described in the preceding paper except that it contained no serine. It will be referred to in this and subsequent papers as Mixture III. Our supply of both

* Aided by grants from the National Research Council and the Graduate School Research Fund of the University of Illinois.

† The experimental data in this paper are taken from a thesis submitted by Ruth H. Ellis in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Physiological Chemistry in the Graduate School of the University of Illinois.

serine and methionine had been exhausted temporarily at the time these experiments were undertaken; but preliminary studies had already shown that the growth rates of rats, under the conditions in question, are not affected by the presence or absence of these two amino acids. This must not be interpreted, of course, as indicating that serine and methionine are not essential, since the proteins used as supplements may have furnished sufficient quantities to meet the needs of the animals.

Each diet had the composition shown in Table I. Inasmuch as 1.405 gm. of Mixture III are equivalent to 1.0 gm. of "effective"

TABLE I

*Composition of Diets**

	gm.
Amino acid Mixture III†	18.3‡
Supplement (gelatin, gliadin, or casein)	5.0
Dextrin	26.7
Sucrose	15.0
Salt mixture§	4.0
Agar	2.0
Lard	24.0
Cod liver oil	5.0
	100.0

* The vitamin B factors were supplied in the form of two pills daily each containing 100 mg. of yeast.

† Identical in composition with Mixture I described in Paper I except that serine was absent.

‡ Equivalent to 13.0 per cent of "effective" amino acids.

§ Osborne and Mendel (1919).

amino acids, it is evident that 18.3 gm. of the mixture provided 13 gm. of "effective" acids. Thus each ration contained 18 per cent of nitrogenous materials in addition to the unnatural enantiomorphs present in the racemic acids. As in the preceding paper, the vitamin B factors were supplied in the form of two pills daily each containing 100 mg. of yeast.

The results of the experiments are summarized in Chart I and Table II. As will be observed, the animals at first lost weight just as if no supplement had been provided. However, the minimal weights were reached in 4 days, after which slow growth en-

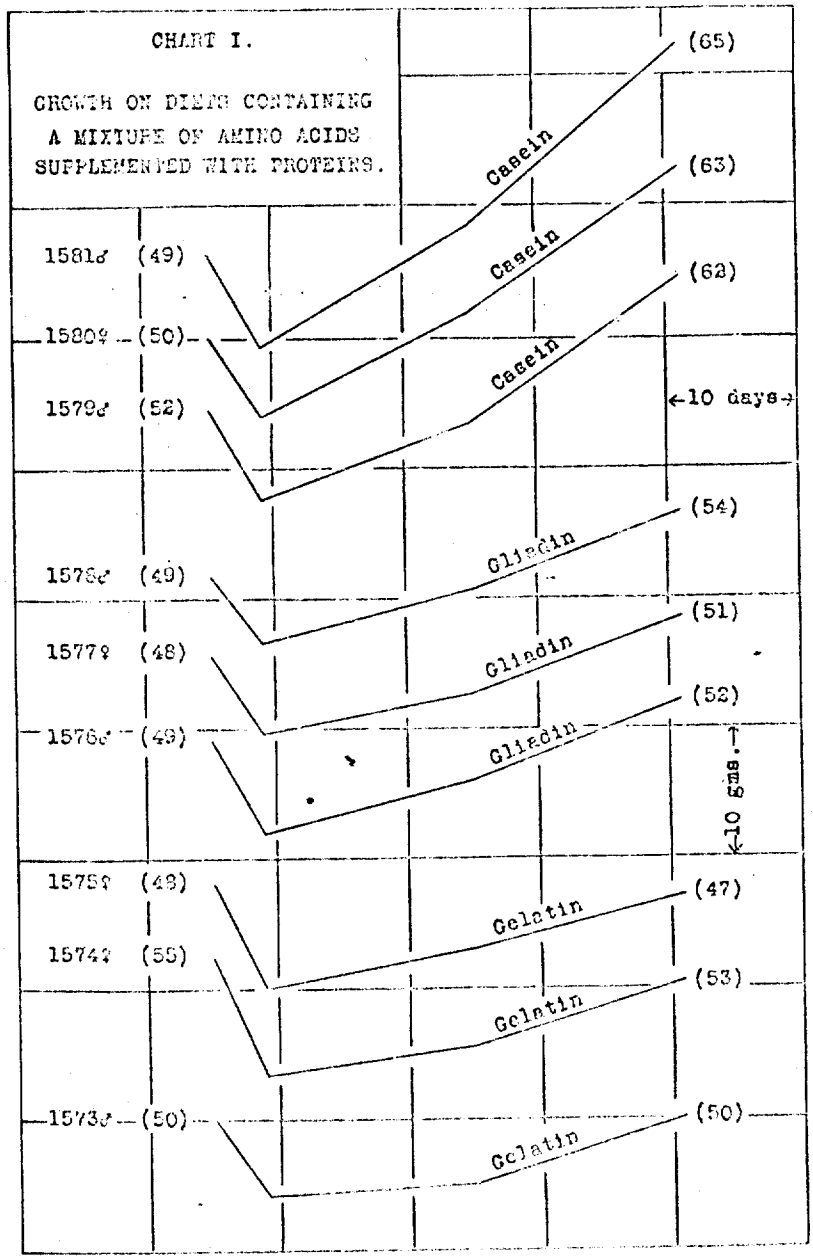

CHART I. The numbers in parentheses denote the initial and final weights of the rats.

sued. In the ca latin, the supplementing effect was scarcely sufficient to enab e rats to regain the losses experienced at the beginning of the experiments. This is a matter of considerable interest in view of the observation of Jackson, Sommer, and Rose (1928) that the addition to a gelatin diet of the eleven known amino acids, which on the basis of the best analyses available seem to be the most likely possible sources of deficiency in this protein, fails to render the food satisfactory for growth. Apparently, gelatin contains very little of the growth essential lacking in our amino acid mixtures. We expect to report further upon this problem in a later paper.

TABLE II

Total Changes in Body Weight and Total Food Intake on Diets Containing a Mixture of Amino Acids Supplemented with 5 Per Cent of Protein

The experiments covered 36 days each.

Rat No. and sex	Supplement	Total change in body weight	Total food intake
		gm.	*gm.*
1573♂	Gelatin	0	93
1574♀	"	−2	110
1575♀	"	−1	90
1576♂	Gliadin	+3	87
1577♀	"	+3	89
1578♂	"	+5	99
1579♂	Casein	+10	103
1580♀	"	+13	107
1581♂	"	+16	114

The supplementing effect of gliadin was only slightly better than that manifested by gelatin. However, each animal which received this protein attained a somewhat greater body weight than that with which it began the experiment. With casein the results were more striking. Upon this ration the rats made total gains of 10 to 16 gm. each. When one recalls that the animals which received the amino acid diets *without* protein supplementation (Paper I) experienced losses of 18 to 26 gm., it is evident that the proteins in question exerted distinctly beneficial effects.

It has been shown that very small quantities of milk proteins (Osborne and Mendel, 1915, and McCollum and Davis, 1915),

or of the proteins of the wheat kernel (Osborne and Mendel, 1920) permit slow growth. In view of these findings one may ask whether the increases in weight of our animals are not to be ascribed entirely to the proteins *per se* rather than to a supplementing effect exerted by them upon the amino acid mixture. Obviously, this question cannot be answered with certainty from the data available. But inasmuch as the amino acid mixture alone supplied all known protein constituents (other than serine, hydroxyglutamic acid, and methionine, which were not limiting factors), it seems logical to assume that *the supplements furnished something which was lacking in the amino acid mixture.* If so, the active material must be a hitherto unrecognized protein component. Certainly, a supplementing effect appears to be the only satisfactory explanation for the results obtained in the gelatin experiments, since all proportions of this material, when used as the sole source of nitrogen, fail to permit growth. Furthermore, since neither gelatin nor gliadin improved the nutritive quality of the ration as effectively as did casein the latter would appear to be the most promising protein of the three to employ in a search for the unknown growth essential.

SUMMARY

Experiments have been conducted to determine the supplementing effect of proteins when added to diets containing a synthetic mixture of amino acids. The results show that when 5 per cent of casein, gliadin, or gelatin is substituted for an equivalent quantity of the amino acid mixture, the animals lose weight for 4 days and then slowly gain. Casein is more effective than either gliadin or gelatin in stimulating growth. This growth stimulation appears to provide additional evidence for the existence in proteins of an unknown dietary essential.

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CHAPTER 11

Gelatin

'Tis the dessert that graces all the feast,
For an ill end disparages the rest.

—W. KING

The above epigraph aptly describes one of the most popular and widely eaten American foods—gelatin desserts. The shimmering, tender texture and brilliant clarity of a quality gelatin dessert has widespread esthetic as well as organoleptic appeal to a broad majority of consumers. And the properties of gelatin that lend themselves to the preparation of such gelled food products are unique and cannot be exactly duplicated by any other hydrocolloid. This has given gelatin a tremendous advantage in the area of packaged dessert mixes, so that today the majority of gelatin produced in this country is used in dessert foods.

Gelatin is defined in the U. S. Pharmacopeia (1965) as "a product obtained by the partial hydrolysis of collagen derived from the skin, white connective tissue and bones of animals." The fact that gelatin is obtained with ease and in relative purity from an abundant starting material probably has much to do with the fact that it has become the classic protein of colloid chemistry, and has been the subject of an enormous amount of experimental work.

Of all the common natural hydrocolloids, gelatin is the only protein of importance, largely because of its novel gelling and thickening properties. Recently, with advances in technology, other protein "gums" are becoming subjects of interest for the hydrocolloid chemist. The most important of these are the soy proteins, which, like gelatin, also have interesting and exploitable gelling, thickening, and

other colloidal properties. Egg albumin, a relatively "old" protein, is being given new functional properties which permit its use in a much wider variety of foods. In addition, the milk protein caseinates have been found to have very effective emulsifying and stabilizing properproperties, while cereal proteins make excellent whipping agents. However, since gelatin is by far the most important protein hydrocolloid, this chapter will be limited to gelatin.

I. Background

"Gelatin," derived from the Latin verb *gelare* meaning "to congeal," has been known for thousands of years. The extraction of glue by cooking hides dates back at least to the time of the ancient Pharaohs of Egypt. Bogue (1922) cites a 3000-year-old stone carving, found in the ancient city of Thebes and belonging to the period of Thotines III, which describes the gluing of a thin piece of rare red wood veneer to a yellow plank of sycamore.

Later, in the Roman era, Pliny, Lucretius, and others referred to the manufacture of glue. Pliny wrote, "Glue is cooked from the hides of bulls." Elsewhere in his writings he referred to glue, which had been mixed with gums, milk, eggs, and wax as a vehicle for paints, used by the ancient Egyptians. Much later, in the Elizabethan period (ca. 1600 A.D.), Shakespeare and Bacon made frequent references to glue in their writings. Indeed, a commercial glue industry appears to have been established in England about 1700. A similar industry developed in the United States within the following century.

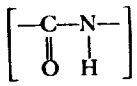
During the early years of the Napoleonic era, gelatin was manufactured on a large scale in an attempt to alleviate the food shortages resulting from the English Naval blockade of Europe. The first manufacture of edible gelatin is credited to Arney, who was granted a patent in 1846 for the preparation of a powdered gelatin "for forming compositions from which may be prepared jellies and blanc manges; also, when mixed with farina, or starch, or starchy vegetable flour, for thickening soups, gravies, etc." (Bogue, 1922). At present, gelatin is manufactured in the United States by at least eleven companies in the amount of about 60–70 million lb annually.

II. Collagen

Collagen, which means "glue-producing material," is the principal protein component of the connective tissues, which serve as the major

stress-bearing elements for all mammals and fishes. Although much of the collagen is located in major tissues such as skin, tendon, and bone, collagen fibers pervade almost every organ and tissue. Collagen is unique among proteins because of its unusual amino acid composition. It owes its distinctive structure to its high content of the cyclic amino acids, proline and hydroxyproline. In addition to these compounds, collagen also contains large quantities of glycine and alanine, the more common nonpolar amino acids with short side chains.

It is now believed that proteins consist of long chains of amino acids connected through their α-amino and α-carboxyl groups to form peptide linkages



These chains can be either stretched out or folded. Additional bonds, between adjacent chains or between adjacent parts of the same chain after folding, become a necessary part of the structure and stabilize the functional protein configuration.

The basic collagen polymer unit, sometimes called tropocollagen, is thought to consist of three helical polypeptide chains wound around each other to form a coiled coil, which behaves as a firm, rigid rod (Rich and Crick, 1961). Recent work indicates that irrespective of source, collagen has a molecular weight of about 350,000, a length of 3000Å, and a diameter of about 14Å (Hannig and Engel, 1961; Boedtker and Doty, 1956). As shown in Fig. 1, it has now been established that two types of bonds contribute to the secondary and tertiary structure of collagen: (1) intramolecular cross-links existing between the individual chains of the collagen molecules, and (2) intermolecular cross-links (Harding, 1965; Veis *et al.*, 1961).

The gross collagen fibers are capable of undergoing considerable mechanical and physical changes which may vary from reversible swelling and partial melting to irreversible disorganization of the entire structure. But all these changes occur without solubilization of the fiber.

III. Transformation of Collagen to Gelatin

Gelatin is the water-soluble product of the dissolution or degradation of water-insoluble collagen fibers. The transformation or transition of collagen to gelatin is the process whereby the highly organized, quasi-crystalline, water-insoluble collagen fiber is transformed from an infinite assymetric network of linked tropocollagen units to a

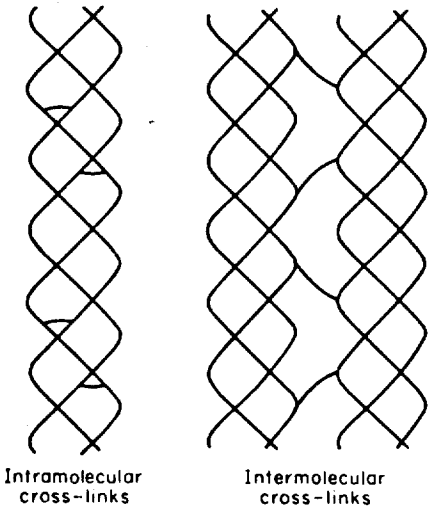


FIG. 1. Types of collagen cross-linking.

system of water-soluble, independent molecules with a much lower degree of internal organization. Since the original collagen structures are not all the same, and since there are many paths by which the structure may be broken down, there are obviously a great many varieties of gelatin formed by the destruction of collagen.

The hydrogen-bonded configuration of the collagen macromolecule can be broken down by heating collagen solutions in acid to about 40°C. The transition is sharp and complete within a few minutes over a small temperature interval, and the disordered molecule falls apart in one of three ways, as shown in Fig. 2 (Veis, 1964):

1. If there are no additional restraining bonds between chains, three randomly coiled single-strand peptide chains result (Path 1). The three chains, known as α-chains, are not of identical composition and probably not of equal molecular weight.

2. In those cases (Path 2) where two chains are joined by one or more covalent cross-linkages, denaturation leads to the appearance of two particles, one an α-chain and the other a two-stranded molecule with approximately twice the molecular weight of the α-chains. The two-stranded β-component may be composed of two similar or two dissimilar α-chains. The weight distribution would be 67% β and 33% α.

3. In the final case, it can be assumed that at least two covalent cross-linkages hold the three chains together. The disordering process

(Path 3) melts out and removes all traces of secondary structure, but the three chains cannot separate and remain as a unit in solution. This three-chain structure is called the γ-component.

It is obvious that there is no reason why every molecule of tropocollagen should have the same number of identically disposed intramolecular cross-linkages, and thus, it is most reasonable to assume that any given preparation is heterogeneous with respect to the degree of intramolecular polymerization. The ideal conversion of the collagen monomer to gelatin is therefore the one via Path 1. The number-average molecular weight of the gelatin system should be one-third the molecular weight of the collagen monomer, and the weight-average molecular weight should be slightly higher due to the nonidentity of the chains. The best values of the collagen monomer molecular weight are substantially above 300,000, and hence the minimum molecular weight of the parent gelatin must be greater than 100,000. Reports of lower molecular weights for gelatin probably indicate that peptide bond hydrolysis was a factor in those studies. Reports of the α-gelatin having molecular weights of about 80,000 are not consistent with recent studies on acid-soluble collagen (Veis, 1964).

Therefore, it can be assumed that native collagen fiber is an ordered array of parallel tropocollagen rods, staggered by approximately one-

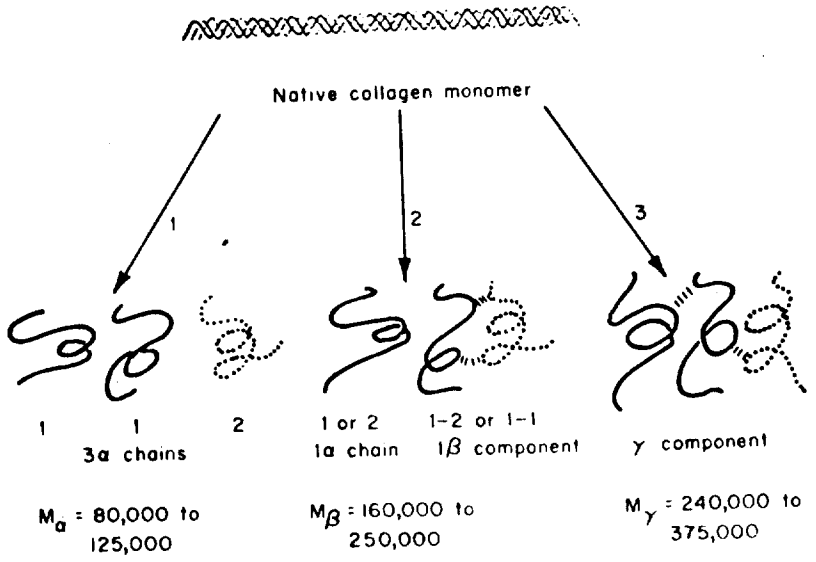


FIG. 2. Possible paths of collagen coversion to gelatin (Veis, 1964).

fourth of their length. Each tropocollagen rod is composed of three chains of α character but probably of different chemical composition, and may contain intra-tropocollagen cross-linkages to produce the β sub-unit of two-chains or the γ units with bonds joining each of the three chains. All γ units may not be of the same degree of intramolecular polymerization and may in addition be bonded together with inter-unit cross-linkages to form γ polymers.

IV. Manufacture of Gelatin

The bulk of gelatin manufactured is derived from three basic sources and consists of two types of finished gelatin product. Type A gelatin is derived from acid-processed materials, primarily pork skin. Type B gelatin is derived from alkaline, or lime-processed, materials, primarily cattle hides and bone (ossein). In Europe, a substantial quantity of type A gelatin is made from ossein.

A. SOURCES

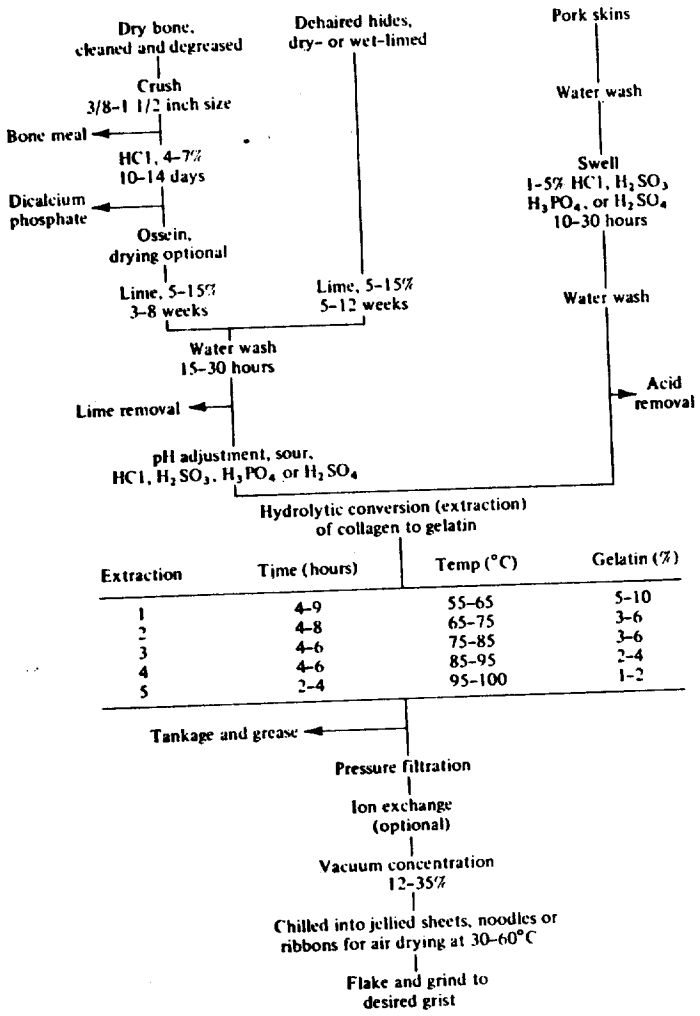
a. Pig Skins. One of the major sources of gelatin in the United States is pig skins. They are frozen just after removal at the meat-packing plants and delivered to the gelatin plants in refrigerated cars. Since some of the fat remains on the skins, it is preferable to process these by acid pre-treatment in order to avoid forming a soapy emulsion that would make extraction of the gelatin extremely difficult.

b. Cattle Hides (Tanner's Stock). These are calf or cattle hide trimmings not usable for the manufacture of leather. The whole hides are shipped to tanners and the trimmings from the hides in various stages of the process, before actual tanning, are used by the gelatin manufacturer.

c. Ossein (Bone). Although this is one of the best known gelatin raw materials, it is the one least used in the United States. Ossein is the residue of dried cattle bones remaining after acid pre-treatment to remove the calcium phosphate. Even though ossein is a very expensive raw material, if properly processed, it makes an excellent gelatin for photographic use and one that can command higher prices.

B. PROCESSING

The manufacture of gelatin is shown in the flow diagram in Fig. 3. More detailed descriptions of this process are available in the follow-



Extraction	Time (hours)	Temp (°C)	Gelatin (%)
1	4–9	55–65	5–10
2	4–8	65–75	3–6
3	4–6	75–85	3–6
4	4–6	85–95	2–4
5	2–4	95–100	1–2

FIG. 3. Flow diagram for gelatin manufacture.

ing articles: Anonymous (1954), Gelatin Manufacturer's Institute of America (1962), Plowright (1963), Kramer (1965, 1967, 1968), and Marks (1966). In general, all gelatin is manufactured by one of the two following processes or modifications thereof.

1. *Acid Processing (Type A)*

Acid processing is usually based on the use of pig skins and ossein, and the most important commercial acid process in the United States

is the preparation of edible gelatin from frozen pig skins. The pig skins are thawed, washed in cold water, and soaked in approximately 5% solutions of inorganic acids. This "plumping" process hydrates and swells the skin without causing appreciable solubilization.

Hydrochloric acid, sulfuric acid, phosphoric acid, and sulfurous acid are the most frequently used acids. The acid soak usually takes 10-30 hours, after which the supernatent acid is removed and cold water is used to wash away the excess acid and raise the pH of the soaked skins to about pH 4. Most of the noncollagenous proteins have isoelectric points in the pH 4-5 range and are thus most readily coagulated and removed. The acid-conditioned skins are then subjected to a series of hot extractions where the two variables are time and temperature. The initial extraction (first run) is carried out for the longest time at the lowest (about 60°C) temperature. The temperature is raised about 5-10°C in each successive extraction and five to ten extractions may be made. In general, the gelatin made from the first extract excels subsequent extracts in gel strength, and each run is kept separate and blended later to match various requirements.

The dilute gelatin extract is pressure-filtered and concentrated by vacuum evaporation. The warm concentrated solution is then cooled almost to its gelling point, poured onto a belt conveyor, and immediately conveyed into a refrigeration chamber where it is chilled rapidly until it gels. Upon emerging from a refrigerator chamber as a continuous sheet, it is cut into suitable lengths and placed on wire frames, which are then placed into a drying room or drying tunnel, where the temperature is carefully controlled. Continuous dryers are now in use where both temperature and humidity are controlled. The rate of drying is closely regulated to avoid melting or surface dehydration. When the moisture has been reduced to approximately 10%, the gelatin is removed and ground or pulverized to form the final product. Each batch of dried gelatin is graded and stored separately. Gel strengths and viscosities are the criteria for grading, and the different grades of gelatin are blended to give a finished product with the desired specifications.

2. *Alkali Processing (Type B)*

Bones are initially demineralized with dilute acid to remove the calcium salts, particularly the phosphate, and then both the ossein and cowhides are treated in a similar manner. The raw collagen stock is washed and thoroughly hydrated in cold water in large tanks or pits. Excess water is drained and lime is added in sufficient amounts so that

when fresh water is added a saturated solution of calcium hydroxide is formed. An excess of calcium hydroxide must be maintained to make up for the amount consumed in the conditioning reactions. The hides are left in the liming pits for periods of 3-12 weeks or longer, depending upon the nature of the stock, ambient temperature, type of operation, alkalinity of the lime liquors, etc.

The lime treatment removes most of the extraneous albuminoids such as globulins, mucopolysaccharides, albumins, as well as carotenes and various other pigments. After liming is complete, the lime is washed from the surface of the stock with running water for a day or so. The residual base is then neutralized by washing with dilute hydrochloric acid until the collagen is deplumped, or until it becomes limp and flaccid. At this point, the collagen has a pH of 5-8 and is ready for conversion to gelatin.

The collagen stock is then loaded into extraction kettles and gelatin is extracted in a series of cooks at successively higher temperatures. The highest quality gelatins are obtained in the first few extractions and the liquors from each cook are filtered, concentrated, and dried separately as for the Type A gelatins. Again, the overall balance of viscosity and gel strength for any particular quality of gelatin is usually achieved by blending gelatins from several different extractions.

C. Final Products

While all gelatins have similar functional properties, there are differences between the Type A and Type B gelatins, which are important in the selection of the appropriate gelatin for any specific application. These differences in physical properties are shown in Table I and will be discussed in more detail in the following sections.

TABLE I
CHARACTERISTICS OF GELATIN

Characteristic	Type A (pork skin, acid treated)	Type B (ossein or calfskin, lime treated)
Moisture	8-12%	8-12%
pH	3.8-5.5	5.0-7.5
Isoelectric point (pH)	7.0-9.0	4.7-5.1
Gel strength (Bloom)	50-300 g	50-275 g
Viscosity	20-70 mps	20-75 mps
Ash	0.3% (mainly Na^+, Cl^-, SO_4^{2-})	0.5-2.0% (mainly Ca^{2+}, PO_4^{2-}, Cl^-)

V. *Chemical Composition*

A. Amino Acids

Gelatin consists of 19 amino acids joined by peptide linkages to form long polymer chains. It gives a typical protein reaction and may be hydrolyzed by any of the proteolytic enzymes to yield its constituent amino acid or peptide components. Gelatin is not a nutritionally complete protein in that it is lacking in the essential amino acid, tryptophan. However, it contains a small amount of the rare amino acid hydroxylysine. Gelatins from different sources may exhibit small variations in amino acid composition, as shown in Table II.

The variations in chemical structure between the Type A and Type B gelatins produce differences in the physical properties, including the isoelectric and isoionic point.

TABLE II
AMINO ACID COMPOSITION OF DIFFERENT GELATINS
(GRAMS/100 GRAMS GELATIN)

Amino acid	Type A[a] (%)	Type B (skin)[b] (%)	Type B (bone)[c] (%)
Alanine	8.6 -10.7	9.3 -11.0	11.3
Arginine	8.3 - 9.1	8.55- 8.8	9.0
Aspartic acid	6.2 - 6.7	6.6 - 6.9	6.7
Cystine	0.1	None-Trace	Trace
Glycine	26.4 -30.5	26.9 -27.5	27.2
Glutamic acid	11.3 -11.7	11.1 -11.4	11.6
Histidine	0.85- 1.0	0.74- 0.78	0.70
Hydroxylysine	1.04	0.91- 1.2	0.76
Hydroxyproline	13.5	14.0 -14.5	13.3
Isoleucine	1.36	1.7 - 1.8	1.54
Leucine	3.1 - 3.34	3.1 - 3.4	3.45
Lysine	4.1 - 5.2	4.5 - 4.6	4.36
Methionine	0.80- 0.92	0.80- 0.90	0.63
Phenylalanine	2.1 - 2.56	2.2 - 2.5	2.49
Proline	16.2 -18.0	14.8 -16.35	15.5
Serine	2.9 - 4.13	3.2 - 4.2	3.73
Threonine	2.2	2.2	2.36
Tyrosine	0.44- 0.91	0.2 - 1.0	0.23
Valine	2.5 - 2.8	2.6 - 3.4	2.77

[a] Neumann (1949), Graham *et al.* (1949), and Eastoe (1955).
[b] Tristram (1953) and Eastoe (1955).
[c] Eastoe (1955).

Gelatin is an amphoteric substance, i.e., one that has both acidic (carboxyl) and basic (amino, guanidino) groups. The overall charge of the molecule depends upon the pH of the solution and other ions present. The pH at which the ionization of both the acidic and basic groups are equal is called the isoionic point (p*I*). At this point, the net charge is zero. If electrolytes are added to the solution, this point may be shifted to a new pH. This new pH, known as the isoelectric point (IEP), is defined as the pH at which gelatin molecules do not migrate in an electrical field (because they are neutrally charged at that point). In some cases, the p*I* and the IEP are identical.

B. Ash

Ash yielded by gelatin varies with the type of raw material and the method of manufacture. Type A or pork skin gelatins contain small amounts of chloride, while ossein gelatin contains principally calcium phosphates. The U.S.P. limit on ash is 2%, but most commercial gelatins have lower ash contents.

C. Metal Content

The metal content of gelatin is rigidly controlled, and the presence of such materials in gelatins is unlikely unless impure acids or chemicals are used in the process of manufacture. The U.S.P. limit for arsenic is 1 ppm and for heavy metals 50 ppm. Most gelatins meet these requirements without difficulty.

D. Sulfur Dioxide Content

The addition of sulfur dioxide gives hard gelatin capsules an increased transparency, greater brilliancy, and improved stability—all qualities that are desired in capsule manufacture. The U.S.P. permits not more than 0.004% sulfur dioxide in most cases, but for gelatin used in capsules, as much as 0.15% is permitted. However, these are special grade gelatins that are not used in normal food consumption.

E. Organic Additives

Organic materials affect gelatin in many ways. Aldehydes tend to cross link gelatin molecules, resulting in polyaldehydes of reduced solubility or increased viscosity. Flavoring agents containing alde-

hydes can be used in gelatin products, but concentrations and stability must be determined by appropriate tests. Inert materials, such as sugars, are usually no problem, but sometimes there is an interaction between saccharides and proteins causing the browning reaction to occur.

VI. Physical Properties

A. SOLUBILITY

Gelatin is insoluble in cold water, but it swells upon contact with water and forms large visible swollen particles known as "fish eyes." If the water is then heated to about 160°F, the hydrated gelatin will go into solution due to the rupture of the molecular aggregates, or micelles. Strictly speaking, a macromolecular colloidal dispersion is formed. In order to prevent gelatin from setting up to a gel, it is necessary to keep the solution hot. If the temperature should drop below 120°F., the gelatin will tend to gel.

Although gelatin is soluble in polyhydric alcohols such as sorbitol, mannitol, propylene glycol, and glycerin, water is usually present as an accessory solvent. Gelatin is practically insoluble in alcohol, acetone, and nonpolar solvents such as carbon tetrachloride, petroleum ether, and carbon disulfide.

B. VISCOSITY

The viscosity produced by gelatin in water solution is one of its most important properties. Commercial gelatins vary in viscosity between 20 and 70 mps. Molecular weight distribution seems to be much more important in its effect on viscosity than on gel strength. Gelatins of higher gel strengths may give lower viscosities than samples of lower gel strength, showing that gel strength and viscosity are not directly related.

In dilute salt-free solutions, the viscosity of gelatin is at a minimum at the isoelectric point, which corresponds to the most tightly folded configuration of the gelatin molecule. As the pH is lowered or raised, the viscosity increases to a maximum which is several times the minimum value. This effect is believed to result from the repulsion caused by the excess of like charges which are now distributed along the chain. Salts reduce this effect substantially, and that is one of the reasons that commercial gelatins give various types of curves where vis-

cosity is plotted against charge and pH. The shape of the curve is also influenced by the type of gelatin, previous thermal history, age of solution, concentration, and other factors.

C. Color

The color of gelatin depends upon the type of raw material used and the order of extraction, i.e., the first, second, or subsequent extraction. Pork skin gelatins usually have less color than those from bone. The final extraction of bone usually contains a great deal of colored material. Generally speaking, however, color does not influence the properties of gelatin, nor does it impair its functionality.

D. Turbidity

Turbidity may be caused by many factors. It may be due to the isoelectric haze, which occurs when the pH of a solution is either at or near the isoelectric point. At this point the hydration of the gelatin particles is minimal and an optically heterogeneous system results. This condition can be eliminated or minimized by adjusting the concentration of the gelatin or adjusting the pH to a lower or higher value outside of the isoelectric range.

Another cause of turbidity stems from the use of mixed gelatins, both Type A and Type B. At pH values between 5 and 7, turbidity may result depending upon the ratio of the mixture and the exact pH value. This phenomenon is due to the formation of a gelatin coacervate between the oppositely charged gelatin micelles. The gel strength and the viscosity at this point is reduced. The addition of neutral salts restores the clarity, viscosity, and gel strength to the normal values, and their quantitative effect is based upon the valence of their constituent ions. Thus, trivalent ions are more effective than divalent ions, which are, in turn, more effective than monovalent ions. If the pH is adjusted to above or below the isoelectric point of both gelatins, a perfectly clear gel results, since both are now mutually compatible. Turbidity may also be due to insoluble impurities if the extract has not been properly filtered.

E. Gel Strength (Bloom)

The formation of heat-reversible gels is probably the most important property of gelatin. Gel formation is believed to result from hy-

drogen bonding of the gelatin molecules to form micelles which results in the formation of a semisolid gel which binds the water component.

Gel strength is usually measured with the Bloom gelometer, the standard instrument for the industry (see Chapter III). The procedure, an essentially simple one, is as follows: A 6.66% gelatin solution is carefully prepared in a standard manner. The solution is then placed in a special wide-mouth Bloom jar, which is cooled in a chill bath maintained at exactly 10°C ± 0.1°C, for 17 ± 1 hours. At this time, the firmness of the gel is measured on the gelometer. The instrument measures the weight of shot plus the weight of the cup required to depress a plunger 1.0 inch in diameter exactly 4 mm into the surface of the gel. The shot is delivered at a controlled rate of 45 gm/5 sec, and the flow is cut off mechanically when the plunger reaches the 4-mm depression. The weight (in grams) of the shot and cup is the bloom rating of the original sample of gelatin. Commercial gelatins vary from 50 to 300 bloom. Zero bloom gelatins have also been made for special applications.

The gel strength of gelatin is affected by a great number of factors, including pH, and the presence of electrolytes, nonelectrolytes, and other additives. Gelatin from acid-treated pork skins shows a more rapid drop in gel strength in the lower pH regions than that from the alkali-treated materials. This is caused by the difference in their isoelectric points. Pork skin gelatin at a pH of 2–3 is so far removed from the isoelectric point that it is much more highly hydrated. This results in a weakening of the forces holding the gelatin polymers together in the gel structure.

Materials such as urea exert a peptizing influence on the formation of gel structures, and in large concentrations, they prevent entirely the formation of a gelatin gel. The effect of urea is probably one of interference with the hydrogen bonding necessary for gel formation.

The effect of acids, alkalis, heat, proteolytic enzymes, etc. is essentially one of hydrolysis: the structure of gelatin is so completely broken down that it will not form a gel.

F. Protective Colloid Action

The protective colloidal action of gelatin was investigated by the original pioneer of this field, Zsigmondy, who introduced the principal of the *gold number*. His procedure permits quantitative comparison of the protective action of different colloidal materials. The ***gold number*** is defined as the number of milligrams of protective substance that just fails to prevent the color change to violet of 10 ml of a

standard red gold colloidal solution on the addition of 1 ml of a 10% sodium chloride solution. It was found that gelatin was the most effective (and hence had the lowest Zsigmondy number) of any colloid. It is believed that the protective action of gelatin for the gold colloid is due to a film of gelatin no more than one molecule thick. The gelatin coats the colloidal particles and prevents their clumping, thus permitting them to maintain their discrete identity. This phenomenon is very important in such gelatin applications as coating silver colloids in the photographic industry and plating of metals in the electroplating industries.

G. Emulsion Stabilizer

An emulsion is a dispersion of one substance in the bulk of another substance. The important emulsions relating to gelatin are liquid systems (emulsions) and gas systems (foams). The ability of gelatin to stabilize emulsions and foams is the result of its protective colloidal action, which allows it to form a semisolid interfacial film around the dispersed globules. The strong gelatin film around the air bubbles or water droplets stabilizes the systems. This stability is probably due to the gelatin groupings within the molecular structure of the system which permits it to orient its micelles both toward water-seeking and water-repelling substances. This activity is dependent upon its structure and is greatly influenced by the pH of the system.

H. Preservation

Both gelatin solutions and gels are subject to the growth of bacteria or microorganisms unless preventive measures are taken. The types of organisms that are likely to grow on gelatin depend primarily upon pH. Depending upon the conditions and constituents of a product, various preservatives can be added to prevent gelatin from deteriorating. Some of the more common preservatives are benzoic acid, alcohol, cetyl pyridinium chloride, and related compounds.

VII. Gelling Properties and Mechanism

A. Gel Structure Hypotheses

Gels are systems in which small proportions of a solid are dispersed in relatively large proportions of a liquid. They have the property of

mechanical rigidity, or the ability to support shearing stress at rest. This characteristic property of rigidity is common to all gels.

A gel is remarkable in that it consists mostly of fluid, behaves as a rigid solid, and yet retains many properties characteristic of the fluid component such as compressibility, vapor pressure, and electrical conductivity.

Many hypotheses (Ferry, 1948) have been proposed to explain the nature of the gel structure; the following three are the most prominent: (1) immobilization of solvent through absorption by the solute, (2) the presence of a three-dimensional network of solute, (3) the operation of long-range forces between solute particles.

The type of structure that most aptly fits the protein gel of gelatin is that of a three-dimensional network. In gelatin gels, the essential structural feature appears to be a three-dimensional network which is held together by primary bonds, by secondary forces localized at certain points on the molecule, or by nonlocalized secondary attractive forces. In the latter case, either fine or coarse networks may be formed depending on the conditions under which the long-chain molecules associate.

When bonds or linkages are gradually introduced into a system of chain molecules, the first appearance of a network is manifested by increase in viscosity, then by mild gelation; but at this point, the rigidity is very small and most of the chains are still unattached. With increasing numbers of linkages, more of the chains become bound and the rigidity increases as the network becomes more closely knit together.

Flexibility of the network strands permits many different configurations and convolutions for each strand and endows gels with rubberlike elastic properties, as well as the ability to swell or absorb solvents. The coarseness of gel networks can be measured by the light-scattering effect.

The distinguishing feature of gelatin gelation is the formation of rigid gels from cooled aqueous solutions of dilute concentrations of the protein. The sol-gel transformation is dependent upon temperature and occurs as the result of the binding together of the individual molecular chains by secondary attractive forces localized at widely separated points. The attractive loci may include several amino acid residues, and hydrogen bonding is probably the primary effective force tying the molecules together.

B. Gelation of Gelatin

Gelatin forms gels similar to those of carbohydrates by forming a microstructural network at concentrations as low as a fraction of 1%.

The water is immobilized within the meshes of the protein structural network. Two different types of linkages between the polypeptide molecules are responsible for the gelation phenomenom (Hamm, 1963). These are:

1. Secondary valences, specifically the hydrogen bonds between the peptide groups. It is possible that the side-chain groups, such as the sulfhydryl or phenol groups, participate in hydrogen bonding (Fig. 4). Other investigators feel that since there are not many sulfhydryl or disulfide groups in gelatin, they are not likely to be responsible for gelation of gelatin.

2. Primary valences, as shown by disulfide cross-linkages, salt bonds between amino and carboxyl groups of side-chains, cross-linkages formed by salt-like bond bivalent cations (Fig. 4). Cross-linking metals may also be tightly built into the protein structure as chelates or clathrates.

In pectins, cross-linking can take place at almost every point of the molecule. In contrast the peptide chains of gelatin are only linked together at distinct, widely separated sites. At these sites, several peptide chains are bundled together forming crystallite regions which can be confirmed by X-ray analyses (Herrmann and Gerngross, 1932; Ferry, 1948). These X-ray diffraction patterns indicate that the gelatin molecules are extended polypeptide chains, which are associated laterally at various places to form bundles. The bundles have a crystalline order and multiple cross-links which tie the chains together in a network, and their presence accounts for the sharp crystalline rings in the X-ray diffraction pattern. The amorphous rings, which are shown by both gels and solutions of gelatin, are considered to be the result of diffraction by the disordered portions of the chains. The gel network is formed by partial crystallization of certain regions of the chain. Its formation is accompanied by the evolution of heat, and contraction in volume, after which an equilibrium is finally established between the crystalline and liquid states of the chain segments.

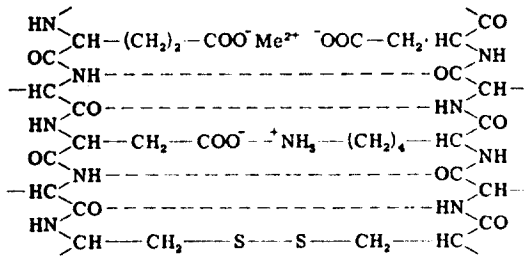


FIG. 4. Types of cross-linking in gelatin gels.

Bello *et al.* (1962) showed in some recent work that the forces which interlink the peptide chains are not of an electrostatic nature because blocking the polar groups by chemical modification does not greatly influence the mechanical properties of gelatin gels. There is no evidence that the nonpolar side chains of gelatin participate in the gelation process. So, cross-linking may be caused only by the peptide groups which are linked together by hydrogen bonds, either with or without participation of water:

)C═O-------H—N(
or
)C═O----HOH----O═C(

It is possible to prevent gelation of gelatin completely by blocking the peptides by forming complexes with cupric ions. The decrease of the ability of gelatin to gel after the addition of certain salts is due apparently to a destruction of intermolecular hydrogen bonds (Courts, 1960). In the isoelectric range of gelatin and at low ionic strengths, however, the polar groups of gelatin participate considerably in the cross-linking of the protein molecules and thus in the process of gelation. This takes place because under these conditions the protein has the maximum number of oppositely charged groups. Particularly in gels with a low concentration of gelatin (about 0.6–0.7%), the interaction between the polar groups of the protein side chains is of importance. A decrease in the ability to gel on the basic side of the isoelectric point may be due to an electrostatic repulsion between carboxyl groups; the same effect on the acidic side of the isoelectric point may be due to repulsion between guanidine groups.

In summation, it can be said that gelatin molecules are highly elongated but of widely varying chain lengths. Many of the properties of gelatin gels can be qualitatively explained on the basis of a three-dimensional network structure formed by the junction of these elongated molecules at widely separated loci of attraction by secondary bonds. That the bonds are due to secondary forces, such as van der Waals forces, is obvious from the fact that they can be disassociated by a small increase in temperature. That they are formed at specific loci of attraction, spaced far apart along the molecules, may be inferred partly from the mechanical properties of the gels and partly from the range of conditions under which the gels are stable.

C. Phenomena Related to Gelling Mechanism

1. Gel Formation

Ferry (1948) pointed out the similarity of the gelation of gelatin to

the gelation of cellulose and other linear polymers. Under suitable conditions, these polymers form random interchain contacts stabilized by either primary or secondary bonds. As long as the number of interchain contacts is below a critical number per polymer chain, the polymer system remains soluble. When the critical number is reached, there is a sharp transition corresponding to the formation of a large network of polymer chains. This network is insoluble, although it may contain many times more solvent than polymer on a weight basis. Immediately upon formation, the polymer network exhibits a well defined rigidity, and the entire polymer-solvent system is immobilized or sets to form a gel. Network systems stabilized by secondary forces, such as hydrogen bonds, rather than by primary covalent bonds, exhibit a strong dependence upon the solvent and upon the temperature of the system.

In general, the structure type of the network gel is determined by the mode of gel formation. If, for example, network formation is brought about rapidly or at high polymer concentrations, random chain contacts and polymer chain entanglements lead to the formation of fine-chain network structures. On the other hand, slow formation of interchain contacts in dilute polymer solutions permits a greater organization of interacting segments, leading to the development of large crystallites in which chains are aligned for relatively long distances and the formation of coarse networks. Tempering a gel formed as a fine network at some point close to its critical melting temperature would permit a gradual rearrangement to a more ordered structure and a consequent gradual transition of the structure to a coarser network (Veis, 1964).

Another theory of gel formation assumes that the initial formation of a gel is due to associations between chains in pairs rather than in bundles, and that the cross-links in gels, when first formed, are individual rather than multiple. As the process continues, further lateral association between paired chains eventually builds up crystallites. But, whether the cross-links are formed in two stages or whether they are all formed by bundles, it is obvious that the chain segment between the cross-links must be fairly flexible to explain the extensibility and elasticity of gelatin gels.

2. *Viscosity*

Recent rheological studies of gelatin solutions show that the effect of changes in the rate of shear on the apparent viscosity of gelatin solutions is small in dilute solutions. In more concentrated solutions above the gelation temperature, the rheological behavior is not substantially different apart from the usual hydrodynamic action. Viscos-

ity increases approximately exponentially with increases in gelatin concentration. The viscosity also increases exponentially with decreases in temperature (Ward and Saunders, 1956).

When a solution of gelatin is cooled from a temperature at which it is liquid to one at which gelation occurs, the viscosity slowly increases with time until the instant of gelation when the viscosity becomes very great.

3. *Rigidity*

The rigidity of the gel increases with time, and as the gel ages, it keeps increasing in strength and becomes much more rigid and tougher. In food products this harder gelatin texture is described as rubbery. The time of onset of gelation depends on the concentration of the gelatin. The higher the concentration, the more rapid the rate of gelation. This phenomenon is accompanied by, and is comparable to, the changes in optical rotation, which change with time in a manner similar to the changes in rigidity. Neither the rigidity nor the specific rotation ever becomes constant, but both slow down asymptotically.

Gelatin gels are elastic and obey Hooke's Law. The rigidity of gelatin gels is approximately proportional to the square of the concentration over a concentration range of about 10-30%. The rigidity is also inversely related to the temperature, and increases rapidly with decreasing temperature.

Rigidity of a gelatin gel increases markedly with increasing molecular weight of the gelatin. For type A pigskin gelatin, pH variation in the range of 4-9, does not have much effect on rigidity, but for type B gelatin there can be a spread of 20-40 Bloom units.

4. *Melting Point*

a. Gelatin Concentration. Melting point increases with increasing concentration of gelatin and is directly related to the molecular weight of the gelatin.

b. pH. Melting point is very little dependent on pH over a rather wide area of pH in the isoionic range, if salt is present or the concentration is quite low. The fact that the melting point is not greatly influenced by sodium chloride concentration or by pH suggests that coulomb forces are unimportant in whatever interaction between gelatin molecules results in gelation.

c. Electrolytes. Salts such as sodium chloride depress the melting points of gelatin gels, while others such as sodium sulfate raise them. The order of effectiveness of the cations and anions in depressing the melting point follows the Hofmeister series. The depression of the melting point is most marked for lithium salts and for thiocyanates.

5. *Turbidity*

Aqueous gels are highly transparent at pH's away from the isoelectric point but exhibit a sharp maximum of turbidity at the isoelectric point. The turbidity increases markedly with decreasing temperature and evidently accompanies gelation.

In summation, it can be said that when gelatin solutions gel, the following important changes in physical properties can be observed: (1) increase in rigidity, (2) change in optical rotation, (3) liberation of heat, (4) change in the X-ray diffraction pattern, (5) change in the amount and character of light scattering, (6) decrease in specific volume. Some of these changes have been reviewed in this chapter, but for more detailed and theoretical discussions, readers are referred to the excellent publications by Ferry (1948), Idson and Braswell (1957), Stainsby (1958), Harrington and Von Hippel (1961), Veis (1964), and Harding (1965).

VIII. Applications

Because of its unique hydrocolloidal properties, gelatin has found tremendous utility, not only in food products, but in some very novel and ingenious nonfood applications. Not too long ago, a bookmaker arrested in Brooklyn was found to be using gelatin paper to record his illegal betting transactions. When the police raided his apartment he tried to destroy the evidence by tossing the betting slips into a bowl of cold water. Unfortunately for him, the gelatin paper congealed in the cold water into a solid, translucent mass, through which the written notations could still be discerned. The gelatin was photographed and the evidence used to put the bookmaker "on ice." At his arraignment, he explained that he had just moved into the apartment and had not had time to buy a pot to heat up water for dissolving the gelatin paper (Anonymous, 1964).

Food applications of gelatin are of a more mundane nature; large quantities are used as hydrocolloidal ingredients in food products. It has been estimated (Idson and Braswell, 1957) that about two-thirds of the gelatin produced in the United States is used in food products such as desserts, marshmallows, confections, jellied meats, consomme, baked goods, ice cream, and dairy products. Of the rest, about 10% is used in pharmaceutical applications and 20% for photographic uses. The remaining 5% is accounted for by specialized industrial applications.

Accurate figures for the amounts of edible gelatin actually consumed are not readily available, but published (Sanders, 1966) and

private estimates indicate that in 1965 about 40,000,000 to 45,000,000 lb of edible grade gelatin was sold in the United States for use in foods. Some estimates have even been as high as 50,000,000 lb. Regardless of the actual amounts, however, it is a fact that the consumption of gelatin continues to show a distinctly increasing trend over the years.

Using the highest figure, the estimated major applications of gelatin can be broken down into areas of utilization as in the tabulation.

Dessert products	37,000,000 lb
Confections (primarily marshmallows, toppings, fillings)	8,000,000
Meat products and ice cream	4,000,000
Other	1,000,000
Total	50,000,000 lb

A. Gelatin Desserts

1. Formulation

The largest use of gelatin is in the preparation of gelatin desserts, a use which is based on the ability of gelatin to form an organoleptically desirable gel with water. Gelatin can retain more than 50 times its weight of water within its gel structure. In addition, the gel is thermally reversible and has the characteristics of melting in the mouth in a pleasant, non-slimy manner and of releasing the flavor constituents in an optimum manner on the tongue and palate.

The forerunner of today's gelatin dessert can be said to have its origins in a patent issued over one hundred years ago to Peter Cooper, the founder of Cooper Union college in New York. In 1845, Cooper was allowed a patent for a "portable gelatine" which consisted in "making a transparent concentrated or solidified jelly containing all the ingredients fitting it for table use, in a portable form, and requiring only the addition of a prescribed quantity of hot water to dissolve it, when it may be poured into glasses or molds, and when cold will be fit for use." This was made by mixing "isinglass or gelatine" with sugar, lemon or lime juice, eggs, and sundry spices "as will suit the tastes of different persons." The mixture was then boiled, filtered, and finally condensed to a jelly or a solid to which "it is only necessary to add a sufficient quantity of hot water to produce at any time a jelly of any consistency that may be required" (Cooper, 1845).

The passage of more than a century has seen great advances in the technology of gelatin manufacture which in turn has been responsible for the development of high quality gelatin dessert mixes that are an integral part of the American diet today. Gelatin dessert mixes are blends of gelatin, sugar, acid, buffer salts, flavor, and color. A typical formulation published by the Gelatin Manufacturer's Institute of America (1962) is the following:

Gelatin	8-12%
Acid	2-3%
Buffer salts	0.6-1%
Salt	0.3%
Flavor	q.s.
Color	q.s.
Sucrose	to 100%

Three ounces of the above mix will gel one pint of hot water after setting at refrigerator temperatures (about 40°F.) for about 3-4 hours.

2. *Gelatin*

The concentration of gelatin in gelatin desserts is variable, since a variety of gelatins of differing gel strengths can be used. The usual gelatin gel strengths in dessert products vary from 175 to 300 Bloom (Borker *et al.* 1966). The setting time and firmness (rigidity) of the finished dessert is dependent upon the gel strength, concentration, viscosity, pH, and type of gelatin used.

The clearest quality gelatins are usually reserved for desserts. A gelatin with a low lime content is particularly desirable. While lime is not always harmful, it can give rise to problems in foods containing appreciable amounts of citric or tartaric acids; lime can cause a precipitate of the calcium salt with resultant impairment of clarity (Selby, 1951).

High Bloom (220-250) gelatins have been used at comparatively high concentrations (12%-15%) by Corben and Hatch (1962) to reduce the setting or gelling time. A similar attempt was made by Cohen (1959), who incorporated a proteolytic enzyme. Excess high Bloom gelatin was used to obtain a faster setting time, and the excessive gel strength was reduced by controlled hydrolysis of the gelatin gel by the enzyme.

3. *Acid*

Traditional food acids, such as citric and tartaric, have been used to impart tartness to the gels, but these acids have been largely sup-

planted in recent years by newer commercially available products such as fumaric, adipic, and malic acids, for which improved properties have been claimed. Some of these properties are greater stability, better performance, and lower cost. Their use is covered by industrial patents (Stokes and Kennedy, 1946; Ferguson, 1953; Pintauro and Hall, 1962; Polya and Lister, 1966). One recent patent (Lauck and Toy, 1962) claimed the use of phenylphosphonic acid as the acidulant to give a gelatin dessert of improved storage stability that will "dissolve in relatively cool water."

4. Buffer Salts

Buffer salts are incorporated into the mix in order to maintain the desired pH and thus control the setting and melting characteristics. Early formulations by Collins (1940) used sodium bitartrate, tartrate, lactate, acetate, or citrate in concentrations sufficient to develop a final pH of 3.0-4.7 necessary to produce smoother, faster-setting gels. Today, the most commonly used buffer salts are sodium phosphates or sodium citrates, and the pH's of the dessert gels are maintained at 3.0-4.0.

5. Flavor

Fixed or encapsulated powdered flavors are used at levels sufficient to impart the characteristic flavor and aroma to the finished gel. Salt (sodium chloride) is usually added to enhance the flavor quality of the dessert, but it is not functionally necessary.

6. Sugar

The sugar used is not necessarily sucrose, but can be dextrose, corn syrup solids, or mixtures thereof. In dietetic or low-calorie desserts, sugar is replaced by artificial sweeteners and some nonnutritive bulking agents.

7. Solubility Improvements

Efforts to improve the solubility of gelatin have been made by coating gelatin with various wetting agents and surfactants. Gunthardt (1958) coated gelatin with about 0.05-0.5% lecithin by grinding gelatin with the lecithin in a ball mill to provide a uniform coating. Steigman (1958) coated gelatin with dispersants of silicones, polyethylene glycols, and other wetting agents by spraying them with the dispersants while the gelatin was tumbled in a ball mill. Hagerty (1957) treated gelatin with 9 parts sugar and 10 parts water and then dried the mixture. After pulverizing and flavoring, the material was stated to be soluble in about 6 parts cold water and claimed to gel upon standing.

Subsequently, Cahn and Hagerty (1958) produced a similar product by spray-drying solutions of sugar and gelatin to obtain a dry powder that would dissolve in cold water at room temperature and set up to form a gel.

Cahn (1960) further modified his cold water-soluble gelatin by making a fluid film of 1 part gelatin, 8-10 parts sugar, and 10 parts water, which was then dried by atmospheric dehydration. The dry, brittle, solid material was cold water soluble.

Reichel (1939) some time ago obtained a cold-water-soluble gelatin by freeze drying an aqueous solution of gelatin. And more recently, it was found that freeze drying a solution of 2.5% gelatin and 4% sugar yielded a product that was immediately soluble upon the addition of cold water (Farbenfabriken Bayer, 1954).

8. Aerated Desserts and Puddings

Chiffon or fluff-type desserts are usually prepared by whipping gelatin solutions that have been flavored with sugar and fruit juices under vigorous, mechanical agitation. Often, whipped cream is folded into the mass, and the setting or gelling of the dessert is accomplished by chilling the final product. The texture is a function of the amount of air whipped into the mass as well as the amount and quality of the gelatin used (Matz, 1962).

Mancuso and Common (1960) patented a fruit-flavored chiffon mix consisting of gelatin, guar gum, soy protein, sugar, and an organic acid. A chocolate chiffon pudding mix, developed by Block (1961), consisted of cocoa powder, soy protein, gelatin, guar gum, and sugar.

Improved whipping compositions were made by the use of combinations of soy protein with gelatin (Gunther Products, 1959).

B. Confections

1. Foamed Products

When air is introduced into a liquid system, a two phase system called a foam is produced. In the confectionery industry, foams of this type can be stabilized by incorporating into the mix certain proteins known as whipping agents. The whipping agents impart special characteristics to the finished confections. The main protein whipping agents used are albumin, gelatin, and processed proteins such as casein, soy albumin, fish albumin, and some specialty ones such as blood plasma albumin.

Gelatin is used for the manufacture of practically all commercial marshmallows. A combination of gelatin and albumin is also used for the production of low density, marshmallow-type toppings known as

marshmallow fluff. Albumin- and casein-base materials are used for nougats and frappes because they have good foaming power in syrups, good coagulation properties, and good resistance to fats and oils. In addition, they give good sets to the finished products (Mallows, 1960).

The type of foam produced by the incorporation of air into sugar syrups consists of spheres of air held in suspension by the air-liquid interfaces containing the liquid. These interfaces in foam formation are increased by supplying energy in various ways. Therefore, the type of machine used and the amount of energy put into the formation of foam will have a great effect upon the entrapment of the air bubbles and the resultant density and texture. In the production of common confectionery foam products, the amount of air incorporated governs the texture of the finished product. With a whipping agent, in general, the more water that is present, the greater is the amount of air that can be incorporated, and the lower the bulk density of the finished product.

As mentioned above, gelatin is used in the production of a great variety of marshmallow-like products which are used as toppings, bakery fillings, confections, and related food products. The use of gelatin in these products is based primarily on its ability to stabilize aerated systems such as gas-solid emulsions and gas-liquid emulsions. The viscosity of gelatin solutions promotes the dispersion of air into the sugar syrups on which these products are based. The surface tension of gelatin solutions prevents the coalescence of the air bubbles and permits the air-liquid or air-solid emulsions to stabilize and retain their aerated state. In addition, the hydrophilic nature of gelatin allows it to bind water in the semirigid structure and to prevent syneresis or weeping of liquid in the finished product. Foamed gelatin systems can be made to vary in density, graininess, moistness, and tenderness.

2. *Marshmallows*

Marshmallows are practically an American tradition, and gelatin is generally recognized as the most important single ingredient in marshmallow production (Anonymous, 1961). This is due to the functional characteristics of gelatin. Gelatin has good whipping properties because it lowers the surface tension of the sugar syrup. Gelatin also stabilizes the marshmallow foam by increasing its viscosity; at the same time, it sets up the foam by gelation into a gelatin gel.

Marshmallows are prepared by whipping a heated syrup consisting of a blend of sugars (usually corn syrup and sucrose) and a whipping and stabilizing agent to form an aerated, stiff product. When the foam

has reached the desired density, it is shaped by pouring into corn starch molds. Setting up of the marshmallow occurs as a result of cooling, drying, and the forming of a firm crust of collapsed cells on the outside surfaces of the piece.

Marshmallows contain only about 1.5-2.5% gelatin, but the quantity and quality of the gelatin are important in determining the degree of whip, the shelf life of the final product, the texture and the overall quality of the finished product.

Two types of marshmallow textures are usually made, ranging from a soft standard type to the harder grained types molded in various novelty shapes. Both kinds are usually molded in starch that must be dried sufficiently to form a durable outer skin while absorbing moisture from the candies to induce proper gel formation.

Bronson (1951) recommended the use of 225 Bloom gelatin for regular, white, cast marshmallows where strength was required for handling and packing. Lower Bloom gelatins of 75-125 were suggested for light, fluffy, grained novelties. Intermediate strength gelatins of 175-225 Bloom were suitable for making low density marshmallows to be deposited on cookies. However, Gorfinkle (1953), and other workers in the field found that the final texture of the candy depended upon other properties as well as the Bloom strength of the gelatin. Martin (1955) reported that the whipping power, which is not related to the gel strength, was equally important.

Taylor (1946) investigated the various parameters of marshmallow production and observed that no matter how the process was modified, the viscosity depended mainly on the concentration of soluble solids. The quantity of gelatin affected the viscosity to only a small degree however when the solids concentration was low. The time and effectiveness of beating also had a marked effect on viscosity. Below a certain critical percentage of gelatin, there was a tendency towards a decrease in the extent of overrun with increasing soluble solids content. Above the critical gelatin concentration, there was an increased expansion within increasing concentration of soluble solids, except when the time of beating was short. The concentration of soluble solids had only a comparatively small effect on the toughness of the marshmallow while the concentration of gelatin had the most powerful influence on toughness. High Bloom gelatins gave tougher marshmallows, but only at excessive concentrations.

On the other hand, Gorfinkle (1953) recommended the use of appropriate quantities of high Bloom gelatins selected for texture characteristics for all types of marshmallows. He believed that the high body gelatins tended to make whiter, more silvery marshmal-

lows. They set faster and had less crust. He also emphasized that gelatins of the same Bloom strength may have different bodying power, some making tougher and others more tender marshmallows.

Steigmann (1944) investigated gel setting time and viscosity of gelatin as possible measures of marshmallow quality. In these measurements, the absorbed differences were magnified by the protein dispersing agents, although the correlations with gel strength and whipping properties were not completely established.

Grettie (1940) found that the addition of sodium hexametaphosphate or other complex phosphates at concentrations of 0.5-5.0% improved the whipping properties of gelatin in marshmallow compositions without decreasing their viscosity or gel strengths.

Improved whipping characteristics were claimed to be the result of the addition of phytic acid to gelatin (Grettie and Young, 1949). Similar improvement was noted by the addition of organic esters such as triethyl citrate, ethyl maleate, and ethyl fumarate (Conrad and Stiles, 1954).

The bulk densities of marshmallows vary between 0.2 and 0.5. The texture may be influenced by the following factors: (1) the type of beater; (2) the temperature at which the marshmallows are deposited; (3) the temperature and the moisture content of the starch into which the marshmallows are deposited; (4) the holding time in starch and the atmospheric conditions during that period; (5) the holding time before packing; and (6) the interval between packing and consumption. Toughness, an important textural defect of marshmallows in many cases, is a function of gelatin quality and content and drying out. If marshmallows are to be kept for lengthy periods before packing it is necessary to use a humectant such as sorbitol to minimize drying out. In addition, close control on changing temperature, starch temperature, and moisture are also necessary (Mallows, 1960). In a thorough study of the qualities and parameters in the production of marshmallows made by Tiemstra (1964 a,b,c,d), the following four basic properties were explored: (1) overrun, (2) viscosity and elasticity, (3) moisture, and (4) set and syneresis.

Investigation of overrun showed that it varied inversely with the viscosity of the sugar syrup used, and that gelatins of higher gel strength did not whip as well as the lower grades of gelatin. However, the amount of gelatin needed to obtain the optimum whip was less with materials of higher gel strengths. Another important factor influencing overrun was the physical and mechanical method of beating and whipping (Tiemstra, 1964a).

Evaluation of the texture of marshmallows showed that the degree of elasticity and viscosity were important parameters; the modulii of elasticity (bulk and rigidity) and many viscosity effects were evident. Tiemstra (1964b) found that the gelatin-to-moisture ratio was directly related to the elastic modulis for any given grade of gelatin as measured by gel strength. And the relationship of gelatin and moisture were the greatest factors affecting the elastic properties of the product.

In summarizing his work, Tiemstra (1964c,d) concluded that no one factor was as important as the amount and type of gelatin used. Water was the second factor of importance, which confirmed the original concept that the ratio of gelatin to water is the most critical parameter.

Modifications of marshmallow products have included such novelties as the preparation of a marshmallow dry mix using powdered gelatin, corn syrup solids, cornstarch, sodium phosphate, sorbitol, and flavor (Downey, 1962).

3. *Other Confections*

Gelatin has been used in many other confectionery products. In the manufacture of caramels, the addition of gelatin has been found to retain the moisture and chew and to give improved caramel-type products (Anonymous, 1960).

Several hydrocolloids have been used for the preparation of jelly confections. At the present time, starch and pectin are preferred, but gelatin is still used to some degree in these products (Williams, 1958). Lees (1960) used 0.25% gelatin as a binder in the production of licorice paste confections, and it is used as a binder in wafer-type confections, e.g. Necco Wafers.

C. Ice Cream Stabilizers

Ice cream is a complex system comprising air, liquid, solid, and oil. Gelatin is the oldest and most widely used protein stabilizer for ice cream, and prior to World War II, it was the most effective stabilizer used. During the war, carboxymethylcellulose and other hydrocolloids gradually took over the market from gelatin, but today there are still many specialty ice cream stabilizers that are composed of gelatin blended with other vegetable and synthetic hydrocolloids. Essentially, gelatin acts to bind a portion of the free water in the ice cream mix and forms a three-dimensional fibral network that retards crystal formation, gives ice cream a good eating texture, and protects it from heat shock. The effectiveness of gelatin as an ice cream stabilizer, by virtue

of its great water-binding capacity and its effect on the freezing characteristics of ice cream, has been compared with the other commonly used hydrocolloids by Wegener (1954), Moncrieff (1954), and Boyle (1959). Its excellent functional properties were recently reconfirmed by Shipe, Roberts, and Blanton (1963).

In the past, the Bloom value of the gelatin was used as a suitable index for performance, until it was found that there was very little correlation between gel strength and performance. Now it is known that gelatins of almost any Bloom strength will effectively stabilize ice creams, but it is more economical to use the higher Bloom strength materials (Tobias and Edman, 1951).

A related product,—a dry baked, toasted edible cone for frozen confections—was made from cereal flour and gelatin. This cone, which did not toughen upon storage, was prepared by baking a mixture of a cereal flour and gelatin at 160–175°C. for about 90 minutes (Washburn, 1954).

D. Dairy Products

Dry dairy mixes containing gelatin have been prepared by Steigmann (1959), who mixed dairy products such as milk and gelatin and dried the mixture to form a dry substance that could be utilized as a powder.

A frozen, ready-to-use, whipped cream product based on butterfat cream, granulated sugar, vanilla, and a stabilizer such as gelatin has been developed. This product is claimed to be stable for three months (Anonymous, 1962).

A nutritious milk beverage combined with fruit juice has been patented by Hughes (1959), who was able effectively (i.e., without curdling) to combine the acidic fruit juice with milk by the use of a gelatin ingredient.

Modified butter can be mixed with gelatin to produce a butter of good keeping and spreading qualities that does not require refrigeration (Bettman, 1953).

E. Meat Products

The meat-packing industry uses large quantities of edible gelatin in the production of deboned hams, meat loaves, sausages, head cheese, scrapple, canned hams, and jellied tongues. The purpose of gelatin is to absorb and hold the meat juices, which separate during the cooking processes, as well as to give form and stability to the product. The ad-

dition of gelatin preserves the natural appearance and texture of the cooked products (Idson and Braswell, 1957).

In processing hams, the normal procedure is to add 1 part dry gelatin to 50–75 parts of deboned ham. The hams are then cooked, chilled, and stored. Hams are dipped in gelatin solutions prior to being inserted in casings, and in this way, the final product retains a pleasing appearance, does not release dripping juices, and maintains its shape for slicing. Jellied meat loaves and sausages are made of parts of different types of cooked meats or poultry suspended in a gelatin solution. Gelatin usually comprises 2–3% of the sausage formulation.

High-viscosity gelatins are useful in glazes because the gelatin has time to set before it runs off the product being glazed. Meat products are sometimes dip-coated in mixtures of starch, gelatin, sodium citrate, and flavoring materials (Lesparre, 1948).

Low lime content is desirable in gelatin for meat products in order to prevent deposits of calcium phosphate, which may be produced from a soluble phosphate in the meat juices and the lime in the gelatin. This is particularly true in the case of meat delicacies such as chicken filet, hams, lamb, and tongues, which are set in gels.

One recent application for gelatin products has been the preparation of edible sausage casings from reconstituted collagen. These new casings are primarily based on a modified collagen, which, of course, is not a complete gelatin product, but somewhere in between. It has certain advantages over most synthetic casing materials and is being investigated for many sausage products (Anonymous, 1963).

F. Bakery Goods

1. Chiffon Pie Fillings

Gelatin is used in various baked goods as a setting and stabilizing agent, as well as a foam-producing material. In chiffon-type pies, it serves a twofold function, producing the desired type of foam in combination with egg white, and acting as a stabilizing agent in preventing syneresis of the filling after the chiffon has been poured into the baked pie shell (Glabau, 1955). The quantity of gelatin used in chiffon pies is inversely proportional to the gel strength, or Bloom. Gelatins of higher Bloom value are used to attain a greater degree of stability and to prevent syneresis. In chiffon cheese cakes, the setting property for foam rigidity is obtained from the gelatin in combination with egg albumin.

2. *Icings*

In icings of various kinds, gelatin is used as a stabilizing material. In icings such as the boiled type, it serves not only as a setting agent but also has some effect on the crystallization of sugar. The cream type of icings – i.e., butter cream – have what is known as a seepage problem during the summer months. When the butter and margarine are whipped up in combination with fine sugar, there is a tendency for the icing to break down. Gelatin, particularly of the high Bloom type, acts not only as a stabilizing agent but also aids in obtaining a larger volume or overrun in the cream-type icing. In cream icings, about 0.5-1% of high Bloom (250 Bloom) gelatin is required, based on the weight of the sugar. Higher levels of gelatin cause structural changes in the icing characteristics.

Icings made with gelatin were compared with icings based on alginates and agar. Under many conditions, the various stabilizers were found to be equally effective (Steiner and Rothe, 1949).

G. Food Coatings

Gelatin has been used as a food coating, not only for protective purposes, but also for esthetic appeal. Bogue, in 1922, wrote that "coffee beans have been dipped into a gelatin solution to impart to them a glaze which has been found to be pleasing to the eye." However, the main applications of gelatin coatings have been for protective purposes.

As early as 1869, Havard and Harmony patented a process for preserving meat, fish, fowl, and other foods in gelatin. Improved gelatin coatings and processes were subsequently developed by Morris and Parker (1895) and Cowin (1911).

More recently, Isherwood (1944) used gelatin to protect dried food materials such as dried milk, eggs, and vegetables against the absorption of moisture, air, or gases. These food products were first compressed into blocks and then coated with a gelatin composition containing a plasticizing agent. In a similar fashion, Hamill (1947) used treated gelatin for coating ripe fruit, dressed poultry and fish, and various dehydrated foods.

Coatings of gelatin in a 3% aqueous solution, either alone or in combination with antioxidants, were found to markedly delay the deterioration of fat components in frozen turkey products (Klose and Mecchi, 1952). Ferguson (1950) preserved other food products by coating them with an aqueous solution of gelatin, gum arabic, and propylene glycol.

Childs (1957) used gelatin in coating sausages. The gelatin was used as the first adherent layer to the meat followed by other sausage casing skins.

Keil (1961) modified gelatin for coating fatty food products such as meats, cheese, margarine, etc. The gelatin was reacted with the acid salt of a metal selected from the group of aluminum and nontoxic heavy metals to give a protective coating that was quite effective.

Forkner (1958) coated dried fruits and nuts with gelatin. The coated products were then mixed with granulated sugar and thoroughly dried. The treatment prevented mold growth and loss of flavor and gave stable products for use in prepared cake mixes.

H. Flavor Fixation

1. Slab Fixation

Gelatin has long been used as a flavor fixative. Olsen *et al.* (1945) invented a method for slab fixation of flavors, wherein the flavor oil was emulsified with a gelatin-water solution and then cooled to form a gel. The gel was then dried and ground to yield a solid powder with the bulk of the oil entrapped in the gelatin particles. In similar fashion, Novak (1957) fixed citrus oils by emulsifying them in an aqueous gelatin solution containing carboxymethyldextran. The solution was then gelled, dried, and ground.

In chewing gum, it was shown that gelatin-encapsulated flavor gave a unique effect. It not only released the flavor at a slower rate, but for a given quantity of flavor, greater overall intensity was obtained than by the use of straight flavor oil addition (Kramer *et al.*, 1959).

2. Macroencapsulation

The use of gelatin for macroencapsulation of flavors has been known for many years. Commercial products have included citrus-flavored pie filling mixes, with the citrus flavor oil encapsulated in a large soft gelatin capsule to protect it against oxidation and the resultant deterioration of the flavor. In a similar manner, a dehydrated chicken noodle soup has been manufactured with a gelatin capsule containing chicken fat to protect it from becoming rancid. Rice products have been sold with incorporated encapsulated seasoning flavors such as curry, paprika, onion, tomato, and other spices (Moxey, 1964). Compositions for encapsulating hygroscopic materials in gelatin capsules have been patented by Stanley and Bradley (1959), and several manufacturing processes for making such commercial gelatin products have been described by Muller (1960).

3. *Microencapsulation*

One of the more sophisticated and technologically advanced applications of gelatin has been in the comparatively new field of microencapsulation. Microencapsulation is a new concept of packaging materials, either liquids or solids, in very tiny, microscopic capsules. Although various size ranges have been specified in definitions of microencapsulation, the most commonly accepted standard is "any size up to 1/125 of an inch in diameter," even though many techniques will yield much larger capsules (Anderson *et al.*, 1963). But in addition to size, microencapsulation implies that the material so encapsulated is held in and protected until such time as it is released under specified conditions. So it is both size and protection that give microencapsulation its uniqueness.

The utilization of gelatin in this field has been through the mechanism of coacervation. Simple coacervation is induced by phasing gelatin out of solution to encapsulate the dispersed liquid oil globules or solid particles with a cohesive, uniform coating. This is done by controlled changing of the pH, temperature, or concentration of the gelatin solution. In practice, this is accomplished by adding to a solution of gelatin containing the dispersed oil globules a solution of sodium sulfate (or other less effective salts). This reduces the solubility of the gelatin and causes it to coacervate around the surfaces of the suspended oil globules and eventually to precipitate out of solution. The microcapsules can then be separated, dried, and treated as granular solids (National Cash Register Co., 1966).

Complex coacervation takes place when two oppositely charged macromolecules react to form an insoluble coacervate that will precipitate from solution. It was found that gelatin in solution will react with gum arabic in solution to form a coacervate that will deposit around dispersed oil droplets to yield individual, microencapsulated, free-flowing spheres. The important parameters are the ratios and concentrations of gelatin, gum arabic, and water (Anonymous, 1962), and complex coacervation can be initiated and controlled by changing the pH or by simply diluting the system with water.

The original application of gelatin coacervates was to National Cash Register Company's "No Carbon Required" transfer paper. A colorless ink was encapsulated in minute gelatin-gum arabic coacervate spheres averaging about 3 μ in diameter and coated on the back of each paper sheet. The front of the sheet was then coated with a clay-like film containing an appropriate reagent. When pressure from a ball-point pen or typewriter was applied, the capsules directly below the striking point burst, releasing the ink onto the sheet below. The

reagent in the coating of the second sheet reacted with the ink causing it to turn blue and create a second or "carbon" copy. This transfer paper eliminated the need for carbon paper, improved handling and cleanliness, and offered other advantages (Green, 1957; Green and Schleicher, 1957).

In food products, gelatin-gum arabic coacervates have been investigated and used as flavor fixatives for inclusion in cake mixes, chewing gum, confections, soluble coffee, and others. It has been suggested for encapsulation of the oil and fat components for dried soups and salad dressings, and has been considered as a method for separating reactive ingredients in dry mix products.

I. MISCELLANEOUS

In the past, gelatin has been used to improve the appearance (sometimes illegally) of certain food products. Bogue (1922) reports that meat extracts and similar preparations have sometimes been given a false body with gelatin. Cream has been occasionally made to appear thicker and richer by added gelatin. Cocoa and chocolate have also been adulterated with gelatin at times for the same purpose.

In the canning industry, gelatin is used in the production of madrilene soups such as jellied tomato consomme, jellied aspic, and jellied beef consomme.

In frozen fruit purees, the amount of stabilizers are important and studies of stabilizers have shown high Bloom gelatin (275 Bloom) to be exceptionally good with respect to overrun, meltdown characteristics, and ease of handling (Lundeen and Worthington, 1951). Apples and other fruits may be preserved without the loss of foam by boiling them for 10-30 minutes in a water solution of gelatin and sugar (Talbert, 1953). Fruit juices may be clarified with gelatins, which tend to precipitate the tannin constituents in these beverages (Koch and Breker, 1949, 1952).

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Ann. Surg. 173:85-90, 1971

A Balanced Fluid Gelatin for the Treatment of Hemorrhage

D. V. Habif, E. Debbas,* J. Migne,** G. G. Nahas

From the Departments of Surgery and Anesthesiology, College of Physicians and Surgeons, Columbia University, New York City

Several authors have advocated the use of balanced electrolyte solutions for the replacement of moderate blood loss, in an amount corresponding to three times the blood loss.[1, 10, 12, 13] The chief advantage of these solutions is "that mortality and morbidity related to transfusions are avoided." However, as pointed out by Powers, a major part of this fluid moves out of the blood stream into interstitial spaces and has to be eventually excreted by the kidney.[9] Good renal function is therefore important. Rapid administration of such solutions to patients with cardiovascular weakness might overload the extracellular fluid space, especially in the lung. Pulmonary edema, even in the healthy young adult, is a distinct possibility following massive administration of electrolyte solutions.[5]

It therefore appears that the adjunction of a macromolecule to such a balanced solution would approximate plasma more closely and provide a more physiological solution for blood replacement. With such a solution one would administer an amount of blood equal to that which was lost. The ideal colloid would be human albumin, but its high cost and short supply preclude general usage as a plasma expander. One should consider the use of the other macromolecules presently available. Dextran is antigenic, alters the plasmatic and platelet factors of coagulation,[2, 3] and impedes the mobilization of the extravascular albumin pool.[4] These side effects are most likely to occur if large amounts of dextran are administered (e.g., over 1 liter in an adult patient).

Modified fluid gelatins developed in the U. S. following the Second World War and widely used in Europe do not seem to produce such side effects, even when administered in amounts as large as 2–5 liters for the treatment of massive hemorrhage. These considerations led us to select a modified succinylated fluid gelatin developed in the United States and used clinically for more than 10 years in continental Europe.[11] The fluid gelatin had been suspended in a 5% glucose solution or in isotonic saline. For the purpose of our experiment, the suspension was modified to approximate the ionic composition of normal plasma. The new modified fluid gelatin was then used to replace, in dogs, volume for volume, blood loss amounting to approximately 50% of blood volume. The influence of this replacement on acid-base equilibrium and ionic and albumin concentration of plasma was studied over a period of 1 to 4 days. Blood volume was also measured as well as gelatin concentration in the plasma and rate of urinary elimination.

In addition, the effect of this balanced fluid gelatin on the coagulation of blood samples from randomly selected healthy human donors was studied.

Submitted for publication February 9, 1970.

This work was supported (in part) by Army Contract DADA-17-67-C-7126, the R. S. Clark Foundation Gift, and the Dolores and Bob Hope Gift for the Study of Shock.

* St. Vincent's Hospital, New York City, N. Y.

** Hôpital Val de Grace, Paris, France.

TABLE 1. *Ionic Composition of Plasma and Electrolyte Solutions Used for Replacement*

mEq./l.	Normal Plasma	Normosol-R Solutions	Lactated Ringer's Solution	Ringer's Solution	Balanced Fluid Gelatin
Na^+	142	140	130	147	152
K^+	5	5	4	4	5
Mg^{++}	2	3	0	0	3
Ca^{++}	5	0	3	5	0
Cl	103	98	109	156	100
HCO_3^-	27, 5 as lactate	27 as acetate, 23 as gluconate	28 as lactate	0	30 as lactate

Methods

1. Experimental Hemorrhage in Animals

Seventeen healthy dogs, age 1 to 4 years, of either sex and weighing 15 to 23 Kg., were studied. The animals were fasted 12 hours and anesthetized with sodium pentobarbital (30 mg./Kg.), intubated and mechanically ventilated to maintain normal oxygen saturation and acid-base equilibrium in arterial blood. Catheters were inserted in the femoral vessels for removal of blood samples and recording of blood pressure. A femoral artery was exposed, a teflon graft securely stitched to the edges of the incision, and suction applied to the upper part of the graft. The vessel was then transected and the aspirated blood collected in a container placed on a scale to obtain a continuous measurement of blood loss through the experiment. The bleeding was stopped when either (a) two successive readings of blood loss were similar and mean blood pressure had fallen below 10 mm. Hg or (b) the EKG recording showed signs of severe conduction failure. The animals were then transfused with 3% modified fluid gelatin suspended in a balanced solution (BFG), administered in an amount equal to that of blood loss. The composition of this solution is shown in Table 1 and compared to that of normal plasma and currently used electrolyte solutions. It should be noted that this gelatin is ionized like plasma proteins are and carries approximately 1 mEq. of Na^+ per gram. BFG approximates the composition of normal plasma more closely than lactated Ringer's (Table 1). It contains 152 mEq. of Na^+ instead of 130, 100 mEq. Cl^- instead of 109. Instead of Ca^{++}, it contains Mg^{++} so that it can be administered along with bank blood.

Samples of blood for measurements of acid-base equilibrium, O_2 saturation, Na^+, K^+, Cl^-, protein and albumin concentrations, hematocrit and blood volume, were taken before bleeding, after bleeding and at regular intervals following transfusions with BFG. The animals were observed for two weeks following the experiment.

2. Coagulation Studies on Human Blood

Forty-eight samples from healthy donors were divided into two aliquots of 10 ml.: A and B. In A, control, to 10 ml. of blood 2 ml. of Owren-Koler solution were added. In B, the test sample, 2 ml. of BFG were added. This 1 to 5 dilution corresponds to that which could occur *in vivo* when 1,000 ml. of BFG are administered to a patient with a 5,000 ml. blood volume. Howell's coagulation test in glass and in siliconized tubes and thrombo-dynamograms were performed on each sample.

Measurements

Plasma volume was measured at the start of the experiment using albumin tagged with I^{131} (RISA—Abbott Laboratories). Blood volume was computed from the hematocrit measurement. A known quantity of tagged albumin was injected and samples withdrawn at 15 min. intervals.

The radioactivity in the plasma was measured with a liquid scintillation spectrometer. Blood pressure, EKG, heart rate, esophageal temperature and blood volume loss were continuously recorded throughout the experiment on a modified Offner-type "R" polygraph recorder, and printed out at 30 sec. intervals during hemorrhage by means of a digital printout system connected to the recorder. This system was specially built by A. St. J. Lee. Acid-base measurements of pH, P_{CO_2} and base excess were made by the triple pH method, using the Astrup micro-electrode and the Siggaard-Andersen nomogram. Gelatin concentration in blood and urine was measured by the hydroxyproline method. Total proteins and albumin in plasma were determined on the Technicon AutoAnalyzer. These were measured at regular intervals following retransfusion and during four days thereafter.

Results

1. Experimental Hemorrhage in Animals (Table 2)

Sixteen out of 17 of the animals survived for at least 2 weeks after the experiments and appeared in good health at that time. Arterial blood pressure and heart rate were restored to normal an hour after transfusion. Hematocrit was less than half of control value, after transfusion with BFG. The metabolic acidosis present immediately after transfusion was restored to normal within an hour.

Plasma volume was not significantly different from controls 24 hours after transfusion. Total protein and albumin plasma concentrations were decreased in proportion to blood loss, immediately after replacement with BFG. However, in the following hours, the concentration of these

TABLE 2. *Effects of Blood Volume Replacement with BFG 3% (Plasmagel-F) Following Hemorrhage in Dogs*

Bleeding Time (min.)	Transf. Time (min.)	Blood Volume (ml.)	Blood Loss (%)	Blood Pressure (mm. Hg)			Heart Rate (beats/min.)			Number of Survivors
				Control	$\bar{p}$ Hem.	1 h $\bar{p}$ Transf.	Control	$\bar{p}$ Hem.	1 h $\bar{p}$ Transf.	
3.7 ±0.6 (17)	3.4 ±0.4 (17)	1880 ±88 (17)	46.9 ±1.1 (17)	150.0 ±3.2 (17)	34.6 ±5.5 (17)	115.2 ±5.0 (17)	168 ±8 (17)	142 ±10 (17)	171 ±8 (17)	16

Arterial pH			Arterial P_{CO_2} (mm. Hg)			Bicarbonate (mEq./l.)		
Control	$\bar{p}$ Hem.	1 h $\bar{p}$ Transf.	Control	$\bar{p}$ Hem.	1 h $\bar{p}$ Transf.	Control	$\bar{p}$ Hem.	1 h $\bar{p}$ Transf.
7.39 ±0.02 (10)	7.32 ±0.02 (11)	7.42 ±0.03 (11)	38.5 ±2.2 (10)	39.0 ±2.1 (11)	36.5 ±2.1 (11)	22.6 ±0.6 (10)	19.3 ±0.9 (11)	23.0 ±0.7 (11)

Plasma Volume (ml)		Hematocrit (%)	
Control	15 h $\bar{p}$ Transf.	Control	15 h $\bar{p}$ Transf.
1201 ±88 (9)	1240 ±82 (9)	39.3 ±1.2 (9)	22.4 ±1.0 (9)

Numbers in parentheses indicate number of animals.
Values are means ± S.E.

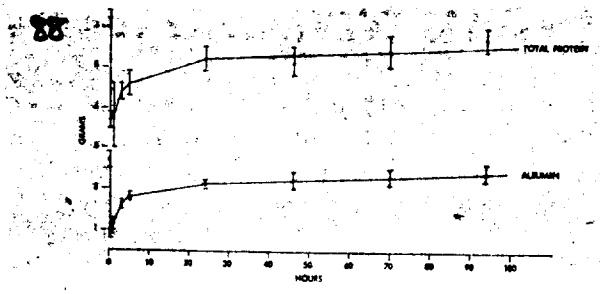

FIG. 1. Total protein and albumin in plasma following replacement of blood loss with balanced fluid gelatin.

substances increased rapidly to reach 82% and 78% of control values after 24 hours, and 90 and 89% after 4 days (Fig. 1).

Urinary elimination and plasma concentration of BFG studied in five animals over a 24-hour period are summarized in Figure 2. The half-life of this substance is 150 minutes in plasma and after 24 hours about 10% remains. Its urinary elimination is rapid, approximately 60% of the administered dose is excreted after 5 hours.

2. Coagulation Studies on Human Blood (Table 3)

These indicate that coagulation time and thrombodynamogram constants are not significantly different in control samples (A) than in samples with BG added (B).

Discussion

These experimental results indicate that replacement of half of the blood volume in the dog with the same volume of BFG is well tolerated and is accompanied by a rapid restoration of cardiovascular function and acid-base equilibrium. This substance remains in the vascular bed long enough to allow a compensatory mobilization of albumin in the extravascular pool to occur. As demonstrated by Moore and co-workers,[6] following hemorrhage there is a rapid refilling of the lost water, electrolytes and albumin. The presence of albumin is essential for the restoration of normal oncotic pressure and of a normal blood volume. Albumin is also important for the transport of many lipo-soluble substances, of hormones, vitamins, and free fatty acids. It is therefore important that this homeostatic mechanism not be impeded by fluid replacement. Moore and co-workers[6] showed that administration of balanced saline did not alter the rate of albumin replacement in the vascular space. BFG does not seem to impede to any great extent the mobilization of the extravascular albumin pool and protein synthesis which follows blood loss. Following hemorrhage, albumin plasma concentration had fallen to 35% of control; 4 days later it had risen to 89% of control.

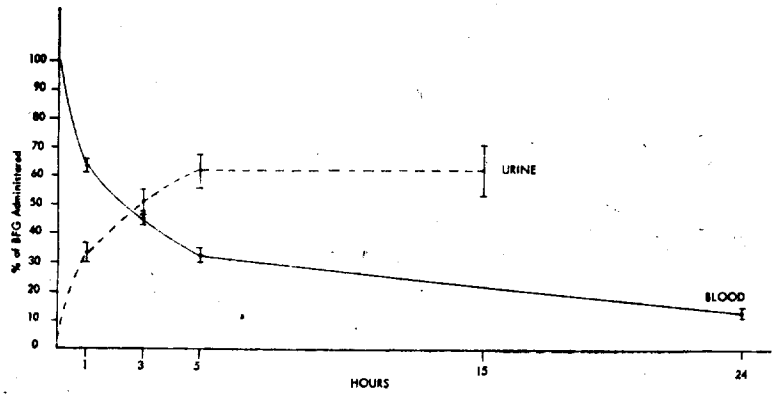

FIG. 2. Plasma concentration and urinary elimination of BFG after replacement of blood loss.

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TABLE 3. *Howell's Coagulation Time and Thrombodynamogram Constants in 48 Samples of Blood from the Same Donors*

	Glass (min.)	Silic. (min.)	r (mm.)	k (mm.)	r + k (mm.)	a (mm.)
A	2.42 ±0.03	7.55 ±0.09	29.3 + 0.9	15.6 ± 0.4	44.9 ± 1.1	57.6 ± 0.5
B	2.44 ±0.02	7.61 ±0.10	29.5 ± 0.9	15.5 ± 0.5	44.8 ± 1.2	57.2 ± 0.4

A — control.
B — with BFG.

In the coagulation tests, in addition to statistical analysis, each pair of samples was studied. In 34 cases no change whatsoever was observed, in two there was hypercoaguability, in five dissociated hypercoagulability, in four hypocoagulability and in three dissociated hypocoagulability. The dissociated results cannot be taken into account in this type of experimentation: Howell's coagulation tests did not change; only the thrombodynamogram was altered. The non-dissociated results are difficult to interpret because they occur in a small percentage of cases and are not consistent: 4% hypercoagulability and 8% hypocoagulability. In summary, BFG in a 1 to 5 dilution does not alter significantly in most cases plasma or platelet factors of coagulation, an observation consistent with that of others.[7] Figure 3 illustrates a thrombodynamogram taken in this study. The tracings of both aliquots of the same blood, control and test, are similar. Figure 4 illustrates a thrombodynamogram of a sample of blood to which dextran 40 in a 1 to 8 dilution was added. In the sample containing dextran 40 there is a significant shortening of "r" and a narrowing of "a" which has been attributed by Dugdale [2] to an alteration of the texture of the clot.

This same BFG was used in amounts varying from 500 to 3,000 ml. during the course of surgical procedures performed in man.[8] Coagulation studies made at that time indicated that this compound did not alter coagulation factors *in vivo*.

Conclusion

The addition of a macromolecule, modified fluid gelatin to a balanced electrolyte solution appears to be an efficient and safe plasma substitute. Replacement of more than 40% of the blood volume in the dog with an equivalent amount of BFG is accompanied by a restoration of adequate circulation and acid-base equilibrium. In man, BFG did not alter coagulation factors. BFG could find a utilization for the re-

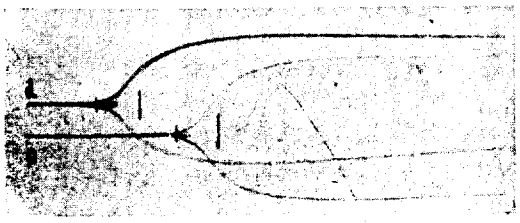

FIG. 3. Thrombodynamograms of the same normal blood. C—after addition of Owren-Koler solution; D—after addition of low-molecular dextran—In both cases, 1 to 5 dilution.

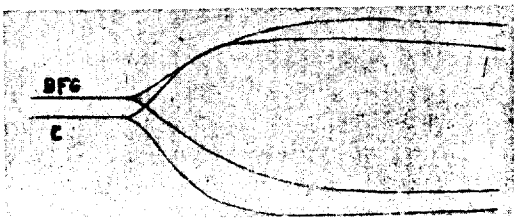

FIG. 4. Thrombodynamograms of the same normal blood. C—after addition of Owren-Koler solution; BFG—after addition of balanced fluid gelatin—In both cases, 1 to 5 dilution.

placement of blood loss amounting to 500–1,000 ml. and for emergency vascular refilling following massive hemorrhage.

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J. Clin. Investigation 25: 575-585, July 1946

THE FATE OF INTRAVENOUSLY INJECTED GELATIN IN HUMAN SUBJECTS[1]

By WILLIAM S. HOFFMAN and DONALD D. KOZOLL[2]

(From the Hektoen Institute for Medical Research of the Cook County Hospital and from the Departments of Surgery of the Cook County Hospital and the Northwestern University Medical School, Chicago)

(Received for publication October 10, 1945)

The value of specially prepared gelatin solutions for intravenous infusion as a plasma substitute in the treatment of shock caused by trauma or hemorrhage is now well recognized. Previous papers from our laboratory (1 to 3) have reviewed the literature on the subject and have presented findings indicating the effectiveness of such solutions in various types of shock encountered in human subjects in a large general hospital. These reports have demonstrated that intravenously injected gelatin is an effective and innocuous hemodiluting agent not only in patients with shock but in the relatively normal "hospital control" subjects.

The present investigation was undertaken to determine the fate of intravenously injected gelatin; the plasma levels achievable; the distribution of gelatin in the blood, tissues, and urine; and the rate of gelatin excretion. An effort was made also to determine to what extent the rate of excretion of gelatin varied with preparations of varying molecular weight.

PLAN OF STUDY

The gelatin preparations[3] used in this study were all 5 per cent solutions of osseous gelatin prepared by electrodialysis of calcium gelatinate. They were of three stages of degradation. Lots No. 39, 45, 65, and 80 were highly degraded gelatins prepared by twice autoclaving for 30 minutes at 15 pounds pressure. These had broad ultracentrifuge patterns and relatively low viscosity. Their weight average molecular weight was of the order of 37,000. It was this type which had been used exclusively in the previous studies from this laboratory and which had been found clinically effective. Lots No. 58–10, 83, and 93 were much less degraded. They were prepared by autoclaving for 20 minutes at a pressure of 10 pounds. Their weight average molecular weight was of the order of 58,000. Lots No. 58–15 and 101 were intermediate with a weight average molecular weight of the order of 47,000. They were autoclaved for 20 minutes at 15 pounds pressure. All of these solutions, it should be recognized, including the least degraded, remained liquid at room temperatures. The latter were slightly more degraded than the Knox preparation employed by Koop (5) and others, which is gelled at room temperatures and must be warmed to body temperature before utilization.

To avoid cumbersome repetition, the least degraded preparations used in this study will be spoken of as heavy gelatin; the most highly degraded, as light gelatin; and the intermediately degraded, as intermediate gelatin.

All preparations except one were in physiological saline solution; Lot No. 39 was 5 per cent gelatin in 5 per cent dextrose solution. These solutions were stable at room temperatures showing no discoloration or other change even after a year. The older as well as the fresh preparations, as in previous studies, produced no reactions, except in the case of one lot (Lot. No. 80), which produced mild pyrogenic reactions that could not be attributed to faulty tubing or glassware.

The subjects chosen for this study were so called hospital controls, patients in the convalescent stage of illnesses not likely to produce appreciable changes in cardiac, circulatory, or renal function. They were for the most part patients recuperating from herniorrhaphy. All were

[1] The studies on which this paper is based were aided by a grant from the Upjohn Company, Kalamazoo, Michigan.

[2] Abbott Fellow in Surgery, Northwestern University Medical School.

[3] The gelatin solutions were kindly furnished by the Upjohn Company. They came in liter bottles prepared for direct use in intravenous infusions. They contained no preservative. The weight average molecular weight (M_w) of the various lots were determined by the Upjohn Company from the intrinsic viscosity (H_o) by the method of Scatchard *et al.* (4). These values are listed in the following table:

Lot. No.	H_o	M_w
B20610–39	0.2202	37,266
B20610–45	0.2100	35,532
B20610–65	0.2176	36,820
B20610–80	0.2248	38,040
B20610–58–15	0.2853	48,270
B20610–101	0.2683	45,400
B20610–58–10	0.3345	56,600
B20610–83	0.3391	57,000
B20610–93	0.3502	59,254

For the sake of convenience, the prefix B20610– will be omitted in references to the individual lots.

males. In a preliminary study a group of 24 subjects were given 2000 ml. of 5 per cent gelatin and the blood and urine gelatin concentration determined after the injection of 1000 ml. and after 2000 ml. and at 24, 48, 72, and 96 hours. Later, it was recognized that a much better insight into the fate of the injected gelatin could be obtained by the following procedure: At 9 a.m. (some 2 hours after breakfast), a control sample of blood was obtained and the patient was asked to empty his bladder. Then 1000 ml. of 5 per cent gelatin were injected at the usual clinical speed of about 330 ml. per hour. At the end of the injection, a blood sample was taken and the accumulated urine collected without catheterization. Then blood and total urine samples were collected at 2 hours, 4 hours, and 6 hours after the end of the injection, and again at 24, 48, 72 and at times 96 and 120 hours after the start of the injection. Gelatin and creatinine determinations were made on all blood and urine samples, thus a comparison of creatinine and gelatin clearances was possible. Besides, the 24-hour creatinine excretions served as a check on the completeness of the urine collections. Those experiments in which there was some doubt as to the accuracy of the collection were discarded. Since the data in the 24-hour preliminary experiments in which 2000 ml. of gelatin were injected do not lend themselves to the same type of analysis as the later experiments, they will not be presented. It should be mentioned, however, that the injection of 2000 ml. was as innocuous as that of 1000 ml. and that the general findings in these experiments, both as to blood levels and rates of excretion, were consonant with the 42 more complete later experiments.

Gelatin was determined in blood and urine by the method of Janota (6) as follows: the total nitrogen (TN) was first determined by micro Kjeldahl method with distillation and microtitration. From this value was subtracted the gelatin plus non-protein (GN) obtained by Kjeldahl determination of a trichloroacetic acid filtrate. The difference is the albumin and globulin nitrogen. The non-protein nitrogen (NPN) was determined in an alcohol filtrate. The albumin and non-protein nitrogen (AN) was obtained by analysis of a filtrate obtained by addition of 21 per cent sodium sulfate (7). Thus

$$TN - GN = \text{Serum Protein N}$$
$$AN - NPN = \text{Albumin N}$$
$$GN - NPN = \text{Gelatin N}$$
$$\text{Serum Protein N} - \text{Albumin N} = \text{Globulin N}.$$

Albumin, globulin, and serum protein N were multiplied by 6.25 to give values as protein, while gelatin N values were multiplied by 5.7 to give gelatin values. As a check on the total nitrogen determinations in the serum, total protein determinations were also made by the falling drop method of Barbour and Hamilton (8). These usually agreed well with the sum of the serum protein plus gelatin concentrations, indicating that for the relatively small concentrations of gelatin, very little error was introduced by calculating the gelatin concentration on the specific gravity as equal to that of the same weight of serum protein. Since it was found that the urine of these subjects never contained any protein precipitable by trichloroacetic acid, urinary gelatin N could be determined by subtracting the NPN from the total N without any appreciable error.

Creatinine determinations were made in plasma and urine by a photoelectric modification (9) of the Folin method. Plasma volume estimations were carried out by the photoelectric Evans Blue method in which a control and single 10-minute sample were taken, as suggested by Gregersen (10). The determinations were made just before the injection of gelatin and immediately after the end of the injection.

RESULTS

Plasma gelatin concentration. The data for 20 cases of heavy gelatin injections, 9 intermediate, and 13 light gelatin injections are given in Tables IA, IB, and IC respectively. The highest plasma gelatin concentration, achieved at the end of the injection, ranged between 0.59 to 1.17 grams per 100 ml. The averages for the three types were almost identical (0.78, 0.79, and 0.78 gram per 100 ml. respectively). There was some indication that the levels were proportional to the speed of the injection; but this impression was not con-

TABLE IA

Plasma gelatin concentration after intravenous injection of 1000 ml. of 5 per cent "heavy" gelatin

Lot no.	Patient	Hours after completion of injection				
		0	2	4	6	24
		grams per 100 ml.	*grams per 100 ml.*	*grams per 100 ml.*	*grams per 100 ml.*	*grams per 100 ml.*
58–10	A.A.	0.82	0.74	0.57		0.29
	J.S.	0.83				0.32
	C.T.	0.72	0.60	0.58	0.51	0.33
	H.M.	0.91	0.75	0.72	0.52	0.43
	G.B.	0.86				0.41
	J.O.	0.77				0.44
	H.H.	0.63	0.50	0.49	0.47	0.24
	O.B.	0.75	0.61	0.48	0.46	0.32
	F.B.	0.76	0.61	0.60	0.53	0.31
	J.D.	0.74	0.64	0.59	0.54	0.35
	A.W.	0.74	0.68	0.62	0.53	0.40
	L.Y.	0.88				0.38
	A.D.	0.75				0.27
	A.E.	0.69				0.38
83	W.H.	0.86	0.82	0.66	0.47	0.43
93	R.R.	0.77	0.74	0.69	0.62	0.40
	D.C.	0.93	0.72	0.71	0.58	0.29
	C.W.	0.79	0.64	0.65	0.58	0.41
	P.W.	0.68		0.55	0.55	0.35
	J.R.	0.74	0.72	0.71		0.29
Average and standard deviation		0.78 ±0.07	0.66 ±0.08	0.64 ±0.08	0.52 ±0.05	0.35 ±0.06

TABLE IB

Plasma gelatin concentration after intravenous injection of 100 ml. of 5 per cent "intermediate" gelatin

Lot no.	Patient	Hours after completion of injection				
		0	2	4	6	24
		grams per 100 ml.	*grams per 100 ml.*	*grams per 100 ml.*	*grams per 100 ml.*	*grams per 100 ml.*
58-15	O.B.	0.82	0.67	0.61	0.58	0.11
	H.C.	0.75	0.59	0.59	0.53	0.16
	S.S.	0.88	0.67	0.65	0.57	0.35
	F.F.	0.92	0.74	0.65	0.62	0.44
101	J.M.	0.95	0.64	0.59	0.55	
	R.Y.	0.73	0.49	0.43	0.33	
	H.M.	0.64	0.38	0.35	0.31	
	F.B.	0.68	0.43	0.33	0.29	0.12
	W.C.	0.74	0.55	0.46	0.41	0.13
Average and standard deviation		0.79 ±0.10	0.57 ±0.11	0.52 ±0.12	0.47 ±0.12	0.22 ±0.13

TABLE IC

Plasma gelatin concentration after intravenous injection of 1000 ml. of 5 per cent "light" gelatin

Lot no.	Patient	Hours after completion of injection				
		0	2	4	6	24
		grams per 100 ml.	*grams per 100 ml.*	*grams per 100 ml.*	*grams per 100 ml.*	*grams per 100 ml.*
39	J.C.	0.59	0.51	0.36	0.36	0.34
	C.U.	0.69	0.51	0.41	0.32	0.20
	L.B.	0.78	0.63	0.45	0.45	0.42
45	S.M.	0.90	0.62	0.56	0.53	0.37
	C.P.	0.84	0.60	0.51	0.47	0.35
65	L.P.	1.17	0.67	0.61	0.51	0.40
	L.M.	0.77	0.55	0.51	0.46	0.33
	Lu.M.	0.68	0.43	0.42	0.27	0.14
	J.W.	0.78				0.48
80	A.T.	0.67	0.52	0.45	0.42	0.34
	H.R.	0.87	0.55	0.52	0.46	0.40
	J.S.	0.88	0.52	0.43	0.27	0.24
	B.C.	0.71	0.54	0.52	0.39	0.08
Average and standard deviation		0.79 ±0.14	0.55 ±0.06	0.48 ±0.07	0.41 ±0.095	0.32 ±0.11
t (heavy-light)		0.3	4.5	6.0	4.3	1.0

firmed, for no attempt was made to vary the speed of injection.

In a few cases plasma gelatin levels were determined during the administration of the gelatin. These values were always lower than those obtained at the end of the injection. Similarly, in [redacted] in which the preliminary series of experiments 2000 ml. were injected, the plasma gelatin concentration reached at the end of 1000 ml. was always lower than that obtained at the end of 2000 ml. Thus it was certain that the rate of removal of gelatin from the blood stream was always slower than that of the ingress, if the injection was made at the common clinical speed.

The plasma gelatin concentrations dropped progressively in 24 hours to 0.35, 0.22, and 0.32 mgm. per 100 ml. respectively for the heavy, intermediate, and light gelatins. There were still appreciable concentrations of gelatin in the plasma at 48 and 72 hours, which finding was confirmed by the demonstrable excretion of gelatin on the third and fourth days. Though a comparison of the levels at the end of the injection and at 24 hours did not reveal any appreciable differences between the heavy and light gelatin (the t value of statistically significant difference [4] being 0.3 for 0 hour and 1.0 for 24 hours), such a comparison made at 2, 4, and 6 hours showed that the rate of fall in plasma gelatin concentration during this period was far greater for the light gelatin than for the heavy variety. This difference is apparent in the curves of plasma gelatin concentration shown in Figure 1. That the difference is not fortuitous is indicated by the t values of 4.5 at 2 hours, 6.0 at 4 hours, and 4.3 at 6 hours. The heavy gelatin thus tended to maintain an effective concentration longer than did the light gelatin. A possible explanation for this phenomenon will become recognizable when the excretion data are analyzed. The plasma gelatin concentrations at 2, 4, and 6 hours for the intermediate types appeared to fall in between those of the other two types, but the differences were less significant chiefly because

[4] The t value of statistically significant difference between the average of two sets of experimental values was determined according to the method of Snedecor (11) as follows:

$$t = \frac{a_1 - a_2}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}}$$

where a_1, σ_1, and n_1 are respectively the mean, the standard deviation, and the number of experiments in the one set of experiments, and a_2, σ_2, and n_2 are those in the second group of experiments. A value of 2.5 or more for t indicates that the difference of the means is at least 4 times as great as the probable error of the difference of the means and is therefore probably a significant difference.

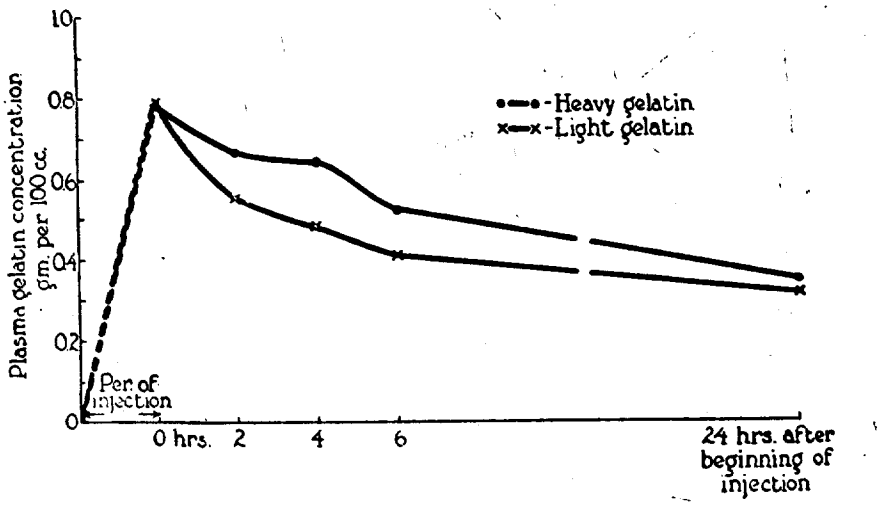

FIG. 1. COMPARISON BETWEEN AVERAGE PLASMA GELATIN CONCENTRATIONS AFTER INJECTION OF HEAVY GELATIN AND THOSE AFTER LIGHT GELATIN

the variations were greater for the intermediate type.

Urinary excretion of gelatin. The data for the excretion of gelatin in the urine for 14 heavy gelatin injections, 9 intermediate and 12 light gelatin injections are shown in Table IIA, IIB, and IIC respectively. It is obvious that the rate of excretion shows a marked variability even in injections of the same lot of gelatin. However a pattern of excretion is discernible. In all cases there was a considerable excretion of gelatin by the time of the end of the injection. At 6 hours after the completion of the injection an average of 22.72 grams of light gelatin had been excreted. The heavy gelatin excretion in this period was significantly less, averaging 14.68 grams. The intermediate gelatin excretion was 21.72 grams. There was much less variation in the individual excretion of the heavy gelatin at 6 hours than in those of light gelatin, the standard deviation being 2.50 while that for the light gelatin was 4.22. These findings were consistent with the differences in plasma levels by the end of 6 hours. That the difference between the excretion of heavy and light gelatin

TABLE IIA

Urinary excretion of "heavy" gelatin after intravenous injection of 1000 ml. of 5 per cent solution

	Lot no. 58–10								Lot no. 93				Lot no. 83		Average	Standard deviation
	C.T.	H.M.	A.H.	F.B.	A.A.	J.D.	A.W.	O.B.	D.C.	R.R.	C.W.	P.W.	J.R.	W.H.		
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
At end of injection	6.36	7.85	1.38	1.85	2.41	7.15	6.47	7.29	6.20	14.63	17.45	8.87	6.89	5.56		
2 hrs. after	4.39	3.78	2.87	4.75	7.92	1.23	5.21	3.76	5.16	5.52	4.13		2.27	3.19		
4 hrs. after	1.81	4.63	4.91	2.98	4.63	1.60	2.90	1.72	2.54	1.81	2.20	0.92	2.52	3.34		
6 hrs. after	3.74	2.69	1.36	3.17	2.41	4.69	1.25	1.07	0.98		1.15	0.88		3.53		
Total to 6 hrs.	16.30	18.95	10.52	12.75	17.37	14.67	15.83	13.84	14.98			10.67		15.62	14.68	±2.50
24 hrs.		7.81	15.55	7.93	8.36		8.89	16.51	5.23	5.92	1.78	14.44	23.38	6.37		
48 hrs.		9.44	7.76	18.15	4.53		16.52	6.64	3.37	5.83			4.00	14.35		
72 hrs.		6.03		2.96	4.33		3.21	5.30	8.62	1.29			0.39			
Total to 72 hours		42.23		41.79	34.59		44.45	42.30	32.20	34.90			39.47		39.00	±4.21

t (Average "heavy" gelatin excretion to 6 hours compared with average "light" gelatin excretion to 6 hours) 5.6.
t (Average "heavy" gelatin excretion to 72 hours compared with average "light" gelatin excretion to 72 hours) 0.7.

TABLE IIB

Urinary excretion of "intermediate" gelatin after intravenous injection of 1000 ml. of 5 per cent solution

	Lot no. 58–15				Lot no. 101					Average	Standard deviation
	U.B.	H.C.	S.S.	F.F.	J.M.	R.Y.	M.H.	F.B.	W.C.		
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
At end of injection	22.06	10.18	7.17	4.63	14.96	7.99	10.29	5.60	8.18		
2 hrs. after	2.31	5.56	4.73	5.75	8.13	9.51		8.81	10.56		
4 hrs. after	2.08	2.41	4.77	7.33		2.56	5.69	2.45	3.41		
6 hrs. after	1.63	1.31	1.17	2.95	5.92	1.61	1.85	1.33	0.58		
Total to 6 hrs.	28.08	19.46	17.84	20.66	29.01	21.67	17.83	18.19	22.73	21.72	±4.00
24 hrs.	2.82	3.24	4.06	3.75	6.72	6.16	7.98	8.09	5.70		
48 hrs.	5.67	1.88	10.94	8.29							
72 hrs.	5.80	7.55	4.91	2.79							
Total to 72 hrs.	42.37	32.13	37.75	35.49						36.69	
96 hrs.	1.15	3.94	8.19	0.54							

TABLE IIC

Urinary excretion of "light" gelatin after intravenous injection of 1000 ml. of 5 per cent solution

	Lot no. 39			Lot no. 65			Lot no. 45			Lot no. 80			Average	Standard deviation
	J.C.	C.U.	L.B.	L.P.	L.M.	LU.M.	S.M.	C.P.	A.F.	H.R.	J.S.	A.D.		
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
At end of injection	18.24	12.97	12.19	7.70	5.65	12.14	14.50	8.84	9.70	12.41	9.23	9.25		
2 hrs. after	7.01	8.70	10.06	9.32	3.04	7.34	6.53	8.90	4.22	3.02	4.65	5.58		
4 hrs. after	2.53	3.41	2.80	4.01	2.14	4.39	3.59	2.66	1.11	2.48	3.10	1.71		
6 hrs. after	3.58	1.51	1.43	1.90	6.41	1.20	1.62	1.01	3.46	0.73	1.59	3.10		
Total to 6 hrs.	31.36	26.59	26.48	22.93	17.24	25.07	26.21	21.41	18.49	18.64	18.57	19.64	22.72*	±4.22
24 hrs.	8.72	4.59	5.79	9.10	6.30	2.33	6.74	6.96	7.02	15.20	23.37	17.60		
48 hrs.	1.73	3.24	2.17		4.61	4.02	3.23	5.74	2.07	2.58	1.54	2.79		
72 hrs.	0.73	2.41			15.81		1.63		8.86	1.04	0.08	2.10		
Total to 72 hrs.	42.54	36.83			43.96		38.84		36.44	37.46	43.56	42.13	40.22	±2.93

* If lot 80 is omitted, average excretion to 6 hours is 24.66.

was significant was shown by the t value of 5.6. By the end of 72 hours, the differences in excretion had largely disappeared. After the 6-hour period, the heavy gelatin began to be excreted more rapidly than the lighter gelatin, so that at 72 hours, an average of 39.00 grams was accounted for in the urine, while for the light gelatin 40.22 grams were excreted. The t value for these differences was only 0.7 which means that the difference was probably statistically insignificant.

For the 9 cases of injection of intermediate gelatin, there were not enough data at 72 hours to allow statistical evaluation, but here, too, it appeared that the final total excretion was of the same order as that for the heavy and light gelatin.

In a few cases determinations of gelatin excretion were made for the fourth day. An appreciable quantity of gelatin was invariably recovered, occasionally as much as 5 grams. Traces of gelatin were found in the urine on the fifth day, but the values often lay within the probable error of the determination. Since some 80 per cent of the injected gelatin could be accounted for in the urine in 72 hours, and since gelatin was still being excreted on the fourth, and presumably on the fifth day, it was probable that little or no gelatin was degraded to products smaller than those precipitated by alcohol. The lack of any significant rise in serum NPN and urea N during or after injection was in agreement with this interpretation, which is contrary to the conclusion arrived at by Brunschwig (12).

In Table IIC, it will be noticed that the excretion of Lot 80 at 6 hours was slower than that of the other light gelatin lots, and that correspondingly the excretion of Lot 80 after 6 hours up to 72 hours was greater than that of the other lots. All 4 of these subjects treated with this lot of gelatin showed a moderate pyrogenic reaction at the end of the excretion. The reactions were not severe, and they occurred so near the end of the injection, that the experiments were carried through to completion. The excretions of gelatin in these cases for the period up to the end of the injection were of the same order as that for the other light lots. But for the next 6 hours there was a markedly diminished gelatin output. That this decrease was due to diminished renal function was indicated by the fact that the creatinine clearances (Table IIIC) were appreciably lower than normal. In three of the four cases, this diminished excretion was compensated for by an unusually large excretion during the remainder of the first day. In the fourth case, the excretion was delayed to the third day (and fourth day, which is not shown in the Table). If Lot 80 is omitted entirely from the calculation of the excretion of light gelatin at 6 hours, the average excretion at 6 hours is increased from 22.72 to 24.66 grams, and the difference between this excretion and the average for heavy gelatin at 6 hours becomes even greater.

Gelatin and creatinine clearances. Tables IIIA, IIIB, and IIIC show the endogenous creatinine and gelatin clearances and gelatin-creatinine ratios for 12 heavy, 9 intermediate, and 11 light gelatin experiments. Though all subjects chosen were thought to have normal renal function, a few showed abnormally low and an occasional one abnormally high creatinine clearances. These abnormalities could for the most part be accounted for by the fact that catheterization was not resorted to, and that volume errors occurred, in one direction in the first two hours and in the reverse direction in the second. Some of the abnormality might have been due to the effect of gelatin injection. On the one hand, the increased plasma volume produced by the gelatin injection should have produced a greater renal flow and a greater glomerular filtration. On the other hand, the excretion of a highly concentrated and viscous gelatin-containing urine might temporarily block a number of tubules and thus temporarily reduce renal function. It is significant that the large ma-

TABLE IIIA

Gelatin and creatinine clearances for injection of "heavy" gelatin

Lot no.	Patient	0 to 2 hrs. after injection			2 to 4 hrs. after injection			4 to 6 hrs. after injection		
		Gelatin clearance G	Creatinine clearance C	G/C (×100)	Gelatin clearance G	Creatinine clearance C	G/C (×100)	Gelatin clearance G	Creatinine clearance C	G/C (×100)
		ml. per min.	*ml. per min.*		*ml. per min.*	*ml. per min.*		*ml. per min.*	*ml. per min.*	
58–10	A.H.	4.33	86.4	5.01	8.26	170	4.85	3.59	86.5	4.15
	J.D.	1.67	43.2	3.87	2.46	83.6	2.94	5.08	133.8	3.80
	A.W.	7.68	136	5.65	3.85	135	2.85	1.50	97.8	1.53
	H.M.	4.03	146	2.74	6.93	135	5.14	4.45	151	2.94
	C.T.	6.63	171	3.88	3.17	177	1.79	6.40	162	3.95
	F.B.	6.03	93.5	6.45	5.09	112.5	4.52	4.59	207	2.21
	O.B.	2.09	61.5	3.40	2.66	112	2.37	1.95	111	1.76
93	C.W.	4.78	134	3.57	3.25	112	2.90	1.45	138	1.05
	R.R.	6.96	182	3.42	2.07	137	1.51			
	J.R.	2.59	133	1.95	2.94	126	2.33			
	D.C.	6.58	122	5.40	3.11	118	2.64	1.89	93.3	2.03
	P.W.	0.636*	36*	1.77*	0.636*	36*	1.77*	1.63	123	1.33
83	W.H.	3.39	86.5	3.92	3.72	134	2.78	5.42	92.3	5.87
Average and standard deviation				3.93 ±1.45			2.95 ±1.13			2.78 ±1.25

t (Mean G/C for 0 to 2 hours compared with mean G/C for 2 to 4 hours) 1.9.
t (Mean G/C for 2 to 4 hours compared with mean G/C for 4 to 6 hours) 0.4.
* Single clearance test over a period of 4 hours.

TABLE IIIB

Gelatin and creatinine clearances for injection of "intermediate" gelatin

Lot no.	Patient	0 to 2 hrs. after injection			2 to 4 hrs. after injection			4 to 6 hrs. after injection		
		Gelatin clearance G	Creatinine clearance C	G/C (×100)	Gelatin clearance G	Creatinine clearance C	G/C (×100)	Gelatin clearance G	Creatinine clearance C	G/C (×100)
		ml. per min.	*ml. per min.*		*ml. per min.*	*ml. per min.*		*ml. per min.*	*ml. per min.*	
58–15	U.B.	2.78	126	2.21	3.16	113	2.79	2.88	116	2.48
	S.S.	5.59	56.2	8.09	6.83	65.4	10.44	2.18	55.5	3.93
	H.C.	7.34	118	6.23	3.40	159	2.14	1.62	119	1.36
	F.F.	5.23	269	1.94	8.31	227	3.66	4.04	99	4.08
101	J.M.	8.18	117	7.02	4.52*	124*	3.65*	4.52*	124*	3.65*
	W.C.	14.4	125	11.50	6.74	111	6.09	1.64	88.5	1.85
	F.B.	14.8	151.5	9.78	6.32	180	3.51	4.18	196	2.13
	H.M.	4.88*	114*	4.28*	4.88*	114*	4.28*	5.31	130	4.09
	R.Y.	12.48	151	8.24	4.64	105	4.41	3.86	111	3.48
Average and standard deviation				6.59 ±3.06			4.55 ±2.32			3.01 ± .99

t (Mean G/C for 0 to 2 hours compared with mean G/C for 2 to 4 hours) 1.6.
t (Mean G/C for 2 to 4 hours compared with mean G/C for 4 to 6 hours) 1.8.
* Single clearance test over a period of 4 hours.

TABLE IIIC

Gelatin and creatinine clearances for injections of "light" gelatin

Lot no.	Patient	0 to 2 hrs. after injection			2 to 4 hrs. after injection			4 to 6 hrs. after injection		
		Gelatin clearance G	Creatinine clearance C	G/C (×100)	Gelatin clearance G	Creatinine clearance C	G/C (×100)	Gelatin clearance G	Creatinine clearance C	G/C (×100)
		ml. per min.	*ml. per min.*		*ml. per min.*	*ml. per min.*		*ml. per min.*	*ml. per min.*	
65	L.M.	4.85	57.1	8.50	3.86	63.3	6.10	6.75	144	4.69
	L.P.	12.72	91.7	13.88	7.02	135.5	5.18	2.84	97.9	2.90
	Lu.M.	12.77	91.3	13.98	10.12	105.1	9.63	3.17	68.3	4.64
39	C.U.	14.4	161	8.95	6.22	110	5.66	3.11	89	3.50
	L.B.	13.3	147	9.05	5.02	131	3.83	2.97	115	2.58
	J.C.	9.18	134	6.85	5.75	192	3.00	8.23	154	5.34
45	C.P.	11.78	102	11.52	4.16	126	3.30	1.80	116	1.55
	S.M.	7.23	89.7	8.06	6.23	92.5	6.73	2.67	70.1	3.81
80	H.R.	3.50	33.0	10.61	3.89	39.8	9.77	1.75	45.3	3.86
	J.S.	6.00	67.2	8.93	4.82	86.4	5.58	5.06	65.3	4.75
	A.D.	7.47	96.5	7.74	3.01	93.5	3.22	5.51	97.5	5.65
Average and standard deviation				9.82 ±2.29			5.64 ±2.26			3.93 ±1.18

t (Mean G/C for 0 to 2 hours compared with mean G/C for 2 to 4 hours) 4.1.
t (Mean G/C for 2 to 4 hours compared with mean G/C for 4 to 6 hours) 2.9.

jority of subjects showed creatinine clearances within 130 ± 25 ml. per minute.

If a comparison is to be made of the gelatin clearances in the various intervals in the same individual, or of those in same interval in the several subjects, it is necessary to nullify the effect of variations in renal functions. This nullification can be accomplished by making use of ratio of the gelatin clearance to the creatinine clearance. This ratio multiplied by 100 is the gelatin clearance in percentage of the creatinine clearance. This value (expressed as G/C in the charts) should be a measure of the relative ease of glomerular filtration of gelatin in any particular ex-

periment as compared with a crystalloid, the clearance of which is supposed to be a measure of glomerular filtration. Though it is known that in man creatinine clearance is larger and more variable than the clearance of a substance like inulin which is not resorbed or excreted by the tubules, in an experiment such as this, where inulin clearances are too inconvenient to perform, creatinine clearances are probably satisfactory as measures of glomerular filtration.

There was a marked difference between the G/C values for heavy gelatin and those for light gelatin as seen in Tables IIIA, IIIB, and IIIC. For the former, for the 0 to 2 hour period, G/C ranged from 1.77 to 6.45 with an average of 3.93, and a standard deviation of $\pm$ 1.45. For the 2 to 4 hour period, the average G/C value was 2.95, with a standard deviation of $\pm$ 1.13; and for the 4 to 6 hour period the average was 2.78 and the standard deviation $\pm$ 1.25. The *t* value when the mean G/C for 0 to 2 hours was compared with the mean G/C for 2 to 4 hours was 1.9; but for the comparison of the 2 to 4 hour and the 4 to 6-hour periods, the *t* value was 0.4. Thus, for the heavy gelatin, though there appeared to be a progressive reduction in G/C value during the 6 hour period following the end of the injection, the change was not significantly great.

On the other hand, in the case of the light gelatin, G/C for the 0 to 2-hour period was much higher. It ranged from 6.85 to 13.98, and averaged 9.82, with a standard deviation of $\pm$ 2.29. There was a significant drop in G/C values in the second period to an average of 5.64, and still further drop in the third 2-hour period to an average of 3.93. That these declines in G/C values were genuine was seen from the *t* values of 4.1 and 2.9. Thus, the light gelatin was much more readily excreted through the glomeruli than the heavy gelatin during the first 2 hours, but by the end of 6 hours, its relative clearance approached that of heavy gelatin (See Figure 2).

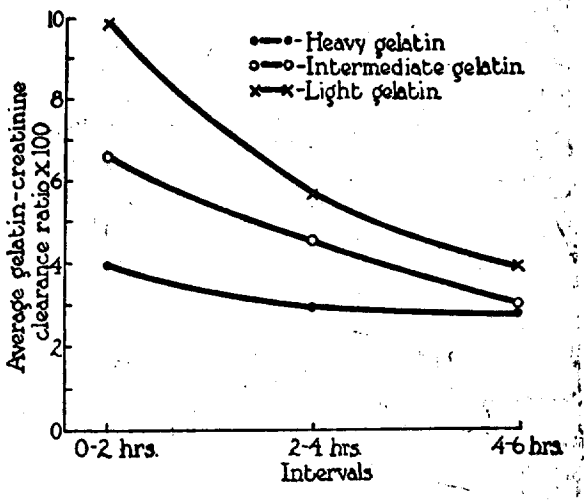

FIG. 2. COMPARISON OF AVERAGE GELATIN-CREATININE CLEARANCE RATIOS IN THREE SUCCESSIVE 2-HOUR PERIODS AFTER THE INJECTION OF HEAVY, INTERMEDIATE, AND LIGHT GELATINS RESPECTIVELY

The intermediate gelatin, as indicated in the other data, showed a much greater variability in excretion, but the values appear to lie in between those of light gelatin and those of heavy gelatin. The mean G/C values for the three periods and their respective standard deviations were 6.59 $\pm$ 3.06, 4.55 $\pm$ 2.32, and 3.01 $\pm$ 0.99. Here too, the progressive drop in G/C until the values were in the range of those of heavy gelatin was apparent.

Plasma volume and the distribution of gelatin. Plasma volume determinations were made in only a few cases toward the end of the investigation. Determinations were made before the beginning of the experiment and at the end of the injection. The data in 8 cases, shown in Table V, are too

TABLE IV

Water excretion and urinary gelatin concentrations for 6 hours following the intravenous injection of 5 per cent gelatin solution

Type of gelatin	Light					Heavy		Intermediate	
Lot nos.	39	65	45	80	83	93	58–10	58–15	101
Average water excretion to 6 hours after injection, ml.	1538	889	621	779	445	955	835	846	1239
Maximal water excretion, ml.	1780	1230	925	1402	445	1577	1493	1542	1725
Minimal water excretion, ml.	1345	510	317	272	445	600	422	325	325
Maximal gelatin concentration, grams per 100 ml.	3.42	10.26	12.06	5.77	4.71	6.18	4.16	7.96	14.96

TABLE V

Distribution of gelatin at the end of injection of 50 grams of gelatin

Subject	Weight	Gelatin type and lot	Initial plasma volume	Plasma volume at end of injection	Plasma gelatin concentration	Total plasma gelatin	Total urinary gelatin	Total tissue gelatin
	kgm.		*ml.*	*ml.*	*grams per ml.*	*grams*	*grams*	*grams*
D.C.	71	Heavy 93	3333	3846	0.929	35.72	6.20	8.08
C.W.	50	Heavy 93	2000	3030	0.792	24.00	17.45	8.55
P.W.	53	Heavy 93	2424	3571	0.684	24.43	8.88	17.69
C.P.	55	Light 45	2410	3125	0.838	26.19	8.84	14.97
J.R.	45	Heavy 93	1887	3704	0.741	27.44	6.89	15.67
R.R.	74	Heavy 93	3125	4273	0.770	32.90	14.63	2.47
J.C.	67	Light 39	2778	3680	0.593	21.82	18.24	9.94
L.B.	50	Light 39	2062	3555	0.780	27.73	12.19	10.08
Average						27.53	11.66	10.87

meager for statistical analysis, but they at least show a trend. They demonstrate the well established hemodiluting effect of injected gelatin. From the plasma volume and the plasma gelatin concentration at the end of the injection, the total circulating gelatin could be calculated. Since the urinary gelatin during the period of injection was known, the difference between the sum of these factors and the total injected (taken as 50 grams in each case [5]) gave the amount of gelatin unaccounted for, which probably was in the interstitial fluids, since it was continuously excreted apparently unchanged during the following days. If the 8 cases were representative of all cases, then only some 55 per cent of the gelatin was still in the blood stream at the end of the injection, some 23 per cent had already been excreted, and some 22 per cent had been filtered off into the tissues. These findings are in close agreement with the findings of Little and Dameron (13), who demonstrated that, after a single injection of a Knox preparation of gelatin (labeled Lot number B 78–1's) into normal dogs, only 50 per cent of gelatin was still present in the blood at the end of the injection and that 23 to 35 per cent had been excreted in the urine.

[5] Analysis of the gelatin preparations showed the presence in all specimens of small but appreciable amounts of non-protein nitrogen which when subtracted from the total nitrogen often reduced the concentrations to slightly below 5 per cent. In addition to this error there was always an error due to loss of some solution in the preparation of the intravenous infusion. However both of these errors were at least partly annulled by the fact that the bottles contained slightly more than 1000 ml.

DISCUSSION

Analysis of the data for the plasma gelatin concentration, urinary gelatin excretions and gelatin-creatinine clearance ratios for the three types of gelatin makes discernible a more or less consistent pattern in spite of great variability in the individual experiments. The higher G/C values for the light gelatin in the first hours must be interpreted as being due to a larger proportion of more easily filterable molecules. These are about 10 per cent as readily filterable as creatinine through the glomeruli (and probably also through the general capillary endothelium). In the second and third 2-hour periods, there being a much smaller porportion of easily filterable molecules, the G/C values drop considerably and approach those for the heavy gelatin experiments. In the later hours the clearances may be of the same order for all types, but the slightly higher plasma concentrations, plus possibly a slightly higher plasma volume, permit the compensatorily increased excretion of the heavy gelatin, so that eventually the total excretions are comparable.

The differences between the plasma gelatin concentrations fit in with this interpretation. With smaller excretions in the early period, and probably with similarly smaller transudations into the interstitial fluids, a relatively high plasma concentration is maintained for the heavy gelatin a longer time than for the light gelatin. Why this phenomenon is not demonstrable at the end of the injection is difficult to determine, but it probably has something to do with the relative amounts of hemodilution that occurred with the two types of gelatin. Unfortunately, an insufficient number of

accurate plasma volume determinations were made in these experiments to permit a comparison between the heavy and light types.

An alternative interpretation of the progressive decline of the gelatin-creatinine clearance ratios presents itself, if one can make the unlikely assumption that gelatin is appreciably resorbed by the renal tubules. Pitts and Alexander (14), studying the renal resorptive mechanism of phosphate, showed that the phosphate-creatinine clearance ratios diminished as the plasma phosphate decreased, because the portion of filtered phosphate that was resorbed increased as less phosphate was filtered. To interpret the gelatin excretions on the same basis, one would have to postulate not only that gelatin is freely resorbed by tubules, but that resorption is the same for all sized molecules of gelatin. Neither of these postulates is likely. It is much more reasonable to assume that of molecules ranging in molecular weight from 20,000 to 80,000, the smaller ones will be filtered more rapidly through the glomerular membranes, even if the molecules are elongated elipsoids with all nearly the same narrow diameter. This concept is further strengthened by the fact that when the ultracentrifuge pattern of the gelatin is narrow there is relatively little change in the successive G/C values, and that the values for the three types of gelatin approach each other.

The magnitude of the gelatin clearance is surprising. Even in the case of heavy gelatin, absolute clearances ranging from 1.7 to 7.7 are much larger than might be anticipated from the knowledge of the behavior of a substance like serum albumin, the clearance of which in intact kidneys approaches 0. The clearance of serum albumin even in nephrotic edema was found by Luetscher (15) to be only about 0.85. The explanation for the difference in excretability of the two proteins is of course not a simple one, but one of the important factors must be the globoid shape of the albumin molecule, as contrasted with the elongated elipsoid character of the gelatin molecule.

That the gelatin concentration in the urine may reach 15 grams per 100 ml. indicates (See Table IV), as might be expected, that tubular resorption of water is not significantly deterred by the osmotic effect of gelatin, which effect is small compared with that of the crystalloids of the urine. Similarly high protein concentrations are not infrequently seen in severe cases of nephrotic syndrome associated with oliguria.

From a clinical point of view, the data point to at least a theoretical superiority of the heavy gelatin over the light gelatin. The plasma gelatin concentrations are maintained better in the case of heavy gelatin during the first few hours after the injection, which is the period of the desired clinical effect, whereas the ultimate excretion is as complete as in the case of the light gelatin. Furthermore the heavy gelatin injections are less likely to produce excessively high concentrations of gelatin in the urine, which might temporarily embarrass renal function. On the other hand, there is unequivocal evidence from this laboratory that the injection of light gelatin is clinically effective in shock and is innocuous. From a practical point of view there may be no appreciable difference between the two types. Surely one cannot extend the results of this study to attempt to prove that still heavier gelatin, the type that gels at room temperature, is for clinical purposes even better than the heavy gelatin utilized here, for many factors other than those discussed here, such as inconvenience of administration and danger of deposition in the tissues, must be taken into consideration.

SUMMARY AND CONCLUSIONS

1. The fate of intravenously injected gelatin was studied in 42 hospital control subjects to whom were administered 1000 ml. of 5 per cent gelatin. Though the solutions were all liquid at room temperatures, they were of three types: heavy, intermediate, and light. The weight average molecular weights were respectively of the order of 58,000, 47,000, and 37,000.

2. The plasma gelatin concentration was highest at the end of the injection. It averaged 0.78 gram per 100 ml., and was the same for all three types. At 24 hours the plasma gelatin concentration had dropped to about 0.3 gram per 100 ml. for all three types. For the first six hours after the injection the plasma levels were maintained significantly higher in the heavy gelatin experiments. At 48 and 72 hours there were still appreciable quantities of gelatin in the plasma.

3. The urinary excretion of gelatin was markedly variable even with the same lot, but a pattern

of excretion was discernible. The heavy gelatin excretion in 6 hours after the end of the injection averaged 14.68 grams, while the light was 22.72 grams. The intermediate gelatin values lay between these. By 72 hours, some 80 per cent of all types of gelatin was excreted. There was still further excretion on the fourth and fifth days. Thus it was unlikely that any considerable quantity of gelatin had been catabolized.

4. The average gelatin-creatinine clearance ratios were much higher for light gelatin than for heavy or intermediate gelatin. During the 3 successive 2-hour periods following the end of the injection, the ratios fell, but much more steeply for the light gelatin, so that at the end of 6 hours the ratios were all of the same order. This phenomenon was interpreted as indicating that the smaller gelatin molecules were rapidly excreted, leaving in the body molecules of the same order of excretability for all three types of gelatin.

5. No diuresis was seen after the injection of gelatin, except possibly where the gelatin was dissolved in 5 per cent dextrose. Concentrations of gelatin in the urine as high as 15 grams per 100 ml. were occasionally encountered which may produce a viscosity great enough to interfere temporarily with renal function. The lowest maximal urinary gelatin concentrations occurred in the heavy gelatin experiments.

6. Plasma volume determinations showed the well established hemodiluting effect of gelatin, and demonstrated that at the end of the injection only about 55 per cent of the gelatin was still in the blood stream, about 23 per cent having been excreted and 22 per cent filtered into the tissues.

7. These results indicate a theoretical clinical superiority of the heavy gelatin over the lighter types. Nevertheless light gelatin has been found to be clinically effective and innocuous in the treatment of shock.

* The authors acknowledge gratefully the assistance of Miss Leah Gluck and Miss Bess Osgood with the chemical analyses, and of Mrs. Betty Vallancey, Miss Margaret Casella, and Mrs. Jeanne Hildebrandt with the infusions and collection of specimens.

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URINARY EXCRETION OF HYDROXYPROLINE-CONTAINING PEPTIDES IN MAN, RAT, HAMSTER, DOG AND MONKEY AFTER FEEDING GELATIN*

H. J. HUECKEL and Q. R. ROGERS

Department of Physiological Sciences, School of Veterinary Medicine, University of California, Davis, California 95616

(*Received* 19 *June* 1969)

Abstract—1. Quantities of free and peptide-bound amino acids were determined in urine of man, rat, hamster, dog and monkey fed normal and gelatin-containing diets.

2. In man dietary gelatin resulted in an increased excretion of bound hydroxyproline without a change in free hydroxyproline excretion.

3. In rats and hamsters both bound and free urinary hydroxyproline was elevated when the diet contained gelatin.

4. The quantities of free and bound urinary hydroxyproline in the dogs and monkeys was the same whether the animals ingested a normal diet or a diet containing gelatin.

INTRODUCTION

IT HAS been shown by Prockop *et al.* (1962) that humans excrete 1·1–1·4 mg of peptide-bound hydroxyproline/min for 1 to 2 hr after eating gelatin. This observation is of interest because, as these authors point out, it indicates that these hydroxyproline-containing peptides were probably absorbed intact and subsequently excreted. As a prelude to the study of the mode of peptide absorption we have conducted a survey to establish whether other mammals behave similarly to humans, in excreting peptide-bound hydroxyproline in the urine after the ingestion of a test dose of gelatin.

MATERIALS AND METHODS

Humans

Four normal male subjects (*Homo sapiens*) between the ages of 20 and 30 years were given either 30 g of casein or 30 g of gelatin in addition to their normal breakfast, and urine was collected 5½ hr following ingestion. The urine (about 200 ml) was adjusted to a pH of 2·0 with HCl to precipitate most of the proteins and was then filtered. The filtrate was concentrated about fivefold under vacuum, and 1 ml of this urine concentrate and 1 ml of 12 N HCl were sealed into a 5-ml ampoule under vacuum and hydrolyzed by heating for 20 hr at 120°C. After hydrolysis the acid was removed under vacuum and samples were made up to 5 ml.

* Supported in part by U.S. Public Health Service Grant Nos. AM-10707 and AM-11066.

Amino acid analyses of both the urine concentrate and its hydrolyzate were done using an automatic amino acid analyzer.

Rats

The experimental diet contained 20 per cent gelatin which was balanced by adding 8·0 per cent essential amino acids. The control diet contained 28 per cent free amino acids in the same proportion as the experimental diet. That is, upon hydrolysis both the control and experimental diets theoretically would have the same quantity of each amino acid. The only amino acid not present in the control diet was hydroxylysine. A third diet contained 26·5 per cent casein. The rest of the ingredients were (per cent): dextrin, 41; sucrose, 20; salt mix, 5 (Rogers & Harper, 1965); corn oil, 5; choline chloride, 0·1; vitamin diet fortification mixture in dextrose, 1·0 (Nutritional Biochemical Corporation); protein or amino acids, 28. The ingredients of these diets were first mixed in a Hobart mixer for 15 min, then an equal weight of hot water was added, containing 2 per cent Agar-Agar; after a short mixing period the slurry was poured into plastic trays and placed in the refrigerator. After the diet had set into a gel it was cut into cubes and fed. The fourth diet contained 50 per cent gelatin and when used was fed only on the day of urine collection. It was prepared by mixing equal amounts of gelatin and sucrose (no vitamins or salts) with a small amount of water to obtain rubbery chunks.

Twelve male Sprague-Dawley rats (*Rattus norvegicus albinicus*) were placed in individual cages with water *ad lib.* and were trained for 1 week to a regimen of feeding for 1 hr twice per day (morning and evening). At the beginning of the experiment the animals were 9 weeks old and weighed an average of 226 g. The rats were placed with their morning meal in stainless steel metabolic cages. Four rats received the amino acid diet, another four rats the 20 per cent gelatin diet and the remaining four rats received the casein diet. On a separate occasion four rats received the 50 per cent gelatin diet in the same manner. After 1 hr the food was removed. Six hr later the urine of each group was pooled, adjusted to pH 2, allowed to stand in the refrigerator overnight and then filtered. The filtrate was concentrated to 5 ml. One ml was hydrolyzed as described above for human urine. The urine concentrate was analyzed for free amino acids before and after hydrolysis.

Hamsters

The strain of hamsters used was derived from the gold hamster (*Mesocricetus auratus*), which has albino white fur except for black-tipped ears and dark-red eyes. The animals used were 6 months old; their basal diet consisted of commercial rat food pellets and fresh vegetables or fruit such as carrots, lettuce or apples. The first of two experiments was conducted using two male hamsters together in one metabolic cage for 4 days. On the first and fourth day they received the pellet diet and on the second and third day they received a diet of 40 per cent sucrose, 40 per cent gelatin and 20 per cent water in the form of a rubbery firm gel block. Twenty-four-hr urine samples were collected and analyzed for free and total amino acids as described earlier.

In the second experiment only one male hamster was used in a metabolic cage. On the first day a 30 per cent casein diet was fed and on the second day a 23 per cent gelatin diet (plus 7 per cent essential amino acids) was fed. Composition of the diets were (per cent): corn starch, 36; sucrose, 18; salt mix, 5 (Rogers & Harper, 1965); corn oil, 10; choline chloride, 0·1; vitamin fortification mixture in dextrose, 1 (Nutritional Biochemical Corporation). The preparation of the diet was the same as for the rat diet.

Dogs

Four pure bred beagles (*Canis familiaris*) in double cages which were divided for feeding were fed their regular diet (basal) for 2 days. On the third and fourth day they were given the basal diet containing 5 per cent of an amino acid mixture resembling gelatin, and on the fifth day they were given the basal diet containing 5 per cent commercial gelatin. The daily

food consumption was 300 g. The basal diet contained (per cent): Kibbles, 60 (Nutro Dog Food Inc., El Monte, California 91733, U.S.A.); water, 18·5; ground meat, 10; meat by-products, 10; vitamin mixture, 1; blood meal, 0·5.

Urine was collected by use of a catheter which was placed into the bladder 6 hr after the daily meal. The amount of urine collected was variable. The reason for choosing 6 hr was that Abderhalden & Buadze (1927) found that the diketopiperazine of DL-leucylglycine reached a maximum concentration in the urine 5 hr after feeding. Amino acid analysis was carried out on the unconcentrated urine before and after acid hydrolysis.

Monkeys

Two monkeys, one adult Rhesus (*Macaca mulatta*) and one adult Capuchin (*Cebus apella*), were housed in individual metabolic cages which enabled the collection of total urine. Water was given *ad lib*. For their two daily meals the monkeys were moved into different cages for 1 hr for each meal so that the food would not contaminate the urine. Urine was collected twice daily at which time a known amount of norvaline was added to the urine (10 μmoles/day for the Rhesus, 5 μmoles/day for the Capuchin). The urine collections were made for 4 days. On the first and third day they received their regular diet of prepared monkey food (semi-solid mixture, prepared by the National Primate Center at Davis). On the second day the monkeys were fed an amino acid mixture resembling gelatin which was added to the monkey diet and on the fourth day gelatin was added to the diet. During the 1-hr feeding period in the morning and afternoon, the Rhesus monkey ate a total of 3·0 g of gelatin and the Capuchin ate 2·4 g per day. The urine was treated the same as that of the four beagles.

RESULTS

Humans

The 30-g test dose of gelatin caused a significant increase in the urinary excretion of peptide-bound proline and hydroxyproline and of free hydroxylysine. The amounts of bound and free amino acids are shown in Table 1. The ingestion

TABLE 1—URINARY EXCRETION OF AMINO ACIDS AFTER THE INGESTION OF EITHER GELATIN OR CASEIN BY MAN

	μmoles/5½ hr			
	Free form		Bound form	
Amino acid	Control	Gelatin	Control	Gelatin
Hydroxyproline	<2*	10*	38 ± 13†	794 ± 126
Aspartic acid	23‡	22‡	160‡	310‡
Glutamic acid	5 ± 1	5 ± 2	600 ± 189	892 ± 295
Proline	3 ± 1	2 ± 1	108 ± 38	649 ± 114
Glycine	307*	1310*	734*	1391*
Alanine	95 ± 44	163 ± 55	33 ± 5	112 ± 45
Lysine	101*	107*	57*	66*
Histidine	337*	349*	136*	145*
Hydroxylysine	2 ± 1	39 ± 8	4 ± 2	17 ± 7

* Average of three values.
† ± Standard error of mean of four values.
‡ Average of two values.

of gelatin had no effect on the amount of free proline or hydroxyproline excreted. Although most of the urinary glutamic acid was peptide-bound, the amount was not influenced by feeding gelatin. There was, however, a slightly greater quantity of bound aspartic acid, glycine and alanine after feeding gelatin. There also was a twentyfold increase in free hydroxylysine in the urine. In contrast to rat urine there is a large quantity of free and bound histidine in human urine.

Rats

It was found that the rat excretes large quantities of free proline and hydroxyproline after eating diets high in proline or hydroxyproline (Fig. 1A, B). The peptide-bound hydroxyproline, proline, glycine and aspartic acid are greatly increased by dietary gelatin while no effect was seen in peptide-bound glutamic acid, alanine, threonine and serine.

Hamsters

Analysis of the hamster urine showed an amino acid pattern similar to that found in rat urine. When the diet contained gelatin the bound proline and hydroxyproline increased in the urine (Table 2). Alanine is the most constant amino acid in the urine and is not affected by dietary gelatin; since we had no other constant parameter to use we have expressed our results as ratios of bound amino acids to that of bound alanine. Alanine also separates completely from other ninhydrin-positive compounds in our analysis making it a dependable standard. There was a fourfold increase in bound hydroxyproline and a twofold increase in bound proline in urine of hamsters fed the 50 per cent gelatin diet. A twofold increase in

TABLE 2—MOLAR RATIOS OF BOUND AMINO ACIDS TO THAT OF BOUND ALANINE IN URINE OF HAMSTERS FED GELATIN DIETS

	Diets					
	First experiment				Second experiment	
Amino acid	Control*	40% Gelatin*	40% Gelatin*	Control*	Casein†	23% Gelatin†
Hydroxyproline	0·16	0·75	0·68	0·18	0·26	0·56
Aspartic acid	0·76	1·65	1·32	1·00	—	—
Threonine	1·55	1·44	1·46	1·29	—	—
Serine	1·40	1·04	0·82	1·36	—	—
Glutamic acid	1·73	2·18	2·28	1·51	1·79	2·28
Proline	0·75	1·53	1·32	0·70	0·94	1·52
Glycine	6·05	6·50	5·55	7·70	3·80	5·05
Alanine	1·00	1·00	1·00	1·00	1·00	1·00

* Pooled urine of two hamsters.
† Urine of one hamster.

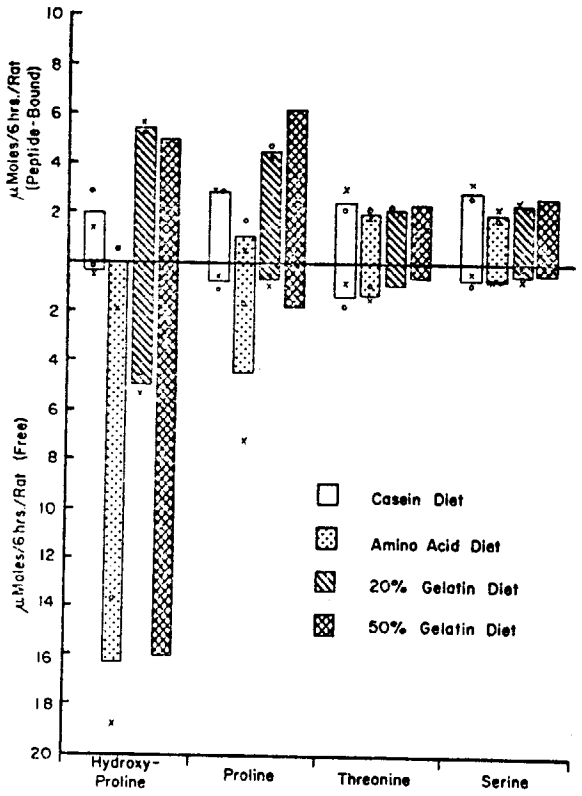

FIG. 1A

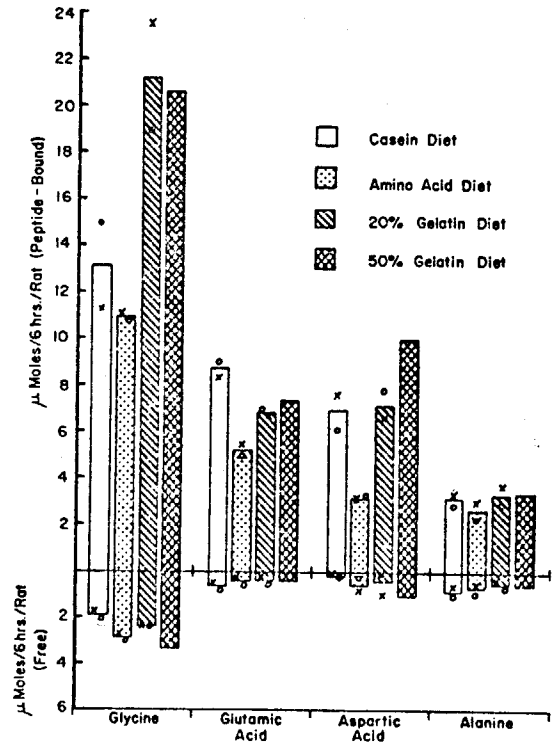

FIG. 1B

FIG. 1A, B. Micromoles of free and bound amino acids from urine of rats. O represents the pooled urine of four rats in a 6-hr period after feeding on the first day of collection. × represents the pooled urine of four rats in a 6-hr period after feeding on the fourth day of collection. For the casein, amino acid and 20 per cent gelatin diets, each bar represents the average of O and × values. For the 50 per cent gelatin diet each bar represents the pooled urine of ten rats in a 6-hr period after feeding. Values below the zero line are the amino acids found free in the urine; values above the zero line are peptide-bound amino acids.

TABLE 3—MOLAR RATIOS OF BOUND AMINO ACIDS TO THAT OF BOUND ALANINE IN URINE OF BEAGLES FED GELATIN

Amino acid	Control diet*	Amino acid diet†	Gelatin diet*
		μmoles/μmole of Ala	
Hydroxyproline	0·12 ± 0·01	0·29 ± 0·06	0·29 ± 0·05
Aspartic acid	1·96 ± 0·09	1·90 ± 0·08	2·39 ± 0·13
Glutamic acid	1·31 ± 0·10	1·18 ± 0·06	1·28 ± 0·04
Proline	0·87 ± 0·09	1·01 ± 0·07	0·99 ± 0·04
Glycine	3·33 ± 0·15	3·54 ± 0·21	3·67 ± 0·05
Alanine	1·00	1·00	1·00
Hydroxylysine	0·10‡	0·16 ± 0·02	0·12 ± 0·01
		μmole/ml of urine	
Alanine	2·28 ± 0·24	1·35 ± 0·29	1·27 ± 0·36

* Average of three values.
† Average of four values.
‡ Average of two values.

TABLE 4—URINARY EXCRETION OF FREE AND BOUND AMINO ACIDS BY CAPUCHIN MONKEY AFTER FEEDING GELATIN

	μmoles/day					
	Control		Amino acids		Gelatin	
Amino acid	Free	Bound	Free	Bound	Free	Bound
HO proline	0	24·2	6·9	45·2	27·8	65·1
Aspartic acid	0	*	0	74·2	1·2	58·8
Threonine	0·5	32·2	9·7	33·4	30·8	32·1
Serine	0·4	33·0	1·0	34·8	54·2	8·3
Glutamic acid	0	190·0	0·3	179·5	11·8	122·8
Proline	0	62·0	0	80·2	1·6	65·6
Glycine	0·3	441·6	111·5	603·0	306·9	490·5
Alanine	0	75·2	2·4	76·7	21·6	45·0
Cystine	4·9	1·5	4·4	5·1	3·8	1·6
Valine	0·3	20·5	0·5	22·3	4·6	14·9
Methionine	0·3	10·2	0·5	11·0	1·9	4·8
Isoleucine	0·3	12·5	0·2	12·9	6·8	9·4
Leucine	1·3	19·8	2·2	21·9	6·8	9·4
Tyrosine	2·2	6·8	2·4	7·4	5·2	1·5
Phenylalanine	0·9	8·9	1·0	14·3	3·0	7·8
HO lysine	0·8	13·4	0·5	15·7	1·5	6·0
Lysine	1·2	39·4	*	*	6·4	22·3
Histidine	10·0	6·1	13·1	3·5	30·0	10·4

* Not determined.

bound hydroxyproline and a 1·5-fold increase in bound proline was found in the urine of hamsters fed the 23 per cent gelatin diet. Only small increases in bound glycine and glutamic acid occurred.

Dogs

The results from the dog studies are presented in Table 3 as bound amino acids in ratios relative to bound alanine which was taken as 1·00. Since variable amounts of dog urine were collected from the bladder, the results were again expressed as the ratio to that of alanine. When the beagle diet contained either the amino acid mixture or gelatin the dogs excreted the same amount of free as well as bound amino acids.

Monkeys

Neither monkey excreted increased quantities of bound amino acids in response to the three diets (Table 4, Fig. 2). The results from the monkey experiments are expressed in μmoles/day. Calculations of total recoveries were based on the known amount of norvaline added. There was a fivefold increase of free hydroxylysine in the Rhesus on the day gelatin was fed. This is of interest insofar as it was

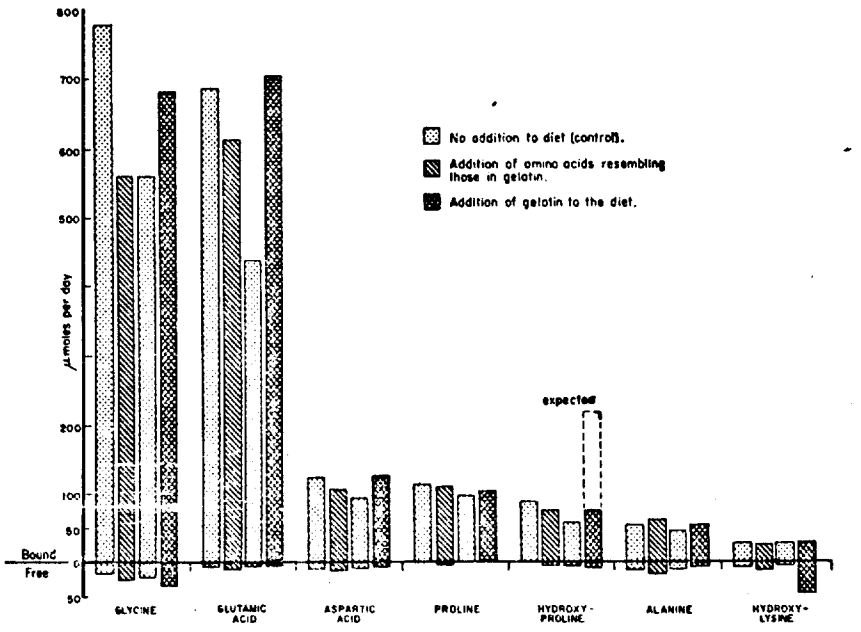

FIG. 2. Micromoles of free and bound amino acids in the urine of a Rhesus monkey fed a diet containing either amino acids or gelatin. Values below the zero line are the amino acids found free in the urine; values above the zero line are peptide-bound amino acids. The broken-line bar indicates the amount of bound hydroxyproline expected if the Rhesus monkey had responded to dietary gelatin in the same way as humans and excreted 7 per cent of the ingested hydroxyproline.

the only positive response to the dietary gelatin. A graphic illustration of the results are presented in Fig. 2, indicating the magnitude of increase of bound hydroxyproline (amounting to 7 per cent of the fed hydroxyproline) to be expected on the day gelatin was fed, if the Rhesus had responded to dietary gelatin in the same way as humans. Results from the Capuchin (Table 4) showed more variation but followed the same trend.

DISCUSSION

The response of the various species with regard to urinary hydroxyproline excretion fell into three categories. (1) The first category, which is represented by humans, showed essentially no change in the excretion of the amount of free hydroxyproline after the ingestion of gelatin while the bound hydroxyproline increased from about 13 to about 600 μmoles during the 5·5-hr period. (2) The second category is characterized by an increase in the excretion of bound hydroxyproline as well as free hydroxyproline after gelatin ingestion and includes the rat and hamster. (3) The third category includes the beagle and the Rhesus and Capuchin monkeys, all of which showed no change in the amount of free or bound hydroxyproline as a response to dietary gelatin.

In humans we found that about 3 per cent of the ingested hydroxyproline was recovered as peptide-bound hydroxyproline in the urine in $5\frac{1}{2}$ hr. Although Prockop *et al.* (1962) recovered approximately 7 per cent of the ingested gelatin, they collected for a 24-hr period. Their maximum peptide excretion rate, however, was 2 hr after the ingestion of the gelatin test dose.

The only other amino acid that responded to dietary gelatin the same as hydroxyproline was proline. This is consistent with the finding of Meilman *et al.* (1963) who have presented evidence that two-thirds of the bound urinary hydroxyproline normally excreted in man is in the form of the dipeptide, prolylhydroxyproline.

In humans dietary gelatin causes only a slight increase in urinary free hydroxyproline. However, when gelatin is fed to rats or hamsters there is a relatively large increase in free hydroxyproline in their urine (Table 2, Fig. 1). Prockop *et al.* (1962) have reported a fourteenfold increase in free hydroxyproline in the plasma of his subjects 2 hr after a test dose of gelatin, indicating that efficient renal reabsorption occurs in man. It is not known to what extent gelatin increases blood hydroxyproline in the rat or hamster.

The amino acid occurring in the greatest amount in the bound form in the urine of all the species tested was glycine. It is quite possible that a major part of this bound glycine is from hippuric acid. Some of the other amino acids such as glutamic acid may also be part of a compound other than peptides. In our study, however, it is important to note that the amount of the bound form of these amino acids was not affected by dietary gelatin.

To make certain that the increase in peptide excretion was not a result of the free amino acid pattern arising from gelatin, we used an amino acid diet resembling the amino acid pattern in gelatin as one of the controls in our animal experiments.

In humans Ziff *et al.* (1956) have fed 28 g of gelatin to a normal male patient and found a four- to fivefold increase in the total urinary hydroxyproline. However, when they fed 4 g of free hydroxyproline (the amount of hydroxyproline in 28 g of gelatin) they observed no increase in the total urinary hydroxyproline.

Although it is often thought that gelatin is easily digested, Rogers *et al.* (1960) have shown that more nitrogen is recovered from the intestine of rats fed gelatin than when casein or egg albumen is fed. The same group, Chen *et al.* (1962), in addition estimated the average peptide length in the trichloracetic acid-soluble fraction of rat intestinal content and found 6·0 amino acid residues/peptide after feeding gelatin and 2·4 and 2·7 after feeding a "nitrogen-free" and casein diet respectively. These results would indicate that gelatin is resistant to digestion and that peptides accumulate in the small intestine.

It would appear from the foregoing results that the beagle and the Rhesus and Capuchin monkeys do not excrete bound hydroxyproline after the ingestion of gelatin. Either they apparently have an enzyme that can readily hydrolyze the hydroxyproline-containing peptides or else they do not absorb such peptides. Abderhalden & Buadze (1927) fed 5 g of DL-leucylglycine anhydride to a 7·7-kg dog. They were able to recover 80 per cent of the anhydride unchanged in the urine. However, when they fed glycyl-D-alanine anhydride they could recover only glycyl-D-alanine in the urine. The above work would suggest that some diketopiperazine linkages withstand enzymatic hydrolysis in the gastrointestinal tract and can be absorbed and excreted intact by the dog. The most probable reason for the lack of bound urinary hydroxyproline would therefore appear to be the presence of a more active peptidase for hydroxyproline-containing peptides. It becomes important therefore to ask the question as to whether the animals which did not excrete bound hydroxyproline received a large enough dose of gelatin. All animals received a greater quantity of gelatin proportional to body weight than humans. Even calculating on the basis of metabolic size (body weight to the three-quarter power), the dose for the monkey was the same as that for man and the quantity fed the beagles was three times higher than for man. From the evidence available, therefore, it appears that hydroxyproline-containing peptides arise from gelatin digestion and that these peptides are resistant to digestion, accumulate in the intestine and are absorbed and excreted intact.

Our studies show that man would clearly be the best experimental model for studying the mechanism of absorption of hydroxyproline-containing peptides. Perhaps with more difficulties, the process of peptide absorption could also be studied in rats or hamsters, but it would appear that dogs or monkeys are not suitable, at least for the hydroxyproline-containing peptides.

SUMMARY

The species tested fall into three categories. (1) The first category is represented by humans which showed essentially no change in the excretion of the amount of free hydroxyproline 5½ hr after the ingestion of gelatin while the bound hydroxyproline increased over fortyfold, from about 13 to about 600 μmoles.

(2) The second category, which includes the rat and the hamster, is characterized by an increase in the excretion of free hydroxyproline from only traces to around 50 μmoles/day. The bound hydroxyproline also increased but only two- or threefold, from 2–3 μmoles to 5–8 μmoles/day. (3) The third category includes the beagle and the Rhesus and Capuchin monkeys, all of which showed no change in the amount of free or bound hydroxyproline as a response to dietary gelatin.

The increased excretion of bound urinary hydroxyproline which is caused by the ingestion of gelatin appears to occur because digestive-resistant hydroxyproline-containing peptides arise during gelatin digestion, these peptides accumulate in the intestine, and are absorbed and excreted intact.

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Key Word Index—Intestinal peptide absorption; urinary hydroxyproline peptides; absorption of peptides; prolylhydroxyproline; gelatin (dietary); hydroxyproline-containing peptides.

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strength of the gel it will form at a given concentration with water. Modern advances are all designed to render skin more readily convertible into gelatin by previous chemical treatment, so as to reduce the time the gelatin is exposed to high temperature. Ames (1949) has treated the subject in detail.

a. Raw Materials. The essential raw material for gelatin is collagen, the principal protein of connective tissue. The major supply of collagen is procured from hides and bones. Muscle tissue, horns, and hoofs are of no value. Hide is, of course, so valuable to the tanner that only those parts which cannot be used find their way to the gelatin manufacturer. The actual materials consist of the leavings of tanneries and slaughter houses, i.e., trimmings, pates, fleshing, cheek-pieces, etc. The stock may be either "dry" or wet and is sometimes degreased and dehaired. The hides are composed primarily of splits and calfskin. When hides are removed from cattle they are run through a splitting machine. The outside of the hide, which is marked with the characteristic grain, is used for the manufacture of leather. This is the so-called top-grain product. The inside portion of the hide does not contain the grain and this is known as a split, and may be obtained in the form of fresh splits, dried splits, and cured or pickled splits. In the United States, a large amount of fresh pigskin is processed which arrives at the gelatin plant in a frozen state. In addition to the species, the age and condition of the animal are of importance.

If green or wet stock is not going to be used within a relatively short time it must be preserved to prevent putrefaction. Salt, lime, and desiccation are most commonly used. However, most stock is immediately "conditioned" by either an alkaline or acid process, after a preliminary water wash to remove dirt and blood, which may interfere with the color of the finished product. The bulk of gelatins are "limed" or treated with a suspension of calcium hydroxide.

Most of the bone is furnished by South America and India; the hide pieces, by the United States, South America, and England; the frozen porkskin is used only in the United States. In Europe, 20% to 35% more gelatin is produced from ossein than from hide pieces. The production of ossein in the United States is small.

A review on the raw materials and production of isinglass, or fish gelatin and fish glue, has appeared (Becher, 1950).

b. "Liming" (Alkaline Process). (1) General. The raw material is soaked in a calcium hydroxide suspension for a period of one to six months at a pH of about 12 to 12.5. The temperature of the lime bath is usually that of the surrounding air and is kept approximately constant by the high humidity present, owing to evaporation of water. As alkali is used

J. Biol. Chem. 80:167-186, Nov. 1928

EXPERIMENTS ON THE NUTRITIVE PROPERTIES OF GELATIN.

BY RICHARD W. JACKSON,* BEATRICE E. SOMMER, AND WILLIAM C. ROSE.

(From the Laboratory of Physiological Chemistry, University of Illinois, Urbana.)

(Received for publication, August 8, 1928.)

Numerous attempts have been made in the past to supplement gelatin with amino acids in such a fashion as to render it capable of maintaining body weight or of preserving nitrogen equilibrium. Most of these efforts have met with complete failure. A few, notably those of Kauffmann (1905) and Abderhalden (1912), appear at first glance to have been somewhat more successful. An examination of the data of these authors, however, reveals the fact that the periods upon gelatin diets during which nitrogen equilibrium was approximated were of exceedingly short duration. Even if it be granted that gelatin with suitable amino acid additions suffices for the maintenance of nitrogen equilibrium in animals for periods of 5 to 10 days, one would scarcely be justified in assuming on the basis of such evidence that the same would be true for longer periods of time. Furthermore, most of the studies of the nutritive properties of gelatin were made before the recognition of the importance of the vitamins as dietary factors.

Since the development of the newer aspects of nutrition during the past two decades very few experiments upon gelatin have been reported. In their classical studies with isolated proteins, Osborne and Mendel (1912–13) showed that this substance is quite incapable of serving as the sole source of nitrogen. Rats placed upon diets of gelatin, starch, lard, and "protein-free milk" rapidly declined in weight, but when half of the gelatin was replaced by casein or gliadin, recovery with maintenance or growth ensued. Osborne and Mendel did not attempt to correct the deficiencies of gelatin by the addition of individual amino acids, but they point out (p. 242) that the "failure to be maintained . . . . is not to be ascribed to a failure in utilization; for nitrogen determinations made on the feces showed that the utilization of the protein was good."

* Most of the experimental data in this paper are taken from a thesis submitted by Richard W. Jackson in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Physiological Chemistry in the Graduate School of the University of Illinois.

Later Totani (1916) attempted to render ge adequate as a source of nitrogen for rats by supplementing the diets with purified amino acids. He reports that the addition of tryptophane alone, or of tryptophane with tyrosine, is without influence upon the quality of the nitrogen. Animals upon gelatin diets, either with or without the supplementing amino acids, suffer 40 per cent losses in body weight in the course of a single month. The author states that hydrolyzed gelatin proved to be a much better source of nitrogen inasmuch as rats receiving this material experienced losses of only 18 per cent in a month. Furthermore, the inclusion of tryptophane in the hydrolyzed gelatin diet led to approximate maintenance. The further addition of tyrosine did not lead to better growth than was secured with tryptophane alone. When tryptophane, tyrosine, cystine, and histidine were incorporated in the ration the results are said to have been "somewhat more satisfactory." The author concludes (p. 397) that, "In the case of the rat, the nutritive efficiency of gelatin is greatly increased by previous hydrolysis. Gelatin when fed intact appears to be badly digested and absorbed." This statement is not in accord with the experience of Osborne and Mendel as outlined above. It is also contrary to the findings of numerous investigators who have studied the utilization of gelatin in animals other than the rat. Thus Reilly, Nolan, and Lusk (1898) report that in diabetic dogs sugar is formed from gelatin in about the same amount as from other proteins. McCollum (1911–12) and Robison (1922), in experiments upon the pig and man respectively, have shown that the ingestion of relatively large quantities of gelatin increases the fecal nitrogen very slightly over the starvation output. Even in the rat, McCollum, Simmonds, and Pitz (1917) have demonstrated that gelatin is capable of supplementing the proteins of rolled oats in a remarkable fashion. These and other experiments reported in the literature would seem to indicate quite conclusively that whatever may be the nutritive deficiencies of gelatin they are not associated with an incapacity of the alimentary enzymes to accomplish its digestion.

In connection with certain investigations in this laboratory we have undertaken a further study of the inadequacies of gelatin as a source of nitrogen for maintenance and growth. On the basis of the most reliable analyses of gelatin it would seem that the amino acid deficiencies are quite evident and could be easily remedied. That the problem is not so simple as it appears will be brought out in the following pages.

Early in our work we observed that the addition of the three conspicuously absent amino acids, cystine, tryptophane, and tyrosine, did not give uniformly the growth desired. It was decided, therefore, to extend the scope of the investigation so as to include not only the amino acid deficiencies, but any other factors

which conceivably might alter the nutritive value of the protein. The following are some of the questions which suggested themselves:

1. Since the very careful and complete analysis of gelatin made by Dakin (1920) reveals the absence of valine, hydroxyglutamic acid, and isoleucine, it seemed not improbable that one or more of these might be limiting factors.

2. Inasmuch as certain other components of gelatin are present in smaller amounts than occur in casein, it appeared necessary to determine the influence upon growth of suitably increasing their intake, either by adding the pure amino acids themselves, or by raising the level of the gelatin in the diet.

3. The pronounced disproportion in the distribution of amino acids in gelatin as compared with casein, particularly as regards the high content of glycine and alanine in the former, suggested that an *improper balance* between the amino acids might exert an influence upon nutrition. Evidence for or against this conception might be secured by incorporating in an otherwise satisfactory diet amounts of glycine and alanine comparable to those present in gelatin.

4. It has been the experience of many that the ingestion by the human subject of large quantities of gelatin is exceedingly difficult. Indeed, depression and nausea almost invariably result. In commenting upon this observation Howell (1925, p. 288) says, "A fact of this kind indicates that the sensory apparatus of the appetite is influenced in some specific way by the metabolism of this particular material." In order to determine whether the peculiar physical or chemical nature of gelatin exerts a deleterious effect upon the organism, we have followed the growth of animals which received a third of their diet in the form of this protein in addition to an abundance of nitrogen of good quality.

5. If gelatin contains amino acid linkages incapable of cleavage by the digestive enzymes, complete hydrolysis of the material before its incorporation in the diet should improve the quality of the nitrogen, as is reported to be the case by Totani (1916). A reinvestigation of this point seemed necessary.

EXPERIMENTAL.

White rats served throughout as the experimental animals. Invariably they were placed upon a casein diet for brief periods at the beginning of the experiments in order to test their growth propensities. In view of experience gained in the course of the

TABLE I.

Composition of Diets.

Diet	A.	B.	C.	D and E.*	F.	G.	H and I.*
	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Casein	18.0	18.0	18.0				
Gelatin			35.0	35.00	30.00	25.00	15.00
Dextrin	34.0	22.0		15.30	16.30	20.90	24.00
Sucrose	15.0	15.0	14.0	15.00	15.00	15.00	15.00
Salt mixture	4.0	4.0	4.0	4.00	4.00	4.00	4.00
Agar	2.0	2.0	2.0	2.00	2.00	2.00	2.00
Cod liver oil	5.0	5.0	5.0	5.00	5.00	5.00	5.00
Lard	22.0	22.0	22.0	22.00	22.00	22.00	22.00
Tyrosine (*l*)				0.90	0.90	0.90	0.90
Cystine (*l*)				0.50	0.50	0.50	0.50
Tryptophane (*l*)				0.35	0.35	0.35	0.30
Histidine monochloride (*l*)					0.26	0.32	0.40
Valine (*dl*)					2.70	2.70	4.00
Phenylalanine (*dl*)					0.60	0.80	2.00
Glutamic acid (*d*)					2.10	2.40	4.00
Aspartic " (*l*)							1.00
Leucine (*dl*)							1.33
Isoleucine (*dl*)							1.33
Norleucine (*dl*)							1.33
Glycine		9.0					
Alanine (*dl*)		6.0					

* Diets E and I contained hydrolyzed gelatin but in other respects were exactly like Diets D and H respectively. With the exception of Diets E and I, all of the gelatin diets contained the protein in an unhydrolyzed form.

investigation to the effect that younger animals do not seem to withstand the change to the gelatin diets as satisfactorily as do older ones, we permitted all rats employed in the experiments reported herein to attain weights of at least 100 gm. before being transferred from the casein ration. In the conduct of the experiments and in the care of the animals essentially the methods of

Osborne and Mendel as described by Ferry (1919-20) were followed.

The composition of the diets is shown in Table I. In addition to the articles of food enumerated, vitamin B was furnished to each animal daily in the form of tablets containing 50 mg. of commercial yeast extract or 200 mg. of whole yeast.[1] The gelatin was a special product[2] which in manufacture had not been subjected to the action of bleaching agents. It consisted of white, opaque flakes, which were thoroughly dried *in vacuo*, ground, and passed through a 40 mesh sieve. A 1 per cent solution exhibited a faint Millon test, and a negative Hopkins-Cole reaction. Hydroferrocyanic acid gave a very faint turbidity.

The hydrolyzed gelatin employed in Diets E and I was prepared exactly according to the procedure followed by Dakin (1920) in the analysis of this protein with a recovery of over 90 per cent of the component amino acids. For this purpose, a mixture composed of 250 gm. of gelatin, 300 gm. of concentrated sulfuric acid, and 650 gm. of water was heated to boiling for 20 hours at atmospheric pressure. The resulting solution was autoclaved at 135–140° for 8 hours. After dilution, the sulfuric acid was removed with barium hydroxide, and the filtrate and washings from the barium sulfate were combined and evaporated to dryness. The final material, after grinding, was light brown in color.

The inorganic portion of the rations was supplied in the form of Osborne and Mendel's (1919) salt mixture. Of the amino acids used in supplementing the diets, tyrosine, cystine, tryptophane, histidine monochloride, glutamic acid, and glycine were prepared in this laboratory from proteins. The aspartic acid was the natural levo variety secured from Kahlbaum. The valine, phenylalanine, leucine, isoleucine, norleucine, and alanine were racemic compounds most of which were synthesized in the organic laboratory of this University. Both the natural and racemic acids were recrystallized until they gave theoretical values for total or

[1] The extract was prepared by the Harris Laboratories, Tuckahoe, N. Y., while the whole yeast was secured from the Northwestern Yeast Company, Chicago. Both the extract and the yeast were thoroughly tested as to their growth-promoting properties before being employed in the gelatin experiments.

[2] Secured from the Wilson Laboratories, Chicago.

amino nitrogen. Whenever a racemic acid was used, twice the calculated amount was incorporated in the ration so as to insure the presence of the active enantiomorph in the quantity desired.

In supplementing the gelatin diets we have used the composition of casein as our guide. On the basis of the most reliable figures for casein composition (*cf.* Mendel, 1923, p. 115), and the analysis of gelatin by Dakin (1920), we have calculated the quantity of each known amino acid[3] present in 100 gm. of food containing 18 per cent of casein and 35 per cent of gelatin respectively. These figures are presented in the first two columns of Table II. In the other columns of this table are shown similar data for the supplemented gelatin diets employed in this investigation.

In most of our experiments gelatin was incorporated in the food at a 35 per cent level. This relatively high proportion was adopted instead of the customary 18 per cent because the protein in question contains several amino acids in quantities far in excess of the accepted values for casein. A comparison of the composition of the two proteins indicates that gelatin yields superfluous amounts of glycine, alanine, hydroxyproline, and arginine. Even if we assume that quantities of these amino acids considerably larger than are supposed to be present in casein are required by the organism, we are still confronted with the necessity of feeding almost double the proportion of gelatin as compared with casein in order to insure the presence of the other amino acids in approximately comparable amounts. A diet containing 35 per cent of gelatin seemed, therefore, a reasonable starting point.

Growth on a Gelatin Diet Supplemented with Three Amino Acids.—The data presented in Table II make clear that in addition to tyrosine, cystine, and tryptophane, gelatin is wanting also in valine, isoleucine, and hydroxyglutamic acid. Furthermore, on a 35 per cent basis, phenylalanine, glutamic acid, and histidine

[3] During the progress of this investigation methionecine, the new sulfur-containing amino acid isolated from casein by Mueller (1923), was identified by Barger and Coyne (1928). For obvious reasons this substance was not taken into consideration in our experiments. Mueller reports its presence in gelatin, but apparently in much smaller quantities than in other proteins. At a later date we expect to determine the relation of methionecine to the nutritive properties of gelatin.

are somewhat below the levels present in the 18 per cent casein diet (Diet A) taken as our standard. In our first experiments, we have supplied only tyrosine, cystine, and tryptophane. Thus the resulting gelatin diet (Diet D) was presumably devoid of valine, isoleucine, and hydroxyglutamic acid. There existed also the possibility that it might be somewhat inadequate in respect

TABLE II.

Amino Acids Present in 100 Gm. of the Various Diets as Compared with 35 Per Cent of Unsupplemented Gelatin.*

	35 per cent gelatin.	Diet A.	Diets D and E.	Diet F.	Diet G.	Diets H and I.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Glycine	8.93	0.08	8.93	7.65	6.38	3.83
Alanine	3.05	0.33	3.05	2.61	2.18	1.31
Valine	0	1.43	0	1.35	1.35	2.00
Leucine	2.49	1.75	2.49	2.13	1.78	1.73
Isoleucine	0		0	0	0	0.66
Norleucine						0.66
Serine	0.14	0.09	0.14	0.12	0.10	0.06
Phenylalanine	0.49	0.70	0.49	0.72	0.75	1.21
Tyrosine	?	0.81	0.90	0.90	0.90	0.90
Proline	3.33	1.37	3.33	2.85	2.38	1.43
Hydroxyproline	4.94	0.04	4.94	4.23	3.52	2.12
Aspartic acid	1.19	0.74	1.19	1.02	0.85	1.51
Glutamic "	2.03	3.92	2.03	3.84	3.85	4.87
Hydroxyglutamic acid	0	1.89	0	0	0	0
Histidine	0.32	0.45	0.32	0.47	0.49	0.46
Arginine	2.87	0.69	2.87	2.46	2.05	1.23
Lysine	2.07	1.37	2.07	1.77	1.48	0.89
Tryptophane	0	0.27	0.35	0.35	0.35	0.30
Cystine	?	Present.	0.50	0.50	0.50	0.50

* The figures are for the *active* amino acids. When racemic acids were employed, only half of each quantity added to a diet is included in the above data.

to phenylalanine, glutamic acid, and histidine. The absence of hydroxyglutamic acid was disregarded in all of our experiments inasmuch as Osborne, Leavenworth, and Nolan (1921) have pointed out that edestin, which does not contain this amino acid, is capable of supporting normal growth.

Of twenty-six rats placed upon Diet D, thirteen died at intervals

varying from 9 to 48 days. The curves representing the body weight changes of two of the animals which failed to survive (Rats 138 and 150) are reproduced in Chart I, and are typical of all except for the length of time which elapsed before death. The curves show profound nutritive failure accompanied by

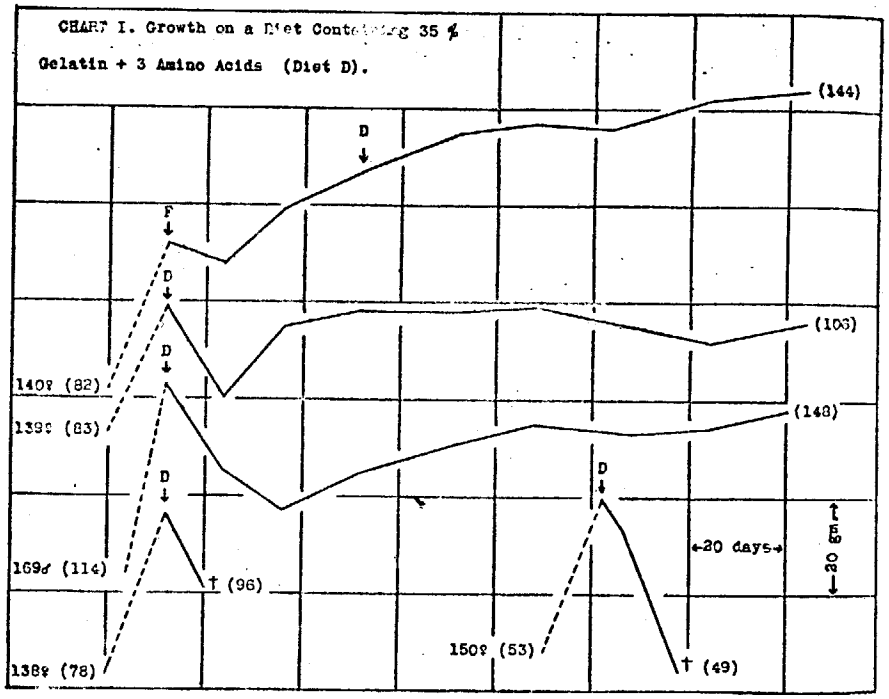

CHART I. The numbers in parentheses signify the initial and final weights of the rats. The broken line portion of each curve represents the growth of the rat during the preliminary period upon Diet A. The solid lines show the growth upon the experimental rations. The arrows denote the points at which the dietary changes were made. The diets are designated by the letters above the arrows.

amazingly rapid losses in weight. In some instances the food consumption dropped almost to zero during the last few days preceding death. At autopsy most of the animals which had died displayed badly deteriorated kidneys. Hemorrhagic lesions were observed in one case, while the exteriors of the kidneys of several

others showed soft, pale, and pitted areas. We are inclined to believe that the renal involvement, rather than the nutritive failure *per se*, was responsible for the early fatal outcome in so many of our experiments. Nor does it seem likely that this can be attributed primarily to the presence of the free amino acids in the ration since numerous experiments in this laboratory (Rose and Huddlestun, 1927) have shown that the incorporation in casein diets of 1.5 per cent of tyrosine, cystine, or tryptophane does not inhibit growth or induce renal failure in rats of 100 gm. weight.[4] Apparently, the effects outlined above are associated with the gelatin factor.

The thirteen animals which survived the transfer to the gelatin ration showed variable but usually very rapid losses in weight immediately after the dietary change, but subsequently improved. We believe that they also experienced kidney injury, but happened to be better adapted to or more successful in adjusting themselves to the adverse conditions than were their fellows who succumbed. The growth curves secured in three long continued experiments are reproduced in Chart I. The typically rapid decreases in weight on the gelatin ration are seen in the curves of Rats 139 and 169. These animals lost 19 and 26 gm. respectively before recovering from the effects of the ration. Subsequently, slow growth followed by practical maintenance ensued. The experiment upon Rat 140 was slightly different from those upon Rats 139 and 169 in that the former animal was transferred from the normal diet to one containing 30 per cent of gelatin supplemented with seven amino acids (Diet F). After it had remained upon this ration for 40 days, Diet D was substituted. This rat showed a smaller decrease followed by a greater increase in weight than either of the others. When the experiment was discontinued at the expiration of 132 days, a net gain of 32 gm. had been made since the transfer from the casein ration.

In Chart II are reproduced growth curves secured in shorter experiments with Diet D. The rats were litter mates. The data are of interest as illustrations of the wide individual differences

[4] Recent unpublished experiments by G. J. Cox of this laboratory have shown that rats weighing 40 to 50 gm. each are rendered nephritic by cystine administration. Under identical conditions, animals of 100 gm. weight manifest no ill effects.

which one encounters in dietary studies involving the use of gelatin. Four of the animals of this litter (curves not shown) died within 2 weeks after the change to Diet D. Two (Rats

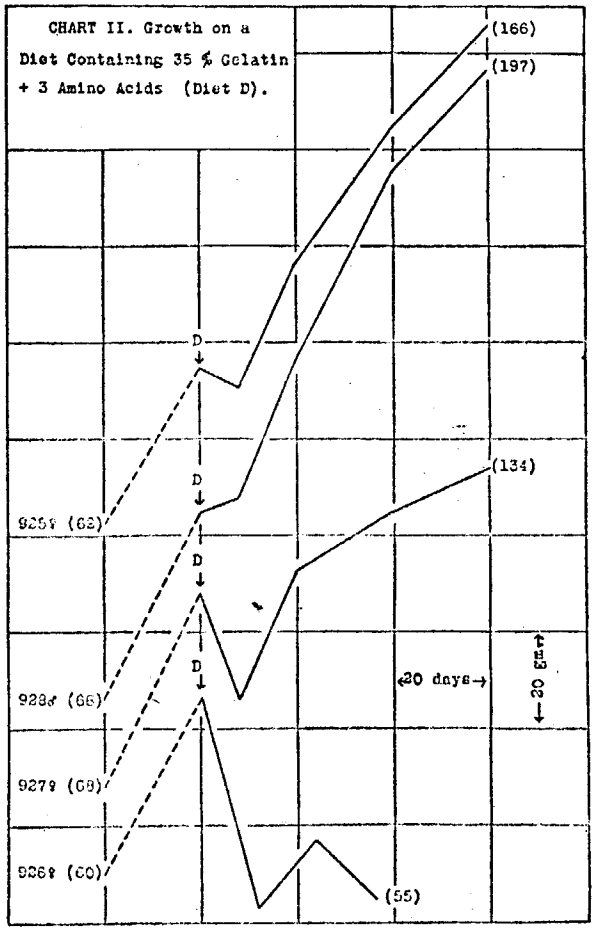

CHART II.

926 and 927, Chart II) underwent profound losses, but Rat 927 partially recovered later. Rats 925 and 928 experienced only slight decreases in body weight followed by almost normal growth. The latter animals, though constituting a small percentage of the

total number employed, are of particular significance in that they prove that very good growth *is possible* when gelatin supplemented with tyrosine, cystine, and tryptophane serves as the sole source of nitrogen except for the small quantity present in 200 mg. of yeast. On the other hand, it is quite evident that Diet D permitted rapid increases in weight in *only two out of a total of twenty-six animals*. This fact led us to try the influence of other proportions of gelatin, and of other supplementing amino acids.

Growth on Gelatin Diets Supplemented with Seven and Eleven Amino Acids.—Inasmuch as tyrosine, cystine, and tryptophane, as stated above, are not the only factors which are low in gelatin diets, attempts were made to improve the quality of the protein by the further addition of histidine, valine, phenylalanine, and glutamic acid. For this purpose, two diets were formulated (Diets F and G, Table I) containing 30 and 25 per cent of gelatin respectively. The data in Table II show that presumably each contained adequate amounts of all of the amino acids definitely known to be present in casein with the exception of isoleucine and hydroxyglutamic acid. Since the latter may be disregarded for reasons already stated, the absence of isoleucine is the only possible deficiency in the rations in so far as our knowledge of the composition of gelatin and casein permits one to judge.

Four rats were placed upon each of these diets after preliminary periods upon Diet A. To our surprise the growth curves were not better than those obtained with Diet D. Of the eight animals two died within 16 days. The experiments upon three others were discontinued after the animals had lost weight very rapidly, and were in a greatly weakened condition. Three recovered from the injurious effects of the gelatin and made slow gains. One of these (Rat 140) was subsequently changed to Diet D and has already been referred to in connection with Chart I. The graph makes evident the fact that the growth of this animal was not altered significantly by the transfer from Diet F containing seven supplementing amino acids to Diet D containing only three. The growth curves of the other animals upon Diets F and G (with the exception of Rat 141) are omitted from the charts as they add nothing to the solution of the problem in question.

Rat 141 was changed from Diet F to Diet H containing 15 per cent of gelatin supplemented with eleven amino acids. An

inspection of Table I reveals the fact that in addition to the seven amino acids included in Diet F, Diet H contained the three leucines and aspartic acid. The growth curve is shown in Chart III. The animal made a slow but fairly consistent gain upon this ration, and when the experiment was discontinued had increased in body weight to the extent of 24 gm. in 72 days. At a later

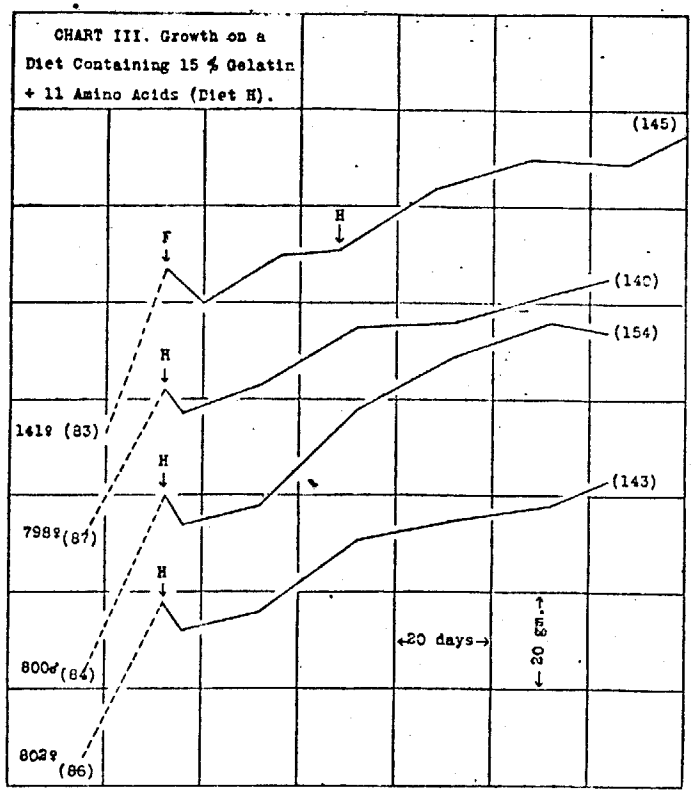

CHART III.

date, three other rats (Rats 798, 800, and 802) of a single litter were transferred directly from the casein ration to Diet H. The growth curves of these animals also are shown in Chart III, and manifest increases in body weight comparable to that attained by Rat 141. The only striking difference between these experiments and those involving the use of the 35 per cent gelatin diet is the fact

that upon the lower level of gelatin none of the animals succumbed. After becoming "adjusted" to Diet H, all of the rats made moderate gains. It should be emphasized that Diet H contained all known amino acids with the exception of hydroxyglutamic acid. Aside from the latter, the only component present in distinctly lower quantity than in an 18 per cent casein diet is lysine. That this was not a limiting factor is made probable by the observation of Osborne and Mendel (1915) that edestin, which contains 1.65 per cent of lysine, supports normal growth when included in the ration at a 15 per cent level. Under such circumstances, 100 gm. of the edestin diet carry only 0.25 gm. of lysine, or less than one-third the amount present in our Diet H.

Attempts to improve the quality of gelatin rations by increasing the proportion of the protein gave even less satisfactory results than were secured with 15 to 35 per cent levels. Of three rats placed upon a diet containing 55 per cent of gelatin supplemented with tyrosine, cystine and tryptophane, two died promptly after very rapid losses in weight, and the third was moribund when the experiment was discontinued. Further studies of this sort seemed very unpromising and were abandoned.

Does Gelatin Exert a Deleterious Action?—In order to determine whether gelatin, or the unusually large quantities of glycine and alanine which it contains, is detrimental to the organism two modifications of Diet A were formulated, one containing 9 per cent of glycine and 6 per cent of racemic alanine (Diet B), and the other containing 35 per cent of gelatin (Diet C). The added components replaced equivalent amounts of carbohydrate without altering the ratios of casein. Thus each ration carried an adequate quantity of protein of suitable quality (casein) in addition to the superfluous nitrogenous materials. After preliminary periods upon Diet A, two rats were placed upon Diet B, and three upon Diet C. The results are shown in Chart IV. All five animals, but especially those upon Diet C, exhibit decreases in growth rates after the transfer from the normal ration. Rat 188, one of those which received the diet containing 35 per cent of gelatin, experienced a loss in weight of 17 gm. in 8 days before becoming adjusted to the new dietary régime. Following recovery, rather rapid growth ensued in all cases. The experiments indicate that neither gelatin nor a mixture of two of its component

amino acids permanently prevents growth when incorporated in an otherwise normal diet. On the other hand, the large proportion of gelatin (35 per cent) may produce a *temporary inhibition*

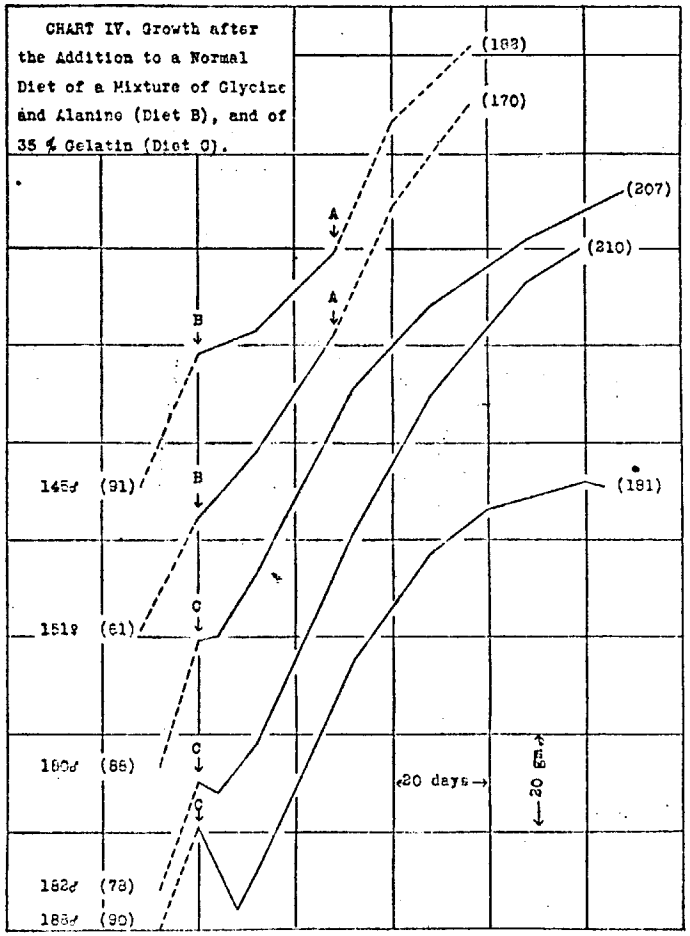

CHART IV.

in growth despite the presence of a suitable amount of adequate protein. This fact, when considered in conjunction with the observation that renal injury frequently followed the administration of the high gelatin diet (Diet D), renders it difficult to

escape the impression that this protein, at the level indicated, may be positively detrimental to as well as negatively inadequate for the organism. Whether this suggested deleterious action is a characteristic of gelatin alone or of other proteins also when taken in excessive amounts (*cf.* Newburgh and associates, 1925) remains to be proved. It should be recalled, however, that Osborne and Mendel (1924) have secured excellent growth, without renal changes other than hypertrophy, with diets containing 95 per cent of protein.

Growth upon Diets of Hydrolyzed Gelatin Supplemented with Three Amino Acids.—In a further attempt to arrive at an explanation for nutritive failure of animals upon gelatin diets, we have reinvestigated the influence of complete hydrolysis of the protein preliminary to its incorporation in the ration. The method of hydrolysis has already been outlined. Two diets were employed. Diet E contained 35 per cent of hydrolyzed gelatin and was exactly analogous in composition to Diet D. Diet I contained 15 per cent of the hydrolyzed protein and is comparable to Diet H. After the usual preliminary periods upon Diet A, three rats were transferred to Diet E and four to Diet I. The results are shown in Chart V. As will be observed, the change from the control to the hydrolyzed gelatin rations was followed by the usual losses in weight. After 12 to 16 days upon Diet E, and 4 to 12 days upon Diet I, recovery appeared to occur, and was succeeded by slow growth until the experiments were discontinued. At the expiration of 80 days upon Diet E, Rat 195 weighed 1 gm. less than when transferred to this ration. The net gains of Rats 192 and 194, upon the same diet, were 10 and 12 gm. respectively.

The four rats upon Diet I made somewhat better net gains. However, this difference is due primarily to the fact that, like the animals upon the unhydrolyzed protein, those which received the higher level of hydrolyzed gelatin usually experienced the greater initial losses in weight.

Although no mortality occurred among the seven animals upon these diets (a fact which may or may not be of significance in connection with the suggested toxicity of unhydrolyzed gelatin), nevertheless the growth curves indicate that hydrolysis occasions very little if any improvement in the growth-promoting properties of this protein (*cf.* Charts I and II with Chart V). It appears

that the inadequacy of gelatin as a source of nitrogen is not to be ascribed to the presence of amino acid linkages which cannot be severed by the digestive fluids of the gastrointestinal tract. In

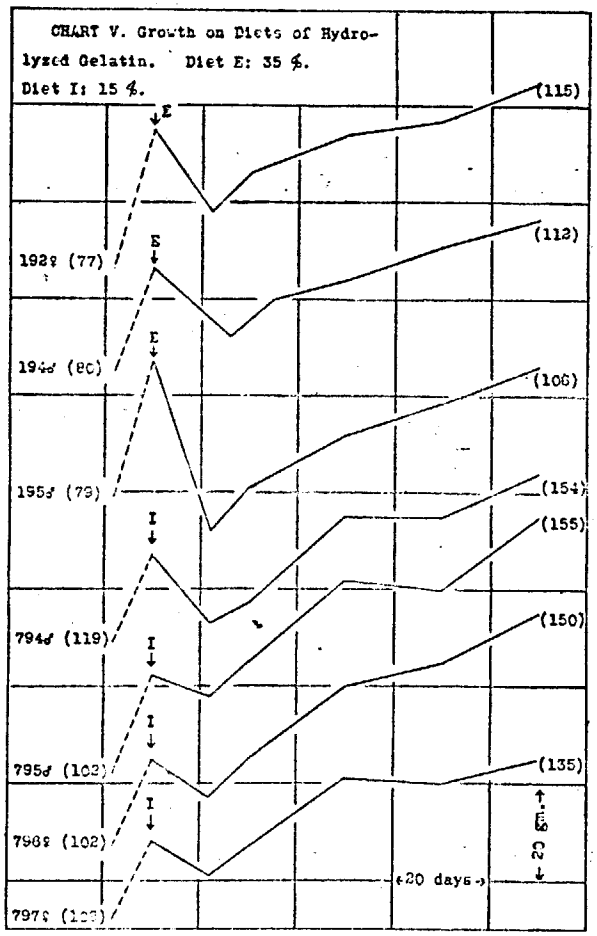

CHART V.

this respect our results are not in accord with those of Totani (1916).

Food Consumption of the Experimental Animals.—In Table III are presented the values representing the average daily food consumption and average daily change in body weight of each rat. For convenience these data are arranged to correspond to the charts.

TABLE III.

Food Consumption and Body Weight Changes of the Experimental Animals.

Chart No.	Rat No.	Diet.	Average daily change in body weight.	Average daily food consumption.	Chart No.	Rat No.	Diet.	Average daily change in body weight.	Average daily food consumption.
			gm.	*gm.*				*gm.*	*gm.*
I	138	A	+2.8	7.8	IV	145	A	+2.3	8.7
		D	−1.9	3.5			B	+0.8	7.0
	139	A	+2.2	7.8			A	+1.5	9.0
		D	0	5.3		151	"	+1.9	5.7
	140	A	+2.5	7.0			B	+1.4	5.4
		F	+0.4	6.7			A	+1.7	8.0
		D	+0.2	6.3		180	"	+3.3	7.6
	150	A	+2.7	5.9			C	+1.1	6.6
		D	−2.3	3.5		182	A	+2.8	6.4
	169	A	+4.9	10.0		—	C	+1.3	6.0
		D	0	5.1		188	A	+2.8	7.5
							C	+0.8	5.6
II	925	A	+1.7	5.9					
		D	+1.2	6.0	V	192	A	+3.5	7.3
	926	A	+1.8	6.9			E	+0.1	4.2
		D	−1.1	3.1		194	A	+2.6	6.0
	927	A	+2.0	6.7			E	+0.1	3.7
		D	+0.4	6.2		195	A	+3.5	7.9
	928	A	+2.0	6.0			E	0	3.8
		D	+1.5	6.5		794	A	+2.3	7.1
							I	+0.2	8.1
III	141	A	+2.8	8.5		795	A	+2.5	8.3
		F	+0.1	6.0			I	+0.[illegible]	7.6
		H	+0.3	5.6		796	A	+2.[illegible]	8.3
	798	A	+1.9	6.4			I	+0.4	7.9
		H	+0.3	7.4		797	A	+2.0	8.3
	800	A	+2.3	6.5			I	+0.2	7.5
		H	+0.4	7.2					
	802	A	+2.0	6.8					
		H	+0.3	7.3					

While admittedly the interpretation of food intakes is sometimes a difficult question, we believe the figures recorded in Table III indicate that the *amount* of food consumed by the individual

animals was not the factor which *determined* whether an increase in weight did or did not occur. It cannot be denied that in some of the experiments a rough correlation between food ingestion and rate of growth appears to exist (*cf.* Rats 138, 150, and 926). On the other hand, with many of the animals the intake upon the gelatin diets was almost as large as upon the casein ration, and in some cases even larger. Rat 140 consumed an average of 7.0 gm. of Diet A daily; and 6.7 and 6.3 gm. respectively of Diets F and D. Thus decreases of approximately 4 and 10 per cent in food ingestion were accompanied by decreases of 84 and 92 per cent in growth rates. Rat 927, with a decrease of a little more than 7 per cent in food intake, showed an 80 per cent diminution in growth rate. Other animals, particularly those upon the lower levels of gelatin (Rats 794, 798, 800, 802), ingested just as much of the experimental rations as of the control diet. Both our experience and the observations of others with food mixtures highly deficient in one or more amino acids have taught us to expect very pronounced failure in appetite when the diets are wanting in some factor or factors essential for the growth process. The high level at which many of our animals consumed the gelatin rations leads us to believe that the supplemented mixtures employed in this investigation were not inadequate *to a pronounced degree* in any necessary amino acid. Probably the diminished food intakes of some of the rats upon the diets containing 35 per cent of gelatin may be attributed to the renal involvement.

It should be emphasized also that average figures, such as are recorded in Table III, may lead to erroneous conclusions in that they do not indicate the *progress* of the decreases in food consumption. This point is of considerable importance in arriving at a correct interpretation. An illustration will serve to make this fact clear. Rat 150, one of those in which the failure in growth might be attributed to the diminished food intake, showed an average daily food consumption of 5.9 gm. on Diet A, and 3.5 gm. upon Diet D (*cf.* Table III). Upon the latter ration the average daily loss in weight (2.3 gm.) was greater than was experienced by any of the other animals. Our records indicate, however, that *during the first 4 days upon Diet D, the daily food ingestion averaged 7.2 gm., or was considerably larger than upon the normal casein diet. During the same 4 day period the animal lost an average of 1.5 gm. daily.* In the three subsequent 4 day periods preceding death, the average daily food consumption amounted to 4.2, 1.8, and 0.8 gm. respectively, accompanied by an increasingly rapid decrease in body weight. In other words, as was observed several years ago by Hopkins (1912) in deficiency studies, the failure in appetite *followed* the failure in growth. For reasons already stated, we are inclined to the belief that renal involvement rather than an amino acid deficiency was the immediate cause of death in many of our experiments, including that upon Rat 150. But whatever may have occasioned the fatal outcome, certainly the initial loss in weight of Rat 150 upon Diet D was not due to insufficient ingestion of food. In a later communication we shall present additional information concerning the relation of food composition to appetite.

SUMMARY.

The above results indicate that diets containing gelatin as the sole protein, at levels of 15 to 55 per cent, are not suitable sources of nitrogen even when supplemented with the amino acids known to be missing, or present in relatively small amounts. About half of the total number of rats placed upon a 35 per cent gelatin diet to which had been added appropriate quantities of cystine, tyrosine, and tryptophane, successfully weathered the change; and after periods of more or less profound "adjustment," maintained body weight or even grew at varying rates.

Reducing the level of gelatin from 35 per cent to 15 per cent of the diet, and incorporating the eleven amino acids which, on the basis of the best available analyses, seem to be the most likely possible sources of deficiency, failed to render the food more satisfactory for growth. It appears that valine and isoleucine, which presumably are absent from gelatin, are not limiting factors in the nutritive properties of this protein. Nor did the increased proportions of phenylalanine, aspartic acid, glutamic acid, and histidine in the supplemented 15 per cent gelatin ration contribute beneficially to the nutritive condition of the animals.

Attempts to improve the quality of gelatin are complicated by what appears to be a deleterious action exerted by the protein when supplied at a 35 per cent level, as manifested by the early fatal outcome of many of the experiments, and the frequent

incidence of severe renal injury. A temporary inhibition in the growth rate of rats upon casein diets may be induced by substituting gelatin for 35 per cent of the carbohydrate.

Diets containing hydrolyzed gelatin supplemented with amino acids do not support growth more successfully than do similar rations containing unhydrolyzed gelatin. Evidently, the peculiar inadequacies of this material for growth purposes are not to be ascribed to the presence in the molecule of linkages which cannot by severed by the digestive mechanism.

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FACTORS WHICH DETERMINE RENAL WEIGHT

XVI. THE NATURE OF THE PROTEIN INTAKE

EATON M. MACKAY AND LOIS LOCKARD MACKAY

The Scripps Metabolic Clinic, La Jolla, California, and Department of Medicine, Stanford University Medical School, San Francisco

ONE FIGURE

(Received for publ : December 13, 1933)

The protein intake has been found to be (Mac Kay, Mac Kay and Addis, '28 a, '28 b; Mac Kay and Mac Kay, '31 a, '31 b) one of the most important of the factors which determine the weight of the kidneys in the albino rat. The relationship between the protein intake and renal weight when casein is the protein, may be expressed for male rats of all ages by the empirical formula,

$$\text{renal weight} = (\text{protein intake} + 2.75) \times 0.0183$$

In the study reported here we were interested in whether or not this relationship was limited to casein or held generally for natural protein products.

Four sources of protein, namely, crude commercial casein, commercial 'egg albumin,' commercial 'blood albumin' and gelatin were used. Except for gelatin these were fed separately. For reasons detailed later gelatin was fed in combination with casein. The method of experiment was identical with that used before (Mac Kay and Mac Kay, '31 a). A group of about twenty male albino rats were fed upon each diet from 26 to 70 days of age. The diets which were used are described in table 1.

Growth on the various diets was interesting. In table 2 are depicted the percentage changes in body weight for each

TABLE 1

INGREDIENT	DIET											
	1A	1B	1C	2A	2B	2C	3A	3B	3C	4A	4B	4C
Casein	16	46	75							16	16	16
Egg albumin				16	46	74						
Blood albumin							16	46	74			
Gelatin										8	30	50
Cornstarch	45	20	1	45	20	2	45	20	2	37	20	5
Lard	15	10	0	15	10	0	15	10	0	15	10	5
Cod liver oil	9	9	9	9	9	9	9	9	9	9	9	9
Salt mixture	4	4	4	4	4	4	4	4	4	4	4	4
Dried yeast	9	9	9	9	9	9	9	9	9	9	9	9
Agar-agar	2	2	2	2	2	2	2	2	2	2	2	2
	Per cent of diet											
Protein	18	43	67	17	41	64	17	42	66	23	44	62
Fat	24	20	11	24	20	11	25	20	11	24	20	16
Carbohydrate	42	20	3	43	22	5	42	21	4	37	20	6
Water	6	6	7	6	6	8	6	6	7	6	6	6
Roughage	5	5	5	5	5	5	5	5	5	5	5	5
Salts	5	6	7	5	6	7	5	6	7	5	5	5
	Per cent of calories											
Protein	16	40	71	15	38	68	15	39	70	20	40	60
Fat	48	41	26	48	42	26	49	42	26	47	41	34
Carbohydrate	36	19	3	37	20	6	36	19	4	33	19	6

TABLE 2

GROUP	WEIGHT 26 DAYS OLD	40 DAYS OLD	50 DAYS OLD	60 DAYS OLD	70 DAYS OLD
	gm.	*Percentage of weight at 26 days*			
1 A	42	193	262	348	426
1 B	42	207	305	393	469
1 C	39	195	277	354	423
2 A	42	195	279	360	444
2 B	44	177	211	245	259
2 C	45	175	187	195	200
3 A	45	191	255	318	317
3 B	48	206	299	393	398
3 C	45	155	213	271	284
4 A	42	190	211	314	328
4 B	52	175	225	295	321
4 C	44	157	169	181	193

group. The increase in body weight was greatest on the casein diets. The 'blood albumin' diets also gave good growth. In both cases the 46 per cent protein diets were optimal. Both the egg albumin (with the exception of the diet with the lowest concentration) and the gelatin diets gave poor growth and the higher the concentration of these proteins the worse the increase in body weight. The significance of the weight changes is borne out by a comparison of the average body lengths (table 3) at death.

Numerous rats receiving the egg white diets developed the 'pink disease' described by Boas ('27) and Findlay and Stern ('29) as occurring when raw egg white (as our product was) is fed to rats. Except for the loss of hair and the thickening and redness of the skin there were no other symptoms and the animals appeared to be in fairly good condition. The more serious symptoms noted by Parsons ('31) were not observed. The affected rats were distributed throughout all three diet groups so that the occurrence of this 'egg white disease' in its mild form was probably without influence upon the results.

We have noted the incorporation of casein in the gelatin diets. Some years ago an attempt was made to feed rats on diets containing gelatin as the sole source of protein. A commercial[1] powdered gelatin was used. Growth was poor but the animals were really in very good shape until they suddenly died in uremia. Further examination disclosed a marked proteinuria before this time and at autopsy the kidneys were twice their normal size and weight. They were firm and gray in color. Histological examination disclosed extreme epithelial degeneration. Later on these findings followed the feeding of a purer gelatin.[2] These brittle sheets were difficult to pulverize and casein was incorporated in one diet to overcome this mechanical difficulty. This diet failed apparently to damage the kidneys. The question of the protection against renal damage from ingested gelatin by the

[1] 'Delft' brand.
[2] 'Gold Label' brand.

TABLE 3

DIET NO.	NUMBER OF RATS	BODY WEIGHT	BODY LENGTH	BODY SURFACE	HEART WEIGHT		LIVER WEIGHT		KIDNEY WEIGHT				RATIO: BODY LENGTH / BODY WEIGHT
					Actual	Per 100 sq. cm. body surface[1]	Actual	Per 100 sq. cm. body surface[1]	Actual	Per 100 sq.cm. body surface[1]			
										Mean	P.E.	Coef. Var.	
		gm.	*mm.*	*sq.cm.*	*mg.*	*mg.*	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*per cent*	
1 A	25	170	192	348	640	184	8.42	2.42	644	185	1.4	6.0	0.89
1 B	21	195	198	381	730	191	8.69	2.28	832	218	2.0	6.9	0.99
1 C	29	159	189	332	644	194	9.07	2.73	964	289	1.8	5.0	0.84
2 A	22	180	194	361	656	183	7.54	2.24	643	178	1.6	6.1	0.93
2 B	24	110	172	259	434	168	5.12	1.98	607	235	2.5	7.6	0.64
2 C	20	86	162	219	347	158	3.95	1.80	585	268	3.0	7.4	0.53
3 A	20	138	186	301	516	171	7.78	2.58	607	202	2.5	7.6	0.74
3 B	22	197	200	384	676	176	9.92	2.58	1054	262	2.5	6.6	0.98
3 C	20	133	181	295	533	181	8.52	2.89	905	307	2.3	4.9	0.74
4 A	20	129	182	289	503	174	5.91	2.04	584	201	3.4	11.9	0.71
4 B	22	173	193	351	648	184	9.16	2.61	1137	322	4.8	10.4	0.90
4 C	21	109	170	258	433	168	6.58	2.55	1148	433	5.3	8.2	0.64

[1] Body surface calculated by the formula of Carman and Mitchell ('26)—$S = 11.36\ W\ 2/3$.

addition of casein to the diet is now the subject of another investigation. In the present experiments the kidneys appeared normal when the rats were killed. Likewise there was no evidence of any renal pathology upon any of the other diets.

The data for the twelve experiments have been summarized in table 3. Among the incidental measurements the small livers in the egg albumin fed rats, particularly on the higher intakes, are noteworthy. On every diet the kidneys became larger when the amount of protein was increased. The kidney weights produced by the gelatin diets are far beyond any of the others and although this may be a specific physiological effect of the gelatin we are prone to believe that it is an early stage in the renal damage from gelatin that has been mentioned. True, the blood urea concentrations of these gelatin fed rats were not abnormal and by routine histological methods no changes were noted in their kidneys. However, the swelling of the epithelial cells which eventually goes on to necrosis is very difficult to discern in the early stages.

In table 4 under the 'A' columns the kidney weights as observed in relation to the body surface calculated by the formula of Carman and Mitchell ('26) have been compared with those calculated from the average protein intake (in relation to the same body surface figure) during the last 10 days of each experiment by means of the casein formula:

$$\text{renal weight} = (\text{protein intake} + 2.75) \div 0.0183$$

Both the egg albumin and the blood albumin diets apparently produced kidneys significantly larger than would be expected from the protein intakes. This extra enlargement may be a pseudo-enlargement due to our method of calculating body surface. Carman and Mitchell's formula was perfectly satisfactory for rats in good condition or where the body surface simply served as a reference standard for the kidney weight and food ingredients in a given group of rats as in earlier studies in this series. When it is desired to compare the relationship with the standard in diverse groups as in the present study, it may not be satisfactory. In our calculations

TABLE 4

GROUP NO.	PROTEIN	INTAKE PER 100 SQ.CM. BODY SURFACE PER DAY				MILLIGRAMS KIDNEY PER 100 SQ.CM. BODY SURFACE				DEVIATION OF OBSERVED FROM CALCULATED				RATIO: KIDNEY WEIGHT / HEART WEIGHT
		Food		Protein		Observed		Calculated						
		A	B	A	B	A	B	A	B	A	B	B^1	B^2	
		gm.	gm.	gm.	gm.					per cent	per cent	per cent	per cent	
1 A	Casein	3.30	4.40	0.61	0.79	185	240	183	193	1.8	24.3			1.01
1 B	Casein	3.02	4.06	1.30	1.74	218	293	221	245	—1.4	16.4	17.6	0.0	1.14
1 C	Casein	3.71	4.76	2.49	3.19	289	370	286	325	1.5	12.2			1.50
2 A	Egg albumin	2.59	3.35	0.44	0.57	178	232	174	181	2.2	22.0			0.98
2 B	Egg albumin	2.32	2.83	0.93	1.15	235	284	202	213	14.0	25.0	19.9	2.3	1.40
2 C	Egg albumin	2.93	3.44	1.89	2.22	268	311	254	271	5.2	12.8			1.68
3 A	Blood albumin	2.14	2.62	0.36	0.44	202	247	175	174	7.8	42.0			1.18
3 B	Blood albumin	3.09	4.04	1.30	1.70	262	366	222	243	15.3	48.2	40.9	23.3	1.56
3 C	Blood albumin	3.68	4.54	2.44	3.04	307	420	284	317	10.8	32.5			1.70
4 A	Gelatin	2.53	3.07	0.60	0.73	201	246	183	190	9.0	29.5			1.16
4 B	Gelatin	3.29	4.26	1.43	1.85	322	418	229	251	28.8	66.6	56.0	38.4	1.76
4 C	Gelatin	3.42	3.67	2.19	2.35	443	478	270	278	39.2	72.0			2.65

REMARKS: A = Body surface calculated by the formula of Carman and Mitchell ('26). B = Body surface calculated by the formula of Lee and Clark ('29). B^1 = Averages for the three groups on each type of protein. B^2 = Averages for the three groups on each type of protein adjusted to the basis of zero deviation of observed from calculated.

body surface depends solely on body weight and the body surface: body weight relationship may be changed by variations in the amount of body fat which seemed to exist or severe stunting which is evidenced by the depressed body length—

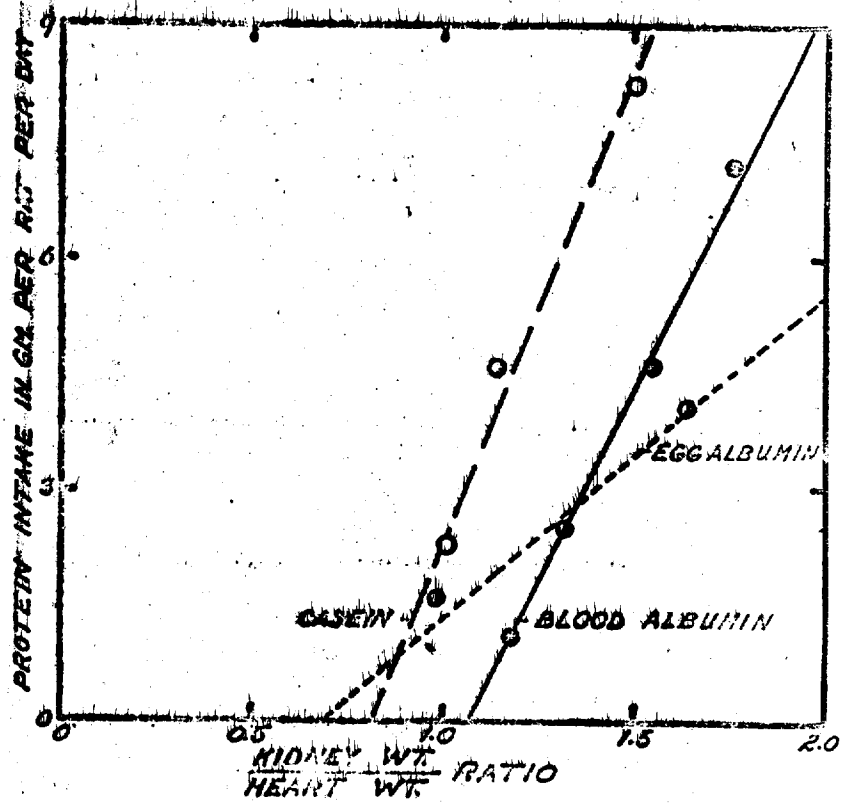

Figure 1

body weight ratios (Jackson, '24) in table 3. Lee and Clark ('29), in their rat body surface formula no. 4, have incorporated the nutritional correction factor used by Cowgill and Drabkin ('27) in their body surface formula for the dog. This formula makes a good correction for variations in body fat and the effects of severe stunting. It is:

$$S = 10.76\, W^{0.67} \left(0.310 + \frac{W^{1/3}}{L}\right)$$

We have recalculated our data on this basis in table 4 under the 'B' columns. Since our protein-renal weight formula is based upon a different body surface even the casein diet groups show a definite deviation of the observed from the calculated kidney weights. When the figures are adjusted to a basis of no deviation for the casein groups we find that egg albumin has the same influence on renal weight as casein. Blood albumin appears to have a significantly greater effect upon the weight of the kidneys than either of the other two proteins.

There is one more possible manner of interpreting the data presented here. In another paper of this series will be considered the use of heart weight in place of body size measurements as a reference standard for the weight of the kidneys. Under some conditions it is useless while in others it seems to have considerable value. The present case is one of the latter. In figure 1 the ratio kidney weight ÷ heart weight has been charted against the protein intake per rat per day. The usual linear relationship is obvious although they diverge significantly from the casein formula. Blood albumin has a slightly greater and egg albumin a far greater influence on renal weight than protein fed as casein. This result is supported in general by the calculations on our old body surface figures.

SUMMARY

In male albino rats an increased protein intake in the form of egg albumin or blood albumin has a greater influence upon renal weight than protein in the form of casein. Gelatin produces a much larger increase in renal weight than any other protein source which has been fed. There is evidence that this may be of a pathological rather than a physiological nature.

ADDENDUM

Shortly after this manuscript was submitted for publication a paper by Wilson (Wilson, H. E. C., Biochem. J., 1933, vol. 27, p. 1348) on the same subject appeared. He reports that gelatin produces a much greater increase in renal weight than other proteins, a finding which we confirm. Wilson does not suggest that this may be of a pathological nature as we believe. His conclusion that glycine and glutamic acid nitrogen have practically the same influence as casein nitrogen coincides with our findi[illegible] (Mac Kay, E. M., J. Nutrition, 1933, vol. 6, p. 157). This author's findings in connection with the addition of phosphate to the diet must be modified by the fact that phosphate renal enlargement is definitely (Mac Kay, E. M., and Oliver, J., Proc. Soc. Exp. Biol. and Med., 1930, vol. 28, p. 321) pathological.

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J. A. M. A. 115:1439-1441, 1940

FAILURE OF GELATIN OR AMINOACETIC ACID TO INCREASE THE WORK ABILITY

OF INDIVIDUAL NORMAL HUMAN MUSCLES

GEORGE L. MAISON, M.D.
DETROIT

The observation that gelatin was an effective agent in postponing human fatigue was made by Ray and his co-workers[1] in a now widely publicized paper which appeared in February 1939. To date no confirmation of this action of gelatin has appeared. Ray theorized that gelatin postponed fatigue by an action on the muscles themselves. He reasoned that creatine was a substance important in muscle chemistry, one of whose precursors in the body may be aminoacetic acid (glycocoll, glycine). Aminoacetic acid makes up 25 per cent by weight of gelatin. An added supply of aminoacetic acid was thought to increase the store of creatine in the muscles. If this is the mechanism by which gelatin acts, the effect should show up in increased work ability of a single human muscle during a period of gelatin or aminoacetic acid ingestion. Further, if gelatin has any action on fatigue its ingestion should increase the ability of individual muscles to do voluntary work, since the work of a single muscle requires cooperation of the circulatory, muscular and nervous systems just as does action of the body as a whole.

Experiments were therefore undertaken to test the work ability of a single human muscle before, during and after the ingestion of gelatin or aminoacetic acid.

METHOD

Male subjects fatigued the extensor digitorum communis muscle daily by doing work at a uniform rate. (The load was constant and the length and rate of contraction were constant.) Fatigue was defined as that condition in which the subject could not maintain the rate of work. In order to differentiate the ability of the neuromuscular system alone from the ability of the combined neuromuscular and circulatory systems, the right arm of each subject did its daily work without blood supply whereas the left arm retained its circulation during work.

A weight ergograph was designed especially for the extensor of the fingers.[2] The ergograph recorded on automatic counters the total distance through which the weight was moved and the total number of individual strokes made. A buzzer was included in the machine and contacts were so arranged that when the load was lifted to a set height the buzzer sounded. The subject was trained to lift the weights until the buzzer sounded and then to relax. Thus the strokes made were of nearly uniform length, about 6 cm. This is a comfortable stroke for the extensor if the metacarpophalangeal joint is used as a fulcrum, and the load is applied over the distal halves of the second phalanges of the fore, middle and ring fingers.

The definition of fatigue mentioned was made possible by the buzzer. The subject makes one contraction per second. When he can no longer ring the buzzer in the allotted time he ceases to work.

The blood supply to the muscle of the right arm was interrupted during its work by means of a sphygmomanometer cuff

From the Department of Physiology, St. Louis University School of Medicine.

1. Ray, G. B.; Johnson, J. R., and Taylor, M. M.: Effect of Gelatin on Muscular Fatigue, Proc. Soc. Exper. Biol. & Med. **40**: 157 (Feb.) 1939.

2. Construction details of the ergograph will be described in a subsequent paper.

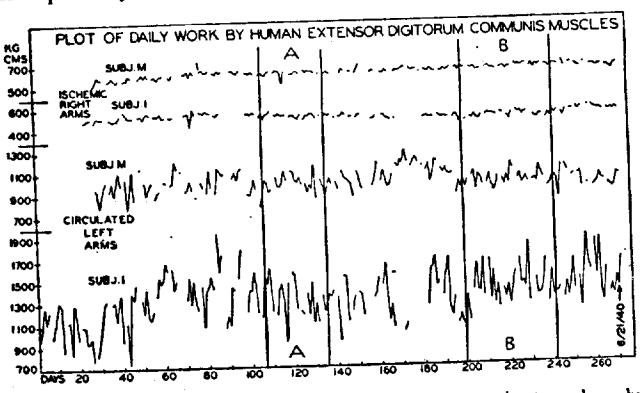
instantaneously inflated to a pressure of 240 mm. of mercury from a pressure reservoir of about 5 gallon capacity. Muscles worked under this condition are hereafter referred to as "ischemic."

RESULTS

The experiments were divided into two groups: The first group shows the effect of gelatin on muscles in the process of rapid training. This is analogous to Ray's procedure, since his subjects had trained for only thirty days before gelatin was taken.

The six subjects included four medical students between 23 and 25 years of age, one mechanician aged 45 and one instructor aged 28 years. All the subjects were in excellent health with normal blood pressures. All work was done with an 800 Gm. load, a 6 cm. stroke and a rate of one contraction per second.

In March 1939, when the experiments began, the circulated (left) muscles were capable of only 300 to 900 Kg. cm. of work. After five weeks of training this ability was doubled or trebled and was still increasing rapidly. Each subject then took 60 Gm. of granular unflavored gelatin [3] in fruit juice, soda water or plain water (divided into two doses) each day. This was the quantity of gelatin suggested and used by Ray.[1]



Each point plotted is the total work in kilogram centimeters done by the extensor digitorum communis muscle on a given day. *A*, aminoacetic acid 15 Gm. daily; *B*, gelatin 60 Gm. daily. The points are connected by lines when the work days follow one another immediately. When work was not done on a particular day, no point is plotted and the line is interrupted. When a subject had to be absent more than two days, a portable ergograph was used each day to maintain training but no point was plotted on the graph. Both subjects had trained their muscles by daily work for more than six months before this graph begins. A fixed rate of work was obtained by using a constant load (1,400 Gm. on circulated muscles, 1,100 Gm. on ischemic muscles), a constant stroke of 6 cm., a constant rate of one stroke per second. Thus the essential variable is the time the subject can continue to work.

Two of the medical students were forced to discontinue gelatin within ten days, owing to apparent gastrointestinal intolerance. The complaint was nausea and a sensation of distention. The remaining four subjects continued the regimen for from twenty-one to thirty-one days. All six subjects continued working daily.

During the gelatin period the ability of all six circulated muscles continued to increase markedly. By the end of May, four weeks after all gelatin was stopped, each of the subjects was able to carry the 800 Gm. load for an indefinite period without fatigue (i. e. steady state). The training during the ingestion of gelatin was indistinguishable from that which occurred before the gelatin or after the gelatin was discontinued.

The ischemic (right) muscle in each case reached maximum ability after three or four weeks of daily work. It showed no further change in its ability (from 200 to 600 Kg. cm.) either as a result of daily work or as a result of the ingestion of gelatin.

3. Swift's "Velvetex" gelatin, purchased from the local office of Swift & Co.

The conclusions derived from this group of experiments were that:

1. Gelatin had no influence on the ability of human muscle working without blood supply.

2. No improvement in training of the circulated muscles was effected by gelatin. This did not prove that gelatin had no effect, as the muscles might have been training so rapidly that the effect of the gelatin would not have been visible.

3. The omission of gelatin from the daily regimen did not decrease the ability of those muscles which had been receiving it.

The second group of experiments was to determine what effect gelatin would have on the thoroughly trained muscle. Only two subjects (mechanician and instructor) were available continuously for the full year required for thorough training. These continued to work daily, using a load of 1,100 Gm. on both the circulated and the ischemic muscles. By the end of September and October respectively the ability of the circulated muscle had mounted from 800 to 1,100 Kg. cm. in June to 2,000 to 4,000 Kg. cm. or very close to steady state ability. The load was therefore increased again, this time on the circulated muscle alone, to 1,400 Gm. The ischemic muscles continued to work with a load of 1,100 Gm. The accompanying graph shows the entire subsequent course.

As can be noted, the ability of both the circulated and the ischemic muscles remained virtually constant for the first three months. Aminoacetic acid [4] 15 Gm. a day was then ingested by each subject for thirty days. This amount was used because it approximates the aminoacetic acid present in 60 Gm. of gelatin. As the graph shows, there was no improvement in work ability during or after the ingestion of aminoacetic acid. Also there was no decline in power after the aminoacetic acid was stopped.

After another two months had elapsed the subjects were given gelatin [5] again at the rate of 60 Gm. a day. After forty-two days on this regimen each subject had taken about 5 pounds of gelatin. The graph shows that neither the circulated nor the ischemic muscles showed any improvement during the ingestion or any decrease of ability after the gelatin was discontinued.

COMMENT

The obvious criticism of these experiments is that experimental work on only two subjects is not often acceptable. It must be remembered, however, that the data from one well trained muscle are more apt to be valid than the data from a dozen incompletely trained ones. Unfortunately, it is difficult to find subjects who are willing to train their muscles by daily work over a period of many months. The total data here presented show that in six trials the ability of the ischemic human muscle was uninfluenced by the ingestion of gelatin. This is probably a measure of the inherent ability of the neuromuscular unit itself irrespective of changes in the circulation. The data show that in six trials no decrease in ability attended the withdrawal of gelatin. Ray reported that the withdrawal was accompanied by a decrease in the summated ability of the many muscles used in operating a bicycle ergometer. The data show conclusively in two thoroughly trained subjects that the ability of the single circulated muscle was not increased by the ingestion of gelatin. This is

4. Glycocoll-Squibb was the aminoacetic acid used in this experiment.

5. Knox's unflavored "sparkling" gelatin was purchased for this purpose from a local distributor.

probably a measure of the ability of the combined neuromuscular and cardiovascular systems.

Most experiments dealing with voluntary fatigue of human muscles are open to the criticism that psychic changes can affect them. Is it probable that the subjects used were biased and consciously or unconsciously decreased their effort during the ingestion of gelatin? When a subject works as hard as he can, though only with a single muscle, certain changes occur. The blood pressure rises markedly, the face flushes and the jaw sets, and new associated movements occur and spread with the rise of the effort required to keep going. Such signs were always seen in these experiments. On a number of occasions the blood pressure was taken on the free arm during the work bout. An increase of 60 to 80 mm. of mercury systolic pressure was always found toward the end of the work. I have no doubt that each of the subjects made the maximum effort of which he was capable on every work occasion.

What influence do the present data have on the hypothesis that gelatin will postpone the occurrence of fatigue in work involving the whole body of normal persons? It is conceivable, though unlikely, that the ability of a single muscle might be differently affected by a given procedure than the ability of the body as a whole. However, the only objective data supporting the increased work ability in normal persons due to gelatin are those of Ray and his co-workers.[1] These authors trained their subjects for only two to four weeks before starting the gelatin. They found the gelatin effective only in male subjects and reported a decrease in work ability within ten days after the ingestion of gelatin stopped. Hellebrandt, Rork and Brogdon [6] tested the influence of gelatin on the ability of female subjects by essentially the same method as that used by Ray. The Wisconsin investigators found no increase in the ability of their women subjects but they pointed out that their subjects could easily maintain a work output two or three times the maximum attained by Ray's male subjects; that the curves presented by Ray could be explained by the process of training without any action of gelatin. Ray's subjects were obviously incompletely trained. They were working daily to complete bodily fatigue. It is unlikely that such fatigue is completely overcome in twenty-four hours. Thus two simultaneous processes might be occurring in these subjects, training tending to increase the work ability and accumulating fatigue (overexercising) tending to decrease the work ability. At first the former process might predominate and ability increase. Later, a plateau might occur and later still staleness would predominate and the work ability decrease. If the gelatin was administered early and discontinued as the second process gains dominance, a curve such as Ray's would result. While all this is theoretical it makes even more imperative the statement of the Council on Foods: [7] "It should be obvious that observations such as those reported [by Ray, Taylor and Johnson] on six men cannot be applied to all men until experimental confirmation is available from other laboratories."

The failure of aminoacetic acid to influence the work ability of the two thoroughly trained subjects suggests that the mechanism which makes aminoacetic acid effective in myasthenia gravis does not operate in normal subjects. Since the average American dietary of protein is usually quite adequate, there is no reason to suppose that a supplement of the amino acid aminoacetic acid would be required for efficient function in normal persons.

6. Hellebrandt, F. A.; Rork, R., and Brogdon, E.: Effect of Gelatin on Power of Women to Perform Maximal Anaerobic Work, Proc. Soc. Exper. Biol. & Med. **43**: 629 (April) 1940.

7. Knox Gelatin Omitted from the List of Accepted Foods, Report of Council on Foods, J. A. M. A. **113**: 127 (July 8) 1939.

SUMMARY

In two subjects trained for one year, neither aminoacetic acid 15 Gm. a day for thirty days nor Gelatin (Knox) 60 Gm. a day for forty days increased the work ability of the extensor digitorum communis muscles working with or without blood supply.

In four subjects in the midst of their training period the ingestion of gelatin for from twenty to thirty days produced no obvious difference between the rate of training of those taking gelatin and the rate of training of controls without gelatin.

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UTILIZATION OF GELATIN, CASEIN AND ZEIN BY ADULT RATS [1]

INEZ D. MASON AND LEROY S. PALMER
Section of Animal Nutrition, Division of Agricultural Biochemistry, University of Minnesota, St. Paul

(Received for publication August 25, 1934)

The relative utilization of gelatin, zein and casein was studied by McCollum ('12) in experiments on the pig. At that time relatively little was known of the mineral and vitamin requirements of animals. The present investigation was undertaken to determine the relative value of these same proteins in the nutrition of the adult rat in the light of the present knowledge of accessory needs. Since McCollum's aim was to determine the influence of a given protein in repairing waste due to endogenous catabolism, he wished to avoid feeding above the endogenous level, and took the urinary nitrogen alone as an index of the nitrogen to be fed. He regarded the fecal nitrogen as accidental in character and independent of the essential tissue metabolism, but of use in determining the thoroughness of absorption.

It is generally recognized that the biological value of a protein for maintenance is underestimated if it is fed above the endogenous level, since a portion of it would then be used for energy requirements. In the case of young growing rats, Mitchell ('23–'24 b) found that corn proteins fed at a 5 per cent level had a biological value of 72, while a 10 per cent level

[1] Part of the data reported in this paper are taken from a thesis presented by Inez D. Mason in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, 1933. The remainder were secured with the aid of a fellowship granted by the Graduate School of the same university. The original data are on file in the Division of Biochemistry, University of Minnesota.

which permitted some growth gave a value of only 60. Whether or not the values are constant for varying levels below that required for maintenance is still a disputed question. Mitchell and Hamilton ('29) consider it logical to suppose that dietary amino acids are oxidized in the tissue ". . . . in proportion to their relative concentration in the intracellular fluids with reference to the other organic nutrients . . . . and also in proportion to their relative oxidizability as compared with glucose, fats, lipoids, etc." Hence the biological value of a protein may be expected to increase as its percentage in the diet decreases.

Widely varying results have been given for the utilization of gelatin when fed at approximately the endogenous level. McCollum ('12), with pigs, found a utilization of 50 to 60 per cent, while Terroine, Fleuret and Stricker ('23), also with pigs, gave nitrogen balance data from which a biological value of 33 may be calculated. Mitchell ('23–'24 b), with rats, got results ranging from 0 to 44.

Casein was given a value of 70 by Thomas ('09), 71 by Mitchell ('23–'24 b) and 76 by Fixsen and Jackson ('32). Rajzmann ('33) found a biological value of 77, 78 and 81 in experiments with the rat, the pig and the rabbit, respectively.

Like gelatin, zein has given variable results. Calculations from Henriques' ('09) nitrogen balance data give a value of 30, while McCollum ('12) records a value of 80.

In this work we used Mitchell's ('23–'24 a) method for determining biological values.

EXPERIMENTAL

Preparations

Since the food intake decreases after a few days on a 'nitrogen free' diet, a study was made of palatability. The inclusion of sugar and a change of fat helped the appetite. The diet chosen contained tapioca dextrin 60 per cent, sugar 19 per cent, Osborne and Mendel's ('19) salt mixture 4 per cent, agar 2 per cent, butter fat 13 per cent, and cod liver oil 2 per cent. This was alternated with a similar diet containing

slightly more lard in the place of butter fat and cod liver oil, and with slightly less sugar. These diets contained 0.22 mg. nitrogen per gram food. Allowance was made for the difference in the energy contents of the two diets in providing for the calculated energy requirements of the different animals.

In order to provide a vitamin supplement low in nitrogen, yeast was extracted as follows:

Five hundred grams of Fleischmann's whole dry yeast were extracted with 50 per cent alcohol three times, the total amount of alcohol used being 5 liters. In each extraction the mixture was heated with constant stirring, to the boiling point, then filtered through a Büchner funnel. The combined filtrates were added to 220 gm. of dextrin, evaporated, ground and

TABLE 1

Nitrogen and water content of casein, zein and gelatin

	WATER	NITROGEN
	per cent	*per cent*
Casein	4.85	15.73
Gelatin	11.11	17.58
Zein	4.37	15.94

made into tablets. Each tablet contained the extract from 500 mg. of yeast and weighed 800 mg. The nitrogen content of these tablets prepared from various yeast extracts was 6.2, 8.2 and 5.5 mg. per tablet, respectively.

The casein was prepared by Palmer and Kennedy ('27), and the zein by Mason and Palmer ('34). A commercial sample of gelatin was used.

Analyses

Samples of these three proteins were analyzed for moisture by drying for 5 hours in a vacuum oven at 100°C. and for total nitrogen by the micro Kjeldahl method. The results are shown in table 1.

All analyses for total nitrogen were made with the Cavett ('31) apparatus, using the regular Kjeldahl method.

The feces were prepared for analysis by air drying, removing loose hair, and grinding. In some cases a large amount of hair was evident after the grinding. This was shaken to the top and the greater portion removed with forceps. Before weighing samples for analysis, the ground feces were again shaken and the top layer containing some hair was brushed aside so that the samples were taken from the lower layer without hair.

In determining the nitrogen distribution in the proteins a few changes were made in Cavett's ('32) modification of the

TABLE 2

Nitrogen distribution of casein, gelatin, zein and the alcohol soluble fecal extract after feeding zein as determined by the Van Slyke analysis. Expressed in per cent of total nitrogen

	CASEIN	GELATIN	ZEIN	ALCOHOLIC FECAL EXTRACT
	per cent	*per cent*	*per cent*	*per cent*
Ammonia N	10.30	3.86	19.68	21.39
Humin N	1.89	0.52	1.28	2.23
Arginine N	7.82	15.17	3.78	2.77
Cystine N	0.26	0.21	0.17	0.23
Histidine N	7.54	4.33	3.26	2.00
Lysine N	9.80	6.85	1.24	2.38
Total N of bases	25.42	26.56	8.45	7.38
Filtrate from bases:				
Amino N	57.71	53.06	62.40	64.24
Non-amino N	5.00	17.12	7.06	4.63
Total recovery	100.32	101.12	98.87	99.87

Van Slyke method. The hydrolysis, distillation of hydrochloric acid and determination of ammonia nitrogen and total humin nitrogen were made in the Patton ('32) Kjeldahl-Claissen flask. Twice the quantity of material was taken throughout, the phosphotungstate separation was made by filtration and the histidine fraction obtained by calculation. The results are shown in table 2.

Experimental technic

Adult male rats weighing 350 to 500 gm. were selected at random from various laboratory strains.

The metabolism cages were made by inverting in a rack a battery of bottle tops serving as funnels. In each funnel was placed a round cage with a removable bottom. A water bottle was inserted at the side, and from the top hung a covered feed cup of the McCollum type. A copper wire screen was placed under the cage bottom in the funnel to retain the feces, while underneath the funnel was a beaker to receive the urine. The copper mesh, funnel and beaker were disinfected with an alcoholic solution of thymol to prevent decomposition of the urine, and as an added precaution against loss of ammonia 5 cc. of a 3 per cent sulfuric acid solution were placed in each beaker.

The plan of the experiment was to use a 3-day preliminary period during which the basal ration was fed from the hanging feed cups, but no collections were made. This was followed by a 2-day period in the metabolism cages. At the end of this period urine and feces were collected and analyzed. The animals were put into other cages and given in addition to the previous diet about 0.4 gm. of the protein to be used in the following period. After analyzing the urine, the amount of the desired protein which contained the same amount of nitrogen was calculated. During each of the three succeeding days each rat was given the amount of nitrogen as protein which had been excreted in urine daily by the same individual during the previous period. At the end of 3 days, collection of urine and feces was again made, and protein feeding was discontinued. After a day's interval the rats were returned to the metabolism cages for a final 2-day 'nitrogen free' feeding period, after which collections and analyses were again made. A yeast extract tablet soaked in cod liver oil was given each rat daily. The animals were kept in individual cages at all times during the experiment.

The accuracy of the collection of urine was tested by trickling a known volume of urine over each of two cage bottoms at intervals during a 3-day period. Thirty cubic centimeters of urine were used in each cage, and at the end of the period the collection was made by thoroughly washing the cage bot-

tom, funnel and copper wire netting, using distilled water and a small brush. About 200 cc. of water were used in each case. This was made up to 250 cc., filtered, and 5 cc. duplicates taken for analysis. Analysis of the original specimen showed a loss of only about 3 per cent in collection.

Since the food intake progressively decreases on a diet very low in nitrogen, it seemed desirable to make the preliminary period as short as possible in the hope of securing an adequate energy intake throughout the experiment. In order to determine the length of time required to bring the urinary nitrogen to the endogenous level, experiments were conducted on six rats fed on a 'nitrogen free' diet. The urine was collected and analyzed daily. Only a very slight decrease was found after the third day, so a 3-day preliminary period was chosen.

It seemed desirable to feed the protein separately, since each rat was given a different amount, and it was impossible to predict from day to day just how much of the ration would be eaten. The problem was to feed the protein in such a form that it would be palatable and not easily scattered, and so that any which was thrown out could be recovered for analysis. With gelatin these conditions were met by adding 1 gm. of sucrose to each day's protein ration and then making it into a stiff gel with water. Casein and zein presented difficulties as they were easily scattered and were unpalatable in their highly purified condition. These proteins were incorporated in a sweetened agar gel. The casein was mixed with 10 cc. of a 1 per cent agar sol containing 1 gm. of sucrose and allowed to gel. Zein did not mix well with the sol, so it was beaten into the gel with a piece of copper wire. Fresh protein ration was prepared each day and at the end of 3 days all material left in the dishes as well as any which was scattered was collected and analyzed for nitrogen.

Although the basal diet contained 2 per cent of agar, the increase of this substance during the protein feeding period raised the question as to whether the increase of indigestible material in the diet altered the amount of fecal nitrogen eliminated. Mendel and Fine ('12) found that the addition of agar

plus bone ash to the diet of a dog increased the nitrogen output. Mitchell ('23–'24 a) thought that the addition of indigestible matter in the form of filter paper was not of great importance in determining biological values, although he found an appreciable increase in metabolic fecal nitrogen.

In order to test the effect of this additional agar, seven rats were carried through the regular experimental periods, except that protein was not given. They were given the regular amount of extra agar as a sweetened gel during the interval and the 3-day collection period. There was no appreciable change in the amount of nitrogen eliminated per gram of food eaten.

Nitrogen balance data

Twenty nitrogen balance experiments were carried out with casein, eighteen with gelatin and twenty with zein. The relative utilization was calculated by the McCollum ('12) method in which the fecal nitrogen is not included. The results are given in table 3, the average values being 74 for casein, 23 for gelatin and 57 for zein.

The nitrogen balance data were studied to determine significant correlations and possible errors inherent in the method.

The increase in urinary nitrogen during the growth of the rat was shown by Hatai ('05). In the present experiment only adult males were used, and since the differences in weight were partly due to fat, the question arose whether there would be a larger excretion of urinary nitrogen in the heavier animals. Table 4, no. 1 shows a highly significant correlation between the weight of the animal and its endogenous urinary nitrogen even with adult rats ranging in weight from 341 to 504 gm. The tests for significance are taken from the table of Wallace and Snedecor ('31).

The following formulae were used:

$$\sigma x = \frac{\sqrt{\Sigma x^2 - (\Sigma x)\,\bar{x}}}{\sqrt{n-1}} \qquad r = \frac{\frac{\Sigma xy - (\Sigma x)\,\bar{y}}{n-1}}{\sigma x\, \sigma y} \qquad t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

During the experiments each animal lost weight. This loss was partly due to a decrease in the contents of the alimentary

canal. Several rats were weighed the day after the completion of the experiment having had stock food for 24 hours. The average gain was 11 gm. The other fraction was probably chiefly loss of fat. This was only gradually regained. In

TABLE 3

Percentage retention of casein, zein and gelatin calculated by McCollum's method

RAT NO.	CASEIN	ZEIN	GELATIN
11		60.7	20.1
13			25.5
14			23.1
16	76.0		22.9
17	78.8	69.0	31.8
17		74.3	23.3
20	67.6	51.5	32.8
27	84.6		35.5
27			16.7
29	73.9	50.9	
30			17.8
33	76.3	47.3	17.1
34			17.0
35	75.0	50.0	
36	82.7		
37	70.7		40.4
38			30.3
39	76.3	49.2	22.7
41	77.7		15.1
42	74.5	58.5	14.9
45	78.2		
50	80.3		
51		72.8	
52	77.0	51.9	
52		72.9	
55	64.0		
56	72.5	59.3	
59	71.7	64.0	
60	70.0	58.9	
61	60.1	47.8	16.1
61		57.1	
63		44.1	
64		46.6	
67		59.9	
Mean	74.4	57.3	23.4
Standard deviation	5.95	9.41	7.69

TABLE 4

Summary of nitrogen balance data

FACTORS CORRELATED	n	$\bar{x}$	$\bar{y}$	σx	σy	r	t
1. x: Weight of rat y: Endogenous urinary N	30	399.8	71.4	38.96	9.25	+ 0.482	2.91
2. x: Percentage retention gelatin N y: Percentage retention zein N	7	21.6	55.9	6.59	8.46	+ 0.213	0.486
3. x: Percentage retention gelatin N y: Percentage retention casein N	10	23.6	74.3	8.41	6.74	— 0.036	0.010
4. x: Percentage retention zein N y: Percentage retention casein N	12	56.4	72.9	7.34	5.06	+ 0.255	0.834
5. x: Mg. N in yeast and basal diet y: Percentage retention zein N	20	8.5	57.3	1.78	9.41	— 0.364	1.660
6. x: Mg. N in yeast and basal diet y: Percentage retention gelatin N	18	7.9	23.4	0.81	7.69	+ 0.268	1.111
7. x: Ingested zein N as percentage endogenous urinary N y: Percentage retention zein N	20	83.4	57.3	17.54	9.41	— 0.444	2.101
8. x: Ingested gelatin N as percentage endogenous urinary N y: Percentage retention gelatin N	18	95.2	23.4	23.41	7.69	— 0.477	2.168
9. x: Ingested casein N as percentage endogenous urinary N y: Percentage retention casein N	20	105.2	74.4	9.61	5.95	— 0.318	1.424
10. x: Percentage ingested zein y: Percentage retention zein N	20	4.24	57.3	1.98	9.41	— 0.344	1.555
11. x: Percentage ingested gelatin y: Percentage retention gelatin N	18	3.98	23.4	1.86	7.69	— 0.471	2.136
12. x: Percentage ingested casein y: Percentage retention casein N	20	5.52	74.4	1.58	5.95	— 0.198	0.859
13. x: Fecal zein N as percentage ingested N y: Urinary zein N as percentage resorbed N	20	20.7	55.1	8.77	15.74	+ 0.738	4.890
14. x. Fecal corn N as percentage ingested N y: Urinary corn N as percentage resorbed N (Mitchell)	21	6.0	40.1	3.15	6.54	+ 0.608	3.334
15. x: Fecal cocoa N as percentage ingested N y: Urinary cocoa N as percentage resorbed N (Mitchell)	13	60.0	59.6	7.92	13.32	+ 0.659	2.907
16. x: Efficiency index y: Fecal zein as percentage ingested N	22	1.71	27.8	0.44	8.26	— 0.251	0.116
17. x: Efficiency index y: Urinary zein N as percentage resorbed N	22	1.71	61.7	0.44	10.65	+ 0.029	0.132
18. x: Efficiency index y: Biological value of zein	22	1.71	38.3	0.44	10.64	— 0.030	0.133
19. x: Fecal zein N as percentage ingested N (rats of known efficiency) y: Urinary zein N as percentage resorbed N	22	27.8	61.7	8.26	10.65	+ 0.102	0.462

determining the endogenous urinary nitrogen of young rats, Mitchell ('23–'24 a) introduces corrections for changes in body weight during the experiment. In order to see whether this correction is necessary with large adult rats, twelve experiments were recalculated using corrections for body weight and compared with the uncorrected values. There was no appreciable difference in the figures.

The greatest difficulty of the experiment lay in securing adequate calorie intake and many animals were discarded temporarily because they ate so little. The surface area was calculated from the modified Meeh-Rubner formula as given by Lee ('29). This for male rats is $S = 12.62 \times W^{0.60}$, where S = body surface in square centimeters, and W = weight in grams. Hitchcock ('27) calculates that inactive mature rats require approximately 855 calories per square meter of body surface. The amount of food required was calculated and when the intake fell far below this amount, the rat was discarded. It seemed possible that with fat mature animals a somewhat lowered food intake for a brief period might not be of great importance, since the animal's supply of fat would furnish energy. One might expect that a lowered intake of energy during the protein feeding period would give a lowered retention of nitrogen, since part of the protein would probably be used for energy requirements. On the other hand, a lowered calorie intake during the 'nitrogen free' periods might be expected to give too high a percentage retention because of giving too high a value for the endogenous nitrogen. This would mean too low a value for the exogenous fraction and in consequence a high percentage retention.

In order to determine the significance of the calorie intake, a study was made of the individual cases. Of the fifty-eight experiments there were nineteen in which the energy intake fell below the theoretical requirements in one or both of the 'nitrogen free' periods, but was adequate during the protein feeding period. This showed no appreciable effect on the percentage retention of nitrogen. Similar findings are reported by Fixsen and Jackson ('32). There were only two

cases in which the intake was inadequate during the protein feeding period, but adequate during the other periods. In these two cases the values were distinctly below the average, though not so low as the lowest values in experiments in which the intake was adequate throughout. It was assumed, therefore, that no serious error was introduced into the average results due to inadequate food intake.

Since in some cases the same animals were used in experiments with more than one protein, it seemed desirable to know whether a rat which utilized one protein well would give similar results with another. Correlations were made between the percentage retention of the nitrogen of gelatin and zein, gelatin and casein, and zein and casein. Nos. 2, 3 and 4 in table 4 show no relation between the retention of the different proteins.

As the 'nitrogen free' diet and the vitamin supplement both contained a small amount of nitrogen, the question arose as to the possibility of a supplementary action between these forms of nitrogen and the protein studied. To test this the supplementary nitrogen ingested was correlated with the percentage retention of nitrogen in the case of each of the inadequate proteins. No relationship was found (table 4, nos. 5 and 6).

The constancy of the biological value of any protein for maintenance when fed in amount less than that required for nitrogen equilibrium is still a debated subject. Martin and Robison ('22) are inclined to the view that it is constant, while Mitchell ('23–'24 b) believes that the animal organism becomes more economical as less protein is supplied, so that higher values are obtained for smaller quantities ingested. He believes that the biological value is related to the percentage of proteins in the food, since a decreased calorie intake would increase the utilization of protein for energy.

In the present work each animal was given in the form of protein the amount of nitrogen which it had excreted in urine during the previous 'nitrogen free' period. Varying amounts of the offered protein were eaten, so we were able to correlate

the ingested nitrogen expressed as its percentage of the endogenous urinary nitrogen with the percentage of nitrogen retention. The average of the two 'nitrogen free' periods is used in the correlations. This explains an ingestion of over 100 per cent in no. 9 (table 4, nos. 7, 8 and 9). Similar calculations were made in which the ingested protein was expressed as percentage of the food intake (table 4, nos. 10, 11 and 12). These two sets of correlations were uniformly negative, two in the first set and one in the second being significant, thus indicating a higher retention when less nitrogen is ingested.

As a check on the digestion of the proteins several fecal analyses were made for nitrogen in the experiments with gelatin and with casein. The digestion of gelatin was found to be complete and that of casein nearly so. In eight cases the biological values were calculated by the method of Mitchell and the results showed no appreciable difference from those calculated by McCollum's retention method.

On the other hand, zein was not well digested, and the digestibility varied with different animals. For this reason nitrogen determinations were made on the feces of all zein fed animals and the biological values were calculated according to Mitchell's method. These values showed an extremely wide variation, ranging from 18 to 72 with a standard deviationof 15.86. In investigating this wide range of values a correlation was run between the exogenous fecal nitrogen expressed as the percentage of the nitrogen ingested and the exogenous urinary nitrogen expressed as the percentage of the nitrogen resorbed. The purpose of this was to determine whether there was any connection between the two types of wastage of nitrogen, i.e., that lost through lack of digestion and resorption and the further loss of part of the resorbed portion through failure to utilize it in metabolism. This gave a highly significant correlation, as shown in table 4, no. 13.

In a further study of this relationship the nitrogen balance data (Mitchell, Beadles and Keith, '26) where cocoa was fed, also Mitchell's ('23–'24 b) experiment in which corn was fed at the 10 per cent level were treated in the same manner. In

the case of cocoa, the correlation between the wastage of food nitrogen in the feces and its wastage in the urine was significant, and in the experiment with corn it was highly significant.

These correlations throw light on the wide range of biological values obtained when the animals vary widely in their ability to digest the protein. However, the fundamental explanation for this relationship remains to be determined.

Composition of undigested zein

Mitchell and Hamilton ('29) believe that the portion of the food protein which escapes digestion may differ markedly in its amino acid yield from the ingested food, since the different linkages in the molecule vary in resistance to digestive enzymes. According to this theory, most of the molecules may be partially broken down in the alimentary canal and the more resistant portion excreted. After feeding zein to rats, Osborne and Mendel ('14) were able to recover from 7.4 per cent to 39.5 per cent of the ingested nitrogen by extracting the feces with 80 per cent alcohol. This indicates that the zein residue is still alcohol soluble in spite of changes which may have taken place in its composition. If certain groups of little or no value in nutrition should be split off and resorbed first, most of this nitrogen would be excreted in the urine. In order to test this possibility, an alcoholic extract of the feces of zein fed rats was analyzed for nitrogen distribution.

After a preliminary period on a diet containing dextrin 54 per cent, sugar 10 per cent, salt mixture 4 per cent, agar 2 per cent, zein 15 per cent and lard 15 per cent, feces were collected from nine rats for 4 days. Samples were analyzed for total nitrogen. The metabolic nitrogen was calculated from the average of thirty previous experiments (1.91 mg. per gram food consumed). According to this calculation, 23 per cent of the food nitrogen was present in the feces. After ether extractions to remove the fat the feces were extracted with 92 per cent alcohol. The alcoholic extract contained 16.4 per cent of the ingested nitrogen, and 71 per cent of the calculated exogenous fecal nitrogen. Analysis of this extract is

given in table 2. The nitrogen distribution, not being markedly different from that of the ingested zein, gave no evidence in support of this theory.

Nitrogen balance experiments with rats of known efficiency index

It seemed possible that the individual variation in ability to digest zein might be related to the inherent food utilization efficiency of the animals. To test this, twenty-two nitrogen balance experiments were carried out as before, using male rats whose efficiency index had been determined during the 6-week period after attaining a weight of 60 gm. These animals were supplied by Palmer and Kennedy ('31) and were from inbred strains having at least nine generations of sib matings and which showed great uniformity in ability to digest synthetic diets. The efficiency indices[2] ranged from 1.13 to 2.44. These indices were correlated with the exogenous fecal nitrogen expressed as the percentage of ingested nitrogen, with the exogenous urinary nitrogen expressed as the percentage of resorbed nitrogen, and with the biological value. The exogenous fecal nitrogen expressed as the percentage of ingested nitrogen was also correlated with the exogenous urinary nitrogen expressed as the percentage of resorbed nitrogen. None of these correlations are significant (table 4, nos. 16, 17, 18 and 19).

We suggest that the insignificant correlation in the latter case may be explained by the use of the advanced inbred strains in contrast to the former case where random sampling of rats resulted in a highly significant correlation between the fecal and urinary wastage of the food.

SUMMARY

Nitrogen balance experiments were carried out with adult male rats, using casein, gelatin and zein. The percentage retention calculated by McCollum's ('12) method averaged 74 for casein, 23 for gelatin and 57 for zein.

[2] The efficiency index is the food required (in grams) per gram of gain per 100 gm. body weight.

Studies were made of the nitrogen balance data, first, to determine possible errors inherent in this type of experiment, and, second, to find significant correlations.

With the large adult male rats used it was found unnecessary to correct the urinary nitrogen for the loss in weight during the experiment.

A fall in calorie intake somewhat below the theoretical requirements during the 'nitrogen free' feeding period was of no apparent significance.

There was no apparent supplementary effect of the nitrogenous compounds of the yeast extract and the dextrin on the inadequate proteins used.

The percentage retention increased as less protein was ingested, even though it was never fed above the endogenous level.

When the same animals were used in experiments with more than one protein, there was no significant correlation between the individual utilization of the different proteins.

Gelatin and casein were so well digested that there was no significant difference between the percentage retention calculated by McCollum's ('12) method and the biological value calculated by Mitchell's ('23–'24 a) method. However, zein showed a poor and variable digestibility, the exogenous fecal nitrogen ranging from 4 to 36 per cent of the ingested zein. The biological values varied widely, from 18 to 72, the average being 45. This wide spread in results led to the discovery of a highly significant correlation between the exogenous fecal nitrogen expressed as the percentage of nitrogen ingested and the exogenous urinary nitrogen expressed as the percentage of nitrogen resorbed.

Further experiments with rats of known efficiency index for growth showed no relation between the inherent efficiency of the animal and the digestibility or utilization of the zein.

Analysis of undigested zein residues for nitrogen distribution furnished no explanation of the highly significant correlation between the food wastage in the feces and that in the urine.

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J. Exper. Med. 100:497-513, Nov. 1954

I. ANTIGENICITY OF OXYPOLYGELATIN AND GELATIN IN MAN*,‡

BY PAUL H. MAURER, PH.D.

(From the Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh)

(Received for publication, June 30, 1954)

As there are reports in the literature (1 *a–d*, 2) that highly purified gelatin is non-antigenic, this substance and some of its chemical derivatives have been suggested and tested as possible blood plasma expanders (3). One of the gelatin derivatives which has been proposed is oxypolygelatin (OPG)[1] (4). The recent observations of Kabat and Berg (5) and Maurer (6) that dextran, another plasma expander, although non-antigenic in the rabbit, is a good antigen in man led to the testing of the antigenicity of OPG in man. The results presented in this paper show that gelatin and its derivatives indeed are antigenic in man. The quantitative immunochemical data used to support this thesis and some of the implications of these findings in man will be the subject of this first paper.

EXPERIMENTAL

Antigens.—Oxypolygelatins (OPG). The clinical OPG tested was kindly furnished by Dr. Arthur Cherkin of Don Baxter, Incorporated, of Pasadena. It was Lot No. S 230 X and had an average molecular weight of about 27,000. The molecular weight distribution as determined by Dr. J. W. Williams was as follows: 10,000, 16 per cent; 10,000–20,000, 33 per cent; 20,000–40,000, 33 per cent; 40,000–60,000, 12 per cent; 60,000–80,000, 4 per cent; 80,000–100,000, 1 per cent; > 100,000, 1 per cent.

The "high molecular weight" fraction of OPG S 230 X was obtained by precipitation with two volumes of methanol. This preparation had an average molecular weight of 36,000, based upon correlation of viscosity and osmotic pressure measurements.

Gelatin Lot No. 2160 was also obtained from Don Baxter, Incorporated. This was the material used to prepare the above OPG preparations. It had a number average molecular weight of 50,000–75,000. All of the above materials were found to be non-pyrogenic and non-antigenic in the guinea pig.

* This investigation was supported by a grant from the Office of the Surgeon General, Department of the Army contract No. DA-49-007-MD-248 upon recommendation of the Subcommittee on Shock of the National Research Council.

‡ Presented in part before the 6th International Congress of Microbiology, Rome, Italy, September, 1953, and before the annual meeting of the American Association of Immunologists, Atlantic City, April 12–17, 1954.

This is reprint No. 34 of the Department of Pathology, University of Pittsburgh School of Medicine.

[1] OPG stands for oxypolygelatin.

Nitrogen analyses of the various protein antigens were performed by the Markham modification of the micro Kjeldhal procedure (7). All antigen solutions were adjusted to pH 7.5 and preserved with 1/10,000 merthiolate.

Immunization Procedure.—Healthy medical volunteers were used. An initial preimmunization sample of 50 ml. of blood was taken. Each subject then received two intracutaneous tests with about 0.01 to 0.02 ml. of the OPG or gelatin preparation used for immunization containing 0.25 per cent phenol and a control of 0.85 per cent saline and phenol. After 15 minutes

TABLE I

Response of Humans to Injections of Clinical Oxypolygelatin S 230 X

Subject	No.	OPG solution injected (9 injections)	Preinjection		3 weeks post-injection	
			μg. antibody N precipitated per ml. of serum from 3 ml. serum treated with			
			3 μg. OPG N	9 μg. OPG N	3 μg. OPG N	9 μg. OPG N
		mg./ml.				
DAV	1	2	0.0	0.5	0.4	4.4
DJF	2	2	1.0	2.0	—	12.2
CAB	3	2	0.2	1.0	1.5	4.6
JZ	4	2	1.3	2.7	2.0	6.0
JAC	5	2	0.3	0.8	2.2	3.9
DGC	6	5	1.8	3.3	3.3	7.5
JHA	7	5	0.8	3.4	0.8	2.9
NS	8	5	0.7	3.1	1.9	7.5
HJ	9	5	1.1	6.7	1.9	4.7
PHM	10	5	0.0	0.0	2.2	3.6
LH	11	10	0.1	1.3	1.5	2.7
TCH	12	10	0.8	3.1	2.3	4.3
WJH	13	10	0.6	6.4	—	10.3
RNL	14	10	0.0	3.4	0.8	3.3
CHL	15	10	0.6	3.6	1.4	—
SW	16	25	0.8	2.0	0.0	—
RAP	17	25	0.8	3.1	0.1	3.7
TBL	18	25	0.5	—	3.6	—
RRB	19	25	1.1	4.0	3.5	9.4
JRL	20	25	0.2	0.2	—	—

the skin reactions were read. For the initial study with OPG the volunteers were divided into four groups. Each group received nine daily injections of clinical OPG containing 2, 5, 10, or 25 mg. protein per ml. of solution. 3 weeks after the last injection a second 50 ml. sample of blood was obtained and intracutaneous tests performed. Half liter portions of blood were obtained from those individuals who showed good antibody responses.

For the repeat experiment additional medical volunteers were divided into three groups of seven per group. The groups received nine daily injections containing 25 mg. per injection of either OPG S 230 X, high molecular weight OPG or gelatin. Post-immunization bleedings were taken 3 and 7 weeks after the last injection. 4 months later the best antibody producers in each group were reinjected twice with 10 mg. of the appropriate antigen. 3 weeks after the last injection 500 ml. portions of blood were again obtained. All serum samples were

handled with sterile precautions, and in addition merthiolate to a concentration of 0.01 per cent and phenol to 0.25 per cent added.

Quantitative Precipitin Studies.—The pre- and postimmunization sera were assayed for antibody N by the quantitative precipitin method of Heidelberger and MacPherson (8) using the Folin-Ciocalteu tyrosine reagent (9), standardized against known amounts of normal human gamma globulin (fractions II).[1] The complete method is repeated here. After remaining in the icebox for about 10 days, each serum sample was centrifuged in the cold overnight, the lipid removed from the top, the serum decanted, and recentrifuged until perfectly clear before use. In the initial studies with clinical OPG 3.0 ml. portions of pre- and

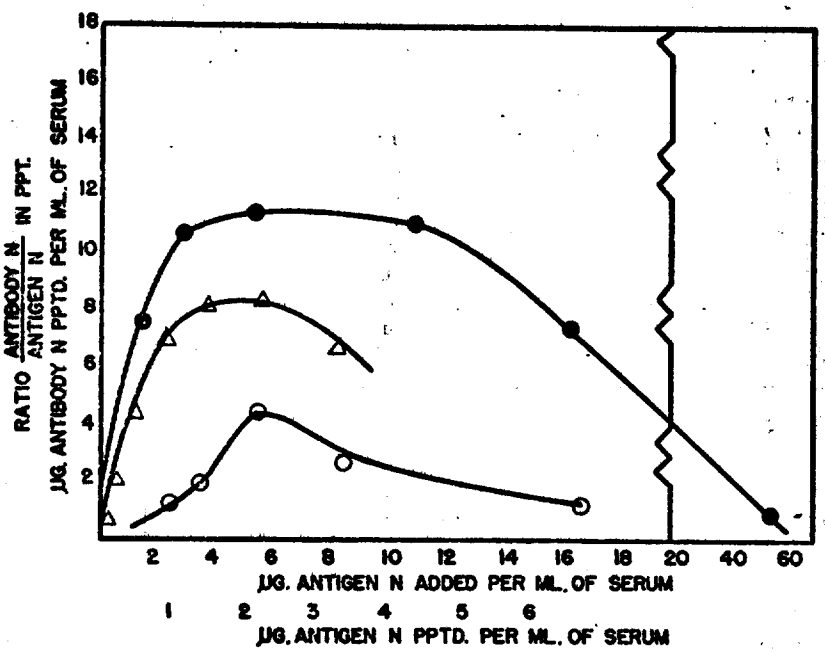

FIG. 1. Calibration curves of human antiserum (RRB.). ○, clinical OPG; △, fractionated OPG; ●, pigskin gelatin.

postimmunization sera were measured into 8 ml. centrifuge tubes calibrated at the 2.5 ml. mark. 3 and 9 μg. of OPG nitrogen in a volume of 1.0 ml. were added to successive tubes. The addition of 1.0 ml. of saline to 3.0 ml. of serum served as the serum control. The tubes were mixed, capped, placed in a water bath at 37°C. for 1 hour and in the refrigerator for from 10 to 14 days with mixing of the tubes daily. The tubes were centrifuged in the cold and the precipitates were washed three times with chilled saline. The dissolution of the precipitates with 0.5 M NaOH at 37°C. was very time consuming. It was later observed that warming the precipitates to 50°C. resulted in immediate solution. This was in accordance with some of the known properties of gelatin which, as will be shown later, was in the precipitate; namely, to dissolve readily upon warming to about 50°C. The dissolved precipitates were made up to the 2.5 ml. mark and 2.0 ml. aliquots analyzed by the Folin-Ciocalteu method at 7500 A using a Beckman model DU spectrophotometer. (Table I).

[1] The author wishes to thank Dr. E. A. Kabat for supplying him with this preparation.

TABLE II

*Response of Human Volunteers to Injection of Oxypolygelatin S 230 X (Group 1)**

Subject	No.	Preimmunization absorption						3 wk. postimmunization absorption‡		7 wk. postimmunization absorption			
		1st		2nd		3rd				1st		2nd§	
		Antigen N added per ml. serum	Antibody N precipitated per ml. serum	Antigen N added per ml. serum	Antibody N precipitated per ml. serum	Antigen N added per ml. serum	Antibody N precipitated per ml. serum	Antigen N added per ml. serum	Antibody N precipitated per ml. serum	Antigen N added per ml. serum	Antibody N precipitated per ml. serum	Antigen N added per ml. serum	Antibody N precipitated per ml. serum
		μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.
RWH	21	1.1 OPG	1.0	5.6 OPG	2.7	1.0 gel	1.9	3.9 OPG	1.6	1.1 OPG	5.4	1.0 gel	1.2
		3.3 "	3.8	—	—	1.5 "	6.5	3.0	5.8*	3.4 "	13.4	1.0 "	3.1
		4.1 gel	14.7‖	3.0 gel	4.8					2.0 gel	19.5	2.0 HOPG	2.0
										3.1 "	22.2	2.0 "	0.0
AK	22	1.1 OPG	0.7	5.6 OPG	6.3	1.0 gel	1.9	3.9 OPG	0.0	1.1 OPG	1.8	1.0 gel	0.0
		3.3 "	5.2	—	—	1.5 "	6.5	3.0 gel	3.2	3.4 "	4.3	1.0 "	0.0
		4.1 gel	11.5‖	—						2.0 gel	7.6	2.0 HOPG	0.0
										3.1 "	7.2	2.0 "	0.0
HM	23	1.1 OPG	1.2	5.6 OPG	3.3	1.0 gel	3.7	1.1 OPG	0.0	1.1 OPG	4.6	1.0 gel	5.3
		3.3 "	4.5	—	—	1.5 "	6.6	4.2 "	3.0	3.4 "	10.7	1.0 "	3.5
		4.1 gel	10.0‖	3.0 gel	4.3			3.0 gel	4.8	2.0 gel	19.0	2.0 HOPG	0.0
										4.1 "	17.8	2.0 "	0.0
HH	24	1.1 OPG	—	5.6 OPG	1.0	1.0 gel	3.0	4.2 OPG	0.0	1.1 OPG	2.2	1.0 gel	3.6
		3.3 "	3.0			1.5 "	5.0	3.0 gel	4.0	3.4 "	6.7	1.0 "	2.4
		4.1 gel	8.8‖	3.0 gel	6.1					2.0 gel	11.8	2.0 HOPG	0.0
										4.1 "	12.8	2.0 "	0.0
TH	25	1.1 OPG	0.5	5.6 OPG	0.4	1.0 gel	1.6	1.1 OPG	0.0	1.1 OPG	2.0	1.0 gel	2.2
		3.3 "	1.4			1.5 "	7.7	4.2 "	0.8	3.4 "	3.3	1.0 "	0.7
		4.1 gel	6.9‖	3.0 gel	3.7			3.0 gel	2.9	2.0 gel	9.0	2.0 HOPG	0.0
										4.1 "	6.3	2.0 "	0.0
JB	26	1.1 OPG	0.7	5.6 OPG	1.6	1.0 gel	2.1	1.1 OPG	1.8	1.1 OPG	3.3	1.0 gel	3.7
		3.3 "	2.1			1.5 "	3.9	4.2 "	4.3	3.4 "	6.6	1.0 "	2.3
		4.1 gel	8.7‖	3.0 gel	4.7			3.0 gel	8.8	2.0 gel	12.6	2.0 HOPG	0.0
										4.1 "	11.0	2.0 "	0.0
JF	27	1.1 OPG	0.0	5.6 OPG	0.0	1.0 gel	3.5	4.2 OPG	4.3	1.1 OPG	3.3	1.0 gel	3.5
		3.3 "	0.2			1.5 "	0.8	3.0 gel	8.8	3.4 OPG	2.2	1.0 "	2.2
		4.1 gel	4.2‖	3.0 gel	4.8					2.0 gel	11.5	2.0 HOPG	0.0
										4.1 "	11.5	2.0 "	0.0

* 3.0 ml. used for analyses except where noted.
‡ Only 1st absorption values reported here.
§ Results of a 3rd absorption with clinical OPG were negative.
‖ 1.5 ml. used for analysis.

In the repeat study when three different antigens were used for immunization, the preimmunization sera were analyzed as follows: Group 1—(clinical OPG); to 3.0 ml. aliquots 3.4 and 10.1 μg. OPG N were added. After incubation and centrifugation as described above, the supernatants were absorbed successively with clinical OPG and gelatin. Additional aliquots were absorbed initially with gelatin.

TABLE III

*Response of Human Volunteers to Injection of High Molecular Weight OPG S 230 X (Group 2)**

Subject	No.	Preimmunization absorption						3 wk. postimmunization absorption‡		7 wk. postimmunization absorption			
		1st		2nd		3rd				1st		2nd§	
		Antigen N added per ml. serum	Antibody N precipitated per ml. serum	Antigen N added per ml. serum	Antibody N precipitated per ml. serum	Antigen N added per ml. serum	Antibody N precipitated per ml. serum	Antigen N added per ml. serum	Antibody N precipitated per ml. serum	Antigen N added per ml. serum	Antibody N precipitated per ml. serum	Antigen N added per ml. serum	Antibody N precipitated per ml. serum
		μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.
CB	31	1.1 HOPG	6.6	1.7 HOPG	7.2	1.0 gel	1.9	1.7 HOPG	6.5	1.7 HOPG	8.7	2.0 HOPG	2.4
		3.8 "	15.3	1.7 "	1.9	1.5 "	0.3	5.6 "	7.7	2.8 "	10.1	2.0 "	1.0
		4.1 gel	15.8‖	3.0 gel	4.6			2.8 gel	9.0	2.0 gel	10.3	1.0 gel	0.6
										4.1 "	7.2		
JF	32	1.1 HOPG	6.1	1.7 HOPG	3.6	1.0 gel	2.5	1.7 HOPG	9.3	1.7 HOPG	10.2	2.0 HOPG	3.0
		3.8 "	13.9	1.7 "	2.2	1.5 "	1.0	5.6 "	13.1	2.8 "	13.8	2.0 "	2.1
		4.1 gel	15.5‖	3.0 gel	5.3			2.8 gel	12.3	2.0 gel	13.5	1.0 gel	1.8
										4.1 "	10.6		
CT	33	1.1 HOPG	2.8	1.7 HOPG	2.4	1.0 gel	0.6	1.7 HOPG	5.4	1.7 HOPG	8.8	2.0 HOPG	2.9
		3.8 "	4.5	1.7 "	0.1	1.5 "	0.8	5.6 "	3.3	2.8 "	10.9	2.0 "	0.3
		4.1 gel	5.8‖	3.0 gel	2.0			2.8 gel	5.5	2.0 gel	11.2	1.0 gel	1.4
										4.1 "	9.3		
FK	34	1.1 HOPG	4.5	1.7 HOPG	4.4	1.0 gel	1.4	1.7 HOPG	10.9	1.7 HOPG	9.5	2.0 HOPG	1.9
		3.8 "	8.4	1.7 "	0.4	1.5 "	0.5	5.6 "	14.6	2.8 gel	12.6	2.0 "	2.8
		4.1 gel	8.8‖	3.0 gel	2.6			2.8 gel	13.4	2.0 "	12.1	1.0 gel	2.2
										4.1 "	11.7		
RSS	35	1.1 HOPG	4.9	1.7 HOPG	7.7	1.0 gel	1.8	1.7 HOPG	9.6				
		3.8 "	12.5	1.7 "	1.4	1.5 "	0.7	5.6 "	10.2				
		4.1 gel	13.3‖	3.0 gel	2.6			2.8 gel	11.0				
CLK	36	1.1 HOPG	4.4	1.7 HOPG	4.6	1.0 gel	1.4	1.7 HOPG	5.3				
		3.8 "	9.3	1.7 "	0.4	1.5 "	0.0	5.6 "	6.4				
		4.1 gel	9.9‖	3.0 gel	3.8			2.8 gel	8.5				
RB	37	1.1 HOPG	4.8	1.7 HOPG	4.6	1.0 gel	1.0	1.7 HOPG	2.9	1.7 HOPG	5.7	2.0 HOPG	1.1
		3.8 "	9.6	1.7 "	0.6	1.5 "	0.0	5.6 "	1.5	2.8 "	6.1	2.0 "	0.7
		4.1 gel	8.8‖	3.0 gel	4.8			2.8 gel	2.6	2.0 gel	8.5	1.0 gel	1.1
										4.1 "	5.0		

* 3.0 ml. used for analyses except where noted.
‡ Only 1st absorption values reported here.
§ Results of a third absorption with clinical OPG were negative.
‖ 1.5 ml. used for analysis.

Group 2 (high molecular weight OPG). 3.0 ml. aliquots were set up with 3.3 and 11.4 μg. N fractionated OPG. The supernatants were successively absorbed with five μg. fractionated OPG N and 3 to 4.5 μg. gelatin N. Here, too, additional aliquots were absorbed with gelatin.

Group 3 (gelatin). 3.0 ml aliquots were set up with 4.1 and 8.3 μg. gelatin N. After analysis of the precipitates, the supernatants were absorbed with 8.3 and 4.1 μg. gelatin N respec-

TABLE IV

*Response of Human Volunteers to Injection of Gelatin L 2160**

Subject	No.	Preimmunization absorption						3 wk. postimmunization absorption‡		7 wk. postimmunization adsorption			
		1st		2nd		3rd				1st		2nd§	
		Antigen N added per ml. serum	Antibody N precipitated per ml. serum	Antigen N added per ml. serum	Antibody N precipitated per ml. serum	Antigen N added per ml. serum	Antibody N precipitated per ml. serum	Antigen N added per ml. serum	Antibody N precipitated per ml. serum	Antigen N added per ml. serum	Antibody N precipitated per ml. serum	Antigen N added per ml. serum	Antibody N precipitated per ml. serum
		μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.
JD	41	1.4 gel	11.9	2.8 gel	3.2	4.2 OPG	0.0	3.1 gel	6.2	2.0 gel	10.0	1.0 gel	1.3
		2.8 "	13.1	1.4 "	0.9	3.3 HOPG	0.0	5.5 "	6.3	4.1 "	—		
		4.5 OPG	6.2‖					4.2 OPG	3.5	3.3 OPG	5.5	1.0 gel	0.0
										2.8 HOPG	9.6	1.0 "	0.0
HH	42	1.4 gel	6.7	2.8 gel	0.0	4.2 OPG	0.0	3.1 gel	6.9				
		2.8 "	6.8	1.4 "	0.0	3.3 HOPG	0.0	5.5 "	7.5				
		4.5 OPG	3.0‖					4.2 OPG	4.8				
HH	43	1.4 gel	9.7	2.8 gel	2.1	4.2 OPG	0.0	3.1 gel	7.9	2.0 gel	10.0	1.0 gel	3.1
		2.8 "	11.5	1.4 "	0.8	3.3 HOPG	0.0	5.5 "	8.5	4.1 "	12.3	1.0 "	3.2
		4.5 OPG	4.0‖					4.2 OPG	4.2	3.3 OPG	4.5	1.0 "	3.5
										2.8 HOPG	12.2	1.0 "	1.3
RD	44	1.4 gel	12.1	2.8 gel	2.4	4.2 OPG	0.0	3.1 gel	8.9	2.0 gel	13.1	1.0 gel	3.6
		2.8 "	13.1	1.4 "	1.2	3.3 HOPG	0.0	5.5 "	9.1	4.1 "	11.2	—	—
		4.5 OPG	5.5‖					4.2 OPG	5.8	3.3 OPG	6.1	1.0 gel	1.9
										2.8 HOPG	15.7	1.0 "	0.0
RC	45	1.4 gel	6.3	2.8 gel	0.4	4.2 OPG	0.0	3.1 gel	8.5	2.0 gel	8.8	1.0 gel	1.7
		2.8 "	6.9	1.4 "	0.0	3.3 HOPG	0.0	5.5 "	8.5	4.1 "	6.8	—	—
		4.5 OPG	2.9‖					4.2 "	4.6	3.3 OPG	3.8		
										2.8 HOPG	8.9		
EH	46	1.4 gel	10.4	2.8 gel	2.6	4.2 OPG	0.0	3.1 gel	3.1	2.0 gel	9.5	1.0 gel	2.4
		2.8 "	11.5	1.4 "	0.9	3.3 HOPG	0.0	5.5 "	3.0	4.1 "	7.4	—	—
		4.5 OPG	4.5‖					4.2 "	2.1	3.3 OPG	3.6	1.0 gel	3.5
										2.8 HOPG	9.6	1.0 "	0.4
RB	47	1.4 gel	12.7	2.8 gel	3.7	4.2 OPG	0.0	3.1 gel	3.9	2.0 gel	9.0	1.0 gel	0.9
		2.8 "	16.5	1.4 "	1.9	3.3 HOPG	0.0	5.5 "	4.7	4.1 "	7.3	1.0 "	0.2
		4.5 OPG	5.9‖					4.2 "	2.8	3.3 OPG	3.2	1.0 "	2.4
										2.8 HOPG	9.2	1.0 "	0.0

* 3.0 ml. used for analyses except where noted.
‡ Only 1st absorption values reported here.
§ Results of a 3rd absorption with clinical OPG were negative.
‖ 1.5 ml. used for analysis.

tively, followed by absorption with either clinical OPG or high molecular weight OPG. Once again this procedure was repeated initially using clinical OPG.

The data giving the amounts of antigen added to the original serum and supernatant for each analysis and the antibody N precipitated during each absorption for the pre-, and the 3, and 7 week postimmunization bleedings are given in Tables II to IV.

Quantitative precipitin curves (9) were set up with the sera obtained from those volunteers who had shown the best antibody responses. The sera were calibrated using all three antigens irrespective of the one used for immunization. Tables V and VI show the data with the sera from subject 19 and the pool in Group 1.

TABLE V

Antibody Nitrogen Precipitated by Gelatin and Its Derivatives from 3.0 ml. Human Antiserum (Anti-OPG) from Subject RRB, no. 19

OPG N added per ml. serum	Antibody N precipitated per ml. serum	HOPG N added per ml. serum	Antibody N precipitated per ml. serum	Gel N added per ml. serum	Antibody N precipitated per ml. serum
μg.	μg.	μg.	μg.	μg.	μg.
2.3	1.4	0.5	2.1	1.4	7.5
3.4	2.0	1.1	4.4	2.8	10.9
5.6	4.5	2.2	7.0	5.5	11.3
8.5	2.7	3.6	8.2	11.0	11.2
11.3	2.6	5.4	8.3	16.5	6.4
16.9	1.4	24.4	6.7	55.0	1.0

TABLE VI

Antibody Nitrogen Precipitated by Gelatin and Its Derivatives from Human Antiserum (Group 1)

OPG N added per ml. serum*	Antibody N precipitated per ml. serum	HOPG N added per ml. serum‡	Antibody N precipitated per ml. serum	Gel N added per ml. serum‡	Antibody N precipitated per ml. serum
μg.	μg.	μg.	μg.	μg.	μg.
0.7	1.0	0.9	1.4	0.7	3.6
1.9	4.5	1.9	6.7	2.0	14.0
2.8	6.0	3.5	10.9	4.0	16.5
4.2	7.7	5.6	15.5	5.5	16.0
7.0	10.2	8.4	15.1	8.3	15.4
11.3	11.1	13.4	10.4	13.7	14.2
16.9	10.4	28.0	7.5	27.5	12.5
28.2	7.5	54.2	0.0	41.3	12.0
33.7	4.3	67.6	0.0	69.3	9.6
56.4	0.6				

* 3.0 ml. serum used for each analysis.
‡ 1.5 ml. serum used for each analysis.

Hydroxyproline Analysis.—In order to measure the OPG and/or gelatin incorporated into the specific precipitates advantage was taken of the high concentration of hydroxyproline in gelatin and its derivatives. The method of Neuman and Logan (10) was employed. Additional precipitates, in triplicate, were analyzed along with standards of both gelatin and hydroxyproline[5] each time. This was necessitated by the fact that the hydroxyproline curves were not the same each day.[6]

[5] The hydroxyproline standards were made from the material supplied by Pfanstiehl Chemical Co., Waukegan, Illinois.

[6] Similar observations have been noted by others (11).

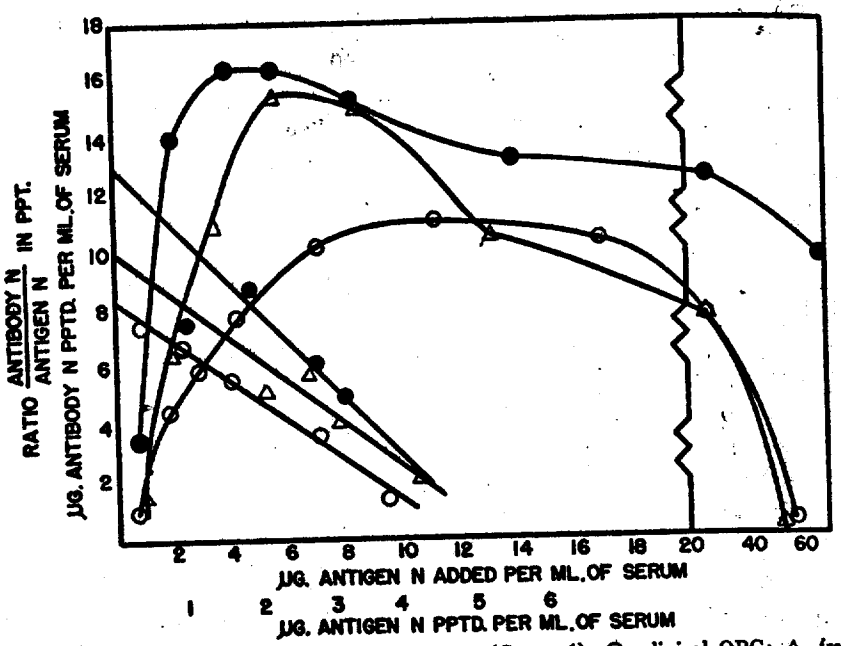

FIG. 2. Calibration curves of human antiserum (Group 1). ○, clinical OPG; △, fractionated OPG; ●, pigskin gelatin.

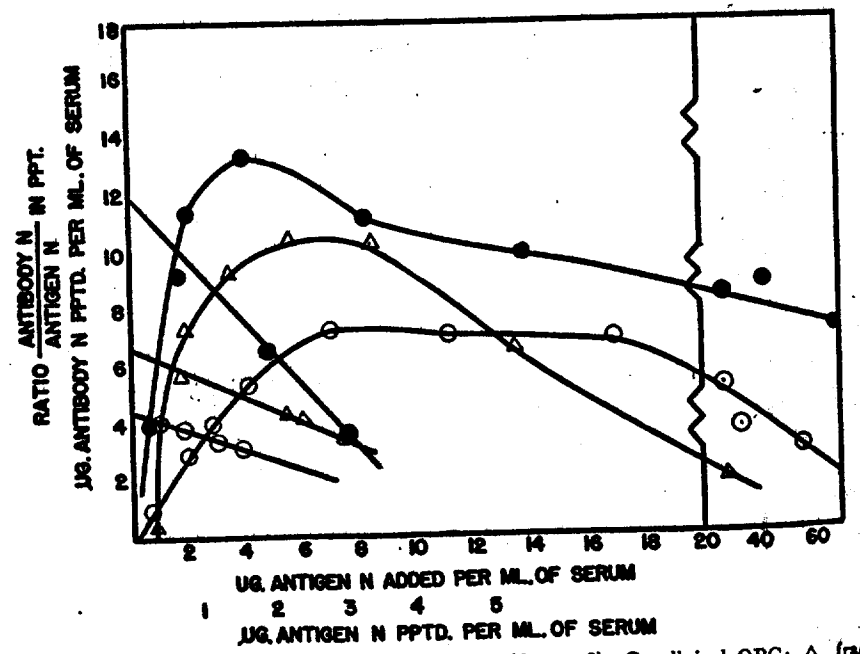

FIG. 3. Calibration curves of human antiserum (Group 2). ○, clinical OPG; △, fractionated OPG; ●, pigskin gelatin.

Although hydroxyproline analyses were performed on many precipitates obtained with preimmunization sera the values presented in Table VII and Figs. 2 and 3 refer only to the postimmunization sera.

Solubility of Specific Precipitates.—In order to determine the solubility of gelatin–antigelatin precipitates in excess antigen additional precipitates were formed. They were washed as described above and then resuspended overnight at room temperature in either 1.0 ml. of gelatin, formalinized gelatin, modified fluid gelatin,[5] bovine serum albumin,[6] ovalbumin,[7] containing 1 mg. N/ml. or in 1 ml. of 0.9 per cent NaCl which served as the control. The precipitates were then centrifuged, washed with chilled saline, dissolved to 2.5 ml. and 2.0 ml.

TABLE VII

Analysis of Precipitates from Group 2 Serum for Presence of Antigen

Antigen N added per ml. serum	Antigen N precipitated from hydroxyproline data	Antigen precipitated	Antibody N precipitated (from precipitin curves)	Antibody N precipitated / Antigen N in precipitate
μg.	μg.	per cent	μg.	
OPG 1.1	0.4	36	1.5	4.1
2.8	0.7	25	2.7	3.8
5.6	1.2	22	4.1	3.4
7.1	1.5	21	4.8	3.2
HOPG 1.1	0.7	66	4.0	5.6
3.5	2.2	63	9.4	4.3
5.6	2.4	43	10.4	4.3
8.4	2.9	35	10.2	3.5
11.2	2.1	18	8.2	3.9
GEL 1.1	0.7	64	6.5	9.3
2.8	1.3	47	12.7	9.8
5.5	1.9	35	12.5	6.6
8.3	3.0	36	11.0	3.7

aliquots taken for analysis of the tyrosine color produced by the Folin-Ciocalteu method. The results were conclusive that the precipitates dissolved only in gelatin or formalin-treated gelatin.

Rabbit Anti-HGG.—Antisera against human gamma globulin were obtained by immunization of rabbits with either Cutter's[8] or Lederle's[9] immune serum globulin as described in previous publications (12).

Resuspension of Specific Precipitates in Anti-HGG Sera. One of the methods used to substantiate the fact that the protein being precipitated by gelatin and/or its derivative was indeed antibody (gamma globulin) was to resuspend precipitates in rabbit anti-HGG sera. Gelatin-antigelatin precipitates were formed by adding increasing amounts of gelatin to

[5] Kindly furnished by Dr. D. Tourtellotte of the Knox Gelatin Company, Camden, New Jersey.

[6] Armour recrystallized BSA Lot No. 128-75, The Armour Laboratories, Chicago.

[7] Prepared according to reference 25.

[8] Cutter Laboratories, Berkeley, California.

[9] Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

varying volumes of human antigelatin sera. The precipitates which formed were washed six times to eliminate the slightest possibility of any serum proteins remaining adsorbed to the precipitates. The precipitates were then resuspended in 1.0 ml. of the rabbit anti-HGG serum at 5°C. Additional precipitates were resuspended in 1.0 ml. of normal rabbit serum which served as the respective controls. Both the normal rabbit serum and rabbit anti-HGG were decomplemented to remove the possibility of complement N interfering with the results (13). After 8 days in the refrigerator, during which time the precipitates were resuspended twice daily, the tubes were centrifuged and washed as mentioned above. The precipitates were dissolved and analyzed for Folin color at 7500 A as described above. The results are given in Table VIII.

Passive Transfer (Anaphylaxis) Studies.—Eight ml. of human serum containing 5 μg. of antibody (antigelatin) N per ml. were injected into each of six guinea pigs weighing about 250 gm. (14). A "shocking" dose of 1 ml. of gelatin containing 1 mg. N was injected intravenously 48 hours later. As no anaphylaxis ensued the guinea pigs were injected with 60 μg. of rabbit antibody N against BSA. This was to see whether the guinea pigs were refractory The challenging dose of BSA was 1 mg.

TABLE VIII

Optical Density at 7500 A of Gelatin-Antigelatin (Human)

Resuspended in	
Normal rabbit serum	Rabbit Anti-HGG sera
0.028	0.040
0.141	0.190
0.164	0.250
0.205	0.300

RESULTS

Table I summarizes the results obtained in 20 individuals in the initial study involving clinical OPG. In all the data presented the exceedingly small contribution of Folin color given by gelatin or its derivatives has been neglected in calculating antibody values. Analyses of varying concentrations of gelatin and OPG were performed by the Folin-Ciocalteu method. As was to be expected, owing to the virtual absence of tyrosine in the proteins, insignificant optical densities were obtained.

Quantities of antibody nitrogen below 2 μg. per ml. are not considered significant. The data in the preimmunization sera show that 13 of the 20 volunteers (65 per cent) had significant amounts of protein precipitable by OPG. In 13 of the postimmunization sera the precipitable antibody had increased by at least 2 μg. N. In one instance (No. 2) there was a rise in antibody N from 2 to 12 μg. per ml. In 3 sera there was no significant change in antibody level whereas one subject actually showed a decrease in antibody level. Three of the postimmunization sera were lost. There were no marked skin reactions (wheal and erythema type) either before or after immunization. The concentration of

OPG injected (2 to 25 mg./ml) had no effect on the magnitude of the antibody response. It was somewhat surprising to find antibody present in the preimmunization sera. The possible sources of this antibody will be discussed later.

The sera of the 500 ml. bleedings obtained from the volunteers (Nos. 2, 6, 13, and 19) were calibrated with clinical OPG, the fractionated OPG and gelatin. Only the data obtained with No. 19 is presented as it was quite representative of the others. From Table V and Fig. 1 it is quite evident that we are dealing with a protein—antiprotein system. The shapes of these curves and those to be presented later are typical of a polydisperse and/or denatured antigen. (For complete discussion of this see reference 9). The precipitation of antibody from the serum by the different antigens increased with the molecular size of the antigen; *i.e.*, gelatin precipitated more antibody than did fractionated OPG which in turn precipitated more antibody than did clinical OPG. This will be reiterated in the results obtained in the repeat experiment.

Tables II, III, and IV present the results of the many analyses of the three different groups of volunteers used in the repeat experiment. Group 1 was injected with clinical OPG, Group 2 with the fractionated (high molecular weight) OPG, and Group 3, was injected with the gelatin from which the OPG was made. For the sake of clarity and to avoid generalization it is best to present the results of each group separately.

Group 1. The analyses of the preimmunization sera with OPG and gelatin indicate that gelatin precipitates much more antibody than does clinical OPG. Although the absorption of the supernatants from the OPG tubes with more OPG precipitates more antibody, still there is considerably less antibody precipitated than by the gelatin alone. In most cases small amounts of gelatin added to the second supernatant precipitated additional antibody. Of considerable interest is the fact that the total antibody N precipitated in two absorptions with OPG (1.1 μg. OPG N/ml. serum followed by 5.6 μg. OPG N/ml) is equal, within experimental error, to the amount precipitated by 3.3 μg. OPG N/ml. serum originally.

In most cases two absorptions with gelatin precipitate significantly more antibody than did the OPG plus small concentrations of gelatin. A third absorption of the sera analyzed with gelatin with OPG gave insignificant precipitates.

The results obtained on the 3 week postimmunization bleedings were somewhat surprising in that five out of the seven individuals had a lower antibody level than before injection of OPG. Further bleedings were therefore taken at the time the 3 week bleeding values were obtained. This, in all cases, was 7 weeks after the last injection. For brevity, only the results of two absorptions will be presented for the 7 week postimmunization bleedings. Of the five individuals who showed decreased antibody in the 3 week postimmunization bleedings four had significant increases in antibody N on the 7 week bleedings.

Group 2.—The analyses of the preimmunization sera with fractionated OPG

and gelatin indicate that gelatin precipitates only a few more μg. of antibody N from the various sera than does the fractionated OPG. Moreover here too the total antibody precipitated by the absorption of antibody first with 1.1 μg. N high molecular weight OPG followed by another precipitation with 1.7 μg. N fractionated OPG was equal to the amount of antibody N precipitated by a single precipitation with 3.8 μg. N fractionated OPG.

The results on the 3 week postimmunization bleedings in this group also indicated that of the seven volunteers injected with fractionated OPG only one showed a significant increase over the preimmunization antibody levels. Three showed a significant decrease and three did not change significantly in their antibody values.

Of the five volunteers from whom serum was obtained 7 weeks after the last injection two showed a significant increase and two showed a decrease in antibody levels. Of interest is subject 32 who showed no significant change in antibody level in any of the three bleedings.

Group 3.—The analyses of the preimmunization sera with gelatin and clinical OPG again showed that gelatin precipitates more antibody than does clinical OPG. The total antibody precipitated by the absorption of antibody first with 1.4 μg. gelatin N followed by another absorption with 2.8 μg. gelatin N was equal to the amount precipitated by 2.8 μg. gelatin N followed by 1.4 μg. gelatin N.

In only 1 individual (No. 45) in the 3 week postimmunization bleeding was there any increase in antibody. No. 42 showed no change and all the others had decreases in antibody level.

Of the six volunteers from whom serum was obtained at the 7 week bleeding three exhibited increases, two showed no change, and one still had antibody levels below the preimmunization values.

The 7 week bleedings in this group were analyzed with gelatin, fractionated OPG, and the clinical OPG. In most cases the antibody N precipitated by gelatin and the high molecular weight OPG were about the same whereas the clinical OPG precipitated ⅓ to ½ as much antibody as the other antigens.

It should be mentioned that in addition to the results on medical volunteers tabulated in this paper, similar results have been obtained in another 40 volunteers. The serum from the 500 ml. bleedings of the two best reactors in each group were pooled into their respective groups. Results of the calibration curves obtained with the pools of Groups 1 and and 2 are presented in Figs. 2 and 3. The data used to prepare Fig. 2 are presented in Table VI. From these data again it is fairly certain that one is dealing with a protein-antiprotein system in which the antigen is polydisperse and/or denatured. That gelatin precipitates more antibody than the fractionated OPG, which in turn precipitates more than the clinical OPG, is further substantiated by these data.

As the precipitates were exceedingly gelatinous in character there was no

doubt that the antigens were in the precipitates. Preliminary analyses of the precipitates from some of the preimmunization sera for hydroxyproline also indicated that gelatin was indeed in the precipitate. However, in order to get a quantitative estimation of the amount of the added antigen which was incorporated into the precipitate, additional precipitates were analyzed for hydroxyproline. The results presented in Table VII (Group 1) indicate that in antibody excess about 33 per cent of the OPG, 65 per cent of the fractionated OPG, and about 65 per cent of the gelatin added are incorporated into the precipitates. Similar results were obtained with the other two pools of human serum. With Group 2 serum 80 per cent of the gelatin was incorporated into the precipitates in antibody excess. As all these antigens are polydisperse one can assume that those molecules of highest molecular weight are present in the antigen-antibody complex. Similar results were recently obtained by Kabat *et al.* (15) using the dextran-antidextran system. When the clinical type and fractionated dextrans containing C^{14} were added to human antidextran sera in the region of antibody excess 60 to 100 per cent of the added radioactivity was precipitated.

From the amounts of antigen added to the serum the theoretical amount of antibody N which should be precipitated can be read off the calibration curves (9). This information allows a calculation of the antibody N/antigen N ratios. When the ratios of antibody N/antigen N in the precipitates were plotted against the antigen N in the precipitates fairly straight lines were obtained. The initial combining ratios (2R) of the different antigens with the Group 1 serum were 8.3 for OPG, 10.2 for the fractionated OPG, and 13 for the gelatin. With the group 2 serum the 2R values were 4.4, 6.7, and 11.7 respectively. That fairly straight lines were obtained would lend credence to the fact that one is dealing with a true antigen-antibody reaction.

The gelatin anti-gelatin precipitates were soluble only in excess gelatin, thus fulfilling a very important criterion of an antigen-antibody precipitate; *i.e.*, that it be soluble in excess antigen. The results obtained with the formalinized gelatin and modified fluid gelatin were in accord with unpublished experiments from this laboratory on the cross-reactivities of various gelatin derivatives with antigelatin sera (16). As a complete report will be submitted shortly it is only necessary to state here that formalinized gelatin cross-reacted extensively with antigelatin sera whereas modified fluid gelatin did not react.

That the protein precipitated with gelatin resembled human gamma globulin is suggested by the fact that the precipitates picked up additional protein material from the rabbit anti-HGG serum (Table VIII). Although offhand one might expect the gamma globulin in the gelatin-antigelatin precipitates would react with more antigamma globulin, it should be remembered that the gelatin may be masking some of the sites in the gamma globulin molecules which are capable of reacting with the antihuman gamma globulin molecules.

Additional proof that the gelatin-antigelatin precipitates contained gamma globulin was furnished by the ultraviolet absorption spectra of the dissolved precipitates which resembled closely that of human gamma globulin.

DISCUSSION

Before discussing any of the implications of these findings on the antigenicity of gelatin in man the obvious question arises as to why had it not been observed heretofore. Previous investigations of this problem did not utilize the micro-precipitin techniques used in this study. Waksman and Mason (2) detected some increase in the nitrogen adsorbed on collagen particles when added to human sera. However, this increase was well within the limits of this method of analysis for nitrogen. In addition, complement fixation tests which were used by previous investigators would be of little value as there were no significant differences in the antibody levels before or after decomplementation with an ovalbumin (Ea)–rabbit anti-Ea system (17). The recent paper of Watson, Rothbard, and Vanamee (18 *a*), which is a more intensive investigation of the work of Loiseleur and Urbain (18 *b*), indicates that rat collagen (acid-soluble form) is antigenic in rabbits. The method for estimation of the antibodies was complement fixation. It is quite possible that the collagen-anticollagen system fixes complement whereas the gelatin-antigelatin precipitate does not. From recent experiments in this laboratory there seem to be some immunochemical differences between gelatin and collagen. Another very likely possibility as to why no precipitation was observed before, is that in previously reported work the reaction was observed only for 48 hours. In most of the cases studied here precipitation of the antigen-antibody aggregates did not occur for 3 to 6 days. At this point it may be worth mentioning that the speed for precipitation was determined not only by the antibody level in the serum but also by the antigen added. Gelatin, in many cases caused precipitation overnight, whereas the clinical OPG set up at the same time did not precipitate for 4 to 5 days. With other sera the gelatin did not precipitate before 4 days.

At this point it might be questioned whether or not we are measuring antibodies to gelatin or to some impurity in the gelatin. Although the presence of hydroxyproline (gelatin) in the precipitates should be conclusive evidence, further evidence was obtained through the use of gelatins from several species (16). These various gelatins were almost equally effective in precipitating antibody from different sera. Complete data on these reactions will be presented in another communication. That all these gelatins would have the same impurity is fairly unlikely. However the possibility that gelatin or its derivative is cross-reacting with antibody to collagen or some degradation product of collagen cannot be overlooked. Experiments are underway to compare by these quantitative immunochemical techniques the "procollagens" (soluble collagen) and collagens from the various species with their respective gelatins.

The next question of importance is what is the source of the antibodies to gelatin in the pre- "immunization" sera. At least two possibilities exist. The individuals may produce antibody against the gelatin present in most diets or else the antibodies may be made to the naturally occurring collagen in the connective tissue. It is quite plausible that by the action of certain enzymes collagen might be converted into a gelatin-like substance which then can induce the formation of antibodies. In order to test these possible explanations it is hoped to analyze sera from the following type individuals: infants who have never been fed gelatin or meat products, true vegetarians, and individuals suffering from what has been termed "collagen" disease such as lupus erythematosis, disseminated lupus, rheumatoid arthritis, and rheumatic fever. Although similar studies were initiated by Waksman and Mason (2), their method of analysis was not sensitive enough to detect 20 μg. of antibody N.

The data presented here to substantiate the fact that there are antibodies to gelatin in human sera may seem a bit voluminous. However, it should be realized that one of the theories concerning the antigenicity of proteins has been based on the fact that gelatin could not be shown to be antigenic. The lack of tyrosine residues in the molecule (19), the denatured state in which gelatin exists (20), and the high concentration of glycine which does not allow the molecule to attain a rigid backbone structure (21) have been said to account for the non-antigenicity of the gelatin molecule. By an entirely different method, namely the hemagglutination technique (22) which can measure as little as 0.006 μg. antibody per ml. serum, Grabar has recently confirmed the findings presented here concerning the presence of significant amounts of antibodies to gelatin in normal sera (23). In view of the data presented here and the confirmation by Grabar, some of the criteria for antigenicity of a protein molecule will have to be revised.

Since gelatin or its derivatives have been proposed as plasma expanders, the question arises as to the advisability of employing a plasma substitute which is antigenic. Under proper medical supervision two good reactors to gelatin were given reinjections of gelatin. No untoward reactions were observed at all.

It is worth recalling here an idea proposed by Grabar that some antibody molecules may not necessarily be functioning as part of a protective mechanism but rather as a transporting system involved in carrying materials to sites for further reaction (24). That the antibodies to gelatin are instrumental in the metabolism of gelatin and/or collagen is quite plausible.

The findings presented here and in the next paper which show that many normal sera have antibodies to gelatin make available immunochemical techniques for studying problems connected with the structure of the gelatins, soluble collagens (procollagens), and insoluble collagens from many species. Applications of these techniques to a study of "collagen" disease can also be visualized. Investigations along these lines are contemplated.

SUMMARY

It has been demonstrated that there are normally occurring antibodies to gelatin in human sera. Immunization with gelatin can in many cases increase the antibody level. The presence of these antibodies does not result in cutaneous reactions of the wheal and erythema type after injection of antigen.

Many of the properties of the gelatin-antigelatin reaction and the precipitates formed are those of a truly specific antigen-antibody aggregate.

Explanations have been advanced both as to the possible sources of the gelatin antibody and its significance.

The author wishes to thank Dr. Arthur Cherkin of Don Baxter, Incorporated, and the staff of the National Research Council Subcommittee on Shock for making available the materials used in this study.

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Acta allergologica, 1952, V, 230–238
5(3): 230-238

From The Wright-Fleming Institute of Microbiology,
St. Mary's Hospital Medical School, London.

CLINICAL SENSITIVITIES TO GELATIN AND OTHER MEAT PRODUCTS

By

R. L. MENDEZ
M.B. Lond.
Late Clinical Assistant, Allergy Dept.

W. HOWARD HUGHES
M.D. Lond.
Reader in Bacteriology, University of London.

Gelatin is generally considered to be non-antigenic in animals and it has therefore been assumed that it is non-antigenic in man also (Spain et al. 1939, Wilson and Miles 1946, Cooke 1947). In consequence, gelatin sensitivity is rarely sought in allergic patients and may well have been overlooked. So certain are allergists on this point that gelatin is now being introduced as a carrier for A.C.T.H. (Gay and Murgatroyd 1952).

Extensive animal work has been done to test whether gelatin can produce anaphylactic shock or stimulate the production of specific antibodies. The most convincing reports are those of Starin (1918), Polettini (1931), Taylor et al. (1943), Pugsley et al. (1943), Robschweit-Robbins et al. (1944), Wormall (1944) and Vanderbrook et al. (1947). None of these authors was able to demonstrate the presence of precipitins or complement-fixing antibodies, nor could they produce anaphylactic responses. The only positive evidence that any response is made by the body to the injection of

gelatin is contained in papers by Arthus (1908), Abderhalden (1910) and Ascoli and Izar (1910). The matter has naturally been carefully investigated in connection with plasma substitutes for transfusion work (Amberson 1937, Taylor and Waters 1941, Waters 1941, Gordon et al. 1942, Parkins et al. 1943, Randolph 1947, Skinsnes 1947). These authors in general consider that gelatin is innocuous and fails to give rise to anaphylactic responses. It is known however that gelatin can act as a hapten and becomes antigenic when coupled to other substances such as diazotised aniline, arsanilic acid, etc. (Landsteiner 1919, Adant 1930, Bruynoghe et al. 1930, Hooker and Boyd 1933, Hopkins and Wormall 1933, Clutton et al. 1938, Mori 1939, Haurowitz et al. 1943, Wormall 1944). Pure gelatin is however thought to be non-antigenic in man and unable to give rise to allergy, and a number of theories have been advanced to explain this peculiarity (Wormall 1937, Haurowitz et al. 1943, Wormall 1944). Cases apparently sensitive to gelatin have been explained as being due not to the gelatin itself but to other proteins of the animal from which the gelatin was derived, small quantities of these being carried over to the finished product (Rowe 1937, Urbach 1946, Randolph 1947). This type of sensitivity undoubtedly exists and is illustrated by *Case b*, although it cannot explain the results obtained in the following case.

Case a.

A young woman of 22 complained of urticaria and angioneurotic oedema associated with some meals. She was engaged to be married and the attacks were particularly marked when she ate in her fiancé's company in restaurants. Attacks came on very soon after the beginning of a meal, the first sign being irritation of the mouth and lips, followed by swelling. She had observed that roast meat did not affect her in this way but that boiled and stewed meats were particularly irritating, and that soups and gravies made from

stock also produced this effect. Skin testing by the prick method with extracts of foods likely to have been the cause, including meats, gave negative responses and it appeared that the condition might be classed as psychogenic. Nevertheless, further questioning showed that she was not particularly "squeamish" about food, having no objection to other substances of slippery texture such as boiled onions, macaroni, etc., and that the attacks did not occur after knowingly eating roast horse meat. One other group of foods also caused symptoms—sweets such as ice-cream, "jellybabies", table jellies and trifles containing jelly. A series of extracts was prepared of gelatin, peptone, ice-cream and also of various proprietary substances which would normally be used in gravies, such as Oxo, Marmite and Bisto. On testing with these six substances positive reactions were obtained to them all, the largest being to gelatin itself. Negative reactions were obtained to the mould groups. Hyposensitisation with gelatin caused a reduction of the skin reaction and a disappearance of clinical symptoms. After four and a half years symptoms began to return, the skin reactions increased in size and it was found necessary to repeat the hyposensitisation. This time she has obtained only partial relief, being able to eat without symptoms small quantities of meats and gravies, although a spoonful of jelly will give rise to swelling of the eyelids, lips and throat within 5 minutes.

Serum was separated from 200 ml. of *Case a* blood and after successfully passing Wassermann and sterility tests was stored in the refrigerator.

This serum undiluted and diluted 1/5, 1/10, 1/20, 1/200, and 1/2,000 was titrated against gelatin solutions diluted 1/100, 1/200, 1/400, 1/800, 1/2,000 and 1/20,000 but precipitins could not be demonstrated.

To investigate the possible presence of circulating skin-sensitising antibodies passive transfer tests were carried out. Owing to the possible occurrence of serum jaundice it was considered unjustifiable to perform such tests, using the Prausnitz-Küstner technique, on normal healthy subjects, and

these tests were therefore performed on 4 non-atopic adult volunteers whose expectation of life was less than the incubation period of serum jaundice. (In fact, no jaundice appeared subsequently in any of them.) All 4 patients gave negative results when prick tested with normal saline, grass pollen, beef, mutton, pork, horse serum and gelatin extracts, and the histamine skin responses were within normal limits.

In 3 patients 0.05 ml. of *Case a* serum was injected intradermally into each of 4 sites on the flexor surface of the forearm, and 2 further sites were sensitised by the intradermal injection of 0.05 ml. of serum from a hay fever subject (W.R. negative) known to possess circulating pollen antibodies. After 48 hours (during which period gelatin-containing foods were forbidden) the first 4 sensitised sites were injected intradermally with 0.02 ml. of normal saline and 0.02 ml. of beef, mutton, and gelatin extracts. The last 2 sites were injected with 0.02 ml. of normal saline and 0.02 ml. of grass pollen extract (positive control). Each injection on a sensitised site was repeated on adjacent normal skin (negative control). Results were read after 25–30 minutes. Positive results were obtained with the gelatin and grass pollen extracts only. In each case the response at the site of the gelatin injection was about half the size of the grass pollen response (positive control), and the smallest response obtained to a gelatin injection consisted of a wheal measuring 0.5 cm. × 0.3 cm. surrounded by a zone of erythema measuring 1.5 cm. × 2.0 cm. At all the other injection sites there occurred either no reaction or else a small flare lasting from 5 to 20 minutes unaccompanied by whealing.

In the fourth patient 4 sites sensitised with *Case a* serum were prick tested 48 hours later with normal saline, beef, mutton and gelatin extracts. No responses were obtained but a positive control test using a known anti-grass pollen serum was not performed.

The gelatin extract was made by breaking up 10 gm. of chemically pure bovine ossein sheet gelatin in 100 ml. of Coca's extracting fluid. This was auto-

claved at 15 lbs. pressure for 20 minutes, thus dissolving the gelatin and sterilising the whole. The gelatin solution so obtained was diluted ten times with sterile normal saline and bottled. This "extract" did not solidify on cooling and was used for all skin testing purposes and (suitably diluted) for the precipitin titration l.c. and for hyposensitisation. It contained 0.01 gm. of gelatin per ml. (equivalent to 10,000 Noon units per ml.) and 1.215 mg. of protein nitrogen per ml.

This case shows that allergy to gelatin can exist with no accompanying sensitivity to other animal proteins, that it behaves like an ordinary protein sensitisation, giving an immediate type of skin response, that desensitisation can be undertaken, and that circulating skin-sensitising antibodies can be demonstrated.

The more common type of allergy, in which there is a sensitivity to an animal protein and also to the gelatin derived from it, is illustrated in the second case. Here it would have been impossible to provide convincing evidence that there was no carry-over of animal protein in the manufacture of the various products which had given rise to symptoms.

Case b.

A widow of 26, in domestic service, gave a history of urticaria and angioneurotic oedema occurring within one to six hours of eating any meat. Twice she had lost consciousness and the attacks were often accompanied by generalised joint stiffness and pain lasting from 24 to 48 hours. From the age of two until she was eighteen the patient had had asthma, but had been free for the past eight years. The present symptoms had begun six years before, during her first pregnancy, and had never occurred except after eating meats and gravies. Pork was especially troublesome. On skin testing by the prick method positive responses were obtained to horse dander, cheese, milk, pork and rabbit flesh. The first three did not appear to be clinically significant. On further testing with gelatin, Oxo, Marmite and Bisto positive responses were obtained to gelatin and Marmite. The mould groups gave negative responses and therefore the skin response to

Marmite was not due to its yeast content. Although negative responses were obtained to beef, mutton and horseflesh the patient could not eat these meats.

A related condition has been found in the following case where there is sensitisation to the products of meat protein digestion without any direct evidence of sensitisation to the meat itself.

Case c.

This case presented a history suggestive of psychogenic asthma. An 18-year-old law student working for examinations had had asthma since childhood. He complained that recently the asthma had become very much worse. Attacks were particularly bad on Sunday afternoon after he had lunch with his family. During the week he was practically a vegetarian, avoiding meat products, and he claimed that any meat meal would give him an asthmatic attack within about 2 hours. Skin prick tests against various meats were negative, but as it had been made clear by *Case a* that sensitivity to parts of meat could occur he was tested with the same series of extracts and positive responses were obtained to Marmite and Oxo but not to gelatin. Testing with the mould groups gave negative results. It was thought that his sensitivity might be due to breakdown products of meat protein digestion, so a tryptic digest of beef muscle was set up at 30°C and samples were withdrawn after 0, 30, 60, 90, 120, 150 and 180 minutes. The samples were immediately heat sterilised (thus inactivating the trypsin) and their pH adjusted to 7.2, and they were then placed in sterile containers on ice until used for skin tests later. Positive skin responses were obtained with the 30-minute to 150-minute beef digest samples only. The first and last beef digest samples gave negative skin responses.

In a sequence of over 1,000 allergic cases, only 4 gave positive skin responses to gelatin with no positive skin responses to meat itself or to meat extracts not containing gelatin. 2 other cases gave positive skin responses to gelatin

and to meats. Of these 6 cases clinical symptoms were present in 2 only (*Cases a* and *b*).

DISCUSSION

In these cases we have therefore encountered 3 types of sensitivity to meat products. The first (recognised for many years) consists of those cases sensitive to meat itself and to its products (where it is probable that traces of unaltered protein or protein not sufficiently denatured to lose its specificity are present in these products). *Case b* is of this type. The second type consists of cases similar to those recorded previously by Stull and Hampton (1941) and by Cooke (1930, 1942), in which products of meat digestion give rise to symptoms but in which there is no direct evidence of sensitisation to the original protein (*Case c*). Finally there is a type, apparently not previously reported, in which there is a sensitisation to gelatin (or possibly to products of gelatin hydrolysis) in the absence of a reaction to the proteins of the parent meat or to meat products not containing gelatin (*Case a*). It is possible that the positive skin responses to gelatin were due to some product of gelatin hydrolysis and the positive response to peptone may be significant in this respect, although even if this were so it would make little difference to the practical applications of skin testing and the assessment of results, as clinically the patient would still be aware of a sensitivity only to gelatin itself.

From previous work already quoted it has been shown that gelatin alone cannot be made to give rise to anaphylaxis in animals, and in man it appears to be a rare allergen.

SUMMARY

3 cases are described illustrating different types of sensitivity to meats and their derivatives, including one case of sensitisation to gelatin in the absence of other demonstrable protein sensitivities. This gelatin sensitivity was confirmed by successful hyposensitisation and by the demonstration of circulating skin-sensitising antibodies.

The incidence of positive skin reactions to gelatin in a series of over 1,000 allergic patients was less than 1 in 150, and the incidence of clinical sensitivity to gelatin was 1 in 500.

ACKNOWLEDGMENTS

Our thanks are due to Mr. C. Jenkins for performing the protein nitrogen estimations.

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U. S. Fish and Wildl. Serv. Res. Rept. 4:1-9, 1942

METABOLISM STUDIES WITH ALGIN AND GELATIN

By Hugo W. Nilson, *Pharmacologist*, and J. M. Lemon, *Technologist*, *Division of Fishery Industries, Fish and Wildlife Service*

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INTRODUCTION

Algin, a salt of alginic acid extracted from kelp, and gelatin, produced from animal bones and skins, have been used for some time as stabilizing agents in the manufacture of ice cream and in similar food products. In ice cream they function through certain of their colloidal properties by affecting the size and nature of ice-crystal formation during the freezing and hardening process and storage; thereby producing a smoother product. Algin also is used to a considerable extent in stabilizing chocolate milk. Members of industries utilizing marine plants are particularly interested in determining some of the metabolic constants of these products.

This investigation was begun in March 1939 in the technological laboratories of the Fish and Wildlife Service. The algin and two grades of gelatin used were purchased from ice cream manufacturers. One grade was a bone gelatin of high gel strength, while the other was manufactured from skins. Gelatin manufactured from skins is used to a greater extent as a stabilizer in ice cream.

JUN 24 1942

EXPERIMENTAL DATA

The algin tested was a sodium alginate product extracted from *Macrocystis pyrifera*, the giant kelp that grows off the coast of California. The product was standardized to uniform colloidal strength with sugar and dextrin and treated with phosphate to make it soluble in milk. Goodman (1935)[1] reported that about 0.15 to 0.25 percent of sodium alginate produced satisfactory stabilization of ice cream. Sommer (1938) pp. 428–433, found that 0.25 percent of sodium alginate was equal or slightly superior to 0.4 percent 225 Bloom gelatin for this purpose. Anderson, Dowd, and Helmboldt (1937) reported an improvement in the sodium alginate mix over a gelatin mix, and that algin

[1] Publications referred to parenthetically by date are listed in the literature cited, p. 9.

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as a stabilizer produced an ice cream with a smoother and creamier melt down.

Chemically, sodium alginate consists of the sodium salt of polymerized d-mannuronic acid in which the carboxyl groups are free and all of the aldehyde groups are shielded by linkages. About 71 percent of algin with sugar and dextrin may be classed as carbohydrate, and it was incorporated into diets in replacement of part of the sugar and dextrin (tables 1 and 2).

TABLE 1.—*Composition of diets used*

Ingredients	Percent by weight								
	Diet No. 1 (control)	Diet No. 2	Diet No. 3	Diet No. 4	Diet No. 5	Diet No. 6	Diet No. 7	Diet No. 8	Diet No. 9
Algin		5.0	10.0	20.0	30.0				
Gelatin						5.0	10.0	20.0	30.0
Sucrose	27.0	26.1	22.4	19.1	15.8	26.0	25.0	23.0	20.4
Dextrin, tapioca	26.0	26.1	21.5	17.7	14.4	25.0	22.2	20.5	19.0
Lard	14.0								
Casein, technical	15.0								
Lactalbumin, technical	5.0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
Brewer's yeast, dried	5.0								
Wheat germ	2.0								
Cod liver oil	2.0								
Salt mixture, regular	4.0					4.0	4.0	4.0	4.0
Salt mixture, No. 1		3.3	.5						
Salt mixture, No. 2			2.6	2.6					
Supplement value:									
Protein equivalent						2.0	5.0	10.0	15.0
Carbohydrate equivalent		2.3	7.1	14.2	21.5	2.4	2.3	5.2	12.6
Mineral equivalent		.3	1.3	2.6	3.8				
Excess moisture		.7	1.4	2.3	4.2	.6	1.2	1.5	2.4

[1] Same in all diets.

TABLE 2.—*The composition of the algin, gelatin, and the salt mixtures used*

Ingredients	Percent by weight					
	Algin with sugar and dextrin	Gelatin		Salt mixture		
		Skin	Bone	Regular	No. 1	No. 2
Moisture	14.0	12.0	12.0			
Mannuronic units	37.0					
Gelatin		86.0	87.5			
Ash	15.0	1.0	.2			
Sucrose	17.0					
Dextrin	17.0					
Calcium lactate				31.0	54.0	65.0
Dicalcium phosphate				11.0		
Dipotassium phosphate				22.0		
Potassium chloride				12.5	15.0	15.0
Sodium chloride				5.0		
Calcium carbonate				7.0	15.0	
Magnesium carbonate				3.0	5.0	5.0
Magnesium sulfate				3.7	1.5	
Ferric citrate				1.3	4.0	1.5
Copper sulfate				.4	1.0	1.3
Manganese sulfate				.1	.5	1.0
Potassium iodide				.01	.01	0.2

as a stabilizer produced an ice cream with a smoother and creamier melt down.

Chemically, sodium alginate consists of the sodium salt of polymerized d-mannuronic acid in which the carboxyl groups are free and all of the aldehyde groups are shielded by linkages. About 71 percent of algin with sugar and dextrin may be classed as carbohydrate, and it was incorporated into diets in replacement of part of the sugar and dextrin (tables 1 and 2).

TABLE 1.—*Composition of diets used*

Ingredients	Percent by weight								
	Diet No. 1 (casein)	Diet No. 2	Diet No. 3	Diet No. 4	Diet No. 5	Diet No. 6	Diet No. 7	Diet No. 8	Diet No. 9
Algin		5.0	10.0	20.0	30.0				
Gelatin						5.0	10.0	20.0	30.0
Sucrose	37.0	33.1	29.4	19.1	15.8	30.0	28.0	23.0	20.4
Dextrin, tapioca	28.6	28.1	21.5	17.7	14.4	28.6	23.3	20.5	19.0
Lard	15.0								
Casein, technical	15.0								
Lactalbumin, technical	5.0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
Brewer's yeast, dried	5.0								
Wheat germ	2.0								
Cod liver oil	2.0								
Salt mixture, regular	5.0					4.0	4.0	4.0	4.0
Salt mixture, No. 1		3.5	.5						
Salt mixture, No. 2			2.0	2.0					
Supplement value:									
Protein equivalent						2.6	5.0	10.0	15.0
Carbohydrate equivalent		3.5	7.1	15.2	21.5	2.4	3.6	8.2	12.6
Mineral equivalent		.5	1.5	3.0	5.0				
Excess moisture		.7	1.4	2.5	4.2	.6	1.2	1.8	2.4

[1] Same in all diets.

TABLE 2.—*The composition of the algin, gelatin, and the salt mixtures used*

Ingredients	Percent by weight					
	Algin with sugar and dextrin	Gelatin		Salt mixture		
		Skin	Bone	Regular	No. 1	No. 2
Moisture	14.0	12.0	12.0			
Mannuronic units	37.0					
Gelatin		88.0	87.5			
Ash	18.0	1.0	.2			
Sucrose	17.0					
Dextrin	12.0					
Calcium lactate				31.0	55.0	65.0
Dicalcium phosphate				11.0		
Dipotassium phosphate				22.0		
Potassium chloride				12.5	15.0	15.0
Sodium chloride				8.0		
Calcium carbonate				7.0	15.0	
Magnesium carbonate				3.0	8.0	8.0
Magnesium sulfate				2.7	1.5	
Ferric citrate				1.3	4.0	5.5
Copper sulfate				.4	1.0	1.5
Manganese sulfate				.1	.5	1.0
Potassium iodide				.01	.01	0.2

Gelatin, a byproduct of the meat-packing industry, is a protein derived from white fibers contained in pig and calf skins, and ossein obtained from bones. Two grades were used in the experimental studies. Since gelatin is a protein, it was incorporated into the experimental diets in partial replacement of the casein and lactalbumin which supplied the protein of the basal diet. Gelatin is an incomplete protein, so enough casein and lactalbumin was included in all diets to at least approximately balance the gelatin protein. In no instance did a diet contain more than 35 percent protein. This higher level of protein has been found satisfactory for normal growth of rats in experiments in which casein was fed (Morris, Palmer, and Kennedy 1933).

ANIMAL-FEEDING EXPERIMENTS

Male albino rats, 4 weeks old, were allotted into nine groups of six animals each, with an average initial live weight per group of 53 to 54 g. The maximum range in individual live weight was 48 to 58 g. The animals were individually housed in screen cages that were fitted with screen floors. Food and water were fed freely and the intake of each animal was recorded. The food intake of the various groups was calculated to the equivalent dry-matter content of the control diet, and the excess was added to the water-intake records.

Groups were allotted to be fed the control diet and diets containing approximately 5, 10, 20, and 30 percent algin with sugar and dextrin, and 5, 10, 20, and 30 percent gelatin (tables 1 and 2). Three rats of each gelatin-fed group received bone gelatin, while the other three received skin gelatin. The data show no significant differences in response, so the results from the subgroups were summarized together in the final computation. The quantities of algin or gelatin fed were chosen arbitrarily, but the smaller amount represents about 20 times the quantity usually incorporated in ice cream.

Only one 9-week test was originally contemplated. In order to obtain accurate apparent digestibility coefficients representing the entire period, paper towels were spread under the screen floors and the feces collected every 2 or 3 days. Feces samples from individual rats were kept in a cool place and later dried to determine the dry-matter content. Preliminary tests soon showed that the algin could not be readily extracted from the dried feces. Consequently another sample was collected during the tenth week for the determination of algin, total nitrogen, and dry-matter content. Data for gain in weight, and food and water requirements cover the 10-week period, while data on the apparent digestibility of algin and the total protein are limited to the tenth week.

The algin content of the feces collected during the tenth week was determined for individual rats by digesting a 3-g. sample with 10 ml. of

0.5N/NaOH and 20 ml. water. The suspension was filtered after the addition of 10 ml. of saturated $(NH_4)_2SO_4$ and the undigested matter was washed with an additional 10 ml. of $(NH_4)_2SO_4$. Twenty-five ml. of a 40 percent solution of $CaCl_2$ and 25 ml. of water were added to the filtrate to precipitate the algin. Filter aid was added and the suspension was filtered through a Buchner filter. The filter cake was washed thoroughly with a saturated $(NH_4)_2SO_4$ solution. The amount of algin was calculated from the difference in weight after drying and then igniting the filter cake. Some difficulty was experienced with occlusion of protein. This procedure probably does not give an absolutely true answer, but it was considered to be the best approximation that could be obtained under the circumstances. The total crude protein content was determined by the modified Kjeldahl method of the A.O.A.C. on samples of feces collected during the tenth week from individual rats fed the control and gelatin diets.

In general, the rats grew very well and at the close of the 10-week period the mean live weight per rat ranged from 332 g. for the group fed the control diet to 123 g. for the group fed the diet containing 30 percent gelatin. The comparative mean live weights were 314, 312, 243, and 208 g. for the algin-fed rats, and 286, 241, 209 and 123 g. for the gelatin-fed rats for the 5, 10, 20, and 30 percent levels, respectively.

Two rats died in each of the groups that were fed algin. These rats sickened and died from 9 to 17 days after being fed the experimental diet. Since these animals died within such a short period, it was considered probable that the bacterial flora of the intestinal tract may have been an important factor. The rats that died may not have become properly infected with a type of bacteria that could grow satisfactorily in the algin media. The problem of physical impaction did not appear to be a factor even with rats fed the high levels of algin.

In order to test the significance of this interpretation, certain rats which had been fed similar diets containing 20 and 30 percent agar during the 10-week period were fed a diet containing 20 percent algin. They were killed at about 4-day intervals and 1-g. samples of the contents of the small intestine and cecum were diluted and plated on nutrient agar.

The data in table 3 show that there was a marked decrease in the bacterial numbers about the twelfth day. The rats that lived through this period showed a subsequent higher bacterial count of the intestinal contents, while those that died showed evidence that the food was not properly digested. Complete differential studies were not made, but it appeared that the grayish-blue colonies were gram negative rods and the small brownish colonies were gram positive cocci. The data are very incomplete, but apparently the bacterial flora of the intestinal tract was an important factor in the efficient utilization of food and in the health of the animals.

No characteristic lesions were found during necropsy of the rats that died when fed the regular experimental diet. The mean total fecal excretion of dry matter per rat during the 10-week period was 26 g. for the control group; 35, 38, 33, and 29 g. for the groups fed algin; and 19, 24, 16, and 12 g. for the groups fed gelatin at the 5, 10, 20, and 30 percent levels. The dry-matter content of the feces varied from 57 to 78 percent.

The data in table 4 show that the rats of all groups except those fed the 30 percent level of gelatin grew rather uniformly, although, only the rats fed the 5 and 10 percent levels of algin grew as well as the control group.

TABLE 3.—*A study of the bacterial flora in the contents of the intestinal tracts of rats fed a diet that contained 20 percent agar prior to June 7*

Rat number and diet fed	Date	Bacterial count in millions per gram		Remarks
		Small intestine	Cecum	
20 percent agar:				
121	June 23	3.3	10	The intestines were fairly well filled with food. The cecum was small and compact. Grayish-blue colonies.
117	July 5	1.5	9	Do.
20 percent algin:				
125	June 11	2.1	628	All grayish-blue colonies.
124	June 15	4.4	21	About 3 times as much food in intestines as for rat No. 125. Grayish-blue and brown colonies.
123	June 19	.2	3	The intestines contained very little food, but the cecum contained considerable solid food. Grayish-blue and brown colonies.
118	June 23	3.0	14	The intestines were filled with what appeared to be algin. The cecum was quite large and well filled with a greenish material. Grayish-blue and brown colonies.
120	do			Killed because he was obviously sick. He did not have any food in the cecum or intestines.
126	June 26			Died No food was found in the cecum or intestines.
122	June 27	16.0	14	The intestines were filled with an algin-like substance. Grayish-blue, brown, and a few white colonies.

TABLE 4.—*Daily gain in weight of rats for the 10-week period*

Group designation	Number of survivors	Mean daily gain	Coefficient of variation	Mean difference from control	Percent level of *t*
		Grams	*Percent*	*Grams*	
Control	6	3.99	13		
Algin:					
5 percent	4	3.72	20	0.27	[1]
10 percent	4	3.53	9	.46	[1]
20 percent	4	2.98	9	1.01	4
30 percent	4	2.20	21	1.79	1
Gelatin:					
5 percent	6	3.31	5	.68	5
10 percent	6	3.10	11	.89	2
20 percent	6	2.22	10	1.77	<1
30 percent	6	.99	31	3.00	<1

[1] Nonsignificant difference.

The data in table 5 are interesting since they show that none of the rats fed algin required more food per gram gain in weight than the control rats. The greater variability of food requirement for the rats

fed 30 percent algin (coefficient of variation equals 17 percent), and the smaller number of animals, made the difference from control of 0.85 g. insignificant. This finding need not be emphasized, since what happens when the lower levels are fed is of greater importance from the standpoint of stabilizers in foods. The groups fed gelatin all required more food per gram gain than did the control rats. There was no effect of increasing levels on the apparent digestibility of the dry matter of the diet, and the diets were digested surprisingly well in all instances.

The water requirement per gram gain in weight varies as may be expected (table 6). It is interesting to note that the rats fed the 5 and 10 percent levels of both algin and gelatin required about the same amount of water per gram gain in weight as the control group. Those fed the greater levels of either material showed a higher requirement which may be explained, at least in part, by the decreased growth rate. The mean, free-choice water consumption for the 10-week period was roughly 1,400 g. for the control group; 1,525, 1,450, 1,750, and 2,000 g. for the algin-fed rats; and 1,300, 1,275, 1,300, and 950 g. for the gelatin-fed rats.

TABLE 5.—*Food required per gram gain in weight and the apparent digestibility of dry matter for the 10-week period*

Group designation	Mean food required	Coefficient of variation	Mean difference from control	Percent level of *t*	Mean apparent digestibility of dry matter
	Grams	*Percent*	*Grams*		*Percent*
Control	3.00	6			96.6
Algin:					
5 percent	3.30	11	0.30	(1)	94.6
10 percent	3.22	8	.22	(1)	94.8
20 percent	3.34	8	.34	(1)	94.8
30 percent	3.85	17	.85	(1)	94.4
Gelatin:					
5 percent	3.31	4	.31	3	97.2
10 percent	3.54	9	.54	2	96.5
20 percent	4.17	16	1.17	1	97.1
30 percent	6.71	17	3.71	<1	97.0

[1] Non-significant difference.

TABLE 6.—*Water required per gram gain in weight for the 10-week period*

Group designation	Mean water required	Coefficient of variation	Mean difference from control	Percent level of *t*
	Grams	*Percent*	*Grams*	
Control	4.91	18		
Algin:				
5 percent	6.22	26	1.31	(1)
10 percent	5.94	8	1.03	(1)
20 percent	8.64	25	3.73	5
30 percent	13.24	8	8.33	<1
Gelatin:				
5 percent	5.67	17	.76	(1)
10 percent	5.98	9	1.07	(1)
20 percent	8.29	10	3.38	<1
30 percent	14.33	21	9.42	<1

[1] Nonsignificant difference.

The tenth week served as a feces-collection period in order to determine the digestibility of the algin and the total crude protein of the control diet and those containing gelatin. Some variation in apparent digestibility may be due to the fact that the food-intake data were taken from the period in which the feces were collected, and day-to-day fluctuations in food intake and feces excretion would have a greater effect during a period of 1 week than for a longer period. This factor may be responsible for part of the rather large coefficients of variation of 79 and 27 percent for the groups fed 5 and 10 percent algin diets (table 7).

The data in table 7 show an increasing apparent digestibility of the algin with increasing level in the diet. There was a statistically significant difference in every case. Either there was an adaptation of the intestinal tract to use a greater amount of the algin in the higher levels in order to conserve food supply, or the micro-organisms responsible for digestion grew better in the intestinal contents containing the higher levels of algin. The algin after digestion was apparently well utilized by the animal, since the data in table 5 show no significantly greater amount of food required per gram gain in weight as the algin content of the diet increased from 0 to 30 percent. Apparently the algin has considerable nutritive value and can be efficiently utilized when properly digested.

TABLE 7.—*Apparent digestibility of algin for a 1-week period*

Group designation	Mean apparent digestibility	Coefficient of variation	Mean difference within series	Percent level of t
Algin:	*Percent*	*Percent*	*Percent*	
5 percent	3.0	79		
10 percent	35.9	27	32.9	<1
20 percent	63.4	7	60.4	<1
30 percent	88.3	3	85.3	<1
10 percent				
20 percent			27.5	2
30 percent			52.4	<1
20 percent				
30 percent			24.9	<1

The data in table 8 show a very uniform apparent digestibility of the total protein of the control diet and the diets containing increasing levels of gelatin. The coefficients of variation were very small, indicating a high degree of uniformity. Apparently the gelatin was very digestible irrespective of the level fed, but the data in table 5 indicate a significantly higher quantity of food required per gram gain in weight when the gelatin was added to the diet and when the level of gelatin in the diet was increased.

TABLE 8.—*Apparent digestibility of the total protein of the diet for a 1-week period*

Group designation	Mean apparent digestibility	Coefficient of variation	Mean difference	Percent level of t
	Percent	*Percent*	*Percent*	
Control	89.2	1.4		
Gelatin:				
5 percent	88.5	1.8	0.7	(1)
10 percent	87.5	1.6	1.7	(1)
20 percent	89.8	1.4	.6	(1)
30 percent	92.1	1.3	2.9	(1)

[1] Nonsignificant difference.

DISCUSSION

These studies are concerned only with the nutritive values of the vegetable gum of algin and the protein of gelatin. In the final analysis, however, both products are used only in very small amounts as stabilizers in food products, because of their physical effects upon them. Although the nutritive values of the products are of secondary importance, both are wholesome. Two rats in each algin-fed group died soon after being fed the diets, but only negative lesions were found on necropsy. The survivors made reasonably satisfactory gains in weight. The gelatin-fed rats grew at a significantly lower rate than the control rats and required more food per gram gain in weight, but all lived. The group fed the control diet, and the combined groups which had previously been fed the 5 and 10 percent levels of algin or gelatin were fed the 5 percent levels of algin and gelatin and these groups have been continued on experiment for over 2 years. Deaths were finally due to pneumonia and malignant tumors, except 1 rat fed algin died of coronary embolism and another of possible digestive disturbance or intestinal infection.

SUMMARY

Male albino rats with an average initial live weight of 53 to 54 g. were fed individually for one 10-week period on a control diet supplemented with 5, 10, 20, and 30 percent of algin with sugar and dextrin, and 5, 10, 20, and 30 percent of gelatin. The apparent digestibility of the algin ranged from 3 to 88 percent, depending upon the level fed. The apparent digestibility of the total protein of the diets containing gelatin varied from about 88 to 92 percent. Both algin and gelatin were found to be wholesome foods.

Ten male rats of similar initial weight, but from another colony, were later fed the basal diet (salt replaced the salt mixture) plus 10 percent of algin with sugar and dextrin. None of the rats died during the 10-week period. The average daily gain was 3.20 g., and 3.32 g. of food and 8.04 g. of water were required for each gram gain in weight.

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Br. J. Nutr. (1970), 24, 241–258

The digestion and absorption of protein in man

2. The form in which digested protein is absorbed

BY S. ELIZABETH NIXON* AND G. E. MAWER

Department of Therapeutics, University of Edinburgh

(*Received* 6 *June* 1969—*Accepted* 2 *October* 1969)

1. Intestinal contents, collected from the human jejunum after a test meal (milk-protein, gelatin or low-protein) were fractionated by centrifugation and gel filtration on G-75 Sephadex. The fractions were hydrolysed and the proportion of the total amino acid in each fraction was determined. The amino acids were measured with an EEL Amino Acid Analyser.

2. The free amino acid concentrations were determined in samples of the contents of the small intestine collected from various levels after the three types of test meal.

3. Intestinal contents collected from two levels of the jejunum after a milk-protein meal, were incubated in vitro at 37° for periods up to 80 min and the rates of release of the individual free amino acids were determined.

4. There was a rapid breakdown of the proteins of the test meals to fragments of molecular weight under 5000. The further breakdown (during incubation in vitro) to free amino acids was sufficiently rapid to account for the absorption in the free form of arginine, lysine, tyrosine, valine, phenylalanine, methionine and leucine. It was not rapid enough to account for the absorption of glycine, threonine, serine, the imino acids or the dicarboxylic amino acids in the free form.

5. The free amino acid concentrations in the intestinal lumen bore very little relationship to the concentrations in hydrolysates of the test meals or to those in hydrolysates of the intestinal contents. Many of the free amino acids in the intestinal samples were present in approximately equimolar concentrations.

6. It is suggested that experiments in which amino acid mixtures, simulating a dietary protein, are fed to experimental animals to determine the rates of amino acid absorption do not present a true picture of the events in the small intestine following the ingestion of a protein meal.

Digestion is not a rate-limiting step in the overall process of digestion and absorption of protein (Dent & Schilling, 1949; Crane & Neuberger, 1960). Because of this, Adibi, Gray & Menden (1966), when studying absorption in man, used amino acid mixtures instead of whole protein. They found that, when amino acid mixtures simulating protein hydrolysates were perfused through the human jejunum, the rates of amino acid absorption depended on the concentrations and characteristic absorption rates of the individual amino acids. Glutamic acid and aspartic acid were absorbed slowly; leucine and methionine were absorbed rapidly. The amount of glutamate and aspartate which was absorbed was less than that of other amino acids, although glutamate and aspartate constituted a larger proportion of the mixture of amino acids perfused.

In a previous paper (Nixon & Mawer, 1970) we have reported the results of a series of experiments in which human volunteers were fed protein-containing test meals (milk protein and gelatin) and net absorption of the amino acid constituents (the differences between the amino acid concentrations in the gut and in the test meal,

* Present address: Stracathro Hospital, Brechin, Angus, Scotland.

related to polyethylene glycol) was determined at various levels of the small intestine. It was found that by a level of 230 cm from the nose about 75% net absorption of each of the amino acids of the milk-protein meal had occurred; absorption appeared to occur at rates proportional only to the concentrations in the meal. Glutamic acid was the principal component (about 18%) of this meal. In the course of this work, intestinal samples which were collected after the ingestion of the test meals were fractionated by precipitation and gel filtration techniques and the individual fractions were analysed for their amino acid content, with an EEL Automatic Amino Acid Analyser, in an attempt to answer the following questions: (1) what is the rate of release of free amino acids in intestinal contents after a protein-containing test meal? and (2) do the amino acids of a protein meal leave the intestinal lumen in the free form?

EXPERIMENTAL

Details of the six human experimental subjects, the intubation technique and the composition of the test meals (milk-protein, gelatin and a low-protein meal) have been given previously (Nixon & Mawer, 1970).

Intestinal samples

The samples of intestinal contents to be fractionated by centrifugation and gel filtration were frozen immediately after collection and were stored at $-25°$ until the analysis was to be done. 5 ml samples were taken from the region 100–190 cm from the nose for fractionation.

After the milk-protein meal five samples were taken during the period 15 min to 1·5 h after the ingestion of the meal, from a level of 110 cm. Although there were variations in the total concentrations of amino acids, the distribution of the various components showed no significant differences with time of collection, and the results of each fractionation experiment were therefore combined.

Three samples taken after the gelatin meal were fractionated; two were from a level of 110 cm and one was from a level of 190 cm. They were collected between 1 and 1·5 h after the ingestion of the meal. No differences were found between the samples from the two levels and the results of each experiment were combined.

Two samples taken from a level of 100 cm 1 h after the ingestion of the low-protein meal were fractionated.

Samples (5 ml) for free amino acid determination were collected as soon as intestinal contents began to siphon after the ingestion of the meal (5–10 min after the ingestion of the meal, from the upper intestinal levels, and 10–20 min, from the lower levels) and again, 1 and 2 h after the ingestion of the meal. The preliminary experiments showed that the free amino acid concentrations in each subject varied little with time of collection and therefore the three samples collected in each experiment were pooled. Immediately after collection, the 5 ml samples were put into 25·0 ml of 1% (w/v) picric acid.

Free amino acids were determined in twelve experiments after the milk-protein meal, at intestinal levels ranging from the proximal part of the duodenum to the

lower jejunum (80–200 cm from the nose), in four experiments after the gelatin meal at levels ranging from 110 to 190 cm and in four experiments after the low-protein meal at levels ranging from 110 to 240 cm.

Fractionation of intestinal contents and protein-containing test meals by centrifugation and gel filtration

G-75 Sephadex was soaked in 0·1% (w/v) NaCl for 24 h. After removal of fines it was made up into a column, 85 cm by 1·5 cm and equilibrated at 4° with ammonium acetate buffer, pH 5·6, ionic strength 0·08. The column was developed with the same buffer; a pressure head of 10–20 cm gave a flow rate of 15 ml/h. Ammonium acetate buffer was chosen so that the electrolyte could be removed later by freeze-drying. The mean pH of the intestinal samples used in these experiments was 5·6. The column was calibrated with Blue Dextran (molecular weight, 2000000), pepsin (molecular weight, 36000; Dixon & Webb, 1964), trypsin (molecular weight, 23800; Dixon & Webb, 1964) and a mixture of free amino acids. The positions of the protein and aromatic amino acid peaks were determined by their extinction at 280 nm. Except where stated the whole of the fractionation procedure was carried out at 4° using a cold room and a refrigerated centrifuge.

Fractionation of the dried milk powder

Humanized Trufood (Trufood Ltd) (700 mg) was suspended in ammonium acetate buffer to give a final volume of 10·0 ml; 5·0 ml were centrifuged for 30 min at 23600 *g*. The supernatant fraction was filtered through Whatman no. 5 filter-paper which was then washed twice with 1 ml portions of buffer. The filtrate with washings was concentrated by rotary evaporation to about 3 ml which was then loaded on to the Sephadex column; 3·5 ml fractions were collected and their extinction at 280 nm was determined.

Fractionation of gelatin

A solution of gelatin (10·0 ml/mg) was made up in ammonium acetate buffer and 4·0 ml was applied to the column at room temperature (at 4° the solution set rapidly to a stiff jelly on the surface of the gel). The fractions were collected and the E_{280} was determined as before.

Fractionation of the intestinal samples

Portions (5·0 ml) of intestinal contents collected after a protein meal were frozen immediately in polyethylene tubes and kept at −25° until the fractionation was to be done. It was then allowed to thaw at 4° and centrifuged at 23600*g* for 30 min. The supernatant fraction was decanted and the residue washed with 1 ml of the acetate buffer. It was then recentrifuged and the washing added to the supernatant fraction. The washing was repeated once.

After centrifugation the supernatant fraction and washings were combined and dialysed against 100 ml of the ammonium acetate buffer for 24 h. The contents of the dialysis sac were filtered through Whatman no. 5 filter-paper which was then washed twice with 1 ml of buffer. The filtrate and dialysate were recombined and freeze-

dried. The residue was dissolved in the buffer and then applied to the column. Intestinal samples taken after the low-protein meal were treated in a similar fashion but, because of the lower protein concentrations in these samples, 20·0 ml portions were fractionated. The samples were applied in volumes of approximately 4 ml as a layer between the surface of the gel and the buffer above the column, by means of a fine catheter attached to a 10 ml syringe.

Gel filtration on G-25 Sephadex

In some experiments, using intestinal contents after the milk-protein meal, Fraction IV (Fig. 3) was subjected to further filtration on G-25 Sephadex. A column, 45 cm by 1·9 cm, was made up and equilibrated with the ammonium acetate buffer. The totally excluded volume was measured with Blue Dextran and the elution positions of tyrosine, phenylalanine and tryptophan were noted.

Fraction IV was freeze-dried and redissolved in ammonium acetate buffer. After application of the sample to the column, fractions were eluted and read at 280 nm as before.

A 5 ml sample of intestinal contents, after precipitation of protein by picric acid, was treated in a similar fashion.

Hydrolysis of the fractions

The eluate from the column of G-75 Sephadex after filtration of the soluble component of the intestinal contents, could be conveniently divided into three fractions on the basis of the extinction at 280 nm (Fig. 3).

The 3·5 ml portions of each fraction were combined, with washings, into a 500 ml flask. They were freeze-dried and the residues were dissolved in 6 N-HCl containing $SnCl_2$, 1·5 mg/ml. Suitable concentrations were obtained when Fractions II and III were made up to 10·0 and 4·0 ml portions were hydrolysed. Fraction IV was made up to 20·0 ml and 5·0 ml portions were hydrolysed.

The residue after centrifugation of the original sample (Fraction I) was suspended in 6 N-HCl, with $SnCl_2$, to give a final volume of 10·0 ml, and 4·0 ml portions were hydrolysed.

The filter-paper, used for filtering the intestinal samples after dialysis, was washed with 6 N-HCl, with $SnCl_2$, to give a final volume of washings of 10·0 and 4·0 ml portions were hydrolysed. The amino acid content of this fraction was found to be very small and the concentration values were added to those of Fraction I.

Hydrolysis was carried out in sealed ampoules under an atmosphere of nitrogen for a period of 18 h at a temperature of 115°.

A separate portion of intestinal contents, prepared for the determination of the free amino acid concentrations by picric acid precipitation of protein, was subjected to acid hydrolysis in the same way as Fraction IV.

Determination of the free amino acid concentrations

The free amino acid concentrations in intestinal contents were determined by the method of Moore & Stein (1954).

After removal of the samples for hydrolysis from Fraction IV, 4·0 ml were freeze-dried directly and the residue was dissolved in 10·0 ml of 0·1 N-HCl for the determination of the free amino acid concentrations in this fraction.

Incubation of fresh intestinal contents

In two experiments, intestinal contents, (*a*) from a level of 110 cm from the nose and (*b*) from a level of 170 cm from the nose, were collected for 2 consecutive hours into polyethylene bottles embedded in crushed ice. The sample which was collected during the 2nd hour was mixed by swirling gently and 5·0 ml portions were transferred to 35 ml stoppered centrifuge tubes. These were fitted into a mechanical shaker and placed in a water bath at 37°. At intervals of 0, 5, 10, 15, 20, 40 and 80 min, 25·0 ml of 1% (w/v) picric acid was added to successive tubes in duplicate and the samples were subsequently treated as for the free amino acids.

RESULTS

Fractions of the intestinal contents and the test meals

Fraction I. Fraction I was the sediment at 23 600 *g*. The microscopic examination of haematoxylin and eosin stained sections showed no evidence of intact mucosal cells in this insoluble fraction of intestinal contents; 50% of the total amino acid of the milk-protein test meal was in this fraction but none of the gelatin was insoluble (Fig. 1).

Constituents containing amino acids were found in this fraction of all the intestinal samples. The mean proportions of the total amino acid content of the intestinal samples varied from 8% after the gelatin meal to 16% after the other two meals. (The total amino acid content in the samples was derived from acid hydrolysis of the unfractionated material.) The total concentrations of amino acids varied from 2·5 μmoles/ml after the low-protein meal to 8·5 μmoles/ml after the milk-protein meal (Fig. 2).

Fraction II. Fraction II was that fraction of the soluble material which was totally excluded from G-75 Sephadex and which was eluted in the volume from 45 to 65 ml (Fig. 3).

Although only 7% of the amino acids of the milk-protein meal and as much as 94% of the amino acids of the gelatin meal were contained in this fraction, in jejunal contents after the three meals the proportion of the total amino acid varied only from 7 to 17% (Fig. 1). The total concentrations of the amino acids varied from 2·5 μmoles/ml after the low-protein meal to 5·5 μmoles/ml after the gelatin meal (Fig. 2).

Fraction III. Fraction III was the fraction which was eluted from the column in the volume from 65 to 110 ml. The reference proteins, pepsin and trypsin, were eluted in this region of the chromatogram (Fig. 3); 20% of the amino acid-containing components of the milk-protein meal and 2% of the components of gelatin were in this fraction.

In jejunal samples after the three meals the proportion of the total amino acid varied from 7% after the milk-protein meal to 18% after the gelatin meal. The total

concentrations of amino acids varied from 2 μmoles/ml after the low-protein meal to 13 μmoles/ml after the gelatin meal.

Fraction IV. Fraction IV was that fraction which was eluted from the column in the volume from 110 to 180 ml. It included that region of the chromatogram in which a standard solution of an amino acid mixture was eluted (Fig. 3).

Only 2% of the total amino acid of the protein test meals was eluted in this region whereas 35–57% of the total amino acid content of jejunal samples after all three meals was contained in this fraction (Fig. 1). The total concentrations of amino acids varied from 6·5 to 41·5 μmoles/ml of intestinal contents (Fig. 2).

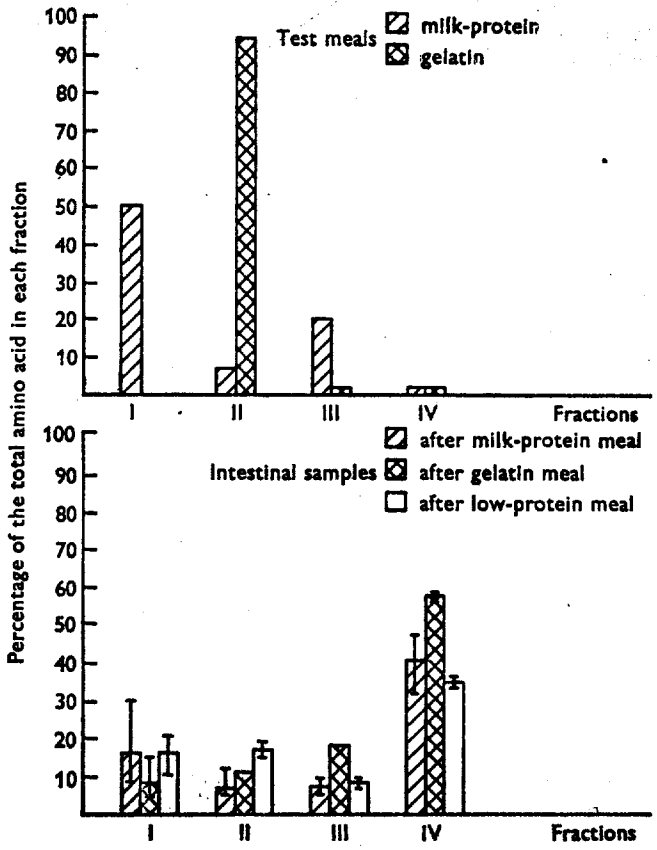

Fig. 1. Proportions of the total amino acid contained in each of the four fractions of the protein-containing test meals and of the intestinal samples from young adults after the test meals. The results are the means of five experiments after the milk-protein meal, of three experiments after the gelatin meal and of two experiments after the low-protein meal. The vertical lines represent the ranges.

Comparison between the test meals and the fractions of the intestinal contents

A rapid breakdown of the large molecules of the protein of the test meals had occurred in the lumen of the small intestine. By 15 min after the ingestion of the test meal, the distribution of the various components in the intestinal samples was similar to that found in samples collected up to 2 h later.

The breakdown was particularly noticeable with gelatin where the bizarre amino acid pattern allowed the processes of digestion to be studied more easily. The relative molar concentrations in the fractions indicate the qualitative changes which had occurred. The molar concentrations of the amino acids relative to leucine in the four fractions show that the whole of the gelatin components in the intestinal contents was contained in Fractions III and IV (Table 1); 75% of the hydroxyproline content of the intestinal samples after the gelatin meal was contained in Fraction IV and the remaining 25% in Fraction III.

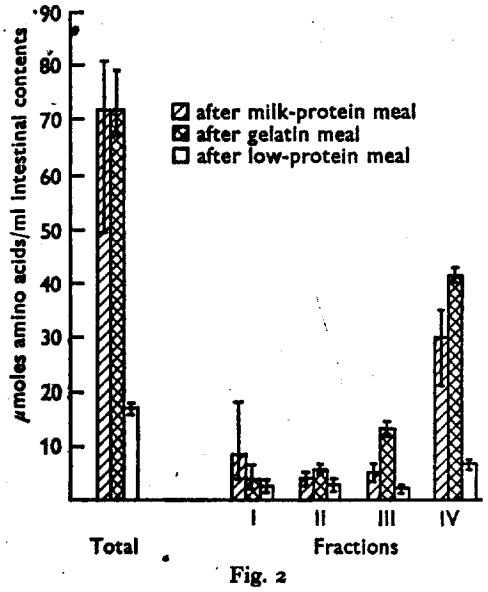

Fig. 2

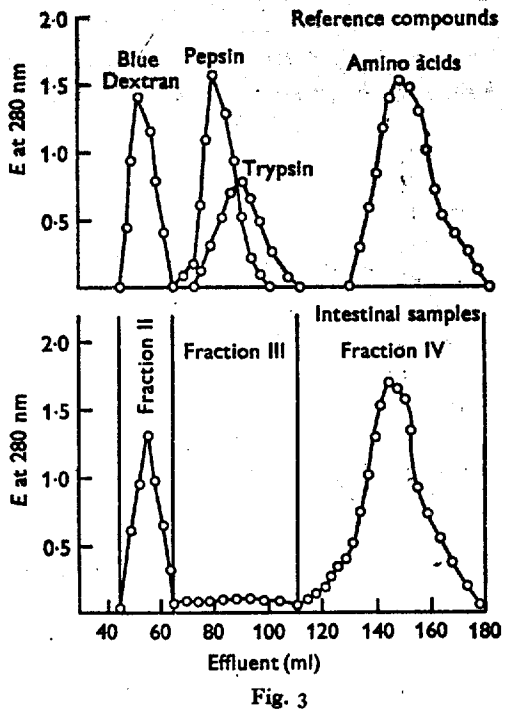

Fig. 3

Fig. 2. Concentrations (μmoles/ml) of the amino acids in hydrolysates of the unfractionated intestinal samples from young adults and of the four fractions of the samples after the three test meals. The results are the means of five experiments after the milk-protein meal, of three experiments after the gelatin meal and of two experiments after the low-protein meal. The vertical lines represent the ranges.

Fig. 3. The three arbitrary fractions of intestinal contents based on the extinction at 280 nm when the soluble component from 5 ml of intestinal contents from young adults was fractionated by gel filtration on a column 85 cm by 1·5 cm of G-75 Sephadex. (Fraction I was the insoluble component of the intestinal contents.) The upper part of the figure shows the elution positions of the reference compounds from G-75 Sephadex.

Even after the low-protein meal approximately one-third of the total amino acid content of the intestinal samples was in the form of amino acids or relatively small peptides.

Further investigation of Fraction IV

To exclude the possibility that Fraction IV was a result of continued enzyme activity during the gel filtration procedure, the amino acid content of this fraction, before and after hydrolysis, was compared with that of intestinal samples after pre-

cipitation of protein with picric acid (the 'picric acid supernatant') immediately on collection (as in the procedure for determination of free amino acids). The percentages of the total of each amino acid in the unfractionated intestinal samples, contained in each of these two fractions are shown in Table 2. With the exception of glycine the percentages are very similar for both fractions. The lower glycine figure in the 'picric acid supernatant' may have been due to loss by adsorption of bile salts on to Dowex 2 during the removal of excess picric acid, taurine, a prominent component of hydrolysates of Fraction IV, was absent from hydrolysates of the 'picric acid supernatant'.

Table 1. *Molar concentrations relative to leucine (leu = 1) of the amino acids in hydrolysates of the fractions of the intestinal contents compared with those in the test meals*

Amino acid	Fraction I			Fraction II			Fraction III			Fraction IV			Test Meals		
	MP	LP	G	MP	LP	G	MP	LP	G	MP	LP	G	MP	LP	G
Asp	1·1	1·7	1·6	1·3	1·4	1·3	1·3	1·5	2·0	1·3	1·5	2·0	0·93	0·67	1·8
Thr	0·75	0·94	0·90	0·88	1·0	1·2	1·3	0·70	1·0	0·78	0·71	0·81	0·68	0·61	0·72
Ser	1·0	1·1	1·3	1·0	1·1	1·0	1·1	1·3	1·7	0·88	0·88	1·5	0·72	0·78	1·4
Pro	0·89	0·89	1·6	0·76	0·93	1·7	1·2	0·90	6·7	0·93	0·71	5·0	0·85	0·81	4·4
Glu	1·5	1·2	1·6	1·2	1·1	1·3	2·3	1·1	3·6	2·3	1·7	3·5	1·8	0·97	2·8
Gly	1·0	2·8	5·0	1·0	1·3	1·9	0·94	4·3	14·1	2·0	7·9	12·8	0·39	0·55	12·6
Ala	0·78	1·0	1·5	1·0	1·0	1·2	0·89	0·90	4·3	0·76	1·1	4·5	0·64	0·48	4·4
Val	0·76	1·1	1·0	0·94	1·1	1·0	1·1	1·0	1·1	0·78	0·88	0·85	0·70	0·84	0·94
Met	0·11	0·12	0·12	0·06	0·05	0·05	0·11	0·10	0·10	0·16	0·18	0·21	0·19	0·19	0·24
Ileu	0·52	0·65	0·64	0·50	0·57	0·57	0·63	0·65	0·55	0·64	0·56	0·50	0·58	0·61	0·78
Tyr	0·29	0·47	0·30	0·44	0·36	0·33	0·26	0·45	0·15	0·25	0·35	0·17	0·28	0·26	0·06
Phe	0·39	0·59	0·42	0·44	0·43	0·46	0·29	0·40	0·45	0·35	0·47	0·42	0·35	0·45	0·59
Lys	0·67	0·65	0·68	0·59	0·50	0·61	0·42	0·55	0·84	0·84	0·79	1·3	0·68	0·65	1·1
His	0·22	0·29	0·27	0·26	0·28	0·30	0·24	0·25	0·21	0·23	0·29	0·26	0·20	0·42	0·42
Arg	0·27	0·47	0·47	0·32	0·36	0·39	0·18	0·30	0·34	0·40	0·44	1·1	0·21	0·36	1·4
Hyp	—	—	—	—	—	—	—	—	5·0	—	—	4·5	—	—	3·1

MP, milk-protein; LP, low-protein; G, gelatin.

In unhydrolysed samples the amino acids in Fraction IV constituted a slightly greater percentage of the total amino acids in the unfractionated intestinal sample compared with those in the 'picric acid supernatant'. This was most noticeable for methionine but was also true of tyrosine, lysine and arginine. Some release of free amino acids within the fraction may, therefore, have occurred without altering the total amino acid concentrations in the fraction.

The proportions of the total amino acids in Fraction IV and in the 'picric acid supernatant' which were present in the free form are shown in the right-hand column of Table 2. For about half the amino acids measured the proportion was less than 15%, but free tyrosine and arginine constituted 50–60% of the total tyrosine and arginine content of this fraction.

Gel filtration of Fraction IV and the 'picric acid supernatant' on Sephadex G-25 gave the elution patterns shown in Fig. 4. For comparison the positions of Blue Dextran (which was totally excluded from the gel), tyrosine, phenylalanine and tryptophan are shown. Neither of the samples contained any components which were excluded from the gel.

The agreement between the results of the analyses of Fraction IV and the 'picric acid supernatant' suggests that these are similar fractions although they were derived by different methods.

Table 2. *Comparison between Fraction IV and the intestinal contents of young adults after removal of protein with picric acid; the percentage in each of these fractions of the total amino acid present in the intestinal sample.*

Amino acid	Hydrolysed (A)		Unhydrolysed (B)		The free amino acids as % of the total in each fraction (B/A × 100)	
	Fraction IV	PSN	Fraction IV	PSN	Fraction IV	PSN
Asp	47	42	5	5	11	8
Thr	43	56	5	4	11	7
Ser	45	48	4	4	8	8
Pro	47	56	—	—	—	—
Glu	51	52	6	8	12	16
Gly	54	21	4	4	7	17
Ala	42	44	6	5	13	12
Val	45	44	6	4	14	9
Met	50	46	14	6	27	13
Ileu	47	48	3	2	7	5
Leu	44	38	5	3	11	8
Tyr	37	35	21	15	56	46
Phe	40	46	13	10	32	24
Lys	52	49	25	17	48	35
His	46	36	13	11	28	32
Arg	43	36	27	21	63	59

Fraction IV was that fraction of the intestinal contents of young adults which was not excluded from Sephadex G–25; the PSN was the fraction of intestinal contents which was soluble in picric acid.

Recovery of the amino acids containing components from the fractionation procedure

The sum of the total amino acids in hydrolysates of each fraction of test meal or of intestinal contents compared with the total amino acid content in the unfractionated material is a measure of the recovery. This varied from 68% from intestinal samples after the milk-protein meal to 98% from gelatin (Table 3). The losses probably occurred before gel filtration; when the amino acid content of the three fractions eluted from the column was compared with that of the material loaded on to the column, 95–100% recovery was observed.

The percentage recovery of amino acids was greater for gelatin and for intestinal samples collected after gelatin than it was for the milk-protein meal or for intestinal samples collected after this meal. This may have been because a greater percentage of the total amino acid was in a soluble form in intestinal contents following gelatin compared with that after the milk protein. Certainly the gelatin itself was more soluble than the casein of the milk protein.

Loss may therefore have occurred (*a*) of insoluble residue, due to non-uniform sampling for hydrolysis, or (*b*) of proteins which may have come out of solution during dialysis and adhered to the dialysis sac.

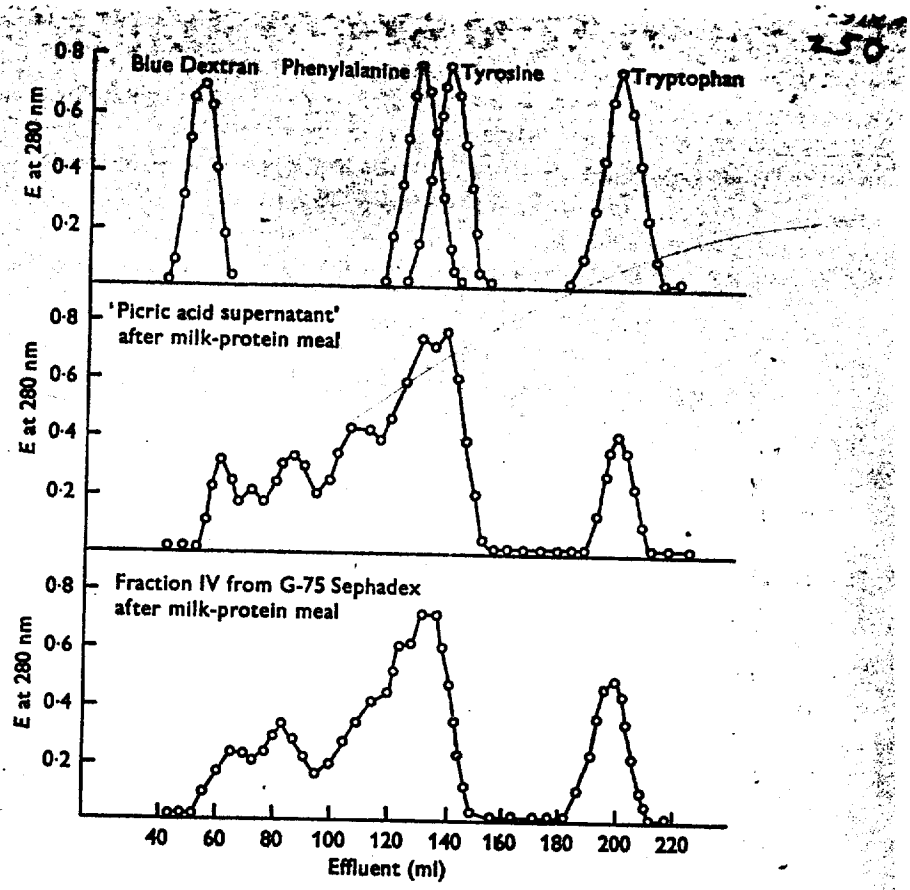

Fig. 4. Elution pattern from G-25 Sephadex of intestinal contents from young adults after removal of protein by precipitation with picric acid ('picric acid supernatant') compared with that of Fraction IV of the intestinal contents. The elution positions of the reference compounds are shown in the upper part of the figure.

Table 3. *Percentage recovery (mean and range) of total amino acid when the test meals and intestinal samples from young adults were fractionated by centrifugation and gel filtration on G-75 Sephadex*

(The single numbers in parentheses show the number of experiments which were done)

Meals		Intestinal samples after		
MP	G	MP	LP	G
80 (2)	98 (2)	62 (56–72) (5)	75 (74–76) (2)	93 (90–98) (3)

Percentage recovery of total amino acid when the soluble component of intestinal contents of young adults after a milk-protein meal was fractionated on G-75 Sephadex:

98 (95–100) (3)

MP, milk-protein; G, gelatin; LP, low-protein meal.

Concentrations of the free amino acids

The determination of the free amino acids in intestinal contents was complicated by the presence of ninhydrin-positive material, probably small peptides, which emerged from the ion exchange column immediately after the buffer peak, corresponding with the change from pH 3·25 to 4·25 buffer. This was particularly noticeable in samples collected after the gelatin meal where the peaks corresponding to cystine, valine, methionine, isoleucine and leucine, which were present in small concentrations, were

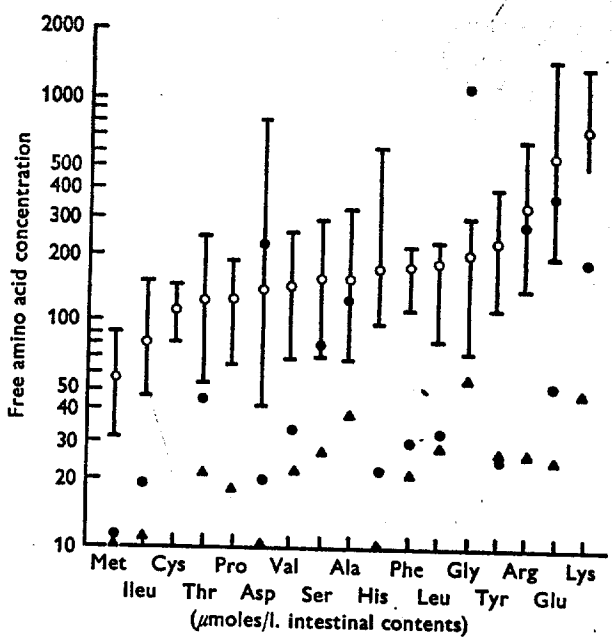

Fig. 5. Free amino acid concentrations (μmoles/l.) in 3 h collections of small intestinal contents from young adults after the three test meals. The values for samples after the milk-protein meal are the means and ranges from experiments at intestinal levels ranging from the proximal part of the duodenum to the lower jejunum (80–200 cm from the nose). The values for samples after the gelatin test meal are the means of four experiments at levels ranging from 110 to 190 cm from the nose. The values for glutamate and aspartate are given separately for the two subjects who took this meal. The values for samples after the low-protein meal are the means from four experiments at levels ranging from 110 to 240 cm from the nose. A log scale has been used. ○, after milk-protein meal; ●, after gelatin meal; ▲, after low-protein meal.

artially obscured by much larger peaks due to unidentified compounds. In many stances it was not possible to determine the actual concentrations of these amino cids.

ree amino acid concentrations showed little variation with level of sampling. oncentrations of aspartate, threonine, serine, alanine and histidine, after the milk- meal were lower in the duodenal samples than in the jejunal samples. Lindberg found that dipeptidase activity was low in the proximal duodenum but rose in the distal part of the duodenum. The concentrations of tyrosine and arginine

were lower in the lower jejunum than in the upper intestinal samples. However, more experiments would be necessary to show significant differences with level of sampling.

Fig. 5 shows the free amino acid concentrations (μmoles/l., mean and range) in samples from all levels in the six subjects after the milk-protein meal. The ranges were greatest for the dicarboxylic amino acids; high concentrations of free glutamate and aspartate in jejunal contents appeared to be a characteristic feature of the subjects B.N. (Fig. 6).

The concentrations in the intestinal contents after ingestion of the other two meals are also shown in Fig. 5. Except for glutamate and aspartate, the values are the means of four values from two subjects. The values for glutamate and aspartate in the intestinal contents after the gelatin meal are given separately for the two subjects, one of whom was the subject B.N.

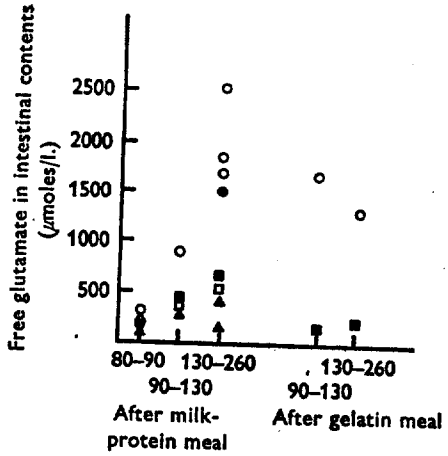

Fig. 6. Concentrations (μmoles/l.) of free glutamate in 3 h collections of intestinal contents from the six young adults. Each value is the result from one experiment. ○, B. N.; ●, B. W.; ▲, G. M.; △, B. H.; □, A. T.; ■, G. H.

The concentrations in the samples after the gelatin and the low-protein meals were, in general, lower than those after the milk-protein meal. The relative concentrations bore little relationship to the total amino acid concentrations in the test meals or to those in the intestinal contents. Proline and hydroxyproline, which together represented 21% of the amino acid content of the gelatin meal, were not detectable in the free state in the intestinal samples taken after this meal. (The limits of detection were approximately 50 μmoles/l. in each instance). Many of the amino acids in each sample were present in approximately equimolar concentrations.

The free amino acid concentrations expressed as percentages of the total amino acids in the intestinal contents were very similar in samples collected after the milk-protein meal and after the low-protein meal. Fig. 7 shows the mean values (and ranges) of the concentrations expressed in this way in samples after the milk-protein meal. Also shown on the figure are the mean values for the samples obtained after the other two meals (the values for glutamate and aspartate are again given separately for the two subjects taking the gelatin meal).

The proportion of the total amino acid which was present in the free form ranged from 2% for proline to 20% for arginine in samples taken after the milk-protein meal.

Release of free amino acids during in vitro incubation of fresh intestinal contents

Samples from two intestinal levels, 110 and 170 cm from the nose, were incubated. The total amino acid concentrations (μmoles/l.) were very similar in both types of sample; therefore the results from each level will be directly compared.

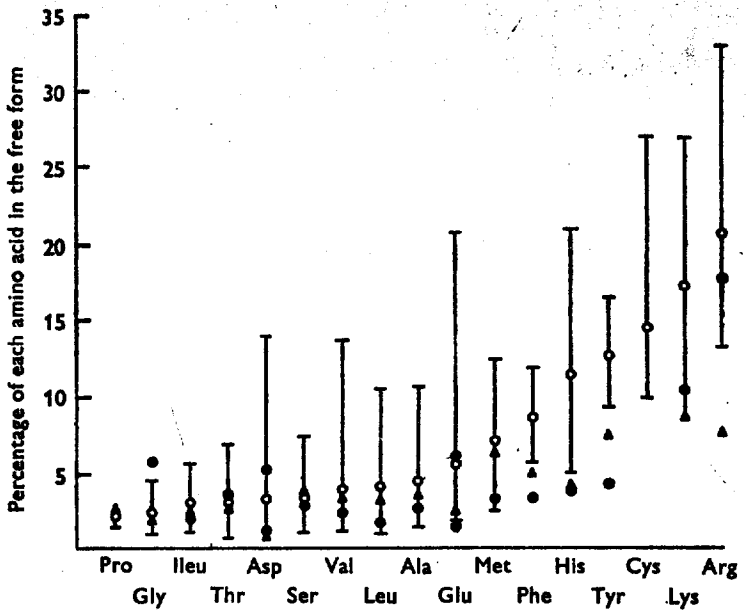

Fig. 7. Free amino acid concentrations in 3 h collections of intestinal contents from young adults expressed as percentages of the corresponding amino acid concentrations in hydrolysates of each intestinal sample (e.g. 2% of the total proline was present in the free form.) The values were obtained as indicated in Fig. 5. ○, after the milk-protein meal; ●, after the gelatin meal; ▲, after the low-protein meal.

The amino acids fell roughly into three groups with regard to the rate of increase in free amino acid concentrations during incubation. In Fig. 8*a*, *b*, the concentrations expressed as percentages of the totals, are plotted against the time of incubation for various representatives of the three groups.

Upper level (*Fig.* 8*a*). Arginine in samples from a level of 110 cm from the nose increased from 380 to 625 moles/l. during the first 10 min of incubation. Thereafter the rate of release declined, the concentration rising only to 775 μmoles/l. over the next 70 min. This concentration amounted to over 60% of the total arginine present. The proportions of lysine, methionine and tyrosine in the free state increased in a similar fashion. In contrast a group of amino acids, including proline, glycine and the acidic amino acids, showed little or no increase in concentrations during the period of incubation. Where an increase was observed it appeared to occur during the time when the rate of release of the rapidly released amino acids had declined. Between these

extremes there was a group, including histidine, alanine, phenylalanine and the branched-chain amino acids, which showed a gradual but continued rise in concentration over the incubation period.

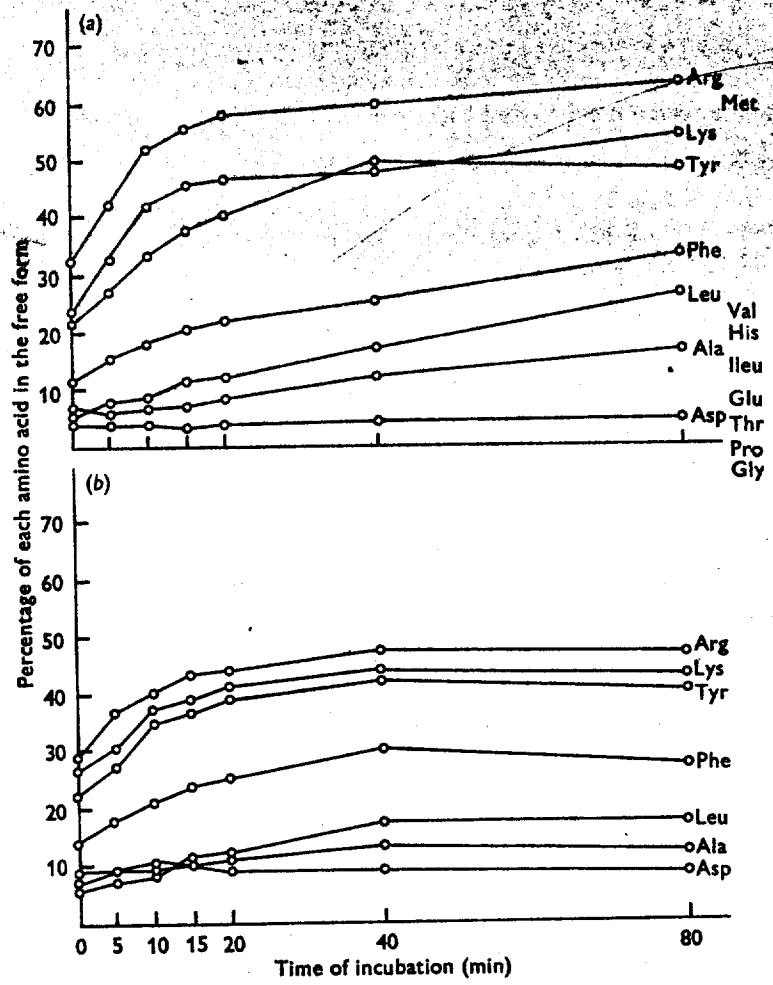

Fig. 8. Increase in the free amino acid concentrations, expressed as percentages of the corresponding amino acid concentrations in hydrolysates of each sample, when small intestinal contents, (*a*) from a level of 110 cm from the nose and (*b*) from a level of 170 cm from the nose, were incubated at 37°. Representative amino acids only are shown in each instance. The approximate final concentrations of the others are shown in (*a*).

Lower level (*Fig.* 8*b*). The same grouping was observed after incubation of the samples from the lower level. The initial free amino acid concentrations were sometimes slightly greater than those in the upper level sample but often were almost identical. The concentrations of free aspartate and glutamate in the sample from the lower level were approximately double those in the sample from the upper level.

The initial rate of release of the rapidly released amino acids was less in the samples from the lower level than in those from the upper level. After 40 min of incubation

there was no further release of any of the amino acids. The results for the group comprising phenylalanine, alanine, histidine and the branched-chain amino acids were almost identical at both levels over the first 40 min.

DISCUSSION

Digestion

There was a rapid breakdown of the food proteins in the small intestinal lumen. It was not possible to make definitive statements about the fate of the milk protein for the following reasons: (1) there was incomplete recovery from the fractionation procedure; (2) there was no way of differentiating between dietary and endogenous protein; (3) by the time the test meal had reached the level of sampling, 110 cm from the nose, a large part of the dietary protein had probably already been absorbed (Nixon & Mawer, 1970). These criticisms did not apply to the same extent to the intestinal samples after the gelatin meal. In these samples, where hydroxyproline could be used as a marker, the dietary protein of molecular weight over 50000 (Table 1; Gelotte, 1964) had been digested to the extent where 75% of the hydroxyproline was present in fragments of under 5000 molecular weight. The close relationship between Fraction IV and intestinal samples in which protein had been precipitated immediately on collection (Table 2) confirmed that the breakdown had occurred in the intestinal lumen.

Table 4. *Estimated rates (μmoles/min) of release of the free amino acids in the jejunal lumen of young adults after a milk-protein meal*

Amino acid	Upper level	Lower level	Amino acid	Upper level	Lower level
Lys	71	40	Ser	6·6	7·1
Val	30	13	Ileu	5·6	5·8
Arg	24	12	Glu	5·1	7·1
Leu	21	22	Thr	3·6	3·7
Tyr	20	21	His	2·1	2·5
Phe	15	12	Asp	1·7	3·0
Met	14	13	Pro	1·5	1·0
Ala	7·0	8·2	Gly	0·5	2·1

Although the samples which were fractionated were collected 1 h after the ingestion of the meal, most of the breakdown probably occurred during the passage of the test meal from the stomach to the sampling level. As test meal (containing polyethylene glycol, the intestinal marker) could be collected from the upper intestinal levels 5–10 min after the ingestion of the meal, the time for transit from the stomach to a level of 110 cm from the nose need be no more than 10 min.

The results of this work are therefore in agreement with the findings of Börgstrom and his colleagues (Borgström, Dahlqvist, Lundh & Sjövall, 1957) who found that the degree of hydrolysis of iodinated albumin rapidly reached 50–60% in the duodenum. The extent of hydrolysis in their experiments was determined by the decrease of ^{131}I

which was precipitable in phosphotungstic acid with a concomitant increase in the ^{131}I associated with the phosphotungstic acid-soluble material.

The incubation experiments showed that, in samples after a milk-protein meal, the rapid breakdown could continue to the free form for some amino acids, e.g. arginine, lysine, methionine, tyrosine and phenylalanine (Fig. 8). The initial slope of the concentration–time curve gives an estimate of the rate of release of the free amino acids in the intestinal contents at the time of removal. Enzyme autolysis, substrate exhaustion and product accumulation each tends to reduce the rate of release of the free amino acids in vitro. The slopes during the first 10 min appeared to be linear and theoretical rates (μmole/min) of amino acid release in vivo were calculated from the rise in free concentrations during this interval. In view of the delay between the contents leaving the lumen and the start of the incubation each value underestimates the actual rate of release in vivo. The estimated rates of amino acid release within the lumen at 110 and 170 cm are shown in Table 4. The amino acids released most slowly showed only minor increases in concentration after 10 min; in these the slope was calculated from the increase over the initial 40 min period.

For many of the amino acids, the rates of release were almost identical at both levels. However, the release of lysine, valine and arginine was approximately 50% slower at the lower level than at the upper level, while glutamatic acid, aspartic acid and glycine were released more rapidly at the lower level.

The mode of release of these rapidly released amino acids by digestive enzymes is well known; lysine and arginine are rapidly released from proteins by the combined actions of trypsin and carboxypeptidase B and tyrosine and phenylalanine by the actions of pepsin, chymotrypsin and carboxypeptidase A (Dixon & Webb, 1964). These were also the amino acids which were present in the free form as greater percentages of the totals in intestinal samples (Fig. 5).

The mode of release of serine, threonine and the dicarboxylic amino acids is not so well documented. These amino acids were released very slowly on incubation and the free forms constituted very small proportions of the totals in the intestinal samples. It is possible that the enzymes normally releasing these amino acids are intimately associated with the jejunal mucosa. Intestinal mucosa contains a number of dipeptidases (Gailey & Johnson, 1941), the most important one being leucine aminopeptidase, isolated in a highly purified form by Spackman, Smith & Brown (1955). A wide variety of synthetic amides and peptides are split by this enzyme although it is most active against dipeptides with an N-terminal leucine. Other dipeptidases have been described, one splitting ala-gly, one splitting gly-gly and one splitting pro-gly (Gailey & Johnson, 1941). Peptides containing these amino acids may therefore be split on the mucosal surface of the intestinal wall (Ugolev, Iesuitova, Timofeeva & Fediushina, 1964) or in the mucosal cells (Newey & Smyth, 1959; Wiggans & Johnston, 1959).

Form in which amino acids are absorbed

Table 5 gives an estimate, based on the theoretical rates of release in vivo (μmoles/min) at the upper jejunal level, of the time required for the release of all the amino

acids of the milk-protein meal. The entire content of seven of the amino acids could probably be released during the 3 h of the experiments. It is therefore not necessary to postulate anything other than complete digestion within the lumen with the subsequent absorption of the free amino acids for lysine, valine, arginine, tyrosine, phenylalanine and methionine; this might also be true of leucine. These are the amino acids which are absorbed most rapidly from mixtures of free amino acids (Orten, 1963; Adibi *et al.* 1966). However, for the amino acids such as proline, glycine and the dicarboxylic amino acids some other mechanism must be postulated. After 80 min incubation, 90% or more of the total acidic amino acids were still peptide-linked.

Table 5. *Estimated time to release all the amino acids of the milk-protein test meal in the upper jejunum of young adults*

Amino acid	Time (min)	Amino acid	Time (h)
Lys	72	Ala	11·6
Val	175	Ser	13·6
Arg	66	Ileu	13·0
Leu	358	Glu	45·5
Tyr	108	Thr	23·6
Phe	175	His	11·9
Met	104	Asp	71·0
		Pro	74·5
		Gly	98·5

In a previous paper (Nixon & Mawer, 1970) we have shown that the rates of net absorption of the amino acids after a milk-protein test meal were proportional to the concentrations of the amino acids in the meal. Thus by the time the meal had reached an intestinal level of about 200 cm from the nose, 70% of the glutamate (amounting to 12 m-moles) and 70% of the arginine (amounting to 1·5 m-moles) had been absorbed. It has been suggested that because of the higher concentrations of glutamate in the meal it can compete effectively for transport mechanisms (Gitler & Martinez-Rojas, 1964). In these experiments however, the free amino acid concentrations bore little relationship to the concentrations in acid hydrolysates of test meals or to those in acid hydrolysates of the intestinal contents. Although the concentrations of the acidic amino acids showed great subject variation they were in many cases similar to, or even smaller than, the concentrations of the basic or neutral amino acids.

Alternative suggestions are (*a*) that these amino acids are absorbed as peptides, or (*b*) that the peptides are hydrolysed at the mucosal surface of the intestinal wall. There is evidence for the absorption of small peptides. Hydroxyproline-containing peptides have been shown to be absorbed as such after a gelatin meal in man (Prockop, Keiser & Sjoerdsma, 1962). In the work being described, almost 50% of the hydroxyproline of the gelatin meal had disappeared by a level of 190 cm from the nose (Nixon & Mawer, 1970) yet free hydroxyproline was never demonstrated in the intestinal contents. Glycyl peptides, which have also been demonstrated to be relatively resistant to enzymic hydrolysis, can be transported across the gut wall as such (Wiggans & Johnson, 1959; Newey & Smyth, 1959).

Either of the suggested mechanisms could result in a more rapid absorption of

certain amino acids after a protein meal than that which occurs from a mixture of amino acids. If absorption of glutamate occurs in the form of peptides then those peptides may compete more effectively than free glutamate for transport mechanisms. If hydrolysis of the peptides occurs on the mucosal surface of the cell then, possibly by binding of the released amino acids at the surface, the subsequent transport of glutamate may be accelerated.

The evidence is therefore consistent with the view that absorption mainly in the free state occurs for lysine, arginine, methionine, tyrosine, phenylalanine and leucine. It is not consistent with the view that glycine, threonine, serine, the imino acids or the dicarboxylic amino acids are released in the lumen before absorption. Experiments where amino acid mixtures, simulating a dietary protein, are fed with the subsequent determination of the rates of amino acid absorption are not presenting a true picture of the events in the small intestine following the ingestion of a protein meal.

This work was supported by a grant from the Distillers Co. Ltd. We are grateful to Trufood Ltd for a large amount of Humanized Trufood. We are also grateful to Professor R. H. Girdwood and to Professor R. B. Fisher for their encouragement and criticism. Miss L. Shearer provided technical assistance.

This work was embodied in a thesis presented by Miss S. E. Nixon for the degree of PhD in the University of Edinburgh, 1967.

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Printed in Great Britain

Laval Med. 11(9): 969-975. 1946

A STUDY OF DIETARIES DURING INANITION IN THE RAT

Edouard Page and Rosaire Gingras

Department of Biochemistry of the Faculty of Medicine
(University of Laval)

Introduction

Inanition is the state of being of an organism that does not receive all the foods necessary to maintain its physiological integrity. According to the nature of this alimentary insufficiency and the duration of this state, the reactions of a pathological nature which can result from it are either reversible or not. A rich and abundant feeding following this period of inanition might either assure a complete or partial return to health, or prove to be vain, due to irreparable organic lesions. From this it follows that, if one foresees the necessity of a period of inanition which might be more or less prolonged, one should endeavor to limit the alimentary deficiency only to the energy factor, and to try to furnish all the essential nutritive factors which assure the normal pursuit of biological reactions. Thus the organism is permitted to use to the maximum the foods placed at its disposition, as well as its own tissular reserves. Thus the chances of survival are increased and the possibilities of organic disorders which are caused by a prolonged deficiency in one or selveral indispensable factors (vitamins or vitagens) are reduced.

Cases of inanition are frequent not only in countries ravaged by famine, but also among hospitalized patients confronted with the physical impossibility of consuming a normal amount of food. Therefore, the composition of their diet deserves the greatest attention, for the choice of foods is even more restricted because they have to be administered parenterally. This is the problem which we studied in the work which we describe below.

Data of the Problem

Until recent years, we have had to be contented with given patients of operations several liters of a physiological glucose solution, the total caloric value of which, per day, rareley amounted to more than a third or a half of the basal metabolism. In order to make up for a deficit both energetic and proteic, the patient has to draw upon his reserves of glycogen

and fats, and to catabolize, moreover, his tissular proteins.

More recently, the use of amino acid solutions or of hydrolysates of protein has been advocated. The clinical results obtained have been most encouraging, although there remain several points to elucidate, and several difficulties to be surmounted. Elman (1), in a long monograph on the subject, notes that besides using mixtures of complete amino acids which are well balanced, it is also necessary to consider the quantitative relationship between these acids and glucose: "In several of the studies, there are indications that glucose should be added to the solutions of hydrolyzed proteins in order to achieve nitrogen balance more effectively. While the importance of glucose seems obvious on theoretical grounds, there is insufficient data to show just how important this consideration may be. It is hoped that further studies will be made with varying proportions of glucose in order to show whether glucose is really required, and if so, what the optimal amounts may be."

Another aspect of the problem lies in the matter of the value of vitamin supplements during inanition. Finally, it remains to be determined what constitutes a mixture of amino acids that is of high biological value during inanition.

The problem therefore re-arises, of drawing out the best possible part of the nutritive elements that can be administered parenterally: hydrolyzed proteins, glucose, vitamins, mineral elements. The total quantity which can be administered daily is obviously limited by the volume of the isotonic solutions of these elements which one can administer in practice.

Experimental Method and Criteria

Richter and his colleagues at Johns Hopkins (1,2) studied the influence on the survival of young rats of diets composed of a single aliment, for example, casein, sucrose, olive oil, etc. The animals invariably died after a more or less prolonged period of inanition. We were inspired by their method and their results in the elaboration of our plan of action. Some ascertainments in particular caught our attention:

1) The animals were fed ad libitum, so that the daily consumption varies not only in the course of one single experiment, but also from one food to another;

2) A diet composed exclusively of protein was shown to be clearly inferior to glucose in the case of young rats (initial weight 65 grams), but guarantees a much longer survival when the rats are older at the outset (initial weight 150 to 250 grams);

3) Mineral supplements, even sodium chloride, have no effect on the length of survival;

4) Of all the vitamins known and studied, only thiamine served to prolong life.

Since growing animals do not react like the adults, we preferred, first of all, to use rats approaching adult age in order to eliminate this factor. Also, from the outset, we limited the amount of aliments offered to the rats to a caloric level equal to about half the basal metabolism. Since this amount

was clearly inferior to the appetite of the animal, we were assured of the fact that most of the rats would eat their entire ration, whatever the nature and duration of the experiment. Since the consumption was identical from the beginning to the end of the experiment and from one food to another, our comparisons were much facilitated. Moreover, This caloric level corresponds closely to the daily amount of calories which a sick person fed intravenously ordinarily receives (3 liters of glucose solution at 5% = 600 calories).

Finally, basing ourselves on the results obtained at Johns Hopkins, we ignored liposoluble vitamins and mineral supplements. It should be noted that, under our conditions of work, the animals do not begin with a deficit of sodium chloride, as may be the case among patients who have undergone operations.

We used to criteria for the interpretation of the results: in the first place, the length of survival, and secondly, the degree of emaciation at the time of death. This latter indicates to what point the organism has been able to use its tissular reserves before a fatal collapse of the biological mechanism occurs. This criterion is therefore above all a measure of the value of the ration from the point of view of its essential principles, such as vitamins and amino acids. The length of survival is an indication of the value of the ration in toto and probably constitutes the most satisfying criterion.

It is important to note that it was not possible to pursue successive experiments in identical environmental conditions. Since a lowering of the exterior temperature hastens death, it is not possible to compare the dietaries of one experiment with another, unless the circumambient temperature was the same, which was not the case. We had, therefore, to stick with the comparison of the diets used in one single experiment.

First experiment. -- The object of this first experiment was verifying whether the manner of procedure which we have just indicated allowed for the disclosure of the differences which must exist between certain diets, either from the point of view of the quality of their protein, or of their vitamin content. The comparison was therefore made between casein and gelatin, the one being clearly superior to the other. These two proteins were given to the experimental animals with and without vitamins, while a last group of rats was submitted to a complete fasting.

Five groups of rats were used, the distribution of rats as regards their weight being similar in all the groups, except for the last (complete fasting). All received water ad libitum. The vitamin supplement consisted of 0.2 cc of "B-Plex" added to the ration each day. This supplement contained the following quantities of vitamins: thiamin, 25; riboflavin, 50; niacin, 250; pyridoxine, 25; panthothenate of calcium, 125; and choline, 600 micrograms, respectively. Moreover, the solution contained traces of unidentified factors of rice extracts.

The amount of aliments given each day was determined for each rat on the basis of the formula of Brody: $Q = 70.5 M^{0.734}$ (Q = basal metabolsim in cal. and M = animal weight in kilos), so that each rat receives an amount of aliment, the caloric value

of which should be equal (theoretically) to half of its basal metabolism. The aliments were weighed at 0.1 grams. Following the example of other researchers, we assigned an equal caloric value to casein and gelatin (as later also to sucrose), amounting to 4 cal. per gram.

Results. -- The results are represented in tables, 1, 2 and 3. It is confirmed that the average length of survival is much greater in the case of rats receiving casein than in that of the rats nourished on gelatin. The rats receiving a vitamin supplement in addition to the casein survived longer than the others, while this same supplement apparently had no effect when it is added to gelatin (table 1). It is probable tha the absence of tryptophane in gelatin leads to death before the effect of the vitamins can manifest itself.

It should also be noted that the loss of weight, at the time of death, is proportional to the length of survival, except when we compare the rats nourished on gelatin with those subjected to complete fasting (table 1). This is a second index of the mediocre alimentary value of gelatin.

Table 2 indicates the length of individual survivals for each group. It is evident that there are considerable variations in each group. This is inevitable when one is working with adult rats. The use of a general formula for determining the caloric supply also contributes to the variation in the length of survival in the case of identical diets, but this factor, being the same in all the cases, should affect considerably the averages of survival for each group, although it may increase the figures within the groups.

It is logical, however, to measure the time passed since the beginning of the experiment until the moment when the rate of mortality reaches 80% of the total. In this way, the individuals that are exceptionally resistant are eliminated. Thus, it is seen in table 3 that a mortality rate of 80% was reached at the end of 12 and 13 days in the case of the rats nourished on gelatin, while this same level was not reached until after 20 days in the case of the first group, and 17 days in the case of the second.

In conclusion, casein is superior to gelatin as a single aliment in the course of inanition and its influence on survival is accentuated by the addition of B-complex vitamins to the diet. This second ascertainment is more original than the first.

Second experiment. -- The first experiment was pursued in the month of march of 1944, when the room was heated and the temperature was maintained at about 70°F. The second experiment took place at the end of May, at a time when there is no heating, and when the external temperature is lower. For this reason, the experimental conditions were less favorable for survival.

The object of this experiment was examining whether the difference in the alimentary value of gelatin and casein would still be shown when these proteins only constitute a fifth of the diet, of the same caloric value as before, and the balance of the diet being composed of sucrose. The same vitamin supplements were used as before, and the process of the experiment was the same.

Results. -- The results were rather disappointing (tables 4, 5 and 6). There was no difference in the average length of survival of the various groups, nor in the loss of weight at the time of death. This may be due to the fact the amounts of protein ingested were not sufficient to affect the length of survival. Especially with the circumambient temperature being relatively low (around 60°F), the gradual slowing down of the metabolism in the course of inanition more rapidly reaches the point where the production of heat does not suffice to maintain the body temperature. The animal dies before having exhausted its reserves as completely as when the temeperature is higher. As the superiority of the better diets is particularly manifested during the last phase of inanition, it could not be evidenced in the conditions of the experiment. All the same, it is confirmed that the mortality level does not reach 80% until two days later in the first group (casein and sucrose) as in the others.

This results illustrate above all the influence of the temperature on the length of survival in the course of inanition.

Third experiment. -- The object of this experiment was the attempt to establish the optimum relation between glucose and casein in the course of inanition. The method followed was the same as before, except that vitamin solutions were substituted for the "B-Plex". However, for purposes of comparison with the preceding experiments, two groups received a diet composed exclusively of casein with an addition, in one case, of "B-Plex" and, in the other, of pure vitamins, at equal rates. The three other diets contained 80, 50 and 20 per cent casein, respectively, and sucrose as a balance.

Results, -- These results are represented in tables 7, 8 and 9. It is interesting first of all to confirm that the two groups receiving 100% casein show identical average weight losses and that the length of survival does not differ by more than 0.4 days. This is a good indication of the value of the method and leads us to believe that the more marked differences are significant, at least as regards survival.

The rats receiving 50% casein survived longer than the others, and also attained a degree of emaciation more advanced. Those receiving 80% casein resisted nearly as well. If we refer to table 9, we see that the mortality rate reached 90% at the end of 24 days in two of the groups, as compared with 18 and 19 days in the case of diets composed exclusively of casein, and with 21 days in the case of the rats receiving 80% sugar and 20% casein.

We can conclude that a certain amount of sugar is indispensable in assuring a maximum length of survival when the caloric level of the dietary reaches around the half of the basal metabolism. The optimum level of casein seems to be located at at least 50% of the diet. This level represents a minimum, since the survival is practically identical when the diet contains 80 percent casein. It is therefore probable that the optimum relations between proteins and sugar is somewhere between 50 and 80 parts protein to 50 and 20 of sugar, respectively.

General Conclusions

The results of these experiments confirm that which was already known about the respective value of casein and gelatin. This latter, in fact, is just as unsatisfactory during inanition as during growth.

When inanition is prolonged, it is advantageous to administer vitamin supplements. Although we have not shown which of these vitamins are useful, there is every reason to believe that thiamin is the most important.

When the caloric level of the diet is clearly inferior to the needs of the organism, it is not advantageous to administer carbohydrates or proteins exclusively. The optimum relation between protein and sugar seems to be about 1:1, and one should preferably administer more protein than carbohydrate. Furthermore, it is evident that the optimum relation between these two substances varies with the caloric level of the diet.

First Experiment

Table 1

Averages of survival and weight losses in the course of inanition

A. GROUPES	B. Poids initial (grammes)	C. Poids final (grammes)	D. Perte de poids — Pourcentage du poids initial	E. Longueur de survie (jours)
1. Caséine et vitamines.....	224	122	46	16.1
2. Caséine seule.....	239	142	41	14.2
3. Gélatine et vitamines.....	240	155	37	10.5
4. Gélatine seule.....	244	152	38	10.0
5. Jeûne complet.....	255	164	36	7.6

Key:
A. = groups B. = initial weight (grams) C. = final wight (g)
D. = weight loss -- percentage of initial weight
E. = length of survival (days)
1. = casein and vitamins
2.= casein only
3.= gelatin and vitamins
4.= gelatin alone
5.= complete fasting

Table 2

Individual survivals

A. GROUPES	B. NOMBRE DE JOURS										
1. Caséine et vit.....	..	..	..	10	12	15	16	16	20	20	20
2. Caséine seule.....	..	9	9	10	13	14	15	15	17	20	20
3. Gélatine et vit.....	..	..	.	8	10	10	10	11	11	12	13
4. Gélatine seule.....	..	..	..	6	8	9	9	9	11	13	15
5. Jeûne complet.....	7	6	7	7	7	7	7	8	9	9	..

B. = number of days

Table 3

Mortality rate in the course of the experiment

A. GROUPES	B JOURS																
	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	Mortalité (pourcentage)																
1. Caséine et vit.	0	0	0	0	0	14	14	25	25	25	37	62	62	62	62	62	100
2. Caséine seule	0	0	0	0	20	30	30	30	40	50	70	70	80	80	80	100	
3. Gélat. et vit..	0	0	0	12	12	50	75	87	100								
4. Gélatine seule	0	12	12	25	62	62	75	75	87	87	100						
5. Jeûne comp..	9	10	70	80	100												

Rats eliminated in the course of the experiment:

		Survival
1. Casein and vitamins:	2 rats with stomach abcesses	11 and 15
2. Gelatin and vitamins:	2 rats died accidentally	3 and 7
3.=Gelatin only:	2 rats that refused to eat at first	7 and 9

Table 4

Average survivals and weight losses in the course of inanition

A GROUPES	B. Poids initial (grammes)	C. Poids final (grammes)	D. Perte de poids — Pourcentage du poids initial	E. Longueur de survie (jours)
1. Caséine (20%) ..... Sucrose (80%)	252	155	39	11.3
2. Gélatine (20%) ..... Sucrose (80%)	252	154	39	11.0
3. Sucrose (100%)	255	156	38	11.0

For key see table 1.

Table 5

Individual Survivals

A. GROUPES	B. NOMBRE DE JOURS										
1. Caséine et sucrose .	8	8	9	9	11	11	11	13	14	15	15
2. Gélatine et sucrose .	8	9	10	10	10	10	11	11	12	13	16
3. Sucrose	7	9	9	10	11	11	12	12	12	13	14

Table 6

Mortality rate in the course of inanition

A. GROUPES	B. JOURS										
	6	7	8	9	10	11	12	13	14	15	16
	MORTALITÉ (pourcentage)										
1. Caséine et sucrose .	0	0	18	32	32	64	64	73	82	100	..
2. Gélatine et sucrose .	0	0	9	18	44	73	82	91	91	91	100
3. Sucrose	0	9	9	27	36	44	88	91	100	..	..

N.B. -- All the groups received a vitamin supplement

Third Experiment

Table 7

Averages of survivals and weight losses in the course of inanition

A. GROUPES	B. Poids initial (grammes)	C. Poids final (grammes)	D. Perte de poids — Pourcentage du poids initial	E. Longueur de survie (jours)
1. Caséine (100%) et B-Plex.....	241	120	50	17.9
2. Caséine (100%) et vitamines.....	240	120	50	17.5
3. Caséine (80%) et vitamines.....	242	114	53	19.5
4. Caséine (50%) et vitamines.....	239	108	55	20.4
5. Caséine (20%) et vitamines.....	243	112	54	18.1

For key see table 1.

Table 8

Individual survivals

A. GROUPES	B. NOMBRE DE JOURS										
1. Caséine et B-Plex..	14	16	17	17	17	18	18	18	19	25	..
2. Caséine et vit.....	..	14	15	16	17	18	18	18	18	18	23
3. Caséine (80%).....	..	14	16	16	17	18	18	23	24	24	25
4. Caséine (50%).....	..	12	16	17	18	22	22	22	24	24	27
5. Caséine (20%).....	..	11	15	15	16	16	20	21	21	21	25

Table 9

Mortality rate in the course of the experiment

A. GROUPES	B. JOURS																
	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
	Mortalité (pourcentage)																
1.....	0	0	0	10	10	20	30	50	80	90	90	90	90	90	100	..	..
2.....	0	0	0	10	20	30	40	90	90	90	90	90	100	..	..	..	..
3.....	0	0	0	10	10	30	40	60	60	60	60	60	70	90	100	..	..
4.....	0	0	10	10	10	20	30	40	40	40	40	70	70	90	90	90	100
5.....	10	10	10	10	40	50	50	50	50	60	90	90	90	90	100	..	..

COMPARATIVE VALUE OF GELATIN AS AN ACCESSORY INFANT FOOD*

By H. H. Perlman, M.D.

Instructor in Diseases of Children, Jefferson Medical College; Formerly Medical Director of the Ocean City (N. J.) Seashore Home for Babies, Philadelphia

This study with edible gelatin was undertaken because of the widespread publicity and popularity accredited to the product as a means of adding weight to infants. Another reason which led to the survey was the many conflicting prevalent reports relative to the heretofore much neglected bovine derivative as a highly advantageous remedy for difficult feeding cases. Many mothers, whose infants failed to gain sufficiently in weight under the usual regime of simple whole mixture dilutions, reported startling weight gains which they observed after the simple addition of gelatin to the infants feeding. Infants in whom gelatin feeding was employed were not uncommonly referred to as "gelatin fed." During the height of gelatin enthusiasm, overzealous mothers requested the use of the artificial protein for those who failed to respond to routine dietetic care. Several parents attempted to employ gelatin for their young ones without physician's advice, while in other communities anxious mothers looked upon the product as the discovery of the age, unprecedented if not unequaled, by any of the common proprietary infant foods. "Habitual spitters" were said to fare well upon gelatinized milk. Regurgitation, ordinarily observed with breast-fed and bottle-fed babies, was minimized and at times entirely overcome by the simple addition of powered gelatin to formulae. For reasons enumerated the gelatin study was deemed both expedient and timely.

In the spring of 1926, with the re-opening of the Ocean City Seashore Home for Babies, an institution devoted primarily to the care of malnourished infants, ample opportunity was afforded the medical attendants for testing the true value of the colloid. A series of 36 male and female infants whose weights, upon admission to the home, were considerably below normal were selected for the research. During the trial period of artificial feeding with gelatinized milk, controls were enacted, careful weekly gains or loses were recorded, and such supplementary data as was

*Read before the Jefferson Society for Clinical Investigation, December 15, 1927.

consistent with the study filed for future reference. The majority of infants studied remained at the home from several weeks to several months so that a fairly accurate conclusion as to the value of gelatinized milk was reached over a comparatively fair period of time.

The controls were exposed to similar conditions of environment as were the gelatin-fed babies. In addition to simple or gelatinized milk feedings, all infants received the benefit of fresh fruit juices, e.g., orange, grape fruit or prune juices once daily, cod liver oil three times daily, and such other foods as are commonly adapted to various ages. Among the latter might be mentioned the use of cooked cereals, green vegetables and vegetable purees, bread, butter, eggs, etc.

SOURCE, COMPOSITION AND PREPARATION OF GELATIN

The U. S. Pharmacopeia defines gelatin (gelatinum) "as the purified, air dried product of the hydrolysis of certain animal tissues, as skin, ligament and bones by treatment with boiling water." The finished product is designated as a collagen. Upon completion of hydrolysis the solution is permitted to dry in the air. As it is commonly seen upon the market, gelatin occurs in shreds or thin transparent sheets, which, when kept dry, remain permanent. Exposed to moisture putrefaction sets in. The connective tissue derivative is colorless, odorless but with a somewhat insipid taste. Gelatin is soluble in boiling water; insoluble but jells in cold water, hence the popular acquired name by which the chemical is known.

The pharmacopeia recognizes gelatin for the purpose of manufacturing glycerinated gelatin, the latter preparation which largely constitutes the basis for most proprietary and standard suppositories prescribed by the profession. Apropos might be mentioned the hard and soft gelatin capsules utilized as a convenient means of disguising objectionable medicaments which are administered per orum. Other uses applicable to gelatin are as a culture medium, and as a popular food for the sick. In surgery, gelatin is sometimes employed as a remedy in the treatment of shock. For the latter purpose a sterilized one per cent. solution is administered hypodermically or intravenously. Aneurisms have also been treated during the past by injecting the colloid into the dilated sacs. Its use for this purpose has since been abandoned.

Arch. Pediat. 45:14-20, Jan. 1928

CHEMISTRY OF GELATIN

Gelatin is, in a sense, a protein yet differing from the common group of foodstuffs, such as milk, eggs and meat. Analytically its structure lacks certain of the amino acid radicles particularly tyrosin, tryptophan and cystin. As is well known these are indispensable in any diet. In contrast to the true proteins, the decomposition of gelatin is not attended by the formation of indol. It is for this reason that gelatin was at one time considered invaluable as a remedy for intestinal putrefaction. Experience has taught the clinician that when gelatin is used to replace other foodstuffs metabolism invariably suffers. The growth producing property attributed to gelatin when fed to animals over a period of time is due to lysin. The latter occurs to the extent of about 5.9 per cent.

The observations of Robinson, which were conducted at the Lister Institute in London, are corroborative evidence that gelatin is not a true protein. It cannot satisfy the entire nitrogenous needs of the body, differing in this respect from the other well known proteins.

Gelatin works much as a carbohydrate in increasing body weight, provided nitrogen equilibrium is maintained. The colloid will not place a child on nitrogen equilibrium unless an adequate amount of protein food, i.e., milk, scraped beef, etc., is added to the diet. As is common to carbohydrates, gelatin also possesses the property of increasing the respiratory quotient and in this respect may be termed a protein sparer.

The statement often attributed to gelatin to the effect that the latter has no caloric value is erroneous. Gelatin gives three and one-half calories per gram fed. Gelatin is an incomplete protein and when ingested functions as such food unless complete protein is supplemented.

MANNER BY WHICH GELATIN INCREASES NUTRITIVE PROPERTY OF MILK

It is commonly believed that when gelatin is added to cow's milk the food is rendered more digestible. The colloidal action is not uncommon to the other members of the same series, such as acacia and starchy decoctions (oatmeal and barley). Mechanically, gelatin serves very much the same purpose as is ordinarily

accomplished by cereals. Through its use the tough, friable and difficultly digested casein curd is broken up into finer masses. In this manner the digestive juices are enabled to exert what might be termed a much stronger play around each floccus, thus assuring better disintegration, digestion and absorption. Furthermore, by nature of its cohesive property, gelatin permits retention of food in the stomach, particularly food of liquid character which would otherwise be regurgitated by a hypersensitive or irritable organ. Because regurgitation less often follows the use of gelatinized milk, the new method of feeding has found adoption in those infants who are the subjects of pylorspasm and pyloric stenosis.

FORMULAS FOR PREPARING GELATINIZED MILK

The prescription for preparing gelatinized milk at the Ocean City Home consisted in adding a definite amount of powdered gelatin to the infant's total quantity of milk for the day's feeding. For infants under six months, one-half teaspoonful was used, while in infants past a half year, one teaspoonful of powdered gelatin was added to the day's feeding. A satisfactory method of dissolving gelatin in the milk and which was the one commonly employed at the home, is as follows: The specified amount of powdered gelatin is added to one ounce of cold milk of the total requirement for the formula. After allowing the gelatin to soak in the milk for ten minutes, one ounce of hot milk is added, solution of the powder being facilitated by constant stirring with a spoon or glass rod. When the gelatin is completely dissolved, the gelatinized milk is added to the remainder of the fomula and the whole is thoroughly mixed. The finished product is then placed on ice until ready for use. Another plan of procedure consists in adding one level tablespoonful of gelatin to four ounces of unheated cow's milk and allowing it to soak for a period of ten minutes. The receptacle containing the milk and gelatin is then placed in a pan of hot water and the mixture stirred until the gelatin powder is dissolved. The latter is then added to 28 ounces of whole milk.

RESULTS OF EXPERIMENT

Thirty-six infants comprised the gelatin study. These were divided into two groups according to ages, viz. those under one year and those between one and two years. Eight babies under

one year and ten between one and two years received gelatinized milk. Of the 18 controls, 17 were under one year while the eighteenth infant was 16 months.

The youngest gelatinzed infant was seven weeks, the oldest 23 months. The youngest infant which served as a control, with simple whole milk dilution, was two months, while the oldest infant's age in this group was 16 months. In all there were 28 males and eight females; 25 under one year, and eleven infants between the ages of one and two years.

Simple dilutions of whole milk according to calories required per pound body weight was the method of feeding infants at the home. The carbohydrates consisted of milk-sugar or dextri-maltose. Conclusions as to the value of gelatinized milk were reached on a comparative basis of calculation. For this purpose the generally accepted standard principles of weight-gain in infants were utilized. As is well recognized, infants upon the breast or bottle should gain approximately from four to five ounces weekly during the first six months. A gain of 14.2 pounds during the first year of life would average 2.7 ounces weekly. Weight as well as growth is not phenomenal during the second year in contrast to the first twelve months. A fair average is six pounds, or 1.8 ounces weekly.

Based upon the foregoing consideration, the following deductions were reached: Of the 17 controls under one year, there were ten infants under six months, of the latter only two gained more than four ounces weekly. The remaining eight infants gained less than four ounces weekly. Considered in toto, ten out of the 17 gained more than 2.7 ounces weekly. The average weekly gain per capita was 4.2 ounces. The sixteen-months infant gained 7.2 ounces weekly. The smallest weekly gain was .7 ounces in a two and one-half months infant, while the greatest weekly gain was 9.4 ounces in an eight months old baby.

Of the eight gelatinized fed babies in the first group under one year, three were under six months. In the latter, only one gained more than four ounces weekly, while one gained less than the standard requirement. Of the remaining five past the half year mark, four gained more than four ounces weekly while one infant showed a weekly loss of 1.5 ounces. Considered as a group, six gained more than 2.7 ounces weekly. The average weekly gain per capita was 6.1 ounces.

The smallest weekly gain was 1.5 ounces, in a seven weeks infant; while the greatest gain of 10.9 ounces occurred in one aged eleven months. In the second subdivision, all of the ten gelatinized fed babies gained more than 1.8 ounces weekly. The average weekly gain per capita in this group was 4.4 ounces. The smallest weekly gain was made by a nineteen-months old infant—2.4 ounces, while the greatest weekly gain was 8.8. ounces in a twenty-three months' infant.

COMPARISON

The gelatinized fed babies showed a weekly gain of 6.1 ounces per capita as contrasted with 4.2 ounces in the infants fed upon simple dilutions of whole milk, a difference of 1.9 ounces greater weight per capita in the gelatin group.

While the infant over one year in the control group gained 7.2 ounces weekly, a much greater gain per capita than those in the gelatin study, it should be remembered that the comparison of one individual as a control against ten infants who were gelatin fed is an unfair evaluation.

It is interesting to observe that while the results obtained through the use of simple dilutions of whole milk were satisfactory those fed upon gelatinized milk formulas exceeded the controls in weight gain. The gelatinized fed babies seemed perfectly satisfied with the number of feedings in twenty-four hours; regurgitation seldom occurred; vomiting was not observed; and the stools varied from one to three daily. The bowel movements were of a light golden yellow color, smooth and salve-like, without a disagreeable odor. At no time during the gelatin feeding were protein curds noticed in the stools.

SUMMARY AND CONCLUSIONS

A series of 36 male and female infants were studied so as to determine the value of gelatin as a means of increasing body weight. The survey was divided into two groups. One division of 18 infants received gelatinized milk for several weeks, while the other group of 18 babies received simple dilutions of whole milk as a control over a similar period of time. The results obtained from the experimental survey may be summed up as follows:

1. Gelatinized fed babies fare far better than infants fed upon simple dilutions of cow's milk.

2. In the series of infants studied, those fed upon cow's milk formulas vomited more frequently in contrast to those fed upon gelatinized milk.

3. Weekly gains in weight per capita were greater in the gelatinized fed babies than in those fed upon simple dilutions of whole milk.

4. Of eight infants between the ages of six months and one year fed upon gelatinized milk, six gained more than 2.7 ounces weekly, one gained less than this amount weekly, while one infant lost weight. Contrasted with the series of 17 controls of the same age, ten gained above 2.7 ounces weekly, seven less than 2.7 ounces weekly.

5. Of the ten infants from one to two years who received gelatinized milk, all gained over 1.8 ounces weekly. Contrasted with the only control baby of similar age, the latter gained 7.2 ounces weekly.

6. The average weekly gain per capita in infants during the first year fed by means of "gelatin milk" was 6.1 ounces while in the controls, 4.2 ounces. The average weekly gain in infants during the second year upon "gelatin milk" was 4.4 ounces as compared to the single case in the control series of 7.2 ounces.

7. Gelatin milk is superior to whole milk dilutions as a means of increasing avoirdupois in artificial malnourished infants.

8. The preparation of gelatinized milk is comparatively simple, palatable, inexpensive, easily retained by the stomach, and its use encouraged in difficult feeding cases.

1426 *Spruce Street.*

THE JOURNAL-LANCET
61: 484-485, Dec. 1941

Effect of Gelatin Feeding on Strength and Weight According to Body Build

Helen B. Pryor, M.D.†
Maud L. Knapp, M.S.‡
Stanford University, California

IN a recent thesis entitled "An Attempt to Determine the Effect of Gelatin Feeding in Relation to Strength, Endurance and Weight of College Women" Miss Florence Tennant[1] observed 211 Stanford women. Half of these took gelatin for 28 days, while the other half, matched for activity programs, were used as a control group.

Gelatin was taken in the amounts recommended by the Knox Gelatin Company who supplied the material. Each girl took one ounce of gelatin daily for the first two weeks and one-half ounce daily for the second two weeks. Weight and strength were measured and recorded before and after the test period for both the experimental and control groups in the same way. One hundred forty-one women finished the study; 71 who took gelatin and 70 in the matched control group who did not.

Results of the objective portion of the study showed that the mean strength of right hands increased with the administration of gelatin from 72.46 to 75.18 kilograms. This increase of 2.72 kilograms represents 3.7 per cent as compared to 2.3 per cent for the control group. The critical ratio of 3.06 indicates the small statistical significance of this finding. The mean strength of left hands increased from 63.88 to 65.96 kilograms. This increase of 2.08 kilograms represents 3.3 per cent as compared to a zero gain in the control group. The mean weight of the group on gelatin increased from 133.15 to 134.60 pounds, a gain of 1.45 pounds as compared to a mean loss of .03 pounds in the control group.

Since it is apparent that very small gains were made in both strength and weight by 71 women who took gelatin, and similar gains were not made by 70 women in a matched control group who did not take gelatin, a further analysis has been undertaken. The present study is an attempt to find out what factors, if any, were common to the groups who showed positive or negative results.

Ray, Johnson and Taylor[2] carried on a series of experiments at the Long Island College of Medicine with gelatin feeding. They showed that adequate amounts of gelatin increased the amount of work performed 38 to 240 per cent before muscular fatigue set in when they used male subjects. They reported that in women gelatin did not increase the amount of work performed before muscular fatigue because of the marked sexual difference in ability of men and women to store creatine, and suggested that the effect of gelatin is particularly concerned with the action of glycine (or glycocoll) in building creatine. In the experiments of Ray, Johnson and Taylor the subjects were trained on the ergometer until the maximum work output was constant. Then 10 to 15 grams of glycocoll were added to the daily diet. The investigators noted that subjects who were of slight build reacted most promptly to glycocoll and showed gain in body weight. Both phosphoric acid and creatine are now assigned an important role in the chemistry of muscular contraction. Boothby[3,4] reported that the onset of muscular fatigue could be delayed by adding enough glycine to the diet of a normal person. He showed also that giving glycine in certain disease conditions as progressive muscular dystrophy helped to restore wasted muscle tissue. Gelatin which contains 25 per cent glycine is an acceptable source of this important amino acid. It is an established fact that glycine is an amino acid which can increase the creatine content of skeletal muscle, and glycine has been used successfully in treating certain myopathies.

Dr. Hellebrandt[5] challenged the conclusions of Dr. Ray that it was the addition of gelatin to the diet that increased endurance 100 per cent in the experimental subjects. Hellebrandt found neither increase nor decrease in athletic improvement when gelatin was added to the normal diet in his experimental study.

Dr. George Maison[6] of Detroit who also failed to confirm Dr. Ray's findings reasoned that if gelatin has any effect on fatigue, its ingestion should increase the work output of individual muscles. He undertook experiments to test work ability before, during and after ingestion of gelatin. Six subjects were trained for five weeks and then given 60 grams of gelatin a day for 21 to 31 days. No obvious difference could be demonstrated in amount of work done and when the gelatin was withdrawn there was no decrease to be seen. Two subjects trained for one year showed no increase in work ability when given gelatin or amino acetic acid.

Miss Tennant's study also failed to demonstrate significant changes in strength and endurance as the result of gelatin feeding. However, the authors were interested in nutritional aspects of gelatin and consequently investigated the role of body build and weight in the Stanford study.

Tabulations were made of strength and weight changes according to the body build of the girls. Build was indicated in terms of the width-length index, or body width, in proportion to standing height as described elsewhere. A small index indicates a slender-built person and a large index indicates a broad-built person. For the tabulation, indices which were more than a standard deviation below the mean for the age-sex group were classified linear; those more than a standard deviation

†Director, Women's Health Service.
‡Director, Women's Physical Education.

TABLE I.
Strength Changes Right Hand According to Body Build

A. Gelatin Group (70 cases)	Linear No. Cases	Pct.	Lateral No. Cases	Pct.	Intermediate No. Cases	Pct.
Gain	9	60	19	62	11	46
Loss	3	20	9	28	10	42
Same	3	20	3	10	3	12
B. Control Group (68 cases)						
Gain	13	56	10	53	11	42
Loss	7	30	4	21	13	50
Same	3	14	5	26	2	8

TABLE II.
Strength Changes Left Hand According to Body Build

A. Gelatin Group (70 cases)	Linear No. Cases	Pct.	Lateral No. Cases	Pct.	Intermediate No. Cases	Pct.
Gain	10	66	22	71	15	63
Loss	4	27	7	24	8	33
Same	1	7	2	5	1	4
B. Control Group (68 cases)						
Gain	10	46	9	49	14	54
Loss	7	31	8	44	11	42
Same	5	23	3	17	1	4

above the mean, lateral; and those between these two, intermediate.

It will be seen in table I that the very small gains in strength of right hands in the gelatin group over the control group were about equally distributed between broad and slender built girls. Table II indicates a slight advantage for broad-built girls over slender-built ones in percentage of girls who gained strength in their left hands after gelatin feeding.

Table III has the most significant finding, in that 12 out of 15 linear type girls gained weight on addition of gelatin to their normal diets. The percentage of linear type girls who gained weight on gelatin was larger than for intermediate or lateral types of girls and much larger than for the control groups.

Because of the well-known and often lamented difficulty of helping very slender-built people to gain weight, the high proportion of success with gelatin in this study deserves comment.

The Council on Foods of the American Medical Association[7] reported gelatin to be a wholesome food, well tolerated and readily digested, containing 85 to 90 per cent pure protein. Addition of gelatin to a normal diet increases the total proportion of protein as well as increasing the important amino acid, glycine. High protein has been shown to cause a consistent rise in metabolism by exerting a specific dynamic action according to Necheles and Mills.[8]

Beard[9] reported increases in body weight of from 7 to 24 pounds in nine underweight people whose main complaints were anorexia, easy fatiguability and nervousness by addition of amino acetic acid (glycine) to their diets. He had been unable to obtain increases in any of these cases by dietary measures without glycine. He reported further that the glycine caused disappearance of anorexia, fatigue and nervousness when weight was increased.

Anorexia, easy fatiguability, high basal rates, nervousness and underweight have all been described as characteristic of linear type people. Perhaps slender-built people need more protein than others because they use up so much maintaining their high basal rates.

TABLE III.
Weight Changes According to Body Build

A. Gelatin Group (70 cases)	Linear No. Cases	Pct.	Lateral No. Cases	Pct.	Intermediate No. Cases	Pct.
Gained Weight	12	80	18	58	13	54
Lost Weight	2	13	5	16	9	37
Maintained Wt.	1	7	8	26	2	9
B. Control Group (68 cases)						
Gained Weight	9	39	7	37	9	35
Lost Weight	7	31	5	26	11	42
Maintained Wt.	7	30	7	37	6	23

Dr. McGuire[12] reported on the value of glycine in treating progressive muscular dystrophy and he said further "Glycine has been proved valuable not only as a therapeutic and diagnostic agent, but also in cases of 'vague fatiguability', and 'chronic nervous exhaustion', neurotic cases, etc. It is possible, therefore, there is a relationship between the latter conditions and the myopathies."

CONCLUSIONS

1. In a control study, 70 college women on definite exercise schedules took gelatin for 28 days and were then compared with 70 others on the same schedules who did not take gelatin.
2. Very small gains in both weight and strength were made by the gelatin group and similar gains were not made by the matched control group.
3. The study failed to demonstrate significant changes in strength or endurance as a result of gelatin feeding.
4. When the role of body build was investigated, it was found that 12 out of 15 slender-built girls made significant weight gains on addition of gelatin to their normal diets.
5. Other girls matched for build and not on gelatin failed to make similar gains.
6. Slender-built girls on gelatin feeding made larger gains in weight than broader-built girls on gelatin feeding.

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Current Sci. (India) 31(2):58-60, 1962

EVALUATION OF DIETARY GELATIN IN TERMS OF LIVER XANTHINE OXIDASE ACTIVITY

LIVER xanthine oxidase activity is found to decrease in animals suffering partial or complete protein deficiency.[1-8] Litwack *et al.*,[9,10] have demonstrated that liver xanthine oxidase activity is related to the quality of dietary protein and have evaluated the 'protein quality' of casein, lactalbumin, meat protein and gliadin in terms of liver xanthine oxidase activity. By 'protein quality' is meant the value of a dietary protein in promoting synthesis of tissue proteins and enzyme proteins in the body.

It is well known from growth studies that gelatin is a poor quality protein as it is deficient in some of the essential amino-acids like methionine. The scope of the present work was to evaluate the 'protein quality' of gelatin in terms of liver xanthine oxidase activity of animals fed gelatin as protein. The enzyme method of evaluation of a dietary protein obviates some of the interferences encountered in the growth method as the hormonal state of

the animals, water intake and water balance, fat deposition, etc.

Liver xanthine oxidase activity was estimated by the method of Litwack *et al.*[11] The comparison of liver xanthine oxidase activity of rats fed gelatin as protein was made with that of rats fed casein in an isonitrogenous level. It was found by semi-micro Kjeldahl estimation that 20% casein in the diet was isonitrogenous with 16·5% gelatin.

Two groups of 4 albino rats with an initial weight of 80 ± 10 gm. were fed two different diets for a period of 25–28 days. The diets were the same in all ingredients except that one had 20% casein and the other 16·5% gelatin instead of casein. The deficiency in the percentage composition in case of the gelatin diet was made good with sucrose. Weights of rats were recorded every week. The rats on gelatin diet lost considerable weight and became very weak in about 20-25 days. Rats on casein diet had a steady growth and were healthy. All the rats were sacrificed at the end of the experimental period for the estimation of liver xanthine oxidase activity.

Table I of the results shows that the average liver xanthine oxidase activity of rats on gelatin diet is 70% less than that of rats on casein diet in the course of 4 weeks. This indicates that the 'protein quality' of gelatin, *i.e.*, the value of gelatin in synthesising an enzyme protein like xanthine oxidase is very poor when compared with casein. This result is corroborated by changes in body weight of the animals during the experimental period.

TABLE I

Body weight changes and liver xanthine oxidase activity in rats due to dietary casein, gelatin and enriched gelatin

Sl. No.	Dietary protein	Change in body weight at the end of 4 weeks (in gm.)		Liver xanthine oxidase activity (in μ Moles of xanthine oxidised per hour per gm. of wet liver)
		av. gain	av. loss	
1	Casein	21	—	*9·9 ± 0·67†
2	Gelatin	—	20	3·0 ± 1·20
3	Gelatin + Methionine	—	21	5·9 ± 0·45

* Average of 4 values.
† Mean deviation from the average.

As gelatin was found to have poor 'protein quality' by the enzyme method of evaluation, it was enriched with the required level of methionine to the level present in casein and the 'protein quality' of this enriched gelatin was investigated. That liver xanthine oxidase activity is very sensitive to amino-acid availability in dietary protein[12] and to deficiencies of essential amino-acids such as methionine[13] has been shown by Williams and co-workers.

A concentration of 532 mg. % of methionine added to a diet containing 16·5% of gelatin will be equivalent to the amount of methionine in 20% casein diet.[14] Hence a similar experiment was performed by feeding a group of 4 albino rats (initial weight 80 ± 10 gm.) with a diet containing 16·5% gelatin and 532 mg.% of DL Methionine for the same period. Table I of results shows that though there is no influence in the growth of rats, the liver xanthine oxidase activity is found to be doubled due to the addition of methionine to gelatin diet. This is in agreement with the finding that addition of small amounts of methionine in the diet improves the activity of liver xanthine oxidase.[12] Methionine seems to be necessary for maintaining xanthine oxidase activity as the active site of the latter is a sulphur-containing portion, namely a system of two ferric mercaptides between two FAD (Flavin Adenine Dinucleotide) moities.[15]

The author thanks Dr. T. M. B. Nedungadi, Professor of Biochemistry, Madurai Medical College, Madurai, Dr. V. Srinivasan, Director, Sarabhai Chemicals Research Institute, Ahmedabad and Prof. V. V. Raman, Head of the Department of Chemistry, Madurai Medical College, Madurai, for their guidance and encouragement.

Department of Biochemistry, S. RAMAKRISHNAN.
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J. Lab. & Clin. Med. 32:1548-1549, Dec. 1947

107. GELATIN AS AN ALLERGEN

THERON G. RANDOLPH, M.D., CHICAGO, ILL.

In view of the reported nonantigenicity of ossein gelatin and the fact that beef gelatin solutions are commonly used intravenously as plasma substitutes, it is of interest to know that even highly refined ossein gelatin preparations may produce symptoms when ingested by beef sensitive patients.

Four patients with beef sensitivity, each diagnosed by the production of acute allergic symptoms during individual food tests with beef, were subjected to individual food tests with commercial Gelatine* and Gelatine* employed for intravenous use.

The experimental ingestion of Gelatine (ossein) dissolved in water and taken fasting in two doses of ¼ ounce each an hour apart was associated with the following reactions:

Controls

Four patients known not to be beef sensitive—no reaction

Beef-Sensitive Patients

Case 1.—No reaction

Case 2.—Nasal stuffiness, chilliness, dizziness, abdominal distention, and cramps

Case 3.—Fatigue, headache, tinnitus, abdominal distention, nausea, vomiting and diarrhea—the latter persisting two days

Case 3.—Violent paroxysms of sneezing, followed by nausea and severe headache

*Supplied through the courtesy of the Chas. B. Knox Gelatine Co.

The experimental ingestion of 150 ml. and one hour later 75 ml. of Gelatine of beef origin prepared for intravenous use was associated with the following reactions:

Case 1.—No reaction

Case 2.—Nasal stuffiness, chilliness, scotomas, somnolence, and abdominal distress

Case 3.—Chilliness, muscle aching, marked fatigue, indigestion, headache, and delayed peripheral edema

Case 4.—Not subjected to this test because of the violent nature of reaction to beef and to commercial gelatin.

It is of interest that the beef sensitivity in each of these cases was completely masked and was not suspected by any patient in spite of the high degree of sensitivity which existed. Inquires of undiagnosed patients as to whether or not they are known to be beef sensitive are usually worthless.

Pork sensitivity did not exist in any of the four patients studied as determined by compatible individual food tests with pork (complete absence of symptoms and a trajectory curve of leucocytosis.) In each instance commercial porcine Gelatine was tolerated in individual food testing without symptoms.

J. Allergy 26: 320-328, 1955

ANAPHYLACTOGENIC PROPERTIES OF GELATIN AND ITS PRECURSORS

BRET RATNER, M.D., AND LLOYD V. CRAWFORD, M.D.* NEW YORK, N. Y.

ON OCCASION, questions have been raised relative to the allergenicity of edible gelatin. Some clinical reports have claimed its occurrence. Generally, however, gelatin has been considered as nonantigenic.

In addition, little attention has been given to the antigenicity of the parent products as distinct from the hydrolyzed gelatin. Traces of contaminants may elicit allergic symptoms, although gelatin itself may not be allergenic. It is for this reason that we included in this study the antigenic properties of bovine plasma, comminuted cooked ossein, and crude gelatin solution derived from the comminuted ossein, as well as food-grade gelatin and the most purified form—that used for intravenous injection.

MATERIALS

Bovine Plasma.†—In some pilot experiments, bovine serum and bovine plasma each reacted when used to challenge animals previously injected with comminuted ossein. Since the blood element reacting with the comminuted ossein was present in both the plasma and serum, bovine plasma was used exclusively in the experiments.

Comminuted Ossein‡.—

Stage A: This preparation is made from bovine bone demineralized with 5 per cent hydrochloride, washed with water, and then treated with saturated limewater solutions at 60° F. for approximately four weeks for removing extraneous materials such as albumins, mucins, and chondrins and also to initiate hydrolysis of collagen to gelatin. The limed stock is then washed with cold water and dilute acid to approximately pH 6.0 to remove lime salts. At this stage the ossein is ready to extract with hot water at approximately 160° F. This is the material which, in its entirety, formed the basis for the preparation of crude gelatin raw material.

From the Departments of Pediatrics and Immunology, New York Medical College and Flower and Fifth Avenue Hospitals, New York.

This study was aided in part by a grant from the Playtex Park Research Institute.

This is the seventh in a series of studies on the Allergenicity of Modified and Processed Foodstuffs.

The technical assistance of Kathryn M. Mayer, Joseph S. Thomas, and Dr. Mary Retsina is hereby acknowledged and deeply appreciated.

Received for publication Sept. 1, 1954.

*Formerly Research Fellow in Pediatric Allergy. Now in Memphis, Tennessee.

†The bovine plasma and serum were generously supplied to us by Dr. M. S. Cooper, bacteriologist, Biological Production Section, Lederle Laboratories Division, American Cyanamid Company.

‡The comminuted ossein, crude, food-grade, and intravenous-grade gelatins were generously supplied to us by Dr. D. Tourtellotte, Director, Research Division, Charles B. Knox Gelatine Company, Inc. The gelatin solution contained 0.0108 gram protein nitrogen per cubic centimeter and the ossein 0.018 gram nitrogen per cubic centimeter.

Stage B: Two hundred thirty grams (40 per cent solids) of the stock was placed in 500 c.c. bottles with 290 grams distilled water added to fill, autoclaved at 15 pounds steam pressure for twenty minutes, then the cooked mix was poured into a Waring blendor and ground to a uniformly comminuted mush. This comminuted material was rebottled, sealed, and reautoclaved at 15 pounds steam pressure for twenty minutes.

Crude Gelatin.—From the extraction of the boiling just described, an amount of crude gelatin liquors was placed in sealed bottles and subjected to autoclaving at 15 pounds steam pressure for twenty minutes. This provided intermediate material, hot water extracted, but not filtered free of the traces of insoluble and particulate organic matrix.

Food-Grade Gelatin.—This preparation represents the finished commercial dry gelatin dissolved in distilled water, 6 per cent w/v with 0.9 per cent sodium chloride added to physiologic concentration. The commercial dry gelatin used in these experiments was that supplied by the Knox Gelatine Company and is known as Knox Sparkling Gelatine, U.S.P.

*Intravenous-Grade Gelatin.**—Since this product is designed entirely for parenteral use, more exacting selection is made of hard bone starting materials to avoid spongy bone, which can carry traces of hemoglobin and plasma protein residues which might become factors in allergenic responses to the finished product. More exacting care is exercised throughout the intravenous gelatin process to insure complete sterility at every stage. This is accomplished by working in completely enclosed equipment and at temperatures and reactions which are inhibitory to even air-borne contamination. In the case of the intravenous solution, all filtrations are made through special asbestos sterilizing pads, followed by immediate autoclaving in the final sealed flasks used for parenteral administration.

METHODS

The precipitin or complement fixation tests cannot be used because the gelatin congeals on cooling and because the comminuted ossein has particulate matter in it. We believe the induction of anaphylaxis in the guinea pig and the use of the Schultz-Dale technique on isolated tissue to be the most suitable for studies on the antigenicity of these materials.

Normal guinea pigs,† weighing approximately 250 grams, which had never been fed beef or gelatin, were used for the anaphylaxis studies.

Anaphylaxis was tested by the usual sensitization and shock procedures, with incubation periods of three to four weeks intervening. The sensitizing injections were made intraperitoneally and shock injections, unless otherwise specified, were given by the intravenous route. Whenever possible and when there was need for determining whether the absence of shock reaction was the result of absence of sensitization to a related material, the substance to which the animal was originally sensitized was employed as a second confirmatory

*Special gelatine solution, intravenous, Council of Pharmacy and Chemistry, J.A.M.A. 142: 32-33, Jan. 7, 1950.

†Supplied by Carworth Farms, New City, N. Y.

shock injection. A negative reaction obtained with a modified form does not justify the conclusion that allergenicity was not present unless proof is at hand that the animal had been sensitized in the first place. We believe this to be a sound method for determining the anaphylactogenicity of a modified protein.

An adequate number of animals must be used in these experiments. By using ten or more animals, chance of missing a low sensitizing potential is reduced.

Because of the character of the comminuted ossein, the particulate bone elements made it impossible for us to use this material for intravenous shock injection.

The Schultz-Dale experiment was used to supplement graphically the anaphylaxis experiments.

EXPERIMENTS

Eleven animals were sensitized with comminuted ossein and challenged with bovine plasma. The comminuted ossein evidently contained some blood element derived from the homologous bovine plasma, as a cross anaphylactic reaction was demonstrated in this series. However, when the order was reversed and the animals were given a sensitizing injection of bovine plasma and challenged with the comminuted ossein, there was no demonstrable evidence of a cross reaction in eighteen animals tested. When a second injection was given, using bovine plasma to which the animals had been sensitized, seventeen of the eighteen animals died in anaphylactic shock. This proved them to be highly sensitive to the plasma and not to the ossein (Table I). This indicates that the bovine blood elements present in ossein were there in extremely small amounts because, although there was enough present to sensitize, there was not enough present to shock plasma-sensitized animals.

TABLE I

NUMBER OF ANIMALS	SENSITIZING INJECTION	SHOCK INJECTION	RESULTS	SECOND SHOCK INJECTION	RESULTS
11	Comminuted ossein 2 c.c. Ip.	Bovine plasma 0.2 c.c. Iv.	2 animals ++++ 4 animals + 5 animals 0		
18	Bovine plasma 1 c.c. Ip.	Comminuted ossein 2 c.c. Ip.	0	Bovine plasma 0.3 c.c. Iv.	17 animals ++++ 1 animal 0

Key to symbols:
+, Moderate dyspnea.
++, Dyspnea and convulsions.
+++, Dyspnea, convulsions, collapse, and recovery.
++++, Anaphylactic death with typically ballooned lungs.
Ip., Intraperitoneal.
Iv., Intravenous.

Table II shows data on attempts to sensitize animals to ossein and to determine whether or not highly purified intravenous-grade gelatin retained any of the blood-contaminating antigenic elements found in the ossein.

There was no evidence of cross reaction with the gelatin in fourteen animals sensitized with comminuted ossein and challenged with intravenous-grade gel-

TABLE II

NUMBER OF ANIMALS	SENSITIZATION COMMINUTED OSSEIN	SHOCK IV.-GRADE GELATIN	RESULT	CONTROL SHOCK BOVINE PLASMA	RESULT
14	2 c.c. Ip.	0.3 c.c. Iv.	0	0.3 c.c. Iv.	1 animal ++++ 1 animal ++++ 6 animals + 6 animals 0

atin. When these same animals were subsequently challenged with bovine plasma, eight proved anaphylactic, showing that the comminuted ossein contained some blood element, whereas the intravenous-grade gelatin was completely devoid of it.

The next grade of purification after the ossein is crude gelatin. Nineteen animals were injected with crude gelatin and challenged with bovine plasma. There was no evidence that the blood elements were present in the crude gelatin (Table III). When these nineteen animals were subsequently challenged with crude gelatin they were all negative.

TABLE III

NUMBER OF ANIMALS	SENSITIZATION CRUDE GELATIN	SHOCK BOVINE PLASMA	RESULT	CONTROL SHOCK CRUDE GELATIN	RESULT
19	3 c.c. Ip.	0.3 c.c. Iv.	0	0.3 c.c. Iv.	0

In the next series (Table IV), nineteen animals were sensitized to bovine plasma and challenged with crude gelatin with negative results. A second challenging dose of bovine plasma showed them to be highly sensitive to bovine plasma.

TABLE IV

NUMBER OF ANIMALS	SENSITIZATION BOVINE PLASMA 1 c.c. Ip.	SHOCK CRUDE GELATIN	RESULT	CONTROL SHOCK BOVINE PLASMA	RESULT
19		0.3 c.c. Iv.	0	0.3 c.c. Iv.	14 animals ++++ 2 animals + 3 animals 0

Food-grade gelatin was then tested for allergenicity (Table V). In the first twenty-six animals (Group I) food-grade gelatin was used as a sensitizing dose and bovine plasma as a challenging dose. There was evidence of the presence of bovine blood element in the food-grade gelatin in only two of these. In Group II, nineteen animals were injected and challenged with the same food-grade gelatin. There was no evidence of sensitization to the gelatin itself, although one showed the presence of some plasma element when the animals were given a second shock dose with bovine plasma.

In Group III, twenty-two animals were sensitized with bovine plasma and challenged with food-grade gelatin. In no instance was a blood element demonstrated in the food-grade gelatin when used as a challenging dose. However,

TABLE V

GROUP	NUMBER OF ANIMALS	SENSITIZATION	FIRST SHOCK	RESULT	SECOND SHOCK	RESULT
I	24	Food-grade gelatin 3 c.c. Ip.	Bovine plasma 0.3 c.c. Iv.	1 animal ++++ 1 animal + 24 animals 0		
II	19	Food-grade gelatin 3 c.c. Ip.	Food-grade gelatin 0.3 c.c. Iv.	0	Bovine plasma 0.3 c.c. Iv.	1 animal ++++ 18 animals 0
III	22	Bovine plasma 1 c.c. Ip.	Food-grade gelatin 0.3 c.c. Iv.	0	Bovine plasma 0.3 c.c. Iv.	20 animals ++++ 1 animal + 1 animal 0

when these twenty-two animals again were challenged with the bovine plasma with which they were originally sensitized, twenty-one demonstrated profound anaphylaxis.

In a total of forty-five animals, therefore, there were only three instances, or 6.6 per cent, where sensitization to the blood elements was demonstrated by the food-grade gelatin. In twenty-two animals, however, when the food-grade gelatin was used as a shock agent in animals exquisitely sensitive to bovine plasma, there was no evidence that the contaminating blood elements were present in sufficient amounts to produce a shock reaction.

Therefore, in none of a relatively large series of animals was food-grade gelatin shown to be anaphylactogenic to itself. However, there was evidence that there is an extremely slight amount of the blood element found in the food-grade gelatin which occasionally was able to sensitize but never was able to produce shock reactions.

TABLE VI

GROUP	NUMBER OF ANIMALS	SENSITIZATION	FIRST SHOCK	RESULT	SECOND SHOCK	RESULT
I	10	Iv.-grade gelatin 2 c.c. Ip. 5 days later, 1 c.c. Ip.	Bovine plasma 0.3 c.c. Iv.	0	Iv.-grade gelatin 0.3 c.c. Iv.	0
II	30	Bovine plasma 1 c.c. Ip.	Iv.-grade gelatin 0.3 c.c. Iv.	0	Bovine plasma 0.3 c.c. Iv.	19 animals ++++ 2 animals +++ 3 animals + 6 animals 0

The most purified form of gelatin is the one used for intravenous injection. In Table VI, Group I, there were ten animals given two sensitizing injections of intravenous-grade gelatin, five days apart, and challenged with bovine plasma. They were consistently negative. When challenged with intravenous-grade gelatin again, they were also proved to be negative, indicating that this gelatin was devoid of allergenicity. In Group II thirty animals were sensitized with bovine plasma and in no instance was there any evidence of cross reaction when challenged with intravenous-grade gelatin. The animals were sensitive to a high degree, for twenty-four of the thirty showed anaphylaxis when challenged with the original bovine plasma.

Schultz-Dale experiments, an example of which is shown in Fig. 1, graphically epitomize the studies of the antigenicity of gelatin. Virgin female guinea pigs sensitized to comminuted ossein were used in these experiments. The sensitized uterus was immersed in oxygenated Tyrode's solution. The uterine strip was challenged with food-grade gelatin (*A*). There was no evidence of reaction. After several minutes, the strip was challenged with bovine plasma (*B*). A very prompt maximal contraction ensued. The bath was changed with fresh Tyrode's solution (*C*). Bovine plasma was again placed in the bath (*D*). An immediate secondary contraction of lower amplitude ensued. The Tyrode's solution was again replaced (*E*). Bovine plasma was again placed in the bath (*F*). There appeared to be a refractoriness, followed several minutes later by a delayed reaction. The bath was changed again (*G* and bovine plasma (*H*) was put in for the fourth time. The muscle showed complete refractoriness. Finally, without further change of bath, the muscle was challenged with histamine acid phosphate (*I*), which produced a maximal contraction.

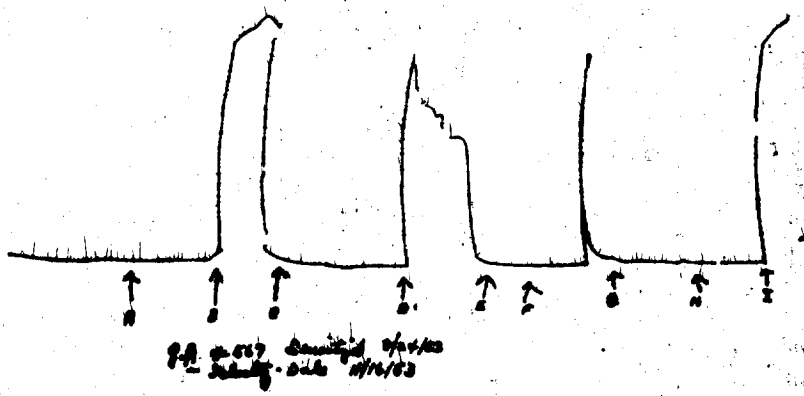

Fig. 1.—Animals sensitized with comminuted ossein and tested with food-grade gelatin and bovine plasma. *A*, Food-grade gelatin; *B*, bovine plasma; *C*, washed; *D*, bovine plasma; *E*, washed; *F*, bovine plasma showing refractoriness and a delayed reaction; *G*, washed; *H*, bovine plasma, desensitization; *I*, histamine acid phosphate.

This experiment shows in a vivid manner that when the precursor (comminuted ossein) of the food-grade gelatin is used as a sensitizing agent, there is no evidence of antigenicity when challenged with food-grade gelatin, but there is definite evidence of sensitization to some element in the blood plasma. The secondary contraction at *D* and the delayed contraction at *F*, when the uterine strip was challenged twice with bovine plasma, suggest that the first challenge dose did not result in a complete refractoriness. This might be due to a multiplicity of antigens in the bovine plasma. The final refractoriness indicates complete desensitization of all the fractions in the bovine plasma. The reaction to histamine proves the validity of the desensitization by showing that the uterine strip retains its viability and optimal functioning.

SUMMARY OF EXPERIMENTS

I. (See Table VII.) Comminuted ossein was found to sensitize against bovine plasma in fourteen of twenty-five animals (56 per cent). Food-grade gelatin, refined from comminuted ossein, sensitized only three of forty-five animals (6.6 per cent) when challenged with bovine plasma. In none of thirty-two animals did intravenous-grade gelatin sensitize against bovine plasma.

TABLE VII. COMPARATIVE REACTIVITY OF CONTAMINATING BOVINE BLOOD ELEMENTS IN CRUDE AND REFINED GELATINE WHEN USED AS A SENSITIZING AGENT

NUMBER OF ANIMALS	SENSITIZATION	SHOCK	RESULTS		
			POSITIVE	NEGATIVE	PER CENT POSITIVE
25	Comminuted ossein	Bovine plasma	14	11	56.0
45	Food-grade gelatin	Bovine plasma	3	42	6.6
32	Iv.-grade gelatin	Bovine plasma	0	32	0.0

II. (See Table VIII.) In none of 141 animals sensitized with bovine plasma, comminuted ossein, crude gelatin, food-grade gelatin, and intravenous-grade gelatin was there any reaction when the animals were challenged with any of the forms of unrefined or refined gelatin.

TABLE VIII. NONREACTIVITY OF CRUDE AND REFINED GELATIN WHEN USED AS ANAPHYLACTIC SHOCK AGENTS

NUMBER OF ANIMALS	SENSITIZATION	SHOCK	RESULT
14	Comminuted ossein	Iv.-grade gelatin	0
9	Crude gelatin	Crude gelatin	0
19	Bovine plasma	Crude gelatin	0
19	Food-grade gelatin	Food-grade gelatin	0
22	Bovine plasma	Food-grade gelatin	0
30	Bovine plasma	Iv.-grade gelatin	0
10	Iv.-grade gelatin	Iv.-grade gelatin	0
18	Bovine plasma	Comminuted ossein	0

III. (See Table IX.) Whether the intravenous-grade gelatin was used as a sensitizing injection or as a shock injection, in no instance in fifty-eight animals was there any sensitization or shock manifested, even when they were cross-tested with bovine plasma.

TABLE IX. ABSENCE OF ANY ANAPHYLACTOGENIC REACTIVITY OF INTRAVENOUS-GRADE GELATIN

NUMBER OF ANIMALS	SENSITIZATION	SHOCK	ANAPHYLAXIS
14	Comminuted ossein	Iv.-grade gelatin	0
24	Bovine plasma	Iv.-grade gelatin	0
10	Iv.-grade gelatin	Bovine plasma	0
10	Iv.-grade gelatin	Iv.-grade gelatin	0

COMMENT

Gelatin does not exist as a natural protein but is derived from hydrolysis of the insoluble connective tissue protein collagen, which exhibits no demonstrable antigenic effects.

The nonantigenicity of gelatin was thought to be due to a deficiency of aromatic groupings of its amino acid constituents, tyrosine and tryptophane. There are several other possible explanations for the immunologic inertness of this protein. It is deficient in carbohydrate groupings. It may be excreted rapidly in the urine after intravenous injection into the animal. It is usually prepared from collagen by prolonged treatment with boiling water or steam and this treatment may well be sufficient to destroy any antigenic power of the preparation.

In explanation of the foregoing are several investigations[2] which showed that the introduction of aromatic groupings, including tyrosine, into gelatin does not produce a compound which has antigenic powers comparable with those of the majority of proteins.

Similarly, the failure does not appear to be due solely to lack of tyrosine even when carbohydrates are added.[3]

With reference to its rapid excretion via the kidneys, Haurowitz and his co-workers[4] believe that the major part of ingested gelatin or gelatin derivatives is so rapidly excreted in the urine that little remains in the body to induce antibody formation. Gelatin and several other proteins with molecular weights of less than 70,000 are rapidly excreted in the urine by anesthetized dogs.[5] Proteins such as egg albumin and Bence-Jones protein, both of which are excreted by the kidneys, are known to be fully antigenic. Loss by excretion in the urine, according to Wormall,[6] cannot be the only factor that determines the nonantigenicity of gelatin.

The last explanation (that is, the effect of prolonged heating in boiling water), we believe, has validity because it has been shown that albumin heated in the presence of water becomes coagulated and does have a definite reduction in antigenicity.[7]

The studies detailed in this article demonstrate that gelatin is a nonantigen and that any antigenicity found in its crude precursor (comminuted ossein) was due solely to contamination with bovine blood elements. In no instance did crude or refined gelatin serve as an anaphylactogen. The presence of contaminating blood elements was found in 56 per cent of the experiments with comminuted ossein and in only 6.6 per cent of the experiments with food-grade gelatin. The anaphylactogenicity of the blood elements present in the comminuted ossein and crude gelatin mixtures persisted despite the heat and hydrolytic treatments during the process of gelatin preparation.

In no instance was any contaminating blood element found in the highly purified gelatin used for intravenous blood replacement.

CONCLUSIONS

1. Within the limits of our experiments, we can state that gelatin is nonanaphylactogenic.

2. The antigenicity attributed to gelatin is related to a contamination by some blood elements of the species from which the gelatin is derived. This antigenic element is present in both the plasma and serum and apparently is highly resistant to heat.

3. The presence of blood contaminants in comminuted ossein was demonstrated in the majority (56 per cent) of tests on animals sensitized to this precursor of refined gelatin. It was found to a negligible degree (6.6 per cent) in sensitization tests with the food-grade gelatin. It was completely eliminated in the intravenous-grade gelatin (Knox).

4. The blood contaminant in the comminuted ossein and the food-grade gelatin was present in so minute an amount that it did not induce any anaphylactic reactions in animals sensitized to the whole plasma.

5. Because no authentic case of gelatin sensitization has been demonstrated in man, we were unable to obtain gelatin antibody-containing blood for human studies. We therefore must assume that because it is nonanaphylactogenic for the animal, it must be nonallergenic for man.

6. Any allergenicity in man that might be attributed to gelatin is due to contamination with blood elements of the species from which it is derived and not to antigenicity of the gelatin itself.

In recent studies, Maurer[8, 9] reports that he found normally occurring antibodies to gelatin in man. He also found them in herbivorous as well as carnivorous animals. This naturally occurring antibody is therefore not related to the diet of the animal. It may arise as a result of some relation between the collagen system and gelatin metabolism but this is uncertain. The antibody is not concerned in the production of the wheal reaction and apparently is not an anaphylactic antibody.

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Proc. Soc. Exptl. Biol. Med. 40:157-161.
1939

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Effect of Gelatine on Muscular Fatigue.

G. B. RAY, J. R. JOHNSON AND M. M. TAYLOR.

From the Department of Physiology and Pharmacology, Long Island College of Medicine, Brooklyn, N. Y.

It has been reported by Boothby[1] that the onset of muscular

[6] Wulzen, R., and Bahrs, A. M., *Physiol. Zool.*, 1936, **9**, 508.

[1] Boothby, W. M., *Proc. Mayo Clin. Staff Meetings*, 1934, **9**, 600.

fatigue can be delayed by the addition of a considerable amount of glycine to the diet of the normal individual. He has further shown that in cases of myasthenia gravis and similar diseases the feeding of glycine tends to restore the wasted muscle tissue, thus indicating an effect upon the physiological state of these tissues. Wilder[2] believed his ability to play tennis increased after 2 weeks on a glycine diet. The possible explanation of these reactions is to be found in the creatinogenic action of glycine and the storage of at least part of this product in the muscles.[3] This is quite in accord with the current concept of the chemistry of muscular contraction wherein creatine in its combination with phosphoric acid plays an important rôle. The concept of storage is confirmed by the work of Rose, Ellis, and Helming,[4] who found a male subject on a creatine diet could store this substance. This was not evident in the case of a female subject.

Unfortunately, Boothby found that normal subjects were unable to take large amounts of glycine without some discomfort.[1] It seemed of interest, therefore, to determine if a food rich in glycine would also increase the amount of work a trained subject was capable of doing before fatigue set in. Among the dietary possibilities, perhaps the most outstanding is gelatine, which contains about 25% glycine, is easily digested, and is readily available as a food substance.

To the authors' knowledge, none of the above papers, concerned with muscular fatigue and glycine, reported quantitative studies on the work output of their subjects. It was felt, therefore, that it was important that the amount of work done be measured as accurately as possible, both during a training period and during a period when gelatine was added to the normal diet. For this purpose a bicycle ergometer of special design was used. The essential part is a small self-exciting 110 volt A. C. generator driven by the rear wheel of a stationary bicycle. The load on this generator depends upon a series of lamps in parallel and the output is read on a watt-meter.* Thus, by observing the output in watts and timing the duration of work, the total energy developed during the exercise period can be calculated.

Ten subjects, 6 men and 4 women, were trained on the ergometer

[2] Wilder, R. M., *Proc. Staff Meetings Mayo Cl.*, 1934, **9**, 608.

[3] Tripoli, C. J., and Beard, H. H., *Southern Med. J.*, 1938, **31**, 662.

[4] Rose, W. C., Ellis, R. H., and Helming, O. C., *J. Biol. Chem.*, 1928, **77**, 171.

* The writers wish to acknowledge with thanks the courtesy of the General Electric Company in supplying the necessary special meter and generator.

until the maximal output of work before fatigue set in was as constant as could be expected under the experimental conditions. After a satisfactory training period, the end of which was determined by the constancy of the daily work, the subjects were placed on a gelatine† diet. Three of the women subjects took 45 g per day, the fourth took 67.5 g. The men were all given 60 g. It is realized that these amounts are, to a degree, arbitrary but do approximate in the case of the men a dose of 15 g of glycine which is within the range used by Adams, *et al.*, for normal subjects.[5] It was

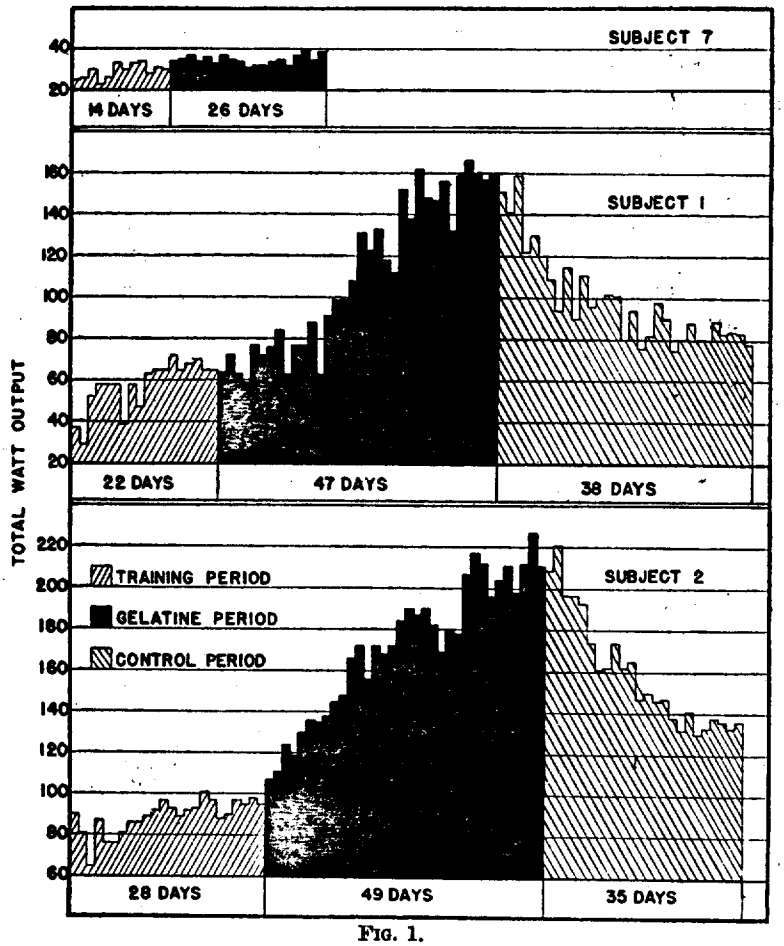

FIG. 1.

† The writers wish to thank the Knox Gelatine Laboratories for their cooperation and the supply of gelatine.

[5] Adams, M., Power, M. H., and Boothby, W. M., *Am. J. Physiol.*, 1935, **111**, 596.

found that the most convenient way of administering these large amounts of gelatine was to mix it with orange and lemon juice. If the juice was well chilled, as much as 30 g of gelatine could be given in 8 ounces of juice and the whole taken before the gelatine swelled appreciably. In this manner it was possible for one to take the entire assigned amount of gelatine without distress.

The details of 3 representative experiments are given in Fig. 1. The cross-hatched area to the left indicates the training period; the solid black, the period during which gelatine was administered, and the area to the right, the control or recovery period during which fruit juice alone was given the subjects. It was felt necessary to include this period in order to demonstrate that any effect was due to the gelatine rather than to the increased intake of vitamins.

The record of Subject 7, at the top of the chart, that of a woman medical student, is presented since it is one of 2 records obtained from women subjects showing a semblance of an increase (3%) during the gelatine diet. She received 45 g of gelatine (the equivalent of 11.25 g of glycine) a day during the experimental period. The slight increase noticed is of dubious significance since it may well be assigned to a possible effect of a lengthened training period.

The second graph, that of Subject 1, represents a complete record on a man 25 years old. After the usual period of training, he received 60 g of gelatine a day. The gelatine was continued until the fatigue level appeared to have reached its maximum. In the case of this individual it was at the end of 47 days. At that point the gelatine was discontinued but the 16 ounces of orange and lemon juice maintained. It will be noted that shortly after the cessation of gelatine, the total work output fell off and at the end of 38 days came to a level. At this point the experiment terminated.

Subject 2, whose complete record is also presented, gives a picture similar to that of Subject 1. There is one notable exception in that the response to gelatine is very prompt. This subject, an instructor, aged 33, was of slight build. It is interesting to record at this time that during the gelatine period be gained 10 pounds, 8 of which were retained to the end of the experiment.

In conclusion, it may be said that men, when given adequate amounts of gelatine, are invariably able to increase the amount of work produced before fatigue sets in. The results varied from 37% to 240% increases above the training level. In by far the greater number of subjects the increase was more than double the pregelatine work level. This is not true in the case of women sub-

jects, where no appreciable effect was noticed. It might be supposed that these effects were due to the considerable amounts of orange juice and its vitamins. When, however, gelatine was discontinued and the same amount of fruit juice maintained, the work output rapidly fell to an approximation of the pregelatine level, thereby indicating that the effect was primarily that of the addition of gelatine to the diet. The sexual variation is also of interest from the point of view of the intepretation of the results. As mentioned above, Rose, *et al.*, found a marked sexual variation in the ability to store creatine. These experiments are most suggestive since they indicate a situation comparable to that found in our own experiments. It would seem as though this action of gelatine were particularly concerned with the creatinogenic action of its amino acids, especially glycine.

Italian Jour. Biochem. 10(1):8-18, 1961

PURINE METABOLISM OF RATS FED ZEIN OR GELATIN AS THE ONLY SOURCE OF AMINO ACIDS (*)

G. RICCERI

Istituti di Fisiologia umana e di Chimica biologica dell'Università di Catania

(Received 1° August 1960)

Studies on the purine metabolism of rats fed deficient diets have shown that the quantity of allantoin eliminated, which is a measure of the metabolism of the purine bases of nucleic acids, decreases progressively when feeding diets deficient in one or several essential amino acids and increases rapidly until normal values are reached, when the diets are supplemented with the missing amino acids. This decrease is not specifically related with the lack of some given amino acids but is observed when any essential amino acid is missing. Hence the deficiency affects also the endogenous metabolism causing slowing down of anabolism and of the successive catabolism of nucleoproteins and purine bases (Ricceri, 1955 and 1956).

This effect of essential amino acids on the metabolism of nucleic acids has been confirmed by further experiments using mixtures of amino acids both in animal organisms (Munro and Clark, 1959) and in bacteria (Gros and Gros, 1956). Thus it is a general phenomenon in nutrition. Finally recent studies (Ricceri and Giuffrida, 1959) using ^{32}P have shown that when essential amino acids are missing, the incorporation of inorganic phosphorus in nucleic acids *in vivo* is markedly decreased.

Studies were thus carried out on the purine metabolism of rats deficient in essential amino acids; the nitrogen content of the diet was supplied by a natural incomplete protein, zein or gelatin. Zein is defi-

(*) Some of the experiments reported in this paper have already formed the object of a communication in the Boll. Accad. Gioenia Sci. nat. Catania, series LV, 1957, 3, 516.

cient in lysine and tryptophan and sulphur containing amino acids, arginine and histidine, are only present in small amounts (Tristram, 1953, p. 223). Gelatin is deficient in tryptophan and cystine and other amino acids such as methionine, histidine, tyrosine and isoleucine are only present in small amounts (Tristram, 1953, p. 221). Hence these proteins were very suitable for studying deficiency phenomena.

Methods

Male adult albino rats weighing 200 to 350 g were used. 4 cycles of experiments were carried out; in 2 which lasted 35 days each, the protein used was zein and in the other two which lasted 62 days each, gelatin was used. In each experiment, 2 rats having the same body weight were used.

The basic diet consisted of the following protein-free mixture according to Sydenstricker, Hale, Hock and Pund (1946): sucrose 90 g, Osborne and Mendel (1918) salt mixture 4 g, cod liver oil, 2 g, cotton seed oil 4 g. The salt mixture was mixed with other mineral constituents (Mo, Cu, Zn). To 82 parts of the protein-free mixture, 18 parts of protein were added. The protein consisted of casein during the initial and final control stages and of zein or gelatin during the deficiency stage or when the diet was supplemented (*).

The following quantities of vitamins were added per 100 g food mixture: thiamin 0.5 mg, riboflavin 0.8 mg, pyridoxin 0.5 mg, nicotinamide 4 mg, Ca pantothenate 0.4 mg, folic acid 0.2 mg, cyanocobalamin 3 μg, axerophtol 15 μg, α-tocopherol 3 mg, choline 100 mg. Owing to the well-known relationship between tryptophan and nicotinic acid, double the amount of nicotinamide was added (8 mg) to mixtures containing zein or gelatin which do not contain tryptophan.

Each cycle of experiment consisted of 4 stages, the first and the 4th were control ones, the 2nd the deficient one and the third the supplemented one. In the first stage the protein added to the diet was casein, in the deficient stage, casein was replaced by zein (experiment 1 and 2) or by gelatin (experiment 3 and 4). In the 3rd stage, the diet was first supplemented with some of the missing amino acids and then every 3 to 4 days with the other essential amino acids until the diet was complete. The amino acids added in the third stage were the following: in the diet with zein, tryptophan, lysine, arginine, histidine, cy-

(*) Casein was obtained from E. Merck, Zein from Nutritional Biochemical Corporation and pure gelatin for bacteriology from C. Erba.

stine and methionine; in that with gelatin, tryptophan, cystine, leucine, isoleucine, methionine and tyrosine (*). Each amino acids was added in such a quantity that it amounted to 5% of the protein content of diet. The cycle of experiments was ended by feeding again the diet containing casein. All diets were administered *ad libitum*.

The animals were kept in metabolic cages and urine was collected so that it was not contaminated and did not ferment (Ricceri, 1956). Urine was collected in a flask containing 5 % acetic acid and toluene. In the morning the urine collector was washed and the flask was changed. For the estimations, the urine excreted and collected in the last 24 hr. or that of the last 48 hr. was used. In the latter case, the urine of the first 24 hr. containing acetic acid and toluene was kept *in the cold store* (**) until use. The urine and the washings collected in 24 hr. or in 48 hr. were made up to 100 ml or to 200 ml respectively. Various fractions were used to estimate the following substances: allantoin which in the rat is the main catabolite of purine origin and which may be taken as a measure of the intensity of the catabolism of nucleic acids; uric acid which is present in small quantities and which has the same physiological meaning as allantoin; total nitrogen to see if the nitrogen supply was absorbed and metabolized and creatinine which is regarded as a measure of endogenous protein metabolism.

The following methods were used; for allantoin the method of Young and Conway (1942) as modified and adapted for the Coleman spectrophotometer ($\lambda = 520$ mμ); for total nitrogen the Kjeldahl method as modified to give a semi-micromethod using a Parnas-Wagner apparatus; for uric acid, the colorimetric method of Folin (1922); for creatinine, the method of Lieb and Zacherl (1934). A Lumetron photoelectric colorimeter ($\lambda = 530$ mμ) was used for the last two methods. Further details have been reported elsewhere (Ricceri, 1955 and 1956).

Results and discussion

All the experimental results are reported as diagrams (fig. 1 to 8). 2 diagrams were made up to represent the results of each experiment, one for allantoin and total nitrogen and the other for uric acid and creatinine.

1) *Experiments with zein.* — When the only source of amino acids was zein, the quantity of allantoin eliminated decreased appreciably and

(*) L-Amino-acids obtained from Nutr. Biochem. Corp., Light a. Co., E. Merck have been used.

(**) *In the cold store* means that the temperature of storage was close to 0°.

progressively from a value of 56-58 mg per day (initial control diet containing casein) to a value of 45-46 mg. The decrease continued even when the diet was supplemented with only some essential amino acids either missing or present in insufficient amounts. Thus when tryptophan, lysine, arginine and histidine were added, the quantity of allantoin eliminated per day decreased further until it reached a value of 38 mg. On the other hand, the quantity of allantoin eliminated increased rapidly until it reached a value of 50 mg per day, when cystine and methionine were also added so that the requirement of essential amino acids was satisfied. Finally when the casein containing diet was fed (final control diet) there was a further slight increase in urinary allantoin which reached a value of 52-53 mg per day (fig. 1 and 2).

The uric acid eliminated per day when the zein diet was given, decreased from an initial value of 5 mg to 3.7 to 4 mg and returned again to 5 to 5.5 mg (fig. 3 and 4) when the diet was supplemented with all the essential amino acids.

The amount of creatinine eliminated showed a slight increase when the zein diet was first fed and then decreased slightly. Finally it showed a further slight increase when all the essential amino acids missing were added to the diet.

2) *Experiments with gelatin.* — When the only source of amino acids was gelatin, the quantity of allantoin eliminated per day decreased progressively from a value of 68 mg (initial control casein diet) to a value of 44-45 mg (fig. 5 and 6). This decrease remained when cystine only or tryptophan only were added to the diet whereas when both these amino acids were supplemented, there was a definite increase in the amount of allantoin eliminated which reached 54 mg per day (fig. 6). Then when the diet was supplemented with the other essential amino acids present in small amounts in gelatin (leucine, isoleucine, methionine and tyrosine) there was a further increase in the amount of allantoin eliminated until a value of 62 to 70 mg was reached (fig. 5 and 6). Finally a further slight increase was observed when the final casein diet was given (fig. 5).

The quantity of uric acid eliminated per day decreased when the gelatin diet was given, from an initial value of 4 to 4.5 mg to 2 to 3 mg. When the diet was supplemented with the amino acids missing or present in insufficient amounts, it increased until a value of about 4 mg was reached (fig. 7 and 8).

The creatinine eliminated showed a slight and variable increase when the gelatin diet was first given followed by a progressive decrease.

When the diet was supplemented with all the missing amino acids, the quantity of creatinine eliminated returned to normal (fig. 7 and 8). This finding is in agreement with the results of experiments carried out with zein.

The slight increase in the amount of creatinine eliminated observed when either the zein or the gelatin diet were first given may easily be accounted for by the behaviour of the endogenous metabolism when a deficient diet is fed. It is known that deficient diets produce at the be-

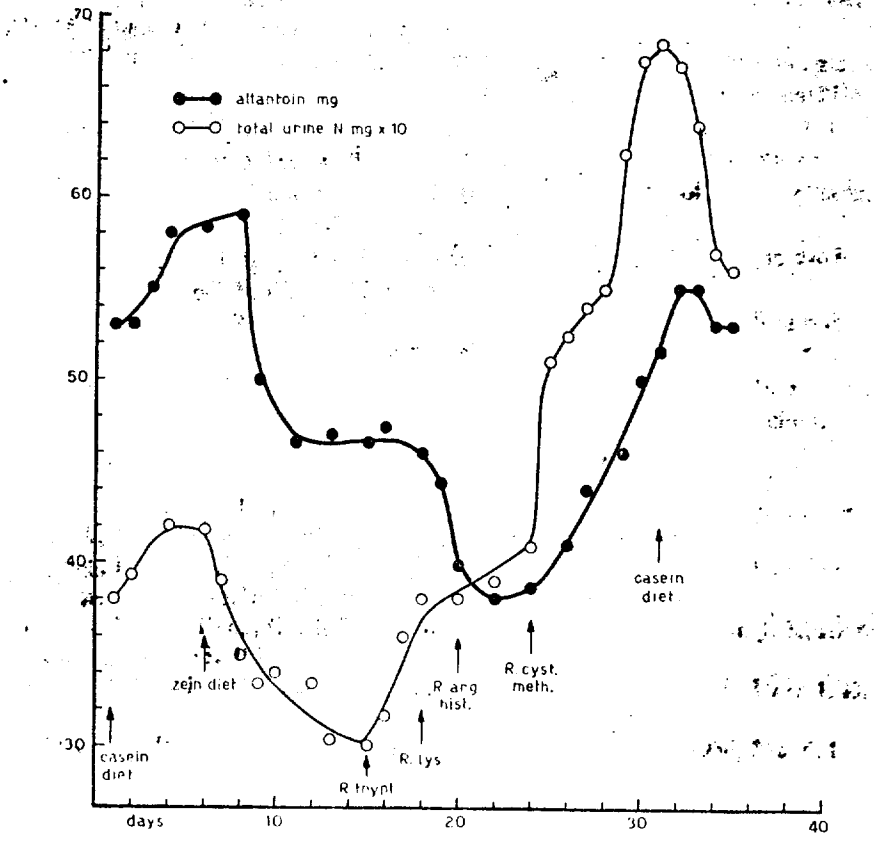

Fig. 1. — Urinary allantoin and total nitrogen eliminated per day by rats fed a diet containing zein as the only source of amino acids and then supplemented with amino acids (rats A and B). aprot. = protein-free diet. R = diet supplemented with amino acids in the following order: tryptophan, lysine, arginine and histidine, cystine and methionine.

ginning an increase in the metabolism of the tissue proteins from which the organism obtains the essential amino acids missing in the diet. However this increase is short since the intensity of the metabolic processes decreases owing to adaptation of the organisms to the new nutritional conditions.

The results of the present experiments confirm fully the conclusions drawn from previous studies (Ricceri, 1955 and 1956; Ricceri and Giuffrida, 1959). When the diet is deficient in essential amino acids, the nucleic acid metabolism is slowed down. I believe that this fact is not due to decreased synthesis of the enzyme systems related to the metabolism of nucleic acids due to lack of the amino acids necessary to form the apoenzyme; the quantity of amino acids required for this synthesis is very small and is available even when the deficient diet is fed since

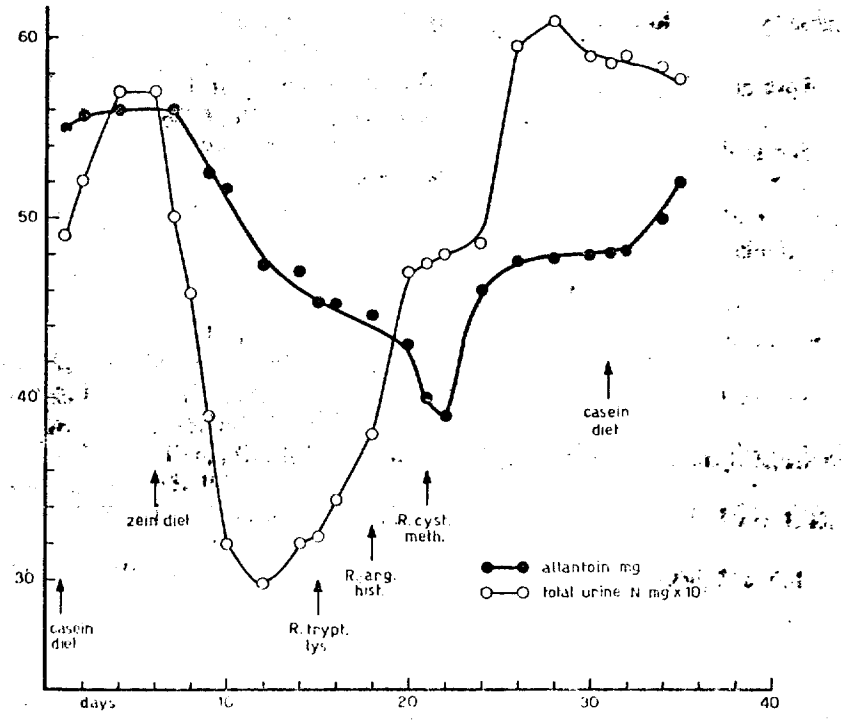

Fig. 2. — Urinary allantoin and total nitrogen eliminated per day by rats fed a diet containing zein as the only source of amino acids and then supplemented with amino acids (rats C and D). For further details see the legend of fig. 1.

they arise from the metabolism of tissue proteins. The biochemical mechanism of this phenomenon is on the other hand clear considering the structural specificity of nucleoproteins. In fact deficiency of one essential amino acid reduces the synthesis of the whole nucleoprotein molecule and thus slows down the anabolism and the successive catabolism of nucleic acids. This view is in keeping with the results of other authors (Sanadi and Greenberg, 1948; Wu, 1954), who found that when essential amino acids are deficient, the incorporation of other amino acids into

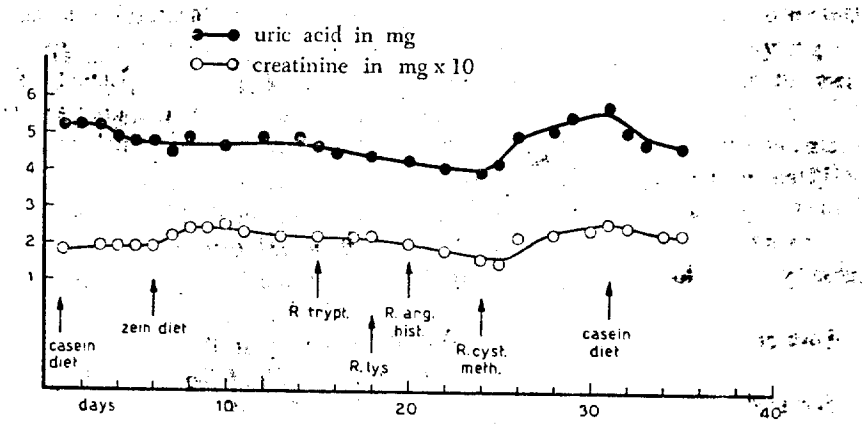

Fig. 3. — Uric acid and creatinine eliminated per day by rats fed a diet containing zein as the only source of amino acids and then supplemented with amino acids (rats A and B). For further details see the legend of fig. 1.

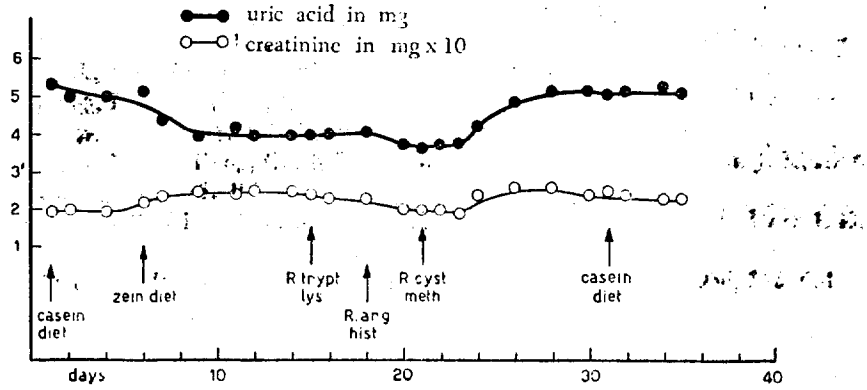

Fig. 4. — Uric acid creatinine eliminated per day by rats fed a diet containing zein as the only source of amino acids and then supplemented with aminoacids (rats C and D). For further details see the legend of fig. 1.

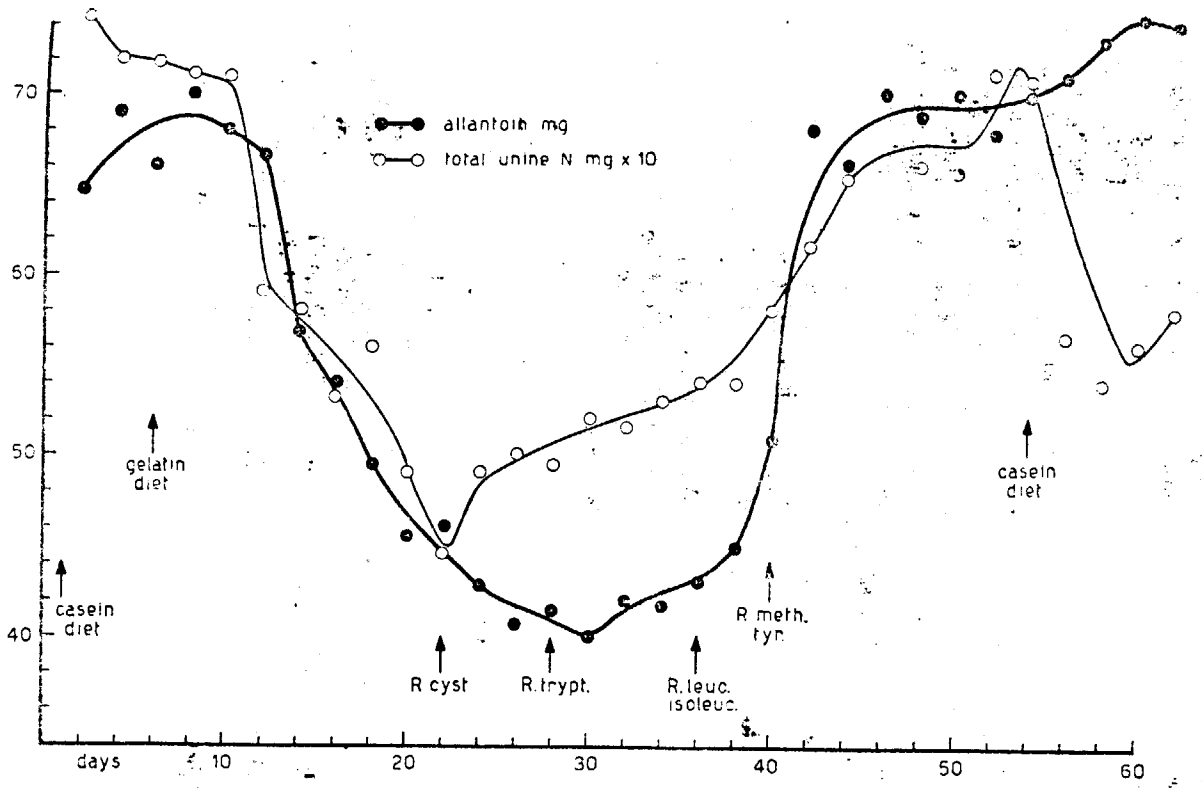

Fig. 5. — Urinary allantoin and total nitrogen eliminated per day by rats fed a diet containing gelatin as the only source of amino acids and then supplemented with amino acids (rats E and F). aprot. = protein-free diet. R = diet supplemented with amino acids in the following order: cystine, tryptophan, leucine and isoleucine, methionine and tyrosine.

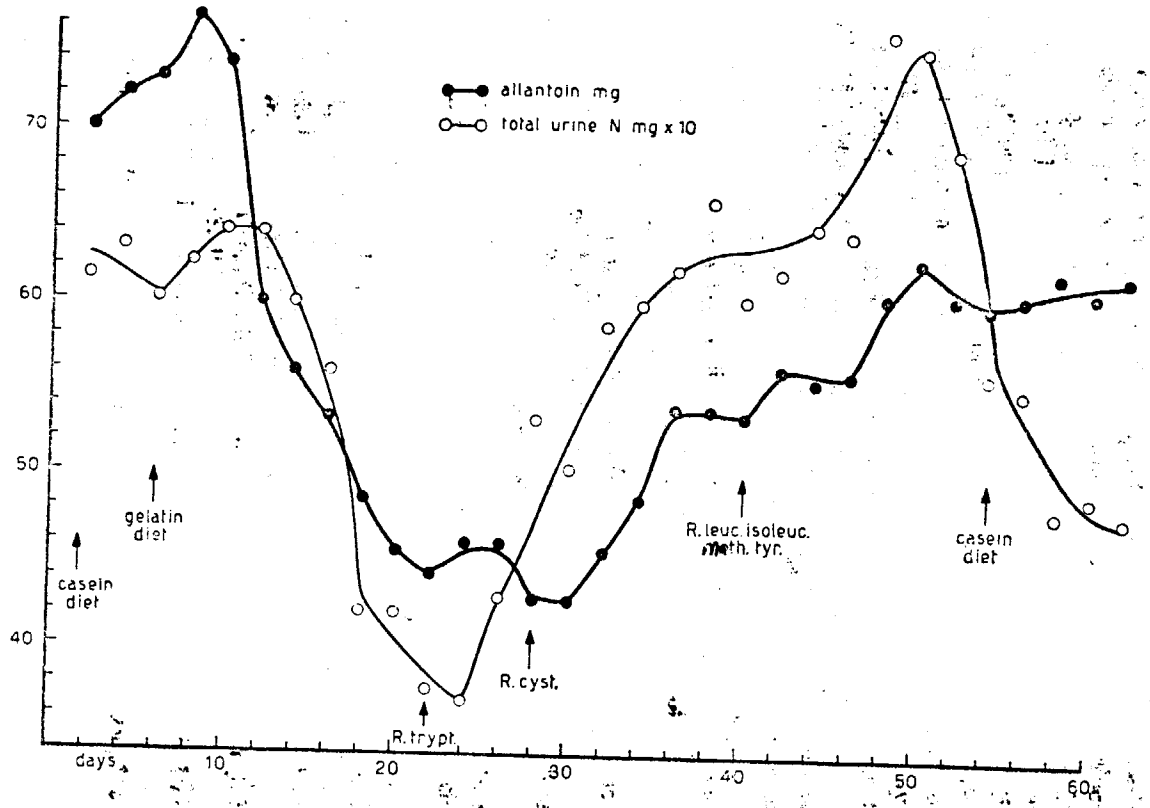

Fig. 6. — Urinary allantoin and total nitrogen eliminated per day by rats fed a diet containing gelatin as the only source of amino acids and then supplemented with amino acids (rats G and H). For further details see the legend of fig. 5.

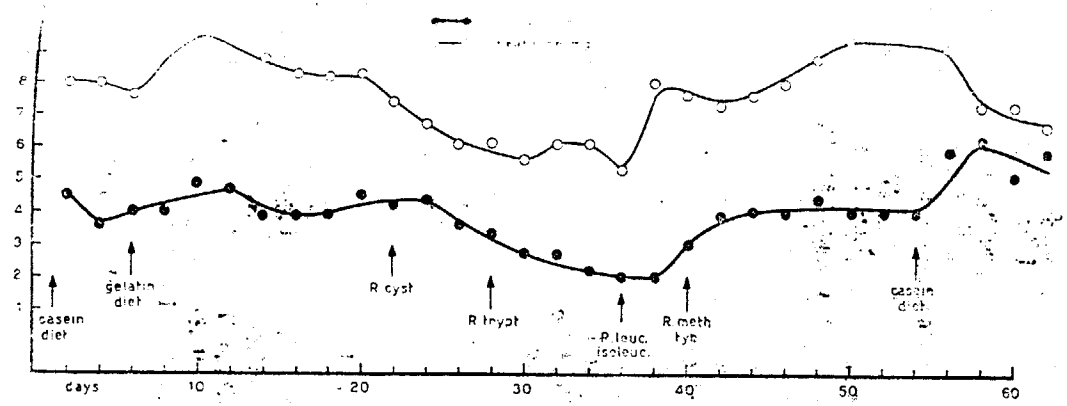

Fig. 7. — Uric acid and creatinine eliminated per day by rats fed a diet containing gelatin as the only source of amino acids and then supplemented with amino acids (rats E and F). For further details see the legend of fig. 5.

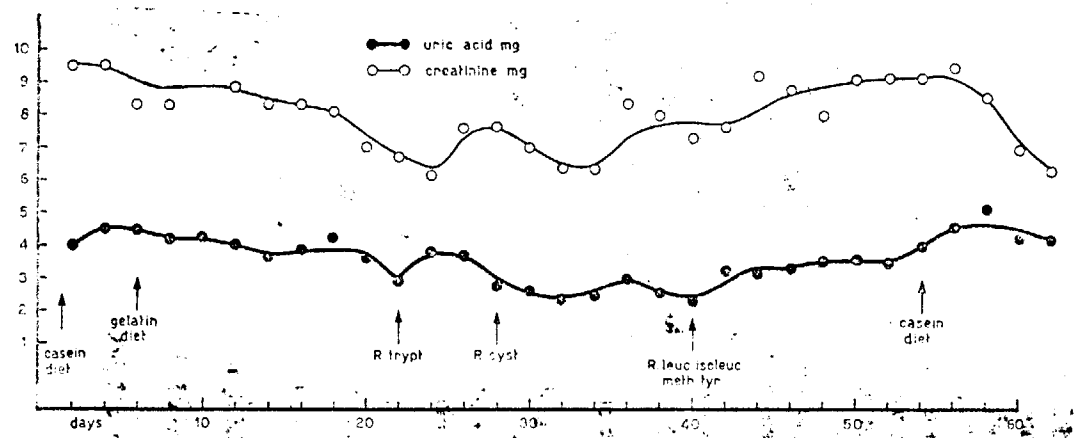

Fig. 8. — Uric acid and creatinine eliminated per day by rats fed a diet containing gelatin as the only source of amino acids and then supplemented with amino acids (rats G and H). For further details see the legend of fig. 5.

tissue proteins is prevented and at the same time there is a decrease of the free amino acids of plasma and urine and elimination of small quantities of amino acids in the bound form.

SUMMARY

1) The quantities of allantoin and uric acid eliminated by adult albino rats which is a measure of the intensity of the catabolism of nucleic acids and thus also of their anabolism decrease markedly and progressively when the nitrogen content of the diets fed the animals consists of zein or gelatin, proteins which are deficient in some amino acids. This decrease remains even when the diets are supplemented only with some of the missing essential amino acids. When the diets are supplemented with all the essential amino acids, the amounts of allantoin and uric acid eliminated return to normal.

2) These results confirm the findings of previous experiments (Ricceri, 1955 and 1956; Ricceri and Giuffrida, 1959). When the diet is deficient in essential amino acids, the nucleic acid metabolism is slowed down.

3) The biochemical mechanism of this phenomenon is put forward in relation to the utilization of essential amino acids for the synthesis of the protein group of nucleoproteins.

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J. Exptl. Med. 80:145-164, 1944

GELATIN—ITS USEFULNESS AND TOXICITY

Blood Protein Production Impaired by Continued Gelatin by Vein*‡

By F. S. ROBSCHEIT-ROBBINS, Ph.D., L. L. MILLER, Ph.D., and G. H. WHIPPLE, M.D.

(From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, New York)

(Received for publication, May 8, 1944)

Gelatin holds the interest of many investigators at this time because it possesses many qualities essential for a plasma protein substitute. This report points out some of its good and bad qualities but in particular *its effect upon plasma protein and hemoglobin production* in standardized depleted dogs. When gelatin in moderate daily doses by vein is continued for 1 to 2 weeks, there is usually impairment of blood protein production, which condition may last for 1 to 3 weeks after gelatin administration has ended.

Administration of gelatin by vein for 2 to 3 days (a total of 1 to 3 gm. per kilo) may not disturb blood protein production nor interfere with the immediate and subsequent utilization of amino acids, casein digests, or food protein to produce new plasma protein and hemoglobin in these standardized dogs. A single 3-day experiment (Table 9) does show intoxication. Evidently dogs vary greatly in their tolerance for gelatin by vein.

This is not the time or the place to review the considerable literature relating to gelatin and its use in clinical emergencies. A great deal of work in various laboratories and clinics is in progress and some of this work was reviewed in a Symposium published by various authors in the *Annals of Surgery*, August, 1943 (4).

Gelatin given intravenously to dogs may cause some changes in blood vessels according to Hueper (3), and dogs may develop some anemia when large amounts are given. Brunschwig, Scott, Corbin, and Moe (1) indicate that dogs may deteriorate when gelatin is given by vein. Gordon, Hoge, and Lawson (2) state that gelatin is non-toxic in dogs in their experiments. Parkins, Koop, Riegel, Vars, and Lockwood (4) gave weekly injections (1.8 gm. per kilo) to normal dogs without any toxic reactions specific to gelatin.

Methods

The majority of the dogs used for these experiments were taken from the anemia colony which represents a white bull terrier and coach strain maintained in the laboratory for many years. Others were mongrels which had been in the laboratory for several months. They

* We are indebted to Eli Lilly and Company for aid in conducting this work.

‡ We are indebted to Merck & Co., Inc., for a generous supply of pure crystalline amino acids.

are accustomed to cage regime and to the technical procedures related to experimental technique. These dogs are conditioned to a very low protein diet prepared in the form of a biscuit. Further details as to care of these animals have been described elsewhere (6).

Blood removal in conjunction with a non-protein diet and abundant iron intake reduce both hemoglobin and plasma proteins to levels which supply the stimulus to form both of these proteins. With abundant iron feeding hemoglobin production is limited only by protein intake. This *double depletion* is a severe strain on the dog and the low protein intake cannot be continued for more than 12 to 14 weeks without obvious disturbance. The experimental history of each dog appears after the related tables.

The *basal protein-free biscuit* has the following ingredients: cornstarch 5000 gm., dextrose 4000 gm., baking powder 300 gm., kaolin 600 gm., bone ash 800 gm., salt mixture 300 gm. (6), lard 2000 gm., crisco 500 gm., cod liver oil 300 cc. The mixture is put into flat pans, cut into squares and baked. The resulting biscuit is similar to a dog biscuit in hardness. The gelatin biscuit represents the above mixture with gelatin added as granular material. Formulas of daily diets are incorporated in the experimental history of each animal.

Vitamin additions consist of dried yeast (Standard Brands, Inc.—Type 200-B), a vitamin B complex powder (5) prepared from liver (Eli Lilly), or a synthetic vitamin preparation.[1] Each 10 cc. dose contains vitamin A 5000 U.S.P. units, vitamin B_1 6.0 mg., riboflavin 6.0 mg., vitamin B_6 hydrochloride 5.0 mg., pantothenic acid (as calcium pantothenate) 5.0 mg., nicotinamide 50.0 mg., inositol 50.0 mg., para-aminobenzoic acid 50.0 mg., ascorbic acid 50.0 mg., 2-methyl-1,4-naphthoquinone 1.0 mg., vitamin D 500 U.S.P. units, rice polish concentrate 1.0 gm., linoleic acid 500.0 mg., choline chloride 300.0 mg., distilled natural tocopherols 50.0 mg. This synthetic vitamin preparation contains practically no protein. At times it was necessary to feed vitamins separately to insure their complete consumption.

Gelatin,[2] Lot P3-20 and P1-20, represent a 6 per cent and 5.75 per cent *bone collagen* solution in 0.9 per cent sodium chloride. The protein content of P3-20 is 6.05 per cent and that of P1-20 is 5.15 per cent. The preparation of both solutions is identical otherwise. P3-180 is a 6 per cent gelatin solution in 0.9 per cent sodium chloride. This gelatin has been degraded by autoclaving so as to give material of a smaller average molecular size than the P3-20 and P1-20 mixtures. The protein content is 5.99 per cent. B121-1 represents dry gelatin in granular form. It is the same type of material as that used for infusion preparations. The protein content is 88.0 gm. per cent. Commercial brand, "pure gelatin, granulated" is one purchased in the open market of unknown origin.

The *gelatin solutions* are warmed to body temperature and after transfer to a sterile drip bottle are injected. In certain experiments, indicated in tables, amino acids added are dissolved in boiling distilled water and then added to gelatin. When completely dissolved, the mixture is filtered and transferred to the injection bottle and the material is given by the drip method. Usually 1 hour or over is allowed for administration. In cases where such fluid intake is too large for a single intravenous injection, the amino acids are completely dissolved in 150 to 200 cc. normal saline solution and injected subcutaneously. *Amino acid mixtures* and their composition are indicated on Table A. Amigen[3] is a casein digest prepared from casein and pork pancreas.

Urinary nitrogen studies are made in many experiments and recorded in the tables. The urine is collected each 24 hours and kept at ice box temperature. Toluene is used as a preservative. Total nitrogen is estimated by macro-Kjeldahl analysis.

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[1] We are indebted to G. B. Walden and A. L. Caldwell of Eli Lilly and Company for this and other valuable materials.

[2] We are indebted for this material to the Knox Gelatin Corporation.

[3] We are indebted to Mead Johnson & Company for this material.

In the following tables for any given dog the periods run consecutively as numbered. Hemoglobin levels are those obtained by sampling 48 hours following the removal of the hemoglobin indicated in the adjacent columns. The plasma protein levels represent the average of samples of all bleedings during the week.

EXPERIMENTAL OBSERVATIONS

These doubly depleted dogs (anemic and hypoproteinemic) have been used in this laboratory for several years and our technique has improved so that an animal is rarely lost during the long continued diet and injection experiments (5). The dogs are very susceptible to infection and must be guarded against such dangers by isolation, aseptic technique, vaccination against distemper and dysentery, uniform temperature control, care in feeding, and attention to any skin abnormalities. These dogs in our eyes are very valuable and when two are lost (Tables 1 and 5), it is not from lack of attention but we believe due to the gelatin given during a 2-week period. Other dogs receiving gelatin were obviously disturbed and showed prolonged periods of convalescence (Tables 2 and 3).

Table 1 indicates that some of the injected gelatin may have been used—total gelatin injected equals 12.4 gm. per kilo. The nitrogen intake (gelatin) and output for total nitrogen (urine) are about in balance and there is a rise in the hemoglobin level from 8.6 to 12.2 gm. per cent, probably an increase of some 20 gm. The plasma protein levels in periods 4 and 5 of 4.8 gm. per cent are in fact 4.0 gm. per cent plasma proteins and 0.8 gm. per cent gelatin. Such plasmas jell at ice box temperature. The persistence of gelatin in the plasma for a week after cessation of injections is of interest (see Experimental History, Table 1).

Amino acid mixture (Vak—Table A) given subcutaneously is utilized only fairly well in the 2nd week and the output of blood proteins is below the expected amount. There is a definite positive nitrogen balance (Table 1, periods 7 and 8).

The salmon diet is not well used to make blood proteins—perhaps about one-half the expected output. The dog is failing and shortly dies of a respiratory infection. This unfavorable response and lessened production of blood proteins we attribute to the gelatin which was given by vein. The very low resistance to infection we believe was due in part to the gelatin.

Experimental History—Table 1.

Dog 43-24. Female young beagle. Plasma protein level 6.34 gm. per cent. A/G ratio 1.7. Sept. 29, 1943—Blood volume 864 cc. Weight 9.1 kilos. Plasma protein and hemoglobin depletion begun. Daily diet of basal protein-free biscuit 200 gm., yeast 5 gm., kennel diet 200 gm. Food consumption 90 per cent and 80 per cent. Oct. 28—Daily diet of basal protein-free biscuit 350 gm., yeast 3 gm., synthetic vitamin B complex 2 gm., reduced iron 600 mg.

Oct. 29 to Nov. 12—Gelatin (P3-20—intravenous (periods 4, 5). Total gelatin 12.4 gm.

per kilo. Daily diet as that of Oct. 28 but synthetic liquid vitamin, 8 cc., replaces yeast and vitamin B complex. Food consumption, 2 weeks, 72 per cent and 86 per cent. Average blood volume 624 cc., average plasma volume 447 cc. Average A/G ratio 0.96. No vomiting. Nov. 3—Urine jells at ice box temperature. Nov. 10—Blood volume 607 cc., plasma volume 408 cc. A/G ratio 0.94. Nov. 11—Daily diet of basal protein-free biscuit 275 gm., yeast 3 gm., vitamin B complex 2 gm., reduced iron 600 mg. Nov. 18—Plasma jells at ice box temperature 1 week after last dose of gelatin.

Nov. 19 (periods 7, 8)—Amino acid mixture Vak (subcutaneous). Table A. Daily diet of basal protein-free biscuit 275 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg.

TABLE 1

Gelatin by Vein—Amino Acids Subcutaneous—Death

Period 1 wk.	Weight	Protein intake		Protein output				Production ratio plasma protein to hemoglobin	Ratio protein output to intake	Total nitrogen	
				Hemoglobin		Plasma protein					
		Type	Weekly	Level	Output per wk.	Level	Output per wk.			Intake	Output urine
Dog 43-24	Gelatin P3-20 by vein followed by standard dose amino acids (Vak—Table A)										
	kg.	*gm.*	*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*
1	9.3	Basal + kennel	—	8.7	21.3	5.3	10.8	51	—	—	—
2	9.0	Basal + kennel	—	9.5	25.1	5.2	13.4	53	—	—	—
3	8.7	Basal	17	8.6	10.9	4.5	5.2	48	—	—	—
4	8.1	Gelatin 54	69	8.6	2.0	4.8	0	—	—	11.4	9.64
5	8.0	Gelatin 54	71	9.3	1.9	4.8	0	—	—	11.7	10.22
6	8.1	Basal	18	12.2	3.8	4.4	—	—	—	2.8	6.55
7	8.2	Amino acids 105	88	8.5	25.6	4.5	6.6	26	37	14.1	8.10
8	7.6	Amino acids 125	106	8.5	9.8	4.4	3.7	38	13	16.9	10.25
9	6.9	Salmon	123	9.8	1.9	4.8	0	—	—	—	—
10	7.6	Salmon	152	11.7	10.4	4.3	4.5	43	10	—	—
11	7.1	Salmon	100	13.9	11.1	4.7	3.1	28	14	—	—

Food consumption 82 per cent. Nov. 24—Plasma and urine do not jell at ice box temperature. Dec. 2—Blood volume 547 cc., plasma volume 395 cc. A/G ratio 0.94. Weight 7.6 kilos.

Dec. 3—Daily diet of canned salmon 100 gm., basal protein-free biscuit 300 gm., yeast 3 gm., vitamin B complex 2 gm. Food consumption 81 per cent. Dec. 8—Weight 6.9 kilos. Dec. 15—Weight 7.0 kilos. Food consumption 100 per cent. Blood volume 592 cc., plasma volume 380 cc. A/G ratio 0.97.

Dec. 17—Dog lies quietly in his cage. Slight salivation. Sulfadiazine treatment for pneumonia begun. Dec. 23—Food consumption 66 per cent. Blood volume 585 cc., plasma volume 353 cc. Daily diet of kennel food 800 gm., liver 100 gm., Klim 30 gm. Period of improvement, Dec. 24 to 27—Food consumption 100 per cent. Weight 7.5 kilos. Dec. 27—Dog left all food and very irritable. Jan. 3 to 10—Slight improvement clinically. Jan. 13—Condition worse. Jan. 14—Dog is moribund, killed with ether.

Autopsy showed bronchitis, bronchopneumonia with abscesses. Other organs normal in gross and histological sections. No evidence of gelatin deposit in any tissues. Dog had been

vaccinated against distemper and this terminal infection we believe was not due to distemper but to a *lowered resistance* due in part to the gelatin by vein and the anemia and hypoproteinemia. Acute respiratory infections must be guarded against in these depleted dogs but any infection once started goes very rapidly.

Table 2, gelatin injections (9 gm. daily for 2 weeks, total 6.4 gm. per kilo) cause obvious *inhibition of blood protein output.* There is no rise in the hemoglobin level and actually a slight fall in plasma protein levels although the figures are 5.2 and 5.0 gm. per cent but 1 gm. per cent is gelatin, making the true levels below the starting level of 4.4 gm. per cent. Gelatin does in fact replace plasma protein and causes some of the plasma protein to move out of

TABLE A

Amino Acid Mixtures Used in Experiments

Amino acids	Vaa	Vak	Vam	Gg	Vj
dl-Threonine	1.4	2.3	1.4	1.4	1.4
dl-Valine	3.0	3.0	3.0	3.0	3.0
dl-Leucine	4.4	3.0	4.4	—	—
l(−)-Leucine	—	—	—	—	3.0
dl-Isoleucine	2.0	2.0	2.4	2.8	2.8
l(+)-Lysine HCl	2.2	3.0	2.2	—	3.0
dl-Tryptophane	0.6	0.4	0.8	0.8	0.8
dl-Phenylalanine	2.0	2.0	2.0	1.6	2.0
dl-Methionine	1.2	1.2	1.2	1.0	1.2
l(+)-Histidine HCl	1.0	1.0	1.0	0.8	1.0
l(+)-Arginine HCl	1.0	1.0	1.0	—	1.0
Glycine	2.0	2.0	2.0	—	2.0
Total	20.8	20.9	21.4	11.4	21.2

the circulation, into what body areas or tissues we do not know. During this time there is a strong positive nitrogen balance and considerable loss of weight.

The inhibition of blood protein output disappears when gelatin injections are discontinued (period 6, Table 2). Continued amino acid injection (periods 7 and 8) shows a good production of hemoglobin and plasma protein and a favorable nitrogen balance. The veins deteriorated during this experiment with gelatin injection and the loss of weight is conspicuous.

Experimental History—Table 2.

Dog 40-34. Male white bull. Born August, 1940. Continuous anemia experiments Apr. 8, 1942, to Feb. 8, 1943. Beginning weight 14.4 kilos. Hemoglobin level 20.3 gm. per cent.

Oct. 28—Regular depletion as usual. Weight 17.5 kilos. A/G ratio 1.35. Plasma protein 5.7 per cent. Daily diet of table scraps 100 gm., basal protein-free biscuit 450 gm., yeast 3 gm., vitamin B complex 2 gm., reduced iron 600 mg. Nov. 2—Table scraps in diet omitted. Food consumption 100 per cent. Nov. 3—Plasma protein 5.2 per cent. Nov. 9—Basal biscuit increased to 500 gm. Nov. 18—Food consumption 100 per cent. A/G ratio 0.92.

Nov. 19—Gelatin (P3-20—vein), amino acid mixture Vaa—Table A, regular standard dose—subcutaneous (periods 4, 5). Total gelatin 6.4 gm. per kilo. Daily diet of basal, protein-free biscuit 435 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg. Food consumption 75 per cent. Nov. 24—Blood volume 1079 cc., plasma volume 912 cc. A/G ratio 0.72. Plasma and urine jell at ice box temperature. Dec. 2—Food consumption 85 per cent. Blood volume 1075 cc., plasma volume 814 cc. A/G ratio 0.62. Dec. 3—Daily diet of basal protein-free biscuit 400 gm., yeast 3 gm., vitamin B complex 2 gm., reduced iron 600 mg. Food consumption 96 per cent. Dec. 9—Blood volume 1156 cc., plasma volume 700 cc. A/G ratio 0.72.

Dec. 10—Amino acid mixture Vaa—Table A, regular standard dose—subcutaneous (periods 7, 8). Daily diet of basal protein-free biscuit 385 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg. Food consumption 72 per cent. Dec. 16—Blood volume 1028 cc., plasma volume 770 cc. A/G ratio 0.83. Basal biscuit decreased to 300 gm. Dec. 21—Blood volume 1020 cc., plasma volume 800 cc. A/G ratio 0.98. Beginning skin lesions. Recovery period.

TABLE 2

Gelatin by Vein—Amino Acid Growth Mixture Subcutaneous

Period 1 wk.	Weight	Protein intake: Type	Protein intake: Weekly	Protein output: Hemoglobin: Level	Protein output: Hemoglobin: Output per wk.	Protein output: Plasma protein: Level	Protein output: Plasma protein: Output per wk.	Production ratio plasma protein to hemoglobin	Ratio protein output to intake	Total nitrogen: Intake	Total nitrogen: Output urine
Dog 40-34	Gelatin P3-20 plus amino acids (Vaa Table A) standard dose										
	kg.	*gm.*	*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*
1	17.0	Basal + kennel	—	9.1	50.5	5.2	32.4	64	—	—	—
2	16.5	Basal	19	7.6	40.9	4.7	22.6	55	—	—	—
3	15.5	Basal	18	6.6	8.5	4.4	5.0	59	—	—	—
4	14.8	Gelatin 45 Amino acids 104	132	6.6	1.4	5.2	0	—	—	22.0	11.17
5	14.1	Gelatin 54 Amino acids 125	183	6.8	1.3	5.0	0	—	—	29.7	15.97
6	13.7	Basal	18	7.3	18.7	5.0	7.8	—	—	2.9	7.15
7	13.7	Amino acids 125	104	6.7	18.3	4.8	12.6	69	30	16.6	8.82
8	12.8	Amino acids 125	104	7.3	9.5	4.6	6.8	72	16	16.6	5.12

Table 3 shows the effects of gelatin by vein in two dogs—one non-toxic after 2 weeks' injection and one toxic after 1 week of gelatin. Dog 41-50 is the only one of our series which took this large dose of gelatin (total 6.7 gm. per kilo) without evidence of intoxication and an after-period of lowered blood protein production. The amino acid mixture given by vein with the gelatin is well used (with or without the gelatin) to make new plasma protein and hemoglobin—in fact the ratio protein output to intake is higher than the expected values and may suggest some use is made of the gelatin. The plasma protein

figures, periods 3 to 5, are about 0.8 gm. per cent too high as this figure represents the amount of gelatin in the plasma—true value for plasma protein level, period 3, is therefore 4.3 gm. per cent. The nitrogen intake and urinary output are fairly close to balance in periods 3 and 4. There is a rapid loss of weight.

TABLE 3

Gelatin by Vein—Amino Acids by Vein

Period 1 wk.	Weight	Protein intake: Type	Protein intake: Weekly	Protein output: Hemoglobin: Level	Protein output: Hemoglobin: Output per wk.	Protein output: Plasma protein: Level	Protein output: Plasma protein: Output per wk.	Production ratio plasma protein to hemoglobin	Ratio protein output to intake	Total nitrogen: Intake	Total nitrogen: Output urine
	kg.	*gm.*	*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*
Dog 41-50	Gelatin P3-20 plus ½ standard dose amino acids (Vaa Table A)										
1	18.2	Basal	17	6.9	51.4	4.8	33.2	65	—	—	—
2	17.6	Basal	16	7.2	1.8	4.3	0	—	—	—	—
3	17.2	Gelatin 55 Amino acids 62	106	8.4	15.8	5.1	8.9	56	24	18.1	17.20
4	16.2	Gelatin 45 Amino acids 52	89	8.1	27.9	4.9	14.0	50	45	15.1	15.65
5	16.2	Gelatin 18 Amino acids 21	36	7.4	11.6	4.1	6.2	53	47	6.0	12.35
6	15.8	Basal	0	7.4	1.7	4.5	0	—	—	0	6.24
7	15.7	Amino acids 62*	52	9.1	19.3	5.1	10.1	52	57	8.3	—
8	15.2	Amino acids 62*	52	8.3	14.9	5.2	6.9	46	42	8.3	8.82
9	15.0	Amino acids 125*	104	7.3	29.4	5.0	18.5	63	46	16.6	17.65
10	14.7	Amino acids 125*	87	7.7	13.9	5.6	7.9	57	21	—	—
11	13.8	Basal	15	7.0	9.0	4.5	5.2	—	—	—	—
Dog 37-82	Gelatin P1-20 by vein—toxic										
1	15.1	Salmon	175	8.5	11.3	4.5	6.2	55	10	—	—
2	15.0	Salmon	182	8.1	13.7	4.6	7.6	55	12	—	—
3	14.3	Gelatin 49	49	8.1	1.7	4.3	0	—	—	—	—
4	13.8	Basal	5	7.9	1.6	4.0	0	—	—	—	—

* Subcutaneous

Amino acids (periods 7 to 10, Table 3) given after the gelatin are very well utilized in full dose and in one-half this amount. The last period 11 in Table 3 shows the usual carry-over from the preceding amino acid period.

Dog 37-82, Table 3, shows the more common reaction following a week of gelatin by vein (total 3.3 gm. per kilo). During the injection period there is complete inhibition of blood protein production—in fact a *fall* in the plasma protein and hemoglobin concentrations if we include period 4.

Following periods 1 and 2 one would expect a slight carry-over from the salmon feeding but the gelatin inhibits this response. In the after-period, if we allow for the gelatin, there is a very low plasma protein level. This dog was obviously intoxicated and made a very slow convalescence on a liberal diet—refer to Experimental History following Table 7, dog 37-82.

Experimental History—Tables 3 and 8.

Dog 41-50. Male bull coach. Born January, 1940. Continuous anemia experiments Feb. 16, 1943, to June 22, 1943. Beginning weight 15.3 kilos. Average blood volume 1230 cc. June 22—Daily diet of basal protein-free biscuit 450 gm., yeast 5 gm., reduced iron 600 mg. Plasma protein 5.2 per cent. Hemoglobin level 5.5 gm. per cent. Blood volume 1430 cc.

July 7 to 24—Gelatin (P3–20) plus amino acids (both by vein). Table 3 (periods 3, 4, 5). Total gelatin 6.7 gm. per kilo. Daily diet of basal protein-free biscuit 450 gm., synthetic liquid vitamin mixture 8 cc., reduced iron 600 mg. Blood volume 1387 cc., plasma volume 1035 cc. A/G ratio 1.2. Amino acid mixture (Vaa—Table A) injected with gelatin was one-half regular standard dose. Food consumption 79 per cent. July 15—Biscuit decreased to 350 gm. Food consumption for 2nd week was 66 per cent. Two injections only were given at the beginning of the 3rd week. Food consumption 88 per cent. July 24—Daily diet of basal protein-free biscuit 350 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg. Blood volume 1225 cc., plasma volume 876 cc. A/G ratio 0.80. Food consumption 77 per cent. Aug. 4—Blood volume 1221 cc.

Aug. 5 to 19 (periods 7, 8)—Amino acids (one-half regular standard dose—subcutaneous). Daily diet as of July 24. Food consumption 65 to 60 per cent. Aug. 18—Blood volume 1160 cc., plasma volume 833 cc. A/G ratio 1.0.

Aug. 19 to Sept. 2—Amino acids (Vaa—Table A) regular standard dose—subcutaneous (periods 9, 10). Daily diet as of July 24. Aug. 25—Blood volume 1168 cc., plasma volume 883 cc. A/G ratio 0.86. Basal biscuit decreased to 300 gm. Food consumption 59 per cent. Sept. 2—Blood volume 1132 cc., plasma volume 878 cc. A/G ratio 0.87. Food consumption 81 per cent. Daily diet of basal protein-free biscuit 300 gm., yeast 2 gm., vitamin B complex 2 gm., reduced iron 600 mg. Food consumption 78 per cent. Sept. 9—Blood volume 1088 cc., plasma volume 836 cc. A/G ratio 0.77. Sept. 16, 1943, to Mar. 16, 1944—Recovery period.

Table 8. Mar. 16 to Apr. 6, 1944—Daily diet of basal protein—free biscuit 450 gm., yeast 3 gm., vitamin B complex 2 gm., reduced iron 600 mg. Blood volume 1795 cc., plasma volume 881 cc. Plasma protein 6.22 per cent. Hemoglobin 18.3 gm. A/G ratio 0.95. Hemoglobin and plasma protein depletion begun. Food consumption 100 per cent.

Apr. 6 to 27—Gelatin (PX10-20) plus Amigen, vein and subcutaneous (periods 4, 5, 6). Total gelatin 1.9 gm. per kilo. Table 8. Daily diet of basal protein-free biscuit 400 gm., synthetic liquid vitamin mixture 8 cc., reduced iron 600 mg. Apr. 12—Blood volume 1380 cc., plasma volume 954 cc. Food consumption 86 per cent. A/G ratio 1.2. Apr. 19—Blood volume 1390 cc., plasma volume 972 cc. Food consumption 100 per cent. Apr. 27—Experiment completed. Food consumption 86 per cent. Blood volume 1413 cc., plasma volume 952 cc. Dog in good condition. Recovery period.

Table 4 shows an interesting experiment in which the production of plasma protein and hemoglobin is *sharply inhibited by gelatin* by vein over a 3-week

period (total 16.8 gm. per kilo). One would expect a considerable output of hemoglobin and plasma protein in the week following the lean beef diet—a carry-over response but this expected reaction is inhibited. There is obviously some intoxication but the dog recovers on a liberal diet and is tested again in Table 8.

Lean beef feeding in liberal amounts causes a large output of plasma protein and hemoglobin with a ratio of protein output to intake of about 20 per cent as is usual for this type of diet protein in this amount. There is 1 kilo gain in weight.

Gelatin plus the amino acids lacking or very poorly represented in gelatin, we thought might yield a favorable output of blood proteins. Perhaps such a combination would produce blood proteins if gelatin by vein was not inhibitory. Refer to oral experiments Table 5, periods 5 and 6. The plasma protein level actually decreased if we allow for the gelatin in the plasma (0.8 to 1.0 gm. per cent) and the hemoglobin level decreases without any significant change in blood volume—see Experimental History. Note also that a smaller dose of gelatin given in a subsequent experiment (Table 8) was non-toxic. During the after-period 7, Table 4, there is a slight rise in hemoglobin levels. There is a great loss of weight during the gelatin periods.

All this means that the gelatin by vein disturbs the protein production even when it is supplemented by its lacking amino acids—the gelatin would seem to upset the blood protein-producing mechanism.

Experimental History—Tables 4 and 8.

Dog 40-32. Male bull. Born August, 1940. Dec. 1, 1941, to May 26, 1942—Regular anemia experiments. Beginning weight 16.5 kilos. Blood volume 1400 cc. Apr. 22, 1942—Periods of blood depletion and standardization.

Mar. 10, 1943—Blood protein depletion begun. Regular experiments to June 24 (Table 4). Blood volume 1214 cc. Hemoglobin level 9.6 gm. per cent. Average plasma protein level 4 per cent. A/G ratio 0.34. June 24 to July 1—Daily diet of lean beef muscle 200 gm., basal protein-free biscuit 250 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg. July 1 to 15—Beef muscle increased to 250 gm.

July 15 to Aug. 6 (Table 4)—*Gelatin* (P3-20—vein), amino acids lacking in gelatin (Gg—Table A)—subcutaneous (periods 4, 5, 6). Total gelatin 16.8 gm. per kilo. Daily diet of basal protein-free biscuit 350 gm., yeast 3 gm., vitamin B complex 2 gm., reduced iron 600 mg. Food consumption 91 per cent, 83 per cent, and 62 per cent each week. Average blood volume 1193 cc., average plasma volume 938 cc. Average A/G ratio 0.90. July 25 and Aug. 5—Plasma jells at ice box temperature. Aug. 6—Daily diet of basal protein-free biscuit 300 gm., yeast 3 gm., vitamin B complex 2 gm., reduced iron 600 mg. Food consumption 81 per cent. Aug. 11—Blood volume 1054 cc., plasma volume 809 cc. Plasma protein level 4.7 per cent. A/G ratio 0.60. Plasma remains liquid at ice box temperature. Aug. 12 to Dec. 2—Recovery period.

Dec. 2—Daily diet of basal protein-free biscuit 500 gm., yeast 3 gm., vitamin B complex 2 gm., reduced iron 600 mg. Blood protein depletion begun. Blood volume 1740 cc., plasma volume 954 cc. Plasma protein level 6.7 per cent. Hemoglobin level 15.9 gm. per cent. Weight 20.5 kilos (Table 8).

Table 8. Dec. 27—*Gelatin* (P3-20) (periods 5, 6) and "Amigen" (vein). Total gelatin 1.2 gm. per kilo. Daily diet as of Dec. 2. Dog vomited after one-half of first dose was given. Balance injected one-half hour later. Repeated vomiting. Dog very restless. Last 50 cc. of mixture was not injected because of vomiting. Dec. 28—Gelatin and "Amigen" by vein allowing 1 hour for injection period. No vomiting. Dec. 29—No gelatin by vein but "Amigen" injection continued. Jan. 5 and 6, 1944—Slight vomiting. Injection time 1¼ hours. Jan. 6—Veins in poor condition. Jan. 8—"Amigen" injection discontinued. Blood volume 1326 cc., plasma volume 910 cc. A/G ratio 1.1. Food consumption 100 per cent.

Jan. 10—*Repetition of gelatin* and "Amigen" experiment (P3-20—both by vein). Table 8 (periods 7, 8, 9). Total gelatin 1.3 gm. per kilo. Gelatin P3-20 and "Amigen" given for 2 days and "Amigen" injection alone continued to Jan. 22. Jan. 13 to 22—Basal protein-free biscuit decreased to 400 gm. Food consumption 92 per cent and 97 per cent. Jan. 19—

TABLE 4

Gelatin by Vein Plus Amino Acids (Gg.) Subcutaneous

Period 1 wk.	Weight	Protein intake		Protein output				Production ratio plasma protein to hemoglobin	Ratio protein output to intake
		Type	Weekly	Hemoglobin		Plasma protein			
				Level	Output per wk.	Level	Output per wk.		
Dog 40-32	Gelatin P3-20 plus amino acids lacking in gelatin (Gg.—Table A)								
	kg.	*gm.*	*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*
1	15.2	Lean beef	296	9.5	40.3	5.4	19.5	48	20
2	15.5	Lean beef	351	8.3	39.0	5.2	22.4	57	17
3	16.2	Lean beef	382	6.5	36.7	5.6	25.9	70	17
4	15.4	Gelatin 90.6 Amino acids 70	133	6.5	1.3	5.1	0	—	—
5	15.0	Gelatin 90.6 Amino acids 70	132	5.0	5.7	5.4	4.9	—	—
6	14.3	Gelatin 90.6 Amino acids 70	129	5.0	1.1	5.2	0	—	—
7	13.6	Basal	15	7.3	1.6	4.7	0	—	—

Blood volume 1290 cc., plasma volume 946 cc. A/G ratio 1.7. Jan. 22 to Feb. 3—Basal period with daily diet as of Jan. 13. General condition of dog other than loss of weight is good.

Table 5 shows a rather poor output of blood proteins due to *gelatin feeding* as compared with several experiments below (Tables 6 and 7). Two weeks of gelatin injection causes chronic intoxication and death follows 4 weeks later. There is a conspicuous weight loss in spite of a strong positive nitrogen balance.

Period 2, Table 5, indicates that some protein reserves remained—an output of 45 gm. plasma protein and hemoglobin. Period 3 with gelatin feeding shows an output of 41 gm. total blood protein and some of this may be derived from protein reserve stores. Period 4 shows a negative response in spite of a strong positive nitrogen balance, supplementing amino acids perhaps not being available.

Gelatin supplemented by the amino acids lacking in gelatin when fed gives no positive response in period 5 but a strong response and considerable blood protein output (total 43 gm.) in period 6. Loss of weight continues.

Gelatin by vein for 2 weeks gives no immediate evidence of intoxication (total 9.2 gm. per kilo). The rise in hemoglobin levels (period 8) may be accounted

TABLE 5

Gelatin by Mouth and by Vein—Death

Period 1 wk.	Weight	Protein intake: Type	Protein intake: Weekly	Protein output: Hemoglobin: Level	Protein output: Hemoglobin: Output per wk.	Protein output: Plasma protein: Level	Protein output: Plasma protein: Output per wk.	Production ratio plasma protein to hemoglobin	Ratio protein output to intake	Total nitrogen: Intake	Total nitrogen: Output urine
Dog 37-87	Gelatin B121-1 oral										
	kg.	gm.		gm.	gm. per cent	gm.	gm. per cent	per cent	per cent	gm.	gm.
1	20.0	Basal	19	8.8	46.1	5.1	26.6	58	—	—	—
2	19.0	Basal	19	9.2	31.6	4.6	14.8	47	—	—	—
3	18.8	Gelatin 280	265	8.6	27.6	4.6	13.4	49	15	47.4	—
4	18.0	Gelatin 234	223	8.6	1.8	4.3	0	—	—	40.1	11.0
		Gelatin B121-1 plus amino acids lacking in gelatin—both by mouth									
5	17.0	Gelatin 210 Amino acids 80	245	8.6	1.8	4.5	0	—	—	43.0	12.0
6	16.0	Gelatin, 168 Amino acids 42	196	7.2	29.6	4.8	13.3	45	22	34.0	12.5
		Gelatin P3-180 by vein									
7	15.4	Gelatin 75	75	7.2	1.6	4.4	0	—	—	13.5	14.5
8	14.9	Gelatin 72	72	10.4	2.2	4.5	0	—	—	13.0	8.3
9	14.3	Soy bean	229	8.3	45.1	4.9	20.9	46	29	—	—
10	14.0	Soy bean	293	7.9	38.7	5.0	21.7	56	21	—	—
11	13.6	Soy bean	293	7.9	2.0	5.2	0	—	—	—	—
12	14.0	Kennel, liver	—	9.8	1.9	5.3	0	—	—	—	—

for in part by some shrinkage in blood volume (Experimental History dog 37-87). Subsequent to the gelatin injection the condition deteriorated in spite of a liberal diet and we believe this chronic intoxication was due to the gelatin. There was a significant output of plasma protein and hemoglobin during periods 9 and 10 put failure in period 11. The lack of blood protein production in periods 11 and 12 we assume is related to the gelatin by vein 4 weeks previously.

Experimental History—Table 5.

Dog 37-87. Male bull coach. Born August, 1936. Continuous anemia experiments Mar. 20, 1939, to June 1, 1943.

Sept. 8, 1943—Blood volume 1740 cc., plasma volume 1089 cc. Hemoglobin 13.6 gm. per cent. Plasma protein 6.5 per cent. Weight 21 kilos. Depletion of plasma protein and hemoglobin begun. Daily diet of basal protein-free biscuit 450 gm., yeast 3 gm., vitamin B complex 2 gm., reduced iron 600 mg.

Sept. 23 to Oct. 8—Gelatin (B121-1—oral). Table 5 (periods 3, 4). Daily diet of gelatin 40 gm., basal protein-free biscuit 450 gm., yeast 3 gm., vitamin B complex 2 gm., sugar 20 gm., reduced iron 600 mg. Food consumption 100 per cent and 90 per cent. Sept. 30—Blood volume 1334 cc., plasma volume 976 cc. A/G ratio 0.53.

Oct. 8 to 21 (periods 5, 6)—Gelatin plus amino acids lacking in gelatin (Gg—Table A). Daily diet of gelatin 30 gm., amino acid mixture 11.4 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg. Food consumption 100 per cent for 1st week, Table 5.

Oct. 21 to Nov. 4—Gelatin (P3-180)—intravenous. Table 5 (periods 7, 8). Total gelatin 9.2 gm. per kilo. Daily diet of basal protein-free biscuit 350 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg. Food consumption 91 per cent, 86 per cent. Oct. 27—Urine and plasma do not jell at ice box temperature. Oct. 28—Beginning skin lesions mainly over joints and hip. Average blood volume 1141 cc., average plasma volume 831 cc. Average A/G ratio 0.50.

Nov. 4 (periods 9, 10, 11)—Daily diet of soy bean meal 75 gm., basal protein-free biscuit 200 gm., yeast 3 gm., vitamin B complex 2 gm., reduced iron 600 mg. Skin lesions of moderate severity. Food consumption 100 per cent for 3 weeks. Nov. 10—Skin lesions are spreading. Nov. 24—Dog very quiet, skin lesions are considerably worse. Weight 13.6 kilos. Blood volume 995 cc., plasma volume 704 cc. Plasma protein level 5.2 per cent. Hemoglobin level 7.9 gm. per cent. A/G ratio 0.50. Nov. 25—(period 12)—Daily diet of table scraps, liver, Klim, and yeast. Dec. 1—Skin lesions definitely ulcerated and some are bleeding. Dec. 8—Dog found dead in cage.

Autopsy.—The skin ulcers on chest and forelegs measure 2 to 5 cm. There are a few early mouth ulcers. Organs in general are normal in gross. Histological sections show much inflammation below the ulcers which obviously were the source of the general infection and related organ injury. Lungs show a little bronchopneumonia and edema. Heart shows focal myocarditis. Liver shows a few small focal necroses, also some small fat droplets in epithelium but no evidence of gelatin deposits. Kidneys show no inflammation but edema and epithelial injury of convoluted tubules—few casts in collecting tubules (? gelatin).

Table 6 shows that *gelatin by mouth* may be responsible for a considerable production of blood proteins, following a long basal period to exhaust protein reserve stores, presumably by the supplementing action of protein nitrogen obtained from body protein or body protein catabolism.

Gelatin by vein supplemented by the growth mixture of essential amino acids also gives a positive output of blood proteins, perhaps a little more than would be expected from the amino acids alone.

Following the *gelatin injection* periods (total 10.2 gm. per kilo) we note a *greatly reduced* plasma protein and hemoglobin production compared with the expected response from the amino acids (period 9) or from the large intake of salmon bread and salmon (period 10). There is rapid weight loss during all gelatin periods in spite of a strong positive nitrogen balance. The salmon bread period shows a rapid gain in weight and the recovery was prompt.

This experiment therefore shows no signs of chronic intoxication but a definite inhibition of plasma protein and hemoglobin production during favorable diet intake, periods 9 and 10. We believe the gelatin by vein is responsible.

Experimental History—Table 6.

Dog 37-85. Male bull coach. Born August, 1936. Continuous anemia experiments Nov. 13, 1940, to Aug. 4, 1943. Beginning weight 17.4 kilos. Average blood volume 1400 cc. Aug. 4—Daily diet of basal protein-free biscuit 400 gm., salmon bread 50 gm., yeast 5 gm., reduced iron 600 mg. Food consumption 100 per cent. Aug. 11—Blood volume 1410 cc., plasma volume 1000 cc. Plasma protein 6.1 per cent. A/G ratio 0.81. Aug. 19—Salmon bread omitted from diet and basal biscuit increased to 450 gm. Sept. 1—Blood volume 1235 cc., plasma volume 965 cc. A/G ratio 0.50.

Sept. 2—Gelatin (B121-1—oral). Table 6 (periods 5, 6). Daily diet of gelatin 30 gm., dextrose 20 gm., basal protein-free biscuit 450 gm., yeast 3 gm., vitamin B complex 2 gm., reduced iron 600 mg. Food consumption 84 per cent. Sept. 9—Blood volume 1239 cc., plasma volume 954 cc. A/G ratio 0.62. Sept. 10—Gelatin made into basal biscuit. Food consumption 76 per cent. Blood volume 1059 cc., plasma volume 792 cc.

TABLE 6

Gelatin by Mouth and by Vein Plus Amino Acids

Period 1 wk.	Weight	Protein intake: Type	Protein intake: Weekly	Protein output: Hemoglobin: Level	Protein output: Hemoglobin: Output per wk.	Protein output: Plasma protein: Level	Protein output: Plasma protein: Output per wk.	Production ratio plasma protein to hemoglobin	Ratio protein output to intake	Total nitrogen: Intake	Total nitrogen: Output urine
Dog 37-85	Gelatin B121-1—oral										
	kg.	*gm.*	*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*
1	19.0	Basal + salmon B.	56	9.8	23.4	6.0	15.3	65	—	—	—
2	18.5	Basal	17	10.2	40.9	5.6	23.3	57	—	—	—
3	18.2	Basal	17	6.9	54.9	5.2	30.9	56	—	—	—
4	17.6	Basal	17	5.8	11.2	4.8	7.8	70	—	—	—
5	15.8	Gelatin 176	163	5.8	15.2	6.1	12.0	79	17	30.1	19.6
6	14.1	Gelatin 160	147	6.1	21.4	6.3	6.8	32	19	27.3	13.5
		Gelatin P3-180 plus ½ dose amino acids (Vam—Table A) both by vein									
7	14.0	Gelatin 72 Amino acids 64	124	6.1	1.4	5.0	0	—	—	21.3	15.4
8	13.2	Gelatin 72 Amino acids 64	124	8.1	22.8	4.9	13.4	59	29	21.3	14.1
		Amino acid mixture (Vam—Table A) full dose, subcutaneous									
9	12.6	Amino acids 128	104	8.1	0.7	5.3	0	—	—	16.6	7.0
10	14.4	Bread + salmon	639	8.3	4.3	5.[illegible]	1.4	—	—	—	—

Sept. 16—Gelatin (P3-180), amino acid mixture Vam incorporated into one mixture given by vein (periods 7, 8). Total gelatin 10.2 gm. per kilo. Amino acids amount to 10.4 gm. daily and equal one-half regular standard dose. Daily diet of basal protein-free biscuit 350 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg. Sept. 23—Food consumption 80 per cent. Blood volume 1090 cc., plasma volume 876 cc. A/G ratio 0.87. Basal biscuit reduced to 300 gm. Sept. 29—Food consumption 72 per cent. Blood volume 990 cc., plasma volume 744 cc. A/G ratio 0.70.

Sept. 30—(period 9)—Amino acids (Vam—Table A)—Full standard dose. Table 6—subcutaneous. Amino acid mixture is twice the amount injected as of Sept. 16. Daily diet

TABLE 7

Production of Hemoglobin and Plasma Protein due to Gelatin Feeding

Period 1 wk.	Weight	Protein intake		Protein output				Production ratio plasma protein to hemoglobin	Ratio protein output to intake
		Type	Weekly	Hemoglobin		Plasma protein			
				Level	Output per wk.	Level	Output per wk.		
Dog 37-82 Gelatin B121-1—oral									
	kg.	*gm.*	*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*
1	17.3	Basal	19	11.5	48.2	4.9	21.9	45	—
2	16.8	Basal	19	8.2	44.1	4.7	19.8	45	—
3	15.9	Basal	17	8.9	13.7	4.5	6.5	47	—
4	15.1	Hb. digest Basal	65	9.7	14.5	4.1	5.8	40	31
5	14.4	Gelatin 180	159	8.3	30.9	4.9	12.4	40	27
6	13.6	Gelatin 219	193	10.1	23.7	4.7	17.8	75	22
		Commercial gelatin—oral							
1	14.6	Salmon	330	10.0	28.9	4.8	15.9	55	14
2	15.2	Salmon	351	9.0	37.2	4.9	17.6	47	16
3	15.5	Salmon	351	8.9	43.1	5.3	23.9	55	19
4	15.8	Salmon	351	9.0	25.1	4.7	12.1	48	11
5	15.4	Gelatin 204	186	8.2	27.2	4.7	13.8	51	22
6	14.8	Gelatin 142	119	7.2	25.7	4.5	14.8	58	34

of dextrose 150 gm., cornstarch 30 gm., bone ash 10 gm., salt mixture 5 gm., cod liver oil 10 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg. Plasma protein 5.3 per cent. Oct. 6—Daily diet of salmon bread 300 gm., canned salmon 300 gm., Klim 30 gm., reduced iron 600 mg. Oct. 9—Blood volume 1019 cc., plasma volume 780 cc. A/G ratio 0.50. Oct. 13—Weight 14.0 kilos. Hemoglobin 8.3 gm. per cent. Plasma protein 5.4 per cent. A/G ratio 0.64. Recovery period without incident.

Table 7 shows two experiments of *gelatin feeding* on the same dog, other experiments (Table 3) intervening between these two experiments. Gelatin consumption is good in both experiments and there is a definite production of new hemoglobin and plasma protein. In fact, the response is excellent and shows 20 to 30 per cent return for the protein ingested, but there is a con-

spicuous loss of weight which may be related to the necessary amino acid supplements to the gelatin. To produce blood proteins due to gelatin feeding the body must supply the necessary supplementary amino acids, presumably from protein stores and protein catabolism. This process cannot go on indefinitely as shown in other experiments (Tables 5 and 6).

This same dog 37-82 following 1 week gelatin by vein (total 3.3 gm. per kilo) (Table 3) shows complete inhibition of blood protein production and intoxication lasting some weeks—see Experimental History, Table 7.

Experimental History—Tables 3 and 7.

Dog 37-82. Male white bull coach. Born August, 1936. Continuous anemia experiments, May 25, 1937, to Sept. 24, 1941. Mar. 17—Blood protein depletion begun. Mar. 27 to Apr. 7, 1943—Regular hemoglobin and plasma protein production experiments with intermittent rest periods. Apr. 7—Blood volume 1073 cc., plasma volume 742 cc. Hemoglobin level 11.7 gm. per cent. Plasma protein 3.51 per cent. Daily diet of canned salmon 250 gm., basal protein-free biscuit 250 gm., yeast 3 gm., vitamin B complex 2 gm., reduced iron 600 mg. continued to May 5. May 5—Blood volume 1027 cc. A/G ratio 0.53.

May 6—Gelatin (commercial brand)—oral. Table 7—2nd half (periods 5, 6). Daily diet of gelatin biscuit (gelatin 30 gm. daily) 160 gm., basal protein-free biscuit 183 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg. Food consumption 97 per cent and 68 per cent each week. May 19—Blood volume 1120 cc., plasma volume 846 cc. A/G ratio 0.80.

May 20—Daily diet of canned salmon 125 gm., basal protein-free biscuit 300 gm., yeast 3 gm., reduced iron 600 mg. Food consumption 94 per cent and 98 per cent. June 2—Blood volume 1100 cc., plasma volume 820 cc. Plasma protein 4.9 per cent. A/G ratio 0.50. Weight 15.0 kilos.

June 2—Gelatin (Pl-20—one dose intraperitoneal, remainder by vein). Table 3 (period 3). Total gelatin 3.3 gm. per kilo. Daily diet of basal protein-free biscuit 400 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg. Moderate reaction followed single intraperitoneal injection of 100 cc. Dog refused food. June 4—Gelatin injection continued by vein. No clinical reaction. June 8—Urine remained liquid at ice box temperature. June 9—Blood volume 1121 cc., plasma volume 847 cc. A/G ratio 0.95. June 10 and 12—Urine jelled at ice box temperature. June 16—Blood volume 1058 cc., plasma volume 800 cc. A/G ratio 0.50. Veins deteriorated. Experiment discontinued (Table 3).

June 17—Kennel diet of table scraps, yeast, Klim, and canned salmon. Dog in very poor physical condition. *Recovery very slow.* Weight gain slow in spite of excellent food intake. Aug. 11—Complete recovery. Sept. 1—Blood volume 1737 cc., plasma volume 908 cc. Hemoglobin level 14.9 gm. per cent. Weight 18.6 kilos. Plasma protein 5.95 per cent.

Daily diet of basal protein-free biscuit 450 gm., yeast 3 gm., vitamin B complex 2 gm., reduced iron 600 mg. Blood protein depletion begun. Sept. 23—Hemoglobin digest 1166 (oral) for 1 week.

Sept. 30—Gelatin (B121-1—oral) (periods 5, 6). Table 7—1st half. Daily diet of gelatin 35 gm., dextrose 150 gm., cornstarch 30 gm., bone ash 10 gm., salt mixture 5 gm., lard 30 gm., Mazola oil 15 cc., cod liver oil 10 cc., synthetic vitamin mixture 8 cc., reduced iron 600 mg. Oct. 5—Gelatin diet was baked in form of a biscuit because of poor food consumption. Food consumption 78 per cent and 92 per cent. Oct. 13—Blood volume 972 cc., plasma volume 716 cc. A/G ratio 0.47. Condition excellent.

Table 8, Dog 40-32, shows two satisfactory experiments in the same dog with no rest interval. Relatively small doses of gelatin by vein during 2 days

(total gelatin 1.2 to 1.3 gm. per kilo) cause no clinical disturbance, no significant loss of weight, and no inhibition of plasma protein and hemoglobin production. In fact, the *output of blood proteins is excellent*, amounting to 30 to 40 per cent of the protein materials given (Amigen). There is a positive

TABLE 8

Gelatin plus Casein Digest by Vein

Period 1 wk.	Weight	Protein intake: Type	Protein intake: Weekly	Protein output: Hemoglobin: Level	Protein output: Hemoglobin: Output per wk.	Protein output: Plasma protein: Level	Protein output: Plasma protein: Output per wk.	Production ratio plasma protein to hemoglobin	Ratio protein output to intake	Total nitrogen: Intake	Total nitrogen: Output urine
Dog 40-32 Gelatin P3-20, 2 days—Amigen, 2 wks.											
	kg.	gm.	gm.	gm. per cent	gm.	gm. per cent	gm.	per cent	per cent	gm.	gm.
1	18.7	Basal	19	12.2	75.1	4.9	26.4	35	—	—	—
2	18.6	Basal	19	10.6	50.3	4.8	20.1	40	—	—	—
3	17.9	Basal	19	8.8	26.3	4.6	11.8	45	—	—	—
4	17.9	Basal	19	9.2	1.3	4.6	0	—	—	—	—
5	18.0	Gelatin 20.6 Amigen 95	103	10.4	29.0	4.8	12.2	42	40	16.8	9.4
6	17.8	Amigen 150	113	9.2	18.8	5.0	8.2	44	24	18.1	11.2
7	18.1	Gelatin 24.2 Amigen 100	117	8.7	27.0	5.9	13.9	51	35	19.2	12.4
8	17.5	Amigen 125	112	7.1	28.0	5.5	15.7	56	39	18.0	11.2
9	17.4	Amigen (3 days) 75	75	6.7	19.7	4.8	12.0	61	42	12.0	9.2
10	17.2	Basal	19	6.8	11.0	4.6	6.4	—	—	3.0	7.0
Dog 41-50 Gelatin PX10-20, 3 days—Amigen, 3 wks.											
1	19.7	Basal	19	10.1	43.6	5.0	19.7	45	—	—	—
2	19.2	Basal	19	9.2	40.8	4.9	19.3	47	—	—	—
3	19.1	Basal	19	10.5	14.5	4.4	6.6	45	—	—	—
4	18.6	Gelatin 36 Amigen 135	138	9.9	19.6	4.9	7.9	41	20	22.7	15.1
5	19.0	Amigen 150	113	10.8	22.6	4.8	8.7	38	28	18.0	11.2
6	18.8	Amigen 159	113	8.9	29.3	4.6	12.7	43	37	18.0	11.0

nitrogen balance. The casein digest (Amigen) by vein in the dog does cause occasional vomiting, restlessness, and discomfort, and it must be given more slowly than a similar solution of crystalline amino acids.

The same dog (40-32) in Table 4 given a larger dose of gelatin shows clearly a complete inhibition of blood protein production and loss of weight. Experimental History, dog 40-32, follows Table 4.

The second dog, 41-50, in Table 8 with a slightly larger dose of gelatin (1.9 gm. per kilo) shows no evidence of intoxication and the normal control production of blood proteins due to the Amigen by vein and subcutaneous. Experimental History, dog 41-50, follows Table 3.

Table 9 shows two experiments in which 2.7 and 2.4 gm. per kilo gave totally different results. The first dog 41-53 gave evidence of inhibition of plasma

TABLE 9

Gelatin plus Amino Acids by Vein

Period 1 wk.	Weight	Protein intake: Type	Protein intake: Weekly	Protein output: Hemoglobin: Level	Protein output: Hemoglobin: Output per wk.	Protein output: Plasma protein: Level	Protein output: Plasma protein: Output per wk.	Production ratio plasma protein to hemoglobin	Ratio protein output to intake	Total nitrogen: Intake	Total nitrogen: Output urine
Dog 41-53 Gelatin (PX10-180) 3 days, plus amino acids (Vj—Table A)											
	kg.	*gm.*	*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*
1	14.4	Des. liver 225	140	8.3	20.6	5.2	14.0	68	25	—	—
2	13.7	Des. liver 287	175	6.7	18.1	5.3	10.8	60	17	—	—
3	13.2	Des. liver 86	53	6.7	8.4	4.9	6.1	73	27	—	—
4	12.4	Basal	21	6.7	0.8	—	0	—	—	—	—
5	13.1	Gelatin 33.4 Amino acids 127	142	6.1	1.9	4.6	0	—	—	23.5	9.4
6	12.9	Obvious intoxication									
Dog 43-250 Gelatin (P3-20) 3 days, plus amino acids (Vj—Table A)											
1	17.3	Basal	16	10.1	161.4	4.9	—	—	—	—	—
2	16.3	Basal	15	8.0	32.0	4.7	16.6	52	—	—	—
3	15.9	Basal + squash	32	7.2	35.4	4.8	18.0	51	—	—	—
4	15.1	Basal	14	6.7	2.2	4.2	0	—	—	—	—
5	15.1	Gelatin 36.4 Amino acids 127	146	7.5	24.7	5.1	15.8	64	28	27.0	18.3
6	14.5	Amino acids 127	109	7.7	11.9	5.2	8.4	70	19	17.5	14.1
7	14.2	Amino acids 127	109	7.7	25.7	5.2	17.4	68	40	17.5	11.8

protein production and obvious intoxication. The second dog, 43-250 showed no intoxication and an ample production of new plasma protein and hemoglobin due to the amino acids given parenterally during a 2-week period.

Experimental History—Table 9.

Dog 41-53. Male bull coach. Born January, 1940. Continuous anemia experiments Feb. 22, 1943, to June 1, 1943. Beginning weight 14.9 kilos. Average blood volume 1060 cc. Dec. 29, 1943, to Feb. 19, 1944—Daily diet of basal protein-free biscuit and depletion of hemoglobin and plasma proteins.

Feb. 19—Table 9—Daily diet of desiccated hog liver 35 gm., basal biscuit 350 gm., yeast 3 gm., vitamin B complex 2 gm., reduced iron 600 mg. Blood volume 1106 cc. Feb. 24—Desiccated hog liver increased to 50 gm. and biscuit to 400 gm. Blood volume 1057 cc., plasma volume 746 cc. A/G ratio 1.1. Food consumption 100 per cent. Mar. 1—Blood volume 1016 cc., plasma volume 709 cc. A/G ratio 1.7. Food consumption 82 per cent. Basal biscuit decreased to 300 gm. Food consumption dropped to 51 per cent. Mar. 7—Desiccated hog liver feeding terminated. Mar. 8—Blood volume 1011 cc., plasma volume 765 cc. A/G ratio 1.4. Mar. 7 to 13—Daily diet of protein-free biscuit 350 gm., yeast 3 gm., vitamin B complex 2 gm., reduced iron 600 mg.

Mar. 13 to 19—*Gelatin* (PX-10-180) plus amino acids (Vj—standard dose—Table A), (Period 5). Total gelatin 2.7 gm. per kilo. Daily diet of protein-free biscuit 350 gm., synthetic liquid vitamin mixture 8 cc., reduced iron 600 mg. Gelatin plus amino acid mixture was injected for 3 days and was followed by the amino acid mixture for 3 days, both by vein. Plasma did not jell. Mar. 13—Hemoglobin level 6.7. Mar. 16—Hemoglobin level 5.7.

TABLE 10

Toxicity of Gelatin by Vein

Dog No.	Table No.	Gelatin total intake	Weight	Gelatin intake per kilo	Type of gelatin molecular size	Toxicity
		gm.	*kilo*	*gm.*		
43-24	1	108	8.7	12.4	Large	Death
40-34	2	99	15.5	6.4	Large	+
41-50	3	118	17.6	6.7	Large	0
37-82	3	49	15.0	3.3	Large	++
40-32	8	20.6	17.9	1.2	Large	0
40-32	8	24.2	18.1	1.3	Large	0
40-32	4	272	16.2	16.8	Large	++
37-87	5	147	16.0	9.2	Small	Death
37-85	6	144	14.1	10.2	Small	+
41-53	9	33.4	12.4	2.7	Small	+
43-250	9	36.3	15.1	2.4	Large	0
41-50	8	36.3	19.1	1.9	Large	0

Blood volume 1015 cc., plasma volume 791 cc. Dog showed signs of intoxication and experiment was discontinued. Subsequent recovery.

Table 9. Dog 43-250. Adult hound. No previous experiments. Feb. 22, 1944—Daily diet of protein-free biscuit 450 gm., yeast 5 gm., reduced iron 600 mg. Beginning blood volume 1560 cc. Hemoglobin level 19.2 gm. per cent. Weight 18 kilos. Plasma protein and hemoglobin depletion. Food consumption average 90 per cent for 2 weeks. Mar. 8—Daily diet of protein-free biscuit plus squash 300 gm., regular protein-free biscuit 100 gm., yeast 5 gm., reduced iron 600 mg. Food consumption 94 per cent. Blood volume 1146 cc. Mar. 16—Daily diet of protein-free biscuit 400 gm., yeast 3 gm., vitamin B complex 2 gm., reduced iron 600 mg. Mar. 23—Food consumption 75 per cent. Blood volume 1136 cc., plasma volume 863 cc. A/G ratio 0.91.

Mar. 23 to April 13—*Gelatin* (P3-20) plus amino acid mixture (Vj—standard dose—Table A). (Periods 5, 6, 7). Total gelatin 2.4 gm. per kilo. Daily diet of protein-free biscuit 300 gm., synthetic liquid vitamin mixture 8 cc., reduced iron 600 mg. Gelatin plus amino acid mixture was injected for 3 days and was followed by the amino acid mixture alone for balance of experimental period. Mar. 25—Plasma jelled. Mar. 30—Food consumption 89 per cent. Blood volume 1141 cc., plasma volume 851 cc. A/G ratio 1.15. April 6—Food

consumption 98 per cent. Blood volume 1060 cc., plasma volume 795 cc. A/G ratio 1.1. April 13—Food consumption 93 per cent. Blood volume 1054 cc., plasma volume 798 cc. A/G ratio 1.2. Experiment terminated. Dog thin but otherwise in good condition.

DISCUSSION

Gelatin as a substitute for plasma proteins has some good and bad qualities. It causes no obvious disturbance when injected intravenously in approximately 6 per cent solution, and we have never noted any anaphylactoid response. It can be sterilized by boiling. In some of the above experiments there appears to be *utilization of the gelatin* to form needed plasma proteins and even hemoglobin when gelatin is given by mouth. Obviously this calls for amino acids derived from body tissues to supplement the gelatin. The production of new blood proteins from gelatin is equivocal when gelatin is given by vein.

To offset these good qualities, our experiments give evidence that *gelatin may be toxic* when given by vein in moderate dosage over a 1- to 2- week period. The condition in such dogs deteriorates and death may follow or the return to normal is slow and there is definite impairment of the capacity of the dog to form blood proteins in these experiments.

Gelatin escapes freely through the kidney, and this reaction in a diseased or damaged kidney might be harmful. The veins used for gelatin injection may show fibrosis and thrombosis, but this may be due to the preservative and if so could be eliminated. There is a rapid loss of weight in the long-term (2 weeks) gelatin injection experiments, and this may carry over into subsequent favorable diet periods—possibly a peculiar type of intoxication.

On the good side we may note that gelatin given for 2 to 3 days accompanied and followed by amino acid mixtures or casein digests by vein usually gives no evidence of intoxication and definite proof of utilization of the amino acids to form blood proteins. This experiment deserves clinical study to show whether gelatin and amino acids can be utilized in the first 2 to 3 days following hemorrhage, trauma, wounds, and shock to tide over the acute emergency and *permit the body to make its own blood proteins* from the administered amino acids and the protein reserve stores.

It may be argued that these doubly depleted dogs (anemic and hypoproteinemic) are poor risks and offer too severe a test for the hypothetical blood plasma substitute. In reply it is obvious that the patient suffering from tissue injury and hemorrhage is not a very good clinical risk but can be benefited and frequently rescued by plasma or whole blood. Our objective should be the perfect plasma substitute whether that goal may ever be attained or not.

SUMMARY

Gelatin given by vein to doubly depleted dogs (anemic and hypoproteinemic) gives no immediate toxic response, no anaphylactoid reactions, and may contribute something to the building of new hemoglobin and plasma protein.

Gelatin given by vein during 1 to 2 weeks (total 3 to 17 gm. per kilo) usually causes serious disturbances—*inhibition of blood protein production*, signs of intoxication, much weight loss, and even death.

Gelatin given by vein for 2 to 3 days (total 1 to 3 gm. per kilo) may not cause any recognizable abnormalities, but dogs vary greatly in their response to gelatin by vein. Some dogs may tolerate a total of 7 gm. per kilo without significant disturbance and other dogs may be seriously intoxicated by 2 to 3 gm. per kilo. No one can predict which animal will be least tolerant.

Some experiments with gelatin by vein for 2 to 3 days (total gelatin 1 to 2 gm. per kilo) given with and followed by amino acids or casein digests do show absence of intoxication and ample production of new hemoglobin and plasma protein during the weeks following the injection of gelatin. This may suggest possible usefulness of gelatin with amino acids or casein digests in acute emergencies (shock, hemorrhage).

These doubly depleted dogs are very susceptible to various injurious agents as compared to normal dogs. They may serve as sensitive testing machines for evaluating plasma substitutes.

Where the gelatin by vein inflicts its damage is not clear and there is little if any significant histological evidence but the disturbance of blood protein production implicates the liver.

Gelatin of smaller molecular weight (degraded by autoclaving) is no less toxic than the standard gelatin.

Gelatin by mouth may contribute to but cannot alone support the production of new hemoglobin and plasma protein.

Gelatin by vein has definite limitations in dogs and, by implication, when used in human cases the amount given should be very carefully watched.

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Amer. Jour. Med. Sci. 222(6):686-696, 1951

EFFECTS OF THE INTRAVENOUS ADMINISTRATION OF GELATIN SOLUTION (10%)

A Laboratory Study on 24 Patients with Chronic Disease

By Roy D. Scott, A.M., M.D.
PROFESSOR OF CLINICAL MEDICINE

AND

Joseph Ryan, M.D.*

WITH THE TECHNICAL ASSISTANCE OF
Ethel Koster

(From the Department of Medicine, Western Reserve University Medical School, and Cleveland City Hospital, Cleveland)

Although gelatin solutions were first administered intravenously in 1897, by Dastre and Floresco, in an attempt to increase the coagulability of the blood after hemorrhage[15], this substance was soon abandoned because of the unsatisfactory preparations then available. More thorough and detailed investigations had to await the development of better physico-chemical and bacteriologic methods which made it possible to prepare pure, stable, sterile solutions for intravenous use. During the past decade, and particularly during the war years, numerous investigators published reports on experimental animals and on clinical subjects. Most of these studies were directed toward establishing the value of gelatin solutions as a substitute for plasma, especially in hemorrhagic shock. The consensus is that gelatin solutions, administered intravenously, have a definite, though limited, usefulness in restoring the blood volume in acute shock from hemorrhage or burns, and merit clinical consideration as an immediate emergency treatment in such cases. Studies to determine the value of gelatin as a nutritional agent in cases of malnutrition and hypoproteinemia have yielded far less conclusive findings. There is also still considerable discrepancy in reported findings and opinion as to the retention and excretion of gelatin following intravenous injection.

The present study was undertaken with the hope of assembling some data that might be useful and helpful in clarifying some of these problems. However, the results are not nearly so definitive as would be desirable. Even though the findings do not permit clear-cut conclusions regarding the metabolism of gelatin, it seems worth while to report the data in detail, so they may be available to others who may be interested in a similar research.†

The purpose of this study was to administer a 10% solution of gelatin to patients chronically ill with malnutri-

* Formerly Chief Resident in Medicine, Cleveland City Hospital.

† All the laboratory tests were made by the same technician, who is extremely well trained and reliable, after a comparative check of numerous methods, with selection of those that proved most accurate in her hands.

tion, liver dysfunction, and edema, and to determine its retention in the blood stream and its excretion in the urine over a 48-hour period, with the hope of elucidating the indications for its use and its clinical value in supportive treatment as a substitute for whole blood or plasma. Approximately half the patients studied had hepatic disease, and a particular point of the investigation was to ascertain the effect of gelatin in patients with ascites and edema.

Materials and Methods. Twenty-four patients with various chronic diseases, including hepatic disease, arteriosclerosis, malnutrition, pulmonary affections and renal disease, received an intravenous injection of 500 cc. of a 10% solution of gelatin prepared from beef bone collagen.†

The day before the infusion was carried out, each patient was subjected to the following laboratory tests: hemoglobin determination, hematocrit, total protein in the blood along with the albumin and globulin fractions, nonprotein nitrogen, blood volume, bromsulphalein retention, and the cephalin flocculation test. After the infusion, total protein, nonprotein nitrogen, hemoglobin and hematocrit, as well as determinations of the gelatin in the blood and urine and measurement of urine volume were made at the conclusion of the injection, and 6, 24, and 48 hours later. Determinations of the blood volume, bromsulphalein retention, and cephalin-cholesterol flocculation were made immediately after the injection. Protein in the urine was determined in 3 patients with evidence of renal damage (Cases 8, 23, and 24). Determination of sedimentation rates was omitted, since all investigators have reported an increase in sedimentation of erythrocytes following the parenteral administration of gelatin.

Janota's method[17] was used in the determination of gelatin in the blood and urine. Nitrogen was determined according to the micro-Kjeldahl method described by Hiller, Plazin and Van Slyke[11]. The separation of nonprotein nitrogen from gelatin and protein nitrogen was performed according to the procedure of Little and Wells[31].

Howe's method was used in the determination of globulin[14]. Barker's[1] urea method was employed. Other procedures used were blood volume, Mitshe and Cohen[illegible], bromsulphalein, Gaebler[8], cephalin-cholesterol flocculation, Hanger[10].

Results. The results of these studies are shown in detail in Tables 1 to 6. The findings are, for the most part, negative, and do not furnish the information we were seeking regarding the metabolism of gelatin injected into the blood stream. There was no change that can be interpreted as significant in the total blood protein, nonprotein nitrogen, or in the liver function, as indicated by bromsulphalein retention and the cephalin-cholesterol flocculation tests. There were some changes in the urea clearance before and after the gelatin injections, but the significance of these is difficult to evaluate; in some instances there was an increase and in others a decrease.

The gelatin apparently disappeared rapidly from the blood, with only small amounts (0.1 gm. per 100 cc. or less) remaining at the end of 48 hours. The gelatin disappeared rapidly from the urine, too, with no measurable quantity detected 48 hours after the injection, in the majority of cases. The average total excretion in the urine during the first 24 hours in 23 cases* was 14.4 gm., with the largest amount 20.5 gm. and the lowest 7.6 and 7.9 gm., respectively. The high value was obtained in a case of cirrhosis and pulmonary emphysema (Case 21). One of the patients with the lowest excretion during the first 24 hours had diabetic neuropathy with edema (probably owing to kidney damage) and the other had kidney disease with edema. It is interesting to note that in these 2 patients (Case 8 and Case 23) and one

† The gelatin used in this study was furnished by the Upjohn Company of Kalamazoo, Michigan.

* The tests were not completed in one case.

TABLE 1.—CLINICAL DATA, ALBUMIN, GLOBULIN AND TOTAL PROTEIN OBSERVATIONS IN 24 PATIENTS GIVEN 500 CC. OF A 10% GELATIN SOLUTION INTRAVENOUSLY

Case	Diagnosis	Sex	Age	Weight lb.	Time for Injection	Albumin gm. per 100 cc.	Globulin gm. per 100 cc.	Total Protein (gm. per 100 cc.) Before	End	6 hr.	24 hr.	48 hr.
1.	Malnutrition	F	22	110	3 hr.	3.6	1.9	5.5	3.9	4.9	5.4	5.0
2.	Malnutrition, pulmonary emphysema, arteriosclerosis	M	72		"	3.1	3.8	6.9	5.8	6.2		6.65
3.	Arteriosclerosis	M	68		4 hr., 15 m.	4.3	2.9	7.2	6.25	5.8	7.1	6.8
4.	Carcinoma of larynx; diabetes	M	60	132		4.2	2.4	6.6	5.8	6.1	6.4	6.7
5.	Bronchogenic carcinoma with metastases	M	60	122	3 hr., 30 m.	3.6	3.4	7.0	6.4	6.6	6.7	7.5
6.	Neoplasm	M	59	148	3 hr., 15 m.	3.6	3.2	6.8	6.0	6.3	7.0	7.3
7.	Dorsolateral spinal degeneration	M	45	134	3 hr., 30 m.	4.9	2.7	7.6	5.8	6.0	6.5	6.5
8.	Diabetic neuropathy	F	58	130	3 hr.	2.6	2.6	5.2	4.0	4.6	5.0	4.4
9.	Cellulitis	M	44	140	3 hr., 30 m.	3.8	3.2	7.0	6.4	6.3	7.3	7.1
10.	Pleural effusion	M		125	2 hr., 30 m.	4.2	4.1	8.3	6.4	7.6	7.8	8.6
11.	Lung lesion	M	62	178	5 hr., 30 m.	5.5	2.8	8.3	7.0	6.9	7.4	7.5
12.	Pneumonia	M	39	110	3 hr., 15 m.	3.6	4.0	7.6	6.1	6.4	6.5	6.5
13.	Laennec's cirrhosis	M	40	143	2 hr., 45 m.	3.0	3.6	6.6	5.7	5.9	6.1	7.3
14.	Laennec's cirrhosis	M	44	153	2 hr., 15 m.	4.3	4.0	8.3	7.2	8.0	8.5	8.1
15.	Hepatomegaly; prob. Laennec's cirrhosis	M	63	130	3 hr.	4.0	3.6	7.6	6.5	7.2	7.0	7.4
16.	Laennec's cirrhosis	M	53	167	3 hr., 20 m.	2.0	4.3	6.3	4.9	5.4	5.8	5.9
17.	Cirrhosis	M	46	232	3 hr., 20 m.	3.3	4.9	8.2	8.1	8.3	8.3	9.0
18.	Laennec's cirrhosis	M	55	144	3 hr., 15 m.	3.2	3.0	6.2	5.2	5.4	5.9	5.9
19.	Liver disease; prob. cirrhosis	M	72	124	2 hr., 45 m.	2.4	4.2	6.6	5.0	5.7	5.8	6.4
20.	Alcoholism; cirrhosis	M	49	185	3 hr., 40 m.	4.0	4.0	8.0	7.7	7.9	7.4	7.7
21.	Cirrhosis	M	63	133	3 hr., 15 m.	4.0	4.2	8.2	6.8	7.0	7.5	Patient discharged
22.	Alcoholism; possible liver disease	M	36	115	3 hr., 15 m.	3.6	3.1	6.7	5.5	5.5	6.6	7.4
23.	Kidney disease with edema	M	65	160	3 hr.	2.2	2.3	4.5	3.5	3.7	4.6	5.1
24.	Intercapillary glomerulosclerosis; diabetes mellitus	M		180	3 hr., 30 m.	2.1	2.3	4.4	3.8	4.4	4.3	4.5

other (Case 24) with diabetes and intercapillary glomerulonephrosis with edema, the excretion of gelatin in the urine at the conclusion of the injection was low and increased during the 48-hour period of study, while in the other cases there was a progressive decrease, with the highest amount excreted at the end of 24 hours. In the last 2 cases (with kidney disease) there was also relatively high excretion at the end of 48 hours, 3.4 and 6.2 gm., respectively.

Since the total amount of gelatin excreted in the urine in 48 hours was less than one-third the quantity of 50 gm. which was injected, the evidence indicates that two-thirds, or more, of the gelatin is retained by the body beyond this period. There has been some speculation that gelatin may be retained in the liver, but if so, it does not produce any change in liver function as shown by the tests of bromsulphalein retention and cephalin-cholesterol flocculation. The evidence of this study would suggest that the gelatin is probably stored in other tissues.

There was usually a decrease in the hematocrit at the end of the injection and 6 hours afterward, with a return to the pre-injection level at 24 and 48 hours.

The blood volume determinations showed an increase in both plasma volume and total volume in all but one

TABLE 2.—NONPROTEIN NITROGEN AND UREA CLEARANCE IN 24 PATIENTS GIVEN 500 CC. OF A 10% SOLUTION OF GELATIN INTRAVENOUSLY

	Nonprotein Nitrogen (mg. per 100 cc.)					*Urea Clearance*		
Case	*Before*	*End*	*6 hr.*	*24 hr.*	*48 hr.*	*Before*	*After*	
1	18	19	17	20	20	8	7	
2	28	35	32		30			
3	25	27	31	33	30	35	38	
						27		
4	33	32	37	32	27	37	63	maximal
							40	
5	61	60	52	51	55	Patient uncooperative		
6	34	32	32	33	31	60	25	
						60	28	
							24	second week
							24	
7	26	26	25	33	23			
8	67	71	71	73	79	21 maximal	12	standard
						16 "		
9	30	32	32	32	32			
10	33	27	31	31	31			
11	29	33	32	30	36			
12	23	26	28	23	23	59	31	
							35	
13	41	41	49	48	50	15	37	
						16	36	
14	30	36	38	39	28	20	55	
						31	43	
15	24	26	28	27	29	45		
						46		
16	35	37	36	35	34	56	57	
						59	50	
17	24	27	28	25	25	58	51	
						52	52	
18	26	25	26	29	24	25	38	
						48	40	
19	36	31	31	43	45	9	26	
						18	23	
20	27	23	28	29	25	47	70	
						83 (maximal	61	
21	23	23	25	25		17 "		
						33 "		
22	14	16	19	19	18	41 "	57	maximal
							51	"
23	45	46	50	49	51			
24	67	73	73	78	83			

case, immediately after the injection, as would be expected. Urine volumes were increased after 24 hours in practically all the cases, and in the majority, the diuresis apparently was sufficient to be of clinical significance. The increase in urine volume was most striking in the patients with edema of renal origin (Cases 8, 23, 24). It was less prominent in patients with cirrhosis and ascites. Unfortunately, the patients were not weighed on the days succeeding the injection, so this check is not available in evaluating the significance of the increased urine output.

Most of the patients felt that they were subjectively improved after the injection of the gelatin. This improvement was particularly striking in Case 24. This patient, who had diabetic glomerular intercapillary sclerosis, demonstrated such an encouraging response to the initial injection that 4 additional infusions were given on succeeding days. The urine volume was determined 24 hours after each injection and showed 2850, 1520, 1540, and

TABLE 3. DATA ON HEMOGLOBIN AND HEMATOCRIT IN 24 PATIENTS GIVEN 500 CC. OF A 10% SOLUTION OF GELATIN INTRAVENOUSLY

	Hemoglobin (gm. per 100 cc.)					*Hematocrit (%)*				
Case	*Before*	*End*	*6 hr.*	*24 hr.*	*48 hr.*	*Before*	*End*	*6 hr.*	*24 hr.*	*48 hr.*
1	13.6	11.2	12.4	13.2	12.0	41	32	39	41	39
2	16.4					47				
3	14.3	14.5	14.5	15.4	15.4	44	42	39.5	44	44
4	14.9	13.4	13.6	13.6	14.1	44	37	38.5	40	40
5	12.0	11.2	11.6	12.0	12.4	35.5	32	35	36	36.5
6	12.8	12.0	14.1	14.1	13.2	38	35	39	39	40
7	Not done					Not done				
8	12.8	11.2	12.8	13.2	10.5	35	30	36	37	32.5
9	14.3	14.3	14.1	15.8	15.4	41.5	41.5	41	44	44
10	Not done					Not done				
11	Not done					Not done				
12	15.8	11.6	12.8	12.0	12.4	49	39	41	41	40
13	10.1	9.0	9.4	10.1	10.5	32	29	30	30.5	33
14	9.4	8.4	10.1	11.2	9.4	28	26	31	32	29
15	14.5	12.0	14.1	14.5	14.5	45	38	43	42	44
16	16.8	13.6	14.3	14.9	14.9	51	43	46.5	49	50
17	14.1		13.2	14.5	14.6	41.5		40	42.5	42
18	9.4	9.4	9.4	9.4	9.4	30	29	29.5	30	30.5
19	8.0	6.7	8.4	7.7	9.4	24.5	21	26	20	28.5
20	16.4	15.4	15.8	14.5	16.4	47.5	43	45	43	45
21	14.1	13.2	13.2	14.1		42.5	38	40.5	42	
22	12.8	11.2	10.9	11.2	11.2	37	32.5	34	34	35
23	9.4	6.7	8.0	10.1	10.1	28.5	21	24	29.5	30
24	8.7	7.4	9.0	9.0	9.0	27	23	28	27	27

TABLE 4.—BLOOD GELATIN AND URINE GELATIN IN 24 PATIENTS GIVEN 500 CC. OF A 10% GELATIN SOLUTION INTRAVENOUSLY

	Blood Gelatin (gm. per 100 cc.)				*Urine Gelatin (gm.)*			
Case	*End*	*6 hr.*	*24 hr.*	*48 hr.*	*End*	*6 hr.*	*24 hr.*	*48 hr.*
1	1.0	0.9	0.5	0.23	5.4	5.4	2.0	1.1
2	0.89	0.56		0.16	2.1	14.8		
3	0.69	0.4	0.25	0.11	10.9	6.7	1.8	None
4	0.8	0.53	0.3	0.15	5.5	10.5	0.5	None
5	0.49	0.3	0.13	0.05	4.9	4.9	None	None
6	0.56	0.35	0.08	0.03	6.0	5.5	2.7	None
7	0.83	0.49	0.28	0.13	9.7	3.9	3.4	
8	0.88	0.65	0.39	0.2	1.5	2.0	4.4	2.0
9	0.6	0.38	0.22	0.09	7.2	7.4	0.9	None
10	0.69	0.42	0.2	0.05	7.5	1.7	1.8	0.3
11	0.47	0.32	0.15	0.04	11.3	2.5	3.5	1.9
12	0.9	0.57	0.29	0.1	8.0	7.5	0.5	
13	0.63	0.42	0.16	0.04	5.0	3.5	2.7	None
14	0.51	0.25	0.11	0.02	9.2	5.0	1.0	None
15	0.74	0.43	0.2	0.07	7.8	5.5	4.9	None
16	0.67	0.45	0.25	0.12	8.8	6.9	1.7	1.1
17	0.43	0.2	0.06	None	8.6	5.0	2.9	None
18	0.6	0.38	0.2	0.07	10.0	3.4	0.9	None
19	0.8	0.54	0.22	0.12	3.7	4.6	3.8	
20	0.55	0.29	0.11	None	6.6	4.8	1.0	None
21	0.79	0.46	0.2		8.8	7.8	3.9	
22	0.79	0.44	0.25	0.1	8.2	3.3	2.5	None
23	0.7	0.5	0.36	0.25	0.9	3.1	3.6	3.4
24	0.52	0.39	0.22	0.12	1.8	2.0	8.0	6.2

TABLE 5.—URINE AND BLOOD VOLUMES AND LIVER FUNCTION TESTS IN 24 PATIENTS GIVEN 500 CC. OF A 10% GELATIN SOLUTION INTRAVENOUSLY

	Urine Volume (cc.)				Blood Volume (cc.) Before		After		Bromsulphalein Retention (%)		Cephalin Flocculation	
Case	End	6 hr.	24 hr.	48 hr.	Plasma	Blood	Plasma	Blood	Before	After	Before	After
1	830	1000	1620	750	1909	3235	Unsuccessful		37		4+	
2	100	200										
3	780	450	1250	3030	2930	5232	5280	9103	4	8	Negative	Negative
4	280	750	1110	1220	2148	3886	3056	4851	3	9	Negative	Negative
5	105	125	320	500	1230	6558	5409	7954	1		3+	3+
6	204	215	815	1185	2777	4180	4230	6508	2	7	Negative	2+*
7	680	260	600									
8	210	250	1570	1475	Not done				1	1	Negative	Negative
9	330	470	1070	1210	3019	5489	3688	6304	6		Not done	
10	475	250	220	940	Not done				Not done		Not done	
11	1025	500	850	1450	Not done				Not done		Not done	
12	305	250	1680		Not successful (sample lost)					6		Negative
13	325	75	440	940	Not done				32	33	Negative	Negative
14	115	120	450	800	3688	5122	4191	6073	17	16	3+	3+
15	105	210	1070	1800	2590	4654	3122	5035	12		2+	
16	240	215	570	725	2777	5067	4230	7421	46	47	3+	3+
17	320	275	1730	2770	7207	12320#	4589	7642	23	16	3+	3+
18	500	135	800		3695	5278	3695	5204	18	16	3+	3+
19	170	105	200		3122	4135	3912	4952	27	36	Negative	Negative
20	305	320	1770	1480	3540	6743	4061	7125	13	13	3+	2+
21	550	200	525		2511	4307	2251	3631	16		2+	
22	163	95	985	1935	Unsuccessful				6	1	Negative	Negative
23	420	700	940	2550	3801	5316	4643	5877	1	2	Negative	Negative
24	565	550	2300	2550	4494	6156	6433	8354	1	1	Negative	Negative

* Negative second week after gelatin injection.
\# Determination evidently in error.

TABLE 6.—URINE PROTEIN IN 3 PATIENTS WITH EDEMA OF RENAL ORIGIN GIVEN 500 CC. OF A 10% GELATIN SOLUTION INTRAVENOUSLY

	Case 8		Case 23		Case 24	
	Urine Volume cc.	Urine Protein gm.	Urine Volume cc.	Urine Protein gm.	Urine Volume cc.	Urine Protein gm.
End	240	2.1	430	1.4	565	1.8
6 hr.	250	2.5	700	3.3	550	1.4
24 hr.	1570	14.0	940	6.0	2300	8.0
48 hr.	1475	11.3	2550	7.7	2350	6.2

1115 cc. (1165 cc. after 48 hours) on these occasions.

Comment. Although the number of patients studied was too small and the data obtained were too equivocal to permit any sweeping conclusions, the findings of this study tend to confirm and extend the observations of Koop and his collaborators[21,23,24] as to the effect of gelatin solutions on patients with edema and ascites. This group from the University of Pennsylvania has reported that in their experience the diuretic effect of gelatin is most pronounced in patients with nephrosis. They have observed some response, though not a consistent one, in patients with ascites, and very little effect in patients with nephritis. Koop's studies and those reported here suggest that additional investigation of the possible role of gelatin in producing diuresis in certain types of cases, particularly those with hypoproteinemia, may be desirable. Experiments with gelatins of various molecular weights and in varying concentrations should be tried to determine, if possible, the best preparation for this purpose. Koop[21] found that the large molecular gelatin produces greater diuresis than the gelatin of small molecular type.

In our studies, we used a more concentrated solution, 10%, than the majority of other workers have employed. Most of them have administered solu-

tions containing 4 to 6% of gelatin. The higher concentration we used may possibly account for the apparently greater retention of gelatin by the patients in our series. The amount of gelatin they excreted in the urine during the first 48 hours was less than that observed by some other authors. The total amount excreted in the urine during the first 48 hours has been reported to be as high as 80%, whereas in our patients, 30% or less was recovered in the urine during a similar period.

Discussion. The numerous investigations that have been made on the intravenous use of gelatin have established some facts about this substance and have resulted in the production of refined, non-toxic products which are safe for clinical administration. Nevertheless, numerous questions remain unanswered as to the utilization and excretion of gelatin in man and concerning the indications for its use in the treatment of patients, and the optimal technique of administration.

The major portion of the studies published during the last 10 years have dealt with experiments on animals[2-5, 9,13,15,18-20, 26-30,33,34,36,38,42,43], particularly on the control of hemorrhagic shock. This experimental work has been done chiefly by teams of investigators at research centers, such as Princeton University[19,42,43], University of Louisville[9, 13,29,26,27], Bowman Gray Medical School[2,29,30], University of Rochester[24,39], University of Pennsylvania[21,23, 24,36], Michael Reese Hospital, Chicago[18,28,33], Northwestern University Medical School[15] and the University of Chicago[3,5].

These experiments have demonstrated conclusively the value of gelatin solutions in restoring the circulating blood volume and in controlling the symptoms of hemorrhagic[9,15,18,26-28,33,34,36] and other types of shock[19,30,42,43]. Various types of gelatin in varying concentrations have been used in these experiments, but all, excepting a 2% solution, proved effective in restoration of blood pressure and blood volume in animals suffering from shock. There is general agreement that gelatin solutions are more effective than saline and glucose solutions for this purpose, but somewhat inferior to plasma or whole blood. The Princeton group has reported that intermittent administration over a 7 or 8 hour period was more effective than a single large infusion[19,42,43]. In experimenting with gelatin preparations of various molecular weights, Knoefel and Lehmann[20] found that the retention of gelatin in normal dogs varies with the molecular weight, but does not increase markedly with molecular weights above 50,000. The fraction of injected gelatin excreted in the urine during the first 4 hours varies inversely with the molecular weight. Those who have experimented with solutions of varying concentrations usually report greater retention of gelatin with more concentrated solutions (6 to 10%) than with those less than 5%[9,28,29].

Brunschwig and his collaborators[3,5] conducted experimental studies of nitrogen balance in dogs maintained on a low protein diet, to determine the possible nutritive value of intravenous gelatin. Daily injections of 150 to 250 cc. of a 10% gelatin solution were administered for several days, followed by a period of observation without gelatin injections. In 3 of 4 dogs, nitrogen balance was maintained after cessation of gelatin injections, suggesting that all the gelatin was not immediately excreted. One dog refused to eat after the 7-day period of gelatin injections and went into marked negative nitrogen balance. An increase in nonprotein nitrogen was noted following the injections. A similar finding was reported in experiments by Janota and her collaborators[18]. In 6 patients with advanced car-

cinomatosis to whom Brunschwig, Corbin and Johnston[3] gave gelatin intravenously, there was an increase in urea nitrogen excretion during and/or following the injections. In a later report on the clinical use of gelatin injections, Brunschwig and Nichols[4] reported results on 23 patients. In 17 patients receiving one injection of 400 to 800 cc. of 8% gelatin solution, there was appreciable retention of gelatin in 11 and little or no retention in 6. In 6 patients receiving repeated daily injections, there was some retention of gelatin in all: 40 to 45% of the gelatin was retained and 60 to 75% was recovered in the urine. The relative amount retained was approximately the same, whether 1 or 8 daily injections were made.

Another experiment on the nutritional value of gelatin solutions was reported by Robscheit-Robbins, Miller and Whipple[38] on dogs that were both anemic and hypoproteinemic (doubly depleted). They concluded that gelatin given by vein to such dogs gives no immediate toxic response and no anaphylactoid reactions and may contribute something to the building of new hemoglobin and plasma protein. Gelatin given by vein during 1 or 2 weeks (total 3 to 17 gm. per kg.) usually causes serious disturbances: inhibition of blood protein production, signs of intoxication, much weight loss, and even death. Gelatin given by vein for 2 to 3 days (total 1 to 3 gm. per kg.) may not cause any recognizable abnormalities, but dogs vary greatly in their individual response. Some may tolerate a total of 7 gm. per kg. without significant disturbance and others may be seriously intoxicated by 2 to 3 gm. per kg. There is no way of predicting which animal will be less tolerant. Where the gelatin by vein inflicts its damage is not clear, but these authors feel that the disturbance of blood protein implicates the liver. Gelatin of smaller molecular weight proved no less toxic than standard gelatin. The final conclusion of this study was that gelatin by mouth may contribute to, but cannot alone support the production of new hemoglobin and plasma protein. The implications are that when gelatin is used in clinical cases the amount given should be carefully controlled.

There is general agreement that properly prepared gelatin in appropriate dosage is safe for intravenous use and has no anaphylactic effect. In some of the work reported, there has been some complaint of thrombosis at the site of injection, but this has been attributed largely to the preservatives used. This difficulty has been corrected in the material prepared more recently. The increased sedimentation of erythrocytes and the tendency to pseudo-agglutination caused by gelatin infusions have been quite consistently cited. To overcome the practical difficulties involved in this phenomenon, Koop and Bullitt[22] described a method for eliminating pseudo-agglutination in typing and cross-matching of blood, following gelatin infusions.

The clinical studies on intravenous gelatin[6,7,12,16,21,23-25,32,37,39-41,44], like the experimental work, have been directed largely toward the treatment of shock from hemorrhage, trauma, and burns[6-16,25,37]; gelatin infusions have been consistently endorsed as a substitute for plasma for emergency use in these conditions. Considerable interest has been shown also in the possible use of intravenous gelatin solutions as supportive treatment in debilitated and undernourished patients being subjected to surgical or medical treatment[12,21,23,24,32,39,41,44]. The consensus is that gelatin solutions have some value in these conditions, but the results reported are not entirely consistent.

The laboratory studies on the effect on the blood proteins are somewhat at variance in the published reports. Some patients apparently show a positive nitrogen balance when gelatin is administered intravenously as a nutritional source, and others do not. The effect on the serum proteins is not entirely clear, though it seems quite generally accepted that there is a decrease in the concentration after the administration of gelatin. This effect was not observed in our laboratory studies. The immediate increase in blood volume after gelatin injections has been noted in all the experimental and clinical studies, with a corresponding decrease in the hematocrit. The evidence regarding the retention of gelatin, as indicated by the excretion in the urine, is most confusing. The amounts excreted during the first 48 hours seem to vary from 25 to 80%. As already mentioned, the retention of gelatin in the body evidently is influenced by the molecular weight of the gelatin, the concentration of the solution, and the metabolic and clinical condition of the individual to whom it is administered.

The diuretic effect of gelatin solutions has not received much attention in the literature, although the results in a few cases reported by Koop and his co-workers[21,23] and in the series reported here suggest that such preparations may have some clinical value for this purpose, especially in cases of nephrotic edema. An experimental study on normal dogs reported by Bridger *et al.*[2] also called attention to the diuretic effect of a 6% solution of gelatin. These findings would seem to indicate that additional studies along this line, with careful selection of cases, might prove fruitful from a clinical standpoint. The relationship of the type and concentration of the gelatin used to the amount retained by the body, and also the relationship of these factors to the diuretic effect in cases of edema of various types, need to be further investigated. It is almost impossible at present to compare the results reported by different workers, because of the lack of standardization or uniformity in the preparations and concentrations used in different investigations, both experimental and clinical.

Summary and Conclusions. Twenty-four patients with non-surgical conditions, more than half of them with evidence of liver damage, received a single injection of 500 cc. of a 10% solution of gelatin intravenously. One patient (Case 24) also received 4 subsequent infusions of the same amount. Detailed laboratory studies, including total proteins, nonprotein nitrogen, urea clearance, hemoglobin, hematocrit, urine volume, blood volume, bromsulphalein retention and cephalin-cholesterol flocculation tests, were performed before and after the injections of gelatin. The gelatin in the blood and urine was also determined at intervals over a 48-hour period.

The findings showed no significant changes following the administration of 50 gm. of gelatin in a 10% solution, except for an immediate increase in blood volume and a decrease in the hematocrit, and an increase in urine volume 24 to 48 hours after the injection. The increase in urinary output was greatest in patients with edema of renal origin and less striking in those with ascites owing to liver disease. Approximately 25 to 30% of the gelatin administered was excreted in the urine during the first 24 hours. Excepting in 3 patients with nephrotic edema, no appreciable quantity of gelatin was found in the urine at the end of 48 hours, and only minute amounts remained in the plasma.

The principal positive conclusion to be drawn from this investigation is that it indicates the need for additional stud-

ies as to the metabolism of gelatin in patients with various clinical conditions. Administration of gelatin solution to patients with nephrosis would seem to offer a worthwhile field for special study, with additional experimentation on optimal methods to be used in such treatment.

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J. Appl. Physiol. 5:814-820, June 1953

Excretion and Retention of Two Modified Gelatins in Normal Human Beings

PENTII K. SIITERI[1] AND GEORGE P. HIGHLAND.[2] *From the Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas*

SINCE HOGAN (1) first reported that gelatin solutions were effective in the treatment of shock in human beings, the results of many additional investigations have established that gelatin is a relatively effective (2, 3), safe (4, 5) and easily prepared plasma volume expander. However, solutions of ordinary gelatin have the serious disadvantage of gelling at ordinary room temperatures. This property would obviously result in difficulty in administration if gelatin were to be used in the field for the treatment of large numbers of military or civilian casualties.

Two methods of depressing the gelling point of gelatin solutions have been used: chemical modification of the gelatin molecule and the addition of various chemicals. By condensation of native gelatin with glyoxal and subsequent oxidation with H_2O_2, Campbell *et al.* (6) have developed a product which, in 5% concentration, is liquid at room temperature. This material has been named oxypolygelatin (OPG). More recently, a so-called 'fluid gelatin' has been produced by treating Knox P-20 ordinary gelatin with a dicarboxylic acid anhydride under specified conditions of pH and temperature. This material, in 3% concentration, is liquid at room temperature and below.

This study was undertaken in order to determine the retention and excretion of OPG and fluid gelatin in normal men.

MATERIALS AND METHODS

OPG[3] was administered as a 5% solution in 0.9% NaCl solution. The number average molecular weight of OPG was approximately 20,000. Two different lots, S141X and S230X, were used. The fluid gelatin,[4] lot MFG-5, was administered as a 3% solution in 0.7% NaCl solution. The number average molecular weight of fluid gelatin was approximately 24,000, and the weight average molecular weight of this material was approximately 87,000.

The subjects were 12 men patients who were convalescing at the Brooke Army Hospital from minor burns. None had cardiovascular or renal disease. The patients were divided into 2 groups. *Group 1* (8 patients) received OPG (*subjects 1* and *3* in *group 1* received lot S141X). *Group 2* (4 patients) received fluid gelatin. In all patients, food containing fat was withheld on the day of infusion. In 2 subjects (*1* and *2* in *group 1*) indwelling catheters were used for the collections of urine; in all the

Received for publication December 29, 1952.

[1] 1st Lt., Medical Service Corps.

[2] Cpl., Army Medical Service. Present address: Ventura Health Department, Ventura, Calif.

[3] The OPG used in these experiments was supplied by Don Baxter Laboratories, Inc., Glendale, Calif.

[4] The fluid gelatin used in these experiments was supplied by the Knox Gelatine Company, Camden, N. J.

others, urine was collected by voluntary voiding. Except for the 2 catheterized patients, all received, by oral administration, 500 ml of water at the beginning of each experiment and 250 ml each half-hour for 6 hr. thereafter.

After obtaining control samples of blood and urine, 500 ml of the gelatin solution were infused over a period of approximately 1 hr. Blood was drawn in heparinized syringes immediately after infusion and at 3, 5 and 20 hr. for the measurement of gelatin and plasma protein concentration, albumin-globulin ratio and hematocrit. The control and immediate postinfusion samples were placed under mineral oil for determination of CO_2 content. The amount of gelatin excreted in the urine was measured at the end of infusion and at 1, 3, 5, 24, 48 and 72 hr.

Control blood volume was estimated from the 10-min. T-1824 dye concentration and the hematocrit, according to the method of Gregerson (7). Concentrations of OPG or fluid gelatin up to 1% did not interfere with the spectrophotometric determination of T-1824 dye concentration. A constant red cell volume was assumed, and the postinfusion plasma volumes were then calculated from this control value and the hematocrit changes. To estimate the validity of these figures, the T-1824 measurement was repeated by reinjection of the dye in all *group 1* subjects. In only 4 of 24 measurements did the calculated values differ by more than 5%. In these 4 instances, the values obtained from the dye measurement appeared more reasonable and, therefore, were used in subsequent calculations. The postinfusion plasma volume of *subject 3* in *group 2* was unreasonable and was therefore excluded from the calculated averages. OPG and fluid gelatin were measured in blood and urine samples as hydroxyproline by a modification of the method of Nueman and Logan (8), as it is described later. Plasma protein was measured by the Biuret method (9), with appropriate correction for gelatin.[5] Plasma CO_2 content was measured by the method of Van Slyke (10). Blood pressure, pulse and temperature were recorded before infusion and at intervals during and after infusion. The subjects were observed for untoward reactions for 20 hr. after infusion.

Method of Gelatin Analysis

Since gelatins contain 12–14% hydroxyproline and constituents of normal body fluids contain very little, it is possible to measure gelatin in the presence of plasma proteins as hydroxyproline.

One-ml samples of urine or plasma containing 0.2–1.5% gelatin are hydrolyzed in sealed tubes with 5.0 cc of 6N HCl for 6 hr. at 150°C. Preinfusion urine and plasma are used to obtain blank values and also for the preparation of standards by the addition of known gelatin solutions. These are hydrolyzed in the same manner as unknowns. The tubes are permitted to cool and the contents are then quantitatively transferred to 100-ml volumetric flasks, neutralized with NaOH, diluted to the mark and filtered, if necessary. The color is developed according to the method of Nueman and Logan. After the specimen stands at room temperature for 25 min., standards and unknowns are read against appropriate preinfusion blanks at 560 mμ in a Beckman model B spectrophotometer. Unknown concentrations are read from the simultaneously prepared standard curve.

Table 1 shows results of analyses performed on standard solutions of OPG[6] in

[5] The color yield with this Biuret reagent for both OPG and fluid gelatin is approximately 60% that of native protein on a weight basis which, therefore, introduces a negligible error in plasma protein estimations with gelatin concentrations below 0.25 gm/100 cc.

[6] These solutions were prepared by Don Baxter Laboratories, Inc., for the purpose of evaluating existing methods of analysis for gelatin. The samples were analyzed after the method, with minor changes, had been in use in this laboratory for more than a year.

plasma and urine, by the use of this method. It is seen that OPG can be measured, in urine, with a precision of 100 ± 3.6%, and an accuracy of 100.4% and, in plasma, with a precision of 100 ± 4.7% and an accuracy of 98.3%. Since satisfactory results were also obtained with solutions of fluid gelatin, presumably any gelatin may be determined by the use of this method. Less accurate results are obtained if a water standard curve is used with appropriate correction for blanks.

Results

Excretion. Figure 1 represents plasma retention and the cumulative urinary excretion of OPG and fluid gelatin, calculated as percentage of infused dose. The curve labeled 'unaccounted for' represents the sum of the other 2 curves subtracted from 100. It will be noted that the most rapid excretion of both gelatins occurred

TABLE 1. RESULTS OF ANALYSES PERFORMED ON STANDARD SOLUTIONS OF OPG (LOT NO. S230X) IN URINE AND PLASMA

W/V OPG, %	Exper. Values	Mean ± SD	Precision	Accuracy, %
Urine				
0.293	0.305, 0.295, 0.305	0.300 ± 0.005	100 ± 3.3	102.4
0.407	0.410, 0.397, 0.400	0.402 ± 0.014	100 ± 3.5	98.8
0.586	0.590, 0.588, 0.572	0.583 ± 0.010	100 ± 3.5	99.5
1.17	1.15, 1.18, 1.13	1.15 ± 0.025	100 ± 4.5	98.3
4.02	4.12, 4.22, 4.08	4.14 ± 0.070	100 ± 3.4	103.0
		Mean	100 ± 3.6	100.4
Plasma				
0.209	0.205, 0.210, 0.203, 0.198	0.204 ± 0.005	100 ± 4.9	98.6
0.314	0.293, 0.310, 0.300, 0.303	0.320 ± 0.007	100 ± 4.6	96.2
0.628	0.640, 0.640, 0.615, 0.642	0.634 ± 0.011	100 ± 3.5	101.0
1.26	1.26, 1.28, 1.20, 1.23	1.24 ± 0.035	100 ± 5.6	98.4
		Mean	100 ± 4.7	98.3

Precision values were calculated by expressing 2 SD as a certain percentage of the mean taken as 100%.

during the first 3 hr., at which time 52–64, or an average of 59% of infused OPG, and 35–39, or an average of 37% of infused fluid gelatin, were excreted. By 5 hr., nearly all of the urinary excretion of OPG had occurred; whereas a considerable amount of fluid gelatin was excreted after that time. By the end of 24 hr., a total average of 67% of OPG and 52% of fluid gelatin was excreted. With but one exception in each group, gelatin was still detectable in the urine up to 72 hr. after infusion.

In figure 1 it is also noted that both materials were rapidly lost from the plasma, so that immediately after the infusion an average of 57% of the OPG and 64% of the fluid gelatin remained in circulation. At 5 hr., these figures are 26 and 33% and at 20 hr., 8 and 15%, respectively.

The 'unaccounted for' fraction averaged 16 and 20% for OPG and fluid gelatin respectively at the end of infusion. At the end of 20 hr., this fraction increased to 27 and 33%, respectively.

Tables 2 and 3 represent a summary of data obtained in *group 1* and *2*, respectively. Plasma protein concentration was diminished after infusion of both gelatins, but returned to near control values at the end of 20 hr. The albumin-globulin ratio remained unchanged. Average total circulating plasma protein was slightly lowered, except for a small increase immediately after infusion in *group 2*.

When plasma volume expansion was calculated as percentage of administered fluid retained in the circulation, the values averaged 82% for OPG and 106% for fluid gelatin immediately after infusion. These values decreased to 17 and 39%, respectively, at the end of 6 hr.

Measurements of the CO_2 content of plasma before and after infusion showed no significant alterations. Neither pyrogenic nor allergic reactions were encountered. In 2 subjects in *group 1* dyspnea was noted during the infusion, but these symptoms disappeared upon reduction of the rate of infusion. Blood pressure and pulse rate showed transient, small elevations in a few subjects.

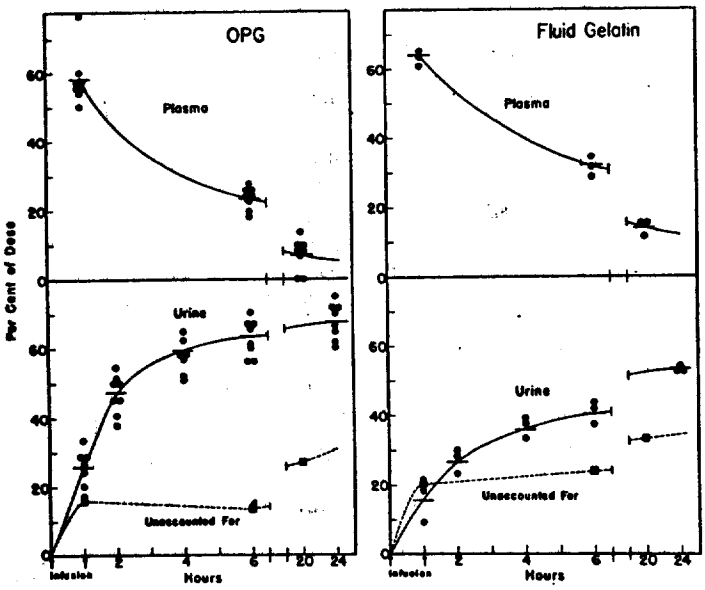

FIG. 1

DISCUSSION

Both OPG and fluid gelatin are heterogeneous mixtures of different sized molecules. The rate of urinary excretion has been shown to be inversely related to molecular weights for gelatins (11, 12) and for other colloids (13, 14) used as plasma volume expanders. The rapid urinary excretion recorded here presumably represents the smaller molecules of the mixture. As was anticipated from the molecular weight data, OPG, which has a smaller average molecular weight, was more rapidly excreted than fluid gelatin. The consistently greater blood retention of fluid gelatin can be explained on the same basis. Although these experiments involve a small number of subjects, the differences in excretion and retention data at the end of 6 hr. for the 2 groups are statistically significant. The level of significance as expressed by P values[7] (15) was found to be less than .001 for excretion and between .01 and .001 for retention at this time.

The fraction not accounted for in the urine and plasma represents material that has escaped into the interstitial fluid, and to a smaller extent, that which has been metabolized or stored in cells. Gropper (16) has demonstrated the presence of OPG

[7] P values were calculated according to the method of F. E. Croxton and D. J. Cowden.

TABLE 2. OXYPOLYGELATIN. SUMMARY OF DATA OBTAINED ON 8 PATIENTS INFUSED WITH 500 ML OPG

Patient	Time Postinfusion	Plasma Vol.	Plasma Protein Conc.	Hct.	Gelatin Conc.	CO_2
		ml	*gm %*		*gm %*	*mEq/l*
1	Preinfusion	2806	6.98	50.0	0	29.2
	Immediately postinfusion	3228	5.78	46.5	0.425	28.9
	5 hr.	2806	6.50	50.0	0.170	
	20 hr.	2861	6.73	49.5	0.060	
2	Preinfusion	3382	6.12	45.5	0	29.0
	Immediately postinfusion	3900	5.33	42.0	0.350	27.9
	5 hr.	3585	5.53	44.5	0.188	
	20 hr.	3452	5.78	45.0	0.060	
3	Preinfusion	2921	6.84	45.5	0	29.1
	Immediately postinfusion	3438	5.57	41.5	0.400	29.7
	5 hr.	2805	6.26	46.5	0.210	
	20 hr.	2980	6.75	45.0	0.050	
4	Preinfusion	2301	6.67	51.0	0	31.0
	Immediately postinfusion	2650	5.28	47.5	0.500	30.8
	5 hr.	2255 (2430)*	6.19	51.5	0.250	
	20 hr.	2492	6.53	49.0	0.095	
5	Prefusion	3056	7.34	48.5	0	30.5
	Immediately postinfusion	3380	5.96	46.0	0.434	29.9
	5 hr.	3182	6.89	47.5	0.195	
	20 hr.	2940	7.43	49.5	0.078	
6	Preinfusion	2639	7.65	50.0	0	30.2
	Immediately postinfusion	3035	6.48	46.5	0.453	27.7
	5 hr.	3160 (2900)*	6.71	45.5	0.195	
	20 hr.	2860	7.09	48.0	0.072	
7	Preinfusion	2797	7.43	51.5	0	29.2
	Immediately postinfusion	3080	6.80	49.0	0.398	25.6
	5 hr.	3215 (2812)*	7.43	48.0	0.192	
	20 hr.	2478 (2600)*	7.55	54.5	0.0	
8	Preinfusion	2973	6.60	45.0	0	28.1
	Immediately postinfusion	3362	6.18	42.0	0.558	28.3
	5 hr.	2973	6.88	45.0	0.152	
	20 hr.	2865	6.94	46.0	0.0	

* T-1824 blood volume.

in lymph in relatively high concentrations during the early postinfusion period. The increase in the unaccountable fraction at the end of 20 hr. is probably the result of metabolism and tissue storage.

In a similar study in normal human subjects, Higgins *et al.* (17) reported a urinary excretion of OPG of approximately 50% in 8 hr., with a corresponding plasma retention of 20%. In dogs, Raisz (18) recorded 50% urinary excretion and 30% plasma retention in 3 hr. In rabbits subjected to hemorrhage and infused with OPG, Raisz (19) found that excretion is much reduced and retention is increased. In

TABLE 3. FLUID GELATIN. SUMMARY OF DATA OBTAINED ON 4 PATIENTS INFUSED WITH 500 ML FLUID GELATIN

Patient	Time Postinfusion	Plasma Vol.	Plasma Protein Conc.	Hct.	Gelatin Conc.	CO_2
		ml	*gm %*		*gm %*	*mEq/l*
1	Preinfusion	2480	8.02	46.5	0	27.6
	Immediately posinfusion	3035	7.05	41.5	0.301	23.5
	3 hr.	2912	6.71	42.5	0.180	
	5 hr.	2685	7.65	44.5	0.190	
	20 hr.	2748	7.95	44.0	0.085	
2	Preinfusion	3090	7.85	45.5	0	21.6
	Immediately postinfusion	3640	6.85	41.5	0.268	30.2
	3 hr.	2970	8.16	46.5	0.185	
	5 hr.	2910	7.93	47.0	0.149	
	20 hr.	3420	7.41	43.0	0.050	
3	Preinfusion	2650	7.67	51.0	0	26.0
	Immediately postinfusion	3610	6.20	43.5	0.320	25.4
	3 hr.	3262	6.79	46.0	0.208	
	5 hr.	3010	6.95	47.5	0.180	
	20 hr.	2620	6.93	51.5	0.068	
4	Preinfusion	3695	6.97	46.5	0	29.1
	Immediately postinfusion	4170	6.33	43.5	0.230	24.5
	3 hr.		6.43		0.138	
	5 hr.	4080	6.51	44.5	0.116	
	20 hr.	3840	6.13	45.5	0.060	

a similar study in dogs, Gropper (16) found comparable values for plasma retention of OPG and dextran at 3 hr. after infusion.

Scanty information is available for fluid gelatin. In preliminary studies in dogs, Parkins *et al.* (20) have reported that fluid gelatin compares favorably with dextran and PVP as a volume replacement agent.

Although greater plasma volume expansion might be expected after infusion of smaller molecular and, therefore, more osmotically active OPG than with fluid gelatin, initially the latter gave a somewhat larger expansion. However, these values showed a wide spread and were not significant.

The use of dicarboxylic acids in the preparation of these materials suggests the possible release of free organic acids or protein molecules with free carboxyl groups

as metabolic products after infusion. The absence of adverse effect on the acid-base equilibrium is indicated by the unaltered plasma CO_2 content.

Summary

Oxypolygelatin and fluid gelatin were given as a 500-ml intravenous infusion in 12 convalescent patients. At the end of 6 hr., fluid gelatin showed a significantly smaller urinary excretion and greater plasma retention than OPG. The greatest urinary excretion of both materials occurred within the first 6 hr. Untoward effects were not noted. A method for accurately measuring gelatin by means of its hydroxyproline content has been presented.

The authors gratefully acknowledge the assistance of Capts. L. G. Raisz and A. L. Gropper, U.S.A. Medical Corps, in performing these experiments and in the preparation of this manuscript.

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GELATIN NEPHROSIS

Renal Tissue Changes in Man Resulting from the Intravenous Administration of Gelatin

OLAF K. SKINSNES, M.D., Ph.D., New York, New York

THE currently increased interest in and greater use of gelatin as a blood substitute in the treatment of peripheral vascular collapse and as a means of parenteral nutrition demands a careful evaluation of any pathological changes for which this substance may be responsible. Three years ago the appearance of striking tubular hydropic degeneration, similar to that seen in sucrose nephrosis (1, 2, 14) but now occurring in the kidneys of patients who had not been subject to sucrose therapy, directed our attention to the possible etiologic rôle of gelatin in the production of these lesions. Recently Popper and associates published a report on the use of gelatin in the treatment of shock, and, noting similar renal tubular swelling in some of their necropsy cases, also suggested that these lesions might be attributable to intravenous gelatin therapy. Continued association with a surgical service on which extensive studies of the use of gelatin intravenously have been carried out (4-7) and on which major surgical procedures are a common occurrence has presented an unusual opportunity for the further study of this lesion. The present study was accordingly undertaken in order to evaluate the rôle of gelatin in the pathogenesis of the lesion, to study the morphological nature of the changes which were produced, and to appraise their functional significance.

MATERIALS AND METHODS

The study is based on the postmortem examinations of 23 patients who received gelatin intravenously in quantities ranging from 100 to 17,600 cubic centimeters with widely varying intervals between the final injection and the death of the patient. The gelatin, derived from pig skin, was given in an 8 per cent solution in physiological saline, usually in doses of 800 cubic centimeters (4).

All the patients, save 2, had neoplastic growths primary in the tissues of the abdominal cavity. Of the exceptions, one suffered with a squamous cell carcinoma of the lower lip and the other from a chronic bleeding gastric ulcer. All but 3 died of their primary disease and its complications or from complications resulting from the surgical procedures, most of which involved extensive regional resections. In most instances the gelatin therapy was given in association with the surgical procedures and either instituted as part of the preoperative preparation or, more commonly, begun at and continued for varying intervals following the operation.

The postmortem examinations were all performed within 17½ hours of death; in the majority of instances the postmortem interval was 2 to 10 hours. Blocks of renal tissue were fixed in Zenker's solution at the time of necropsy, embedded in celloidin and sectioned at 8 microns. The tissues were stained with Heidenhain's iron hematoxylin, Heidenhain's modification of Mallory's phosphotungstic acid hematoxylin, and Delafield's hematoxylin-eosin stain. In 1 case, No. 0507, alcohol fixed tissues were stained with Best's carmine for glycogen.

PATHOLOGICAL RENAL CHANGES

Grossly, kidneys with well developed gelatin nephrosis are swollen and the cut surfaces tend to bulge. The average weight of 17 otherwise relatively normal kidneys from 10 patients (excluding the kidneys of these patients which showed any neoplastic infiltration) in this series who demonstrated well developed microscopic lesions was 210 grams.

Microscopically the lesions are similar to those described for sucrose nephrosis (Fig. 1)

From the Department of Pathology, The University of Chicago.

Fig. 1. Case 6558. The general pattern of the renal lesion in sucrose nephrosis. The patient received 80 cubic centimeters of 50 per cent sucrose solution intravenously 29 hours before death. There is marked vacuolization of the convoluted renal tubules. Compare with Figure 3. Heidenhain's modification of Mallory's connective tissue stain. ×160.

(1, 2, 14). Variations in the extent and the degree of change can be roughly correlated with the time elapsing between the injection of the gelatin solution and the examination of the kidney, and with the amount of gelatin given (Table I). The epithelial cells of the proximal convoluted tubules present a granular, finely vacuolated swelling, often so severe that the tubular lumens are evident only as slits (Fig. 2). Occasionally the affected cells are necrotic but this change is minimal and the cells are, on the whole, well preserved. In fresh, well fixed tissues the brush border remains distinct and is not sloughed. These changes are largely confined to the proximal convoluted tubules and the portions of these tubules which lie in the medullary rays and extend into the renal medulla show the earliest and most pronounced swelling and vacuolization. The distal convoluted tubules may also be affected, though to a lesser degree, but the loops of Henle and the collecting tubules remain free from this change even in the severest gelatin nephrosis encountered (Figs. 3 and 4). Best's carmine stain on the alcohol fixed kidney sections of Case 0507 gave no evidence of glycogen in the hydropic tubular cells.

The lumens of the convoluted tubules and of the glomerular spaces contain much amorphous granular precipitate. This may be indicative of dysfunction, tissue necrosis, or of the presence of gelatin in the tubular lumens, but its significance is obscured by the fact that such precipitates are routinely encountered in the tubules of otherwise normal kidneys examined at necropsy.

No significant glomerular changes have been noted which are not attributable to other renal changes. Changes such as the leucocyte infiltration and shrinkage of glomerular tufts described by Anderson and Berthea (2) for sucrose nephrosis have not been seen. One case (Case 6412, Fig. 5), having well developed gelatin nephrosis together with a complicating icteric nephrosis, displayed a striking cuboidal epithelial metaplasia of the parietal layer of Bowman's capsule adjacent to the neck of the convoluted tubules. This change is morphologically similar to that which has been described by Anderson and Berthea for sucrose nephrosis and to that which has been described by Bywaters and Dible as occurring in association with the renal lesion of the crush syndrome.

In a number of instances large amounts of gelatin were administered over long periods of time, the greatest amount being 17,600 cubic centimeters administered in 23 doses over a period of 34 days. One case only (Case 6305, Fig. 6) showed moderate focal colloidal droplet degeneration of the tubular epithelium. In other instances where varying amounts of gelatin were given but discontinued sufficiently long before death to allow the hydropic swelling to subside (Table I) no changes definitely attributable to the previously existing gelatin nephrosis were demonstrable. The changes seen were such as are routinely encountered in relatively normal kidneys of patients dying with high fever or other generalized disturbances. Only occasional tubules were necrotic, displaying pyknotic nuclei and isolated foci of regenerating tubular cells.

FACTORS INFLUENCING THE DEVELOPMENT OF GELATIN NEPHROSIS

Table I summarizes the major findings pertaining to the degree of gelatin nephrosis, the extent of gelatin therapy, other hypertonic intravenous fluids given, and the basic pathological condition of the patient as well as any renal change other than the gelatin nephrosis.

Fig. 2. Case 6206. There is marked hydropic swelling of the illustrated proximal convoluted tubule. The brush border is intact. On the whole the cells are well preserved. Heidenhain's modification of Mallory's connective tissue stain. ×655.

Fig. 3. Case 6206. The general pattern of the lesion of gelatin nephrosis in the renal cortex. The proximal convoluted tubules display marked vacuolization; the loops of Henle remain free from such change. Heidenhain's modification of Mallory's connective tissue stain. ×160.

It reports all instances of patients receiving gelatin which were encountered in this study.

The most important single factor determining whether or not gelatin nephrosis was present was the time interval between gelatin administration and death. All cases examined within 67 hours of receiving their last gelatin injection showed gelatin nephrosis. No patient expiring more than 120 hours after receiving the last gelatin injection displayed the change. In this connection it is interesting to recall the recent work of Little and Dameron indicating that in dogs given gelatin intravenously only traces of gelatin can be found in the urine after 72 hours. The shortest interval between gelatin administration and death was seen in Case 6317 where the elapsed time was given as 5 minutes in the nursing notes. Though there is basis for questioning the exactness of this figure it is certain that the gelatin was given less than 30 minutes before death. The lesions in this instance were well developed but present only focally in portions of the proximal convoluted tubules lying in the medulla (Fig. 7). The spotty distribution of these lesions is in harmony with recent studies indicating that the individual nephrons each function only part of the time. Their appearance so shortly after the injection of gelatin agrees well with the observation of Little and Dameron that in dogs 50 per cent of the gelatin injected intravenously disappeared from the plasma within 30 minutes. By the third postinjection hour the proximal convoluted tubules exhibited well developed and widespread lesions. These convoluted tubules then persisted as long as gelatin was administered.

Inspection of Table I reveals that some correlation is possible between the severity of the lesion and the amount of gelatin given in that the severest lesions (4 plus) tend to be present in the instances where the greatest amount of gelatin was given. This correlation is not absolute (see Case 6206), factors of length of postinjection interval and individual variability tending to obscure the picture.

Fig. 4. Case 6206. The general pattern of the lesion in the cortex and medullary rays in gelatin nephrosis. The proximal convoluted tubules show marked vacuolization and many are filled with granular debris. The longitudinal section of a loop of Henle illustrated shows no vacuolization. Heidenhain's modification of Mallory's connective tissue stain. ×100.

Fig. 5. Case 6412. Cuboidal metaplasia of the parietal cells of Bowman's capsule. The kidneys showed combined gelatin and icteric nephrosis. Heidenhain's modification of Mallory's connective tissue stain. ×220.

Fig. 6. Case 6305. Colloid droplet degeneration of proximal convoluted tubules; 10,630 cubic centimeters gelatin given intravenously in 13 injections in 16 days. Heidenhain's modification Mallory's connective tissue stain. ×460.

Rigdon and Cardwell suggested that the state of hydration of the patient is of importance in determining the occurrence of sucrose nephrosis. The material available did not permit an evaluation of this factor in the pathogenesis of gelatin nephrosis. This series is also not sufficiently extensive to permit judgment as to whether or not previous renal disease will enhance the lesion.

EFFECT OF GELATIN NEPHROSIS ON RENAL FUNCTION

No consistent, accurate measure of renal function was made on these patients clinically since they did not evince clinical renal dysfunction. The occasional pertinent blood and urine studies recorded gave no substantial evidence of impaired renal function. A careful study of the fluid balance of the patients was made from the clinical records. These studies were frequently complicated by the fact that in most instances the gelatin therapy was begun at the time of operation. Concomitantly increased fluid administration and fluid and blood loss through inadequately measured emesis and drainage and from surgical loss of blood often obscured possible changes such as the early diuresis noted by Bridger and associates in dogs receiving intravenous gelatin.

Case 0532 (Fig. 8) demonstrated the most striking change which might possibly be attributed to gelatin therapy. A marked diuresis occurred 12 days after laparotomy, on the third and last day of gelatin administration. This persisted for 6 days and then subsided. The only other intravenous fluid administered during this time was 5 per cent glucose solution and no other medication was given which might be held accountable for the diuresis. Comparable quantities of glucose were given daily from the third postoperative day (14 days before the diuresis developed) through the twenty-second postoperative day (the day the diuresis subsided). A second patient (Case 6542) had a satisfactory urine output of 1000 cubic centimeters on the second postoperative day. On the third day he was given a first injection of 400 cubic centimeters of gelatin solution intravenously. His urine output on this day dropped to 50 cubic centimeters despite a total of 4000 cubic centimeters of intravenous fluid given. On the 2 succeeding days he received a total of 6,650 cubic centimeters of fluid intravenously, including 400 cubic centimeters of gelatin, but put out only 100 cubic centimeters of urine per day and expired on the sixth postoperative day without having established adequate urine output. The day before his death blood chemistry studies showed serum chloride 108.5 millimoles per liter, serum p_H 7.32, serum carbon dioxide 18.5 millimoles per liter and blood nonprotein nitrogen of 117.0 milligrams per cent. The only significant lesion in the kidneys at necropsy was gelatin nephrosis. A complicating factor in the interpretation of these findings was the myocardial infarction

TABLE I. SUMMARY OF THE SEVERITY OF GELATIN NEPHROSIS, THE AMOUNT OF GELATIN THERAPY GIVEN, OTHER INTRAVENOUS THERAPY EMPLOYED, AND THE BASIC PATHOLOGICAL CONDITION OF THE PATIENTS STUDIED.

Case No.	Gelatin nephrosis	Interval between last gelatin and death	Total c.c. gelatin given	No. of doses	Total days of gelatin therapy	Other i.v. hypertonic fluids[1]	Remarks[2]
Gelatin Given—Gelatin Nephrosis Present							
6317	+	5 min.	100	1	1	50% glucose	Resected left hypernephroma. Acute dilatation of the stomach. Death: 3rd day.
6257	+ + +	3 hr.	400	1	1	Amigen	Resected esophageal carcinoma. Death: 11th day.
6172	+ + +	3 hr.	5,200	6	10	50% glucose	Resected ovarian carcinoma. Peritonitis. Arteriolonephrosclerosis. Death: 10th day.
6266	+ + + +	8 hr.	1,600	2	2	Amigen	Carcinoma of the lip. Coronary thrombosis.
6361	+ +	16 hr.	800	2	4	Amigen	Resected esophageal carcinoma. Death: 4th day.
0725	+ + +	16 hr.	1,600	3	2	none	Resected esophageal carcinoma. Hemorrhage from duodenal ulcer. Death: 35th day.
0507	+ + +	16 hr.	2,320	3	3	Amigen	Resected gastric carcinoma. Death: 4th day.
6443	+ + +	17 hr.	2,400	3	5	none	Resected carcinoma of cervix uteri. Colostomy repair. Fecal peritonitis. Death: 4th day.
6426	+ +	24 hr.	11,200	14	10	none	Resected gastric carcinoma. Fecal peritonitis. Death: 20th day.
6485	+ + + +	24 hr.	11,600	15	20	none	Linitis plastica. Jejunostomy. Pulmonary embolism. Death: 17th day.
0498	+	32 hr.	400	1	1	Amigen	Resected esophageal carcinoma. Death: 2nd day.
6303	+ + + +	33 hr.	16,040	13	10	Amigen	Gastric carcinoma with carcinomatosis. Peritonitis. Death: 12th day.
0220	+ + + +	48 hr.	12,000	15	24	none	Resected retroperitoneal lymphosarcoma. Renal metastases. Death: 10th day.
6412	+ + +	50 hr.	800	1	1	Amigen	Resected carcinoma of gall bladder. Peritonitis. Icteric nephrosis. Death: 17th day.
0542	+ + +	67 hr.	800	2	2	50% glucose	Resected rectal carcinoma. Myocardial infarction. Death: 7th day.
0524	+ + +	119 hr.	800	1	1	50% glucose	Resected gastric ulcer. Peritonitis. Death: 5th day.
6337	+ +	23 days	8,835	12	20	Amigen	Lymphoblastic sarcoma. Extensive abdominal resection. Right hydronephrosis. Death: 54th day.
Gelatin Given—No Gelatin Nephrosis							
6201	....	80 hr.	800	1	1	none	Resected rectal carcinoma. Death: 4th day.
6233	....	90 hr.	7,000	10	15	50% glucose Amigen	Resected duodenal carcinoma. Bronchopneumonia. Death: 8th day.
6402	....	19 days	17,020	23	34	none	Resected carcinoma of colon. Bronchopneumonia. Death: 52nd day.
6191	....	45 days	6,665	18	27	none	Resected gastric carcinoma with metastases and emaciation. Death: 71st day.
6380	....	102 days	800	1	1	Amigen	Resected pancreatic carcinoma with metastases and emaciation. Death: 102 days.
6532	....	147 days	2,400	3	3	none	Resected mammary carcinoma. Abdominal carcinomatosis. Death: 158th day.

[1]Includes only fluids given within the last 7 days preceding death. [2]Day of death given is the postoperative day.

Fig. 7. Case 6317. Early focal gelatin nephrosis. There is well developed vacuolization of the convoluted tubules. The patient received 100 cubic centimeters of gelatin intravenously within half an hour of his death. Heidenhain's modification of Mallory's connective tissue stain. ×160.

developing on the third postoperative day. His blood pressure subsequently remained at about 86/60 to 100/80 (preoperative pressure, 145/80) till the day of death.

DISCUSSION

Three of the cases presented raise the question of another possible effect of gelatin injection. Case 6206 had suffered from several attacks of "indigestion" during the past few years. On April 5, 1944, at 2:30 p.m. he was given 800 cubic centimeters of gelatin intravenously. Throughout the following day he had vague abdominal distress and mild substernal pain, felt nauseated, and noted excessive sweating. At 2:45 p.m. of this day intravenous injection of 800 cubic centimeters of gelatin solution was begun. After receiving about 200 cubic centimeters he complained of severe substernal pain, became cyanotic and his breathing became shallow. Two hours later he complained of severe epigastric pain boring to the back, his blood pressure was unobtainable and at 10:35 p.m. he expired with extensive myocardial infarction demonstrated at necropsy. Another patient (Case 6542) was found cold and sweating on the morning of his third postoperative day. His blood pressure was 70/60. Saline, blood, amigen, and 400 cubic centimeters of gelatin solution were given intravenously. Seventeen and a half hours later he was given an additional 400 cubic centimeters of gelatin and 1000 cubic centimeters of amigen; 67 hours later he expired with extensive myocardial infarction. Both patients had severe coronary arteriosclerosis. The third (Case 6465) developed thrombosis of left iliac vein and artery postoperatively while receiving gelatin and other fluids intravenously and died of pulmonary embolism 24 hours after the last gelatin injection.

Intravenous injection of gelatin is known to produce circulatory changes. Fletcher and associates as well as Holt and Knoefel demonstrated an increase in blood volume greater than the volume of gelatin injected, this apparently being due to the effect of gelatin in drawing additional fluid into the circulation. The former investigators did not note any excessive strain on the cardiovascular systems of their patients or volunteer subjects though they did note a marked increase in cardiac output and a sustained hemodilution. They urged caution in the use of gelatin and other macromolecular substances in chronically ill patients. Hanzlik and Karsner demonstrated pseudoagglutination of erythrocytes in the form of conglutinated thrombi in postmortem sections of a guinea pig receiving 0.5 per cent gelatin intravenously. Ivy and associates noted similar marked pseudoagglutination and an increase in the sedimentation rate in dogs given 5 per cent gelatin solution intravenously. Similar observations on patients have been reported by Kozoll and associates (19, 25). Earlier reports (24) indicated that gelatin given intravenously increases the viscosity and coagulability of the blood. Subcutaneous injections of gelatin have been used in the past to increase the coagulability of the blood in the treatment of aneurysms. Recently Koop and associates following an extensive investigation, reported that the clotting time after gelatin infusion is uniformly prolonged in dogs but was unaffected in 6 of 7 patients studied. The seventh patient demonstrated an appreciably prolonged clotting time. Steigmann and associates found no pathological changes in bleeding time, clotting time, or platelet counts of patients given gelatin infusion and Gordon and associates found no postmortem evidence of thrombosis, hemolysis, embolism, or capillary damage in dogs given 8 to 10 per cent solutions of gelatin intravenously.

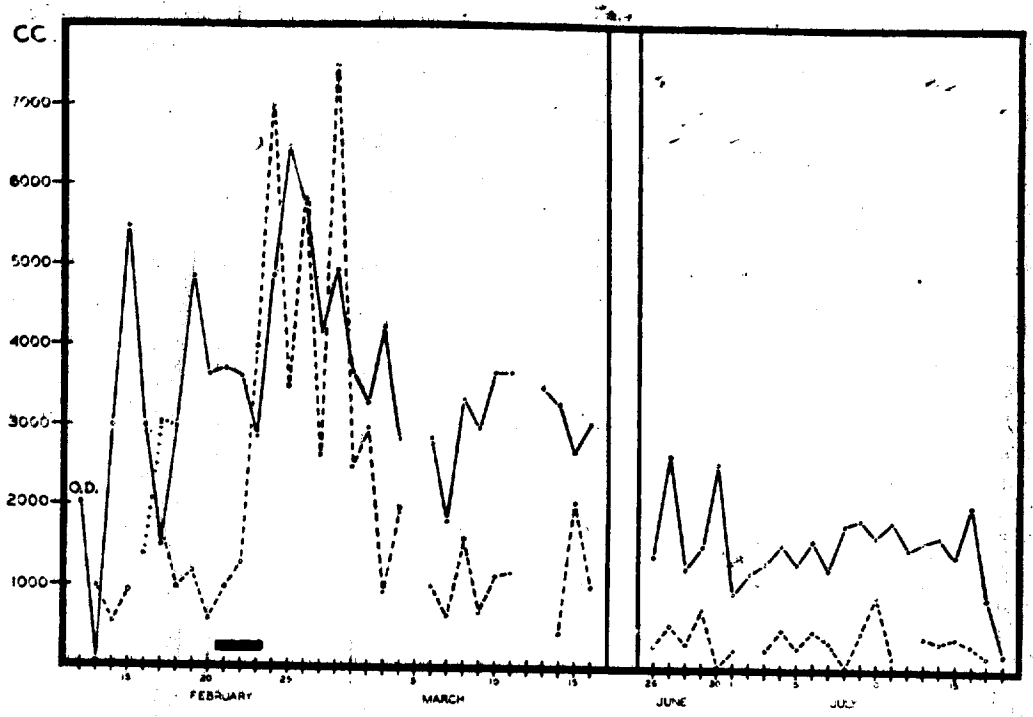

Fig. 8.—Case 0532-L.B. Fluid balance record. Total intake, o ——o; total output, o+++o; urine output, o——o; gelatin given, ———.

In the light of these reports, conflicting though the conclusions sometimes are, the 3 cases reported above, though not conclusively implicating gelatin, suggest the need for caution against the indiscriminate use of gelatin intravenously administered to patients with circulatory disturbances and vascular disease. It may be that some of the conflicting reports may be due to the variability of gelatin preparations. A report by the National Research Council indicates that at least some of the local thrombotic effects reported may be due to added preservatives.

This series of necropsy studies would seem clearly to establish the etiological rôle of gelatin in the production of hydropic swelling of renal tubules. Well developed lesions were found in all postmortem examinations of kidneys of patients receiving gelatin intravenously within 67 hours of the time of death. Heuper found similar lesions in dogs given gelatin solution intravenously. Similar well developed lesions were not found in over 400 consecutive cases studied except in those instances in which sucrose therapy had been employed. Minimal lesions were occasionally encountered which corresponded to the minimal lesions seen in some of the patients who had received gelatin so long before death that the residual changes could only be attributed to gelatin by inference. The etiology of these changes was not clearly demonstrated but the changes were such as might be attributed to other intravenous fluids.

The effect of other intravenous fluids was evaluated. Cutler reported finding hydropic changes in the kidneys of patients receiving hypertonic solutions of dextrose (10-15 per cent solutions). Nearly all the patients in our series received dextrose intravenously, some receiving relatively large amounts of 10 per cent solution over long periods. This may in some instances have enhanced the effect of the gelatin but other patients receiving similar amounts of dextrose without concomitant gelatin showed only minimal or equivocal tubular vacuolization. Five of these patients received 50 per cent dextrose intravenously,

usually in 1 or 2 doses of 50 cubic centimeters each during the week preceding their demise. No tubular lesions were found. One patient (Case 62,3) received 250 cubic centimeters of 50 per cent dextrose during the 48 hours before death but displayed no renal lesion at necropsy. Amigen was frequently administered together with gelatin. A number of control cases receiving amigen without concomitant gelatin failed to demonstrate nephrotic renal changes.

The pathogenesis of gelatin nephrosis is not demonstrated by this study. Wilmer in discussing the mechanism of sucrose damage to the kidneys concluded that this effect was osmotic, the sucrose diffusing into the renal tubular cells and drawing in water. He noted that rats and rabbits phloridzinized to prevent sucrose reabsorption still developed hydropic changes of the renal tubules. It is probable, as also suggested by Popper and associates that gelatin nephrosis develops in much the same manner, the gelatin arriving in the tubular cells by diffusion or active absorption, or both, and causing in turn the taking up of water by increased osmotic pressure exerted by the gelatin. The ready reversibility of the change in both cases speaks in favor of this concept.

Though no uniform, clearly demonstrable harmful effects have as yet been attributed to gelatin nephrosis, it is well to bear in mind the growing feeling that the morphologically similar sucrose lesion is not innocuous. Feher and associates reported that in dogs having an initial large renal reserve, no demonstrable effect on the blood nonprotein nitrogen was found when sucrose was injected though the ability of the kidneys to concentrate urine was reduced. In dogs with relative renal excretory insufficiency, produced by Goldblatt's method of producing hypertension or by temporarily occluding the renal arteries for 1 hour, marked reduction in the ability to concentrate urine and a transient rise in blood nonprotein nitrogen occurred when sucrose was injected intravenously. A report by the National Research Council points out that it is not known whether the use of gelatin will impair functional return to normal in kidneys in sustained ischemia or severe burns or in the crush syndrome. Anderson and Berthea (2) recommend that sucrose be withheld from patients with renal lesions and state that it should in no instance be given in large or repeated doses. The 2 cases reported as showing diuresis and oliguria in the presence of gelatin injection, warrant a similar caution in the use of gelatin.

SUMMARY

1. The postmortem findings of 23 patients receiving intravenous gelatin therapy, who expired as a result of medical and neoplastic disease or from complications of therapeutic surgical measures, are reported as demonstrating the etiologic rôle of gelatin in the pathogenesis of renal hydropic changes morphologically similar to those produced by the intravenous injection of sucrose.

2. The morphology of the renal lesion produced by gelatin is described and compared with that of sucrose nephrosis.

3. Gelatin nephrosis is shown to be a reversible change occurring within half an hour after the intravenous injection of gelatin and disappearing within 120 hours following the last injection.

4. Studies of the effect of the renal lesion on renal function are reported as inconclusive but 2 suggestive cases are discussed, one showing diuresis and the other oliguria following the intravenous administration of gelatin. Caution is suggested in the use of this substance in patients exhibiting previous renal impairment.

5. Attention is directed to the possible rôle of gelatin in promoting circulatory disturbances and 3 cases are reported as suggesting gelatin as a potential precipitating factor in coronary occlusion and vascular thrombosis in the presence of circulatory disturbances and vascular disease.

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ALLEGED HEMOSTATIC ACTION OF GELATIN, COAGULEN, FIBROGEN AND HISTIDINE ADMINISTERED BY MOUTH

By M. L. Tainter, M.D.; A. H. Throndson, D.D.S., and A. P. Richardson, M.D., San Francisco, Calif.

IN a previous paper, we discussed the alleged hemostatic value of calcium. viosterol, styptysate and ceanothyn, when used orally under conditions of dental practice.[1] It was found that prolonged administration of these agents in maximum doses failed to shorten the blood coagulation time.

From the Department of Physiology and Pharmacology, College of Physicians and Surgeons, A School of Dentistry, and the Department of Pharmacology, Stanford Medical School. The greater part of this work was done at the dental school and supported, in part, by grants from the Research Commission of the American Dental Association and the California State Dental Association.

J.A.D.A., Vol. 26, March 1939 26: 420-427

The present study is a continuation of work with hemostatic agents of animal origin. The methods and procedure were substantially the same as those of the previous paper, and the details need not be repeated here. Briefly, the procedure consisted of having subjects take the agents by mouth in maximum or recommended dosage each day, and measuring the coagulation time twice daily during the period of medication, and during similar periods before and afterwards. The drug administration was continued from ten days to two weeks, and the average coagulation time for this period calculated. This was compared with the

average coagulation time observed in the same subject during the two weeks preceding and immediately following the medication. These averages were analyzed statistically to determine whether the differences observed were of sufficient magnitude to be beyond the range of spontaneous or chance fluctuation. The reasoning involved in these statistical considerations has been discussed in the previous paper.[1]

It is sufficient to point out that, if the critical ratio between the periods being compared is greater than 2, there is about a 21 to 1 chance against such a difference arising from chance alone, and therefore it can reasonably be assumed that the difference is a true one. When such a reliable difference is found, the second question may then be considered as to whether the change observed is of sufficient magnitude, or in the proper direction, to be of therapeutic value under clinical conditions.

The agents studied are currently used by mouth as hemostatics, and are asserted to be definitely valuable. As these agents have been used by us solely by mouth, as in dental practice, we are not concerned here with their possible value when applied locally, or perhaps injected hypodermically. There have been so many papers written about the hemostatic value of these materials that it is not possible to recapitulate the substance of these reports. The literature has been thoroughly considered and critically reviewed by Gold,[2] and those interested are referred to that summary. Only the reports pertinent to the agents investigated by us are considered here with each agent.

GELATIN

Gelatin has had a considerable vogue as a hemostatic for preoperative use in tonsillectomies, extractions and other operations where excessive bleeding might be anticipated. It is commonly mentioned in dental textbooks as a drug which may be given before operations to minimize or prevent bleeding. Such administration is almost exclusively made orally, since the hypodermic injection of this agent is not a simple procedure, nor is it suited to the ordinary conditions of dental practice. It is difficult to understand why gelatin should act as a hemostatic when taken by mouth, since it is a food protein, the composition of which is the same as that of most other protein foods. Therefore, administration of gelatin should give about the same result as that of any protein meal. However, because of the hemostatic claims, it was thought necessary to make the trial under clinical conditions.

Six subjects made control observations on themselves twice daily for from ten days to two weeks. Then they took, for a similar length of time, 15 gm. of powdered gelatin three times a day, continuing the observation of coagulation time. After the period of medication, there was a similar observation period. The average coagulation time for each subject is shown graphically in Figure 1.

It can be seen that in one subject, the coagulation time was prolonged; in two, it was unchanged, and in three, it was slightly decreased as compared with the control periods. The accompanying table contains a summary of the actual values and their statistical validity. For the entire group of six subjects, the average coagulation time during all control periods was 196 seconds. During gelatin administration, the value was 194 seconds, or a difference of only two seconds. The averages show that, in three trials, there were definite decreases, the critical ratios being larger than 2, which was arbitrarily selected as being reliable. In one trial, there was an equally valid difference in the opposite direction, and in the two others, the differences were not beyond the range of chance fluctuation. In those subjects in which valid decreases were observed, these were only of the order of magnitude of from twenty to thirty seconds out of a total time of about

three minutes. It is extremely doubtful whether a change so small could be of any therapeutic value. Therefore, the changes were not of such size or uniformity of direction as to justify expectation of clinical effects from gelatin.

COAGULEN

Coagulen is a tissue extract prepared from bovine blood, and marketed by the Ciba Company, to whom we are indebted for the material used in this study. It is said to contain natural coagulating ferments present in blood and is claimed to be effective when administered by mouth in the firm's advertising literature and in some clinical reports.[3] However, this claim has not been allowed by the Council on Pharmacy and Chemistry of the American Medical Association.[4]

In the present trials, 5 gm. of coagulen was taken daily by mouth by each of seven subjects, as recommended by the manufacturer; that is, the contents of a 5-gm. bottle were dissolved in 200 cc. of distilled water and taken in three portions during twenty-four hours. The subjects in this group made control observations for from nine to sixteen days before and after the medication, which was continued for ten days. The average coagulation time was decreased eighteen seconds, as shown in the table. However, Figure 1 shows that in only two of seven subjects was the decrease of any considerable magnitude. The greatest average decrease was only fifty seconds, and, in at least four of the seven subjects, the changes were so small as to be within the range of spontaneous variation, as shown by the low critical ratios in the table. In

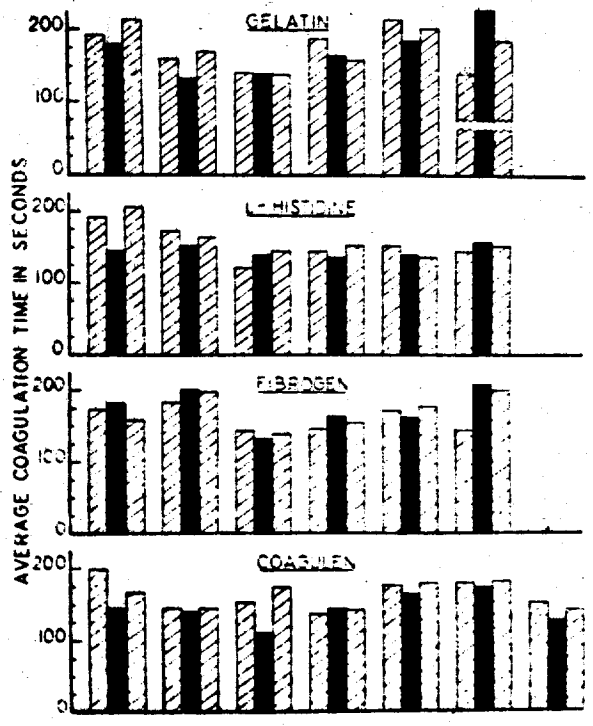

Fig. 1.—Average coagulation times of blood in study of alleged hemostatics administered by mouth. The solid columns represent the average time in each subject during the period of medication, and the cross-hatched columns the average values in the control periods, before (left column) and after (right column) medication.

only two of the subjects were the changes great enough to be both beyond the range of chance fluctuation and large enough to indicate some possible, if only moderate, clinical value. However, since ten days of continued medication, which is longer than commonly practiced clinically, produced no useful effects on blood coagulation in five of seven subjects, it seems clear that the use of coagulen by mouth cannot be relied on for controlling postoperative hemorrhage.

AVERAGE COAGULATION TIMES OF BLOOD IN COMBINED CONTROL PERIODS AS COMPARED WITH PERIODS OF MEDICATION WITH DIFFERENT ALLEGED HEMOSTATICS*

Subject	Control Period				Medication Period					
	Days	Observations	Coagulation Time Seconds	SD Means Seconds	Days	Observations	Coagulation Time Seconds	SD Means Seconds	SD Diff. Means Seconds	Critical Ratio
			Gelatin (15 gm. three times daily)							
Tr.	31	61	164	5.0	16	31	130	6.5	8.2	4.1
Fy.	20	40	209	2.6	10	20	185	2.8	3.8	6.3
Hi.	20	40	141	5.3	15	30	141	5.1	7.3	0.0
Cs.	20	39	177	5.0	10	20	167	6.3	8.0	1.25
Br.	27	53	283	6.7	11	22	357	12.0	13.7	5.4
Tn.	30	59	204	3.2	14	28	181	2.8	4.2	5.5
Average			196				194			
			Coagulen (5 gm. daily)							
Fy.	23	45	149	5.2	10	20	126	7.3	8.95	3.9
Br.	19	38	146	4.8	10	20	140	7.0	8.5	0.71
Cs.	20	40	163	3.5	10	20	113	4.9	6.0	8.4
Rn.	32	59	140	3.1	10	20	145	6.2	6.9	0.72
Tn.	20	40	181	6.1	10	20	175	4.5	7.5	0.93
Fy.$_2$	17	33	181	4.4	10	20	142	5.1	6.7	5.8
Cs.$_2$	20	40	177	4.4	10	20	164	9.3	10.2	1.3
Average			162				144			
			Fibrogen (15 cc. daily)							
Er.	14	28	164	4.2	7	14	181	3.0	5.2	3.3
Tn.	29	58	190	3.3	16	32	201	3.7	4.9	2.2
Fy.	20	39	165	6.1	10	20	209	9.5	11.2	3.9
Br.	19	37	152	4.3	10	20	164	9.8	10.7	1.12
Cs.	20	40	175	3.8	10	20	163	5.2	6.4	1.9
Rn.	30	60	141	3.3	10	20	132	5.2	6.1	1.5
Average			165				175			
			l—Histidine (0.2 gm.) (5 cc. 4 per cent twice daily)							
Tr.	30	59	168	4.6	15	30	152	6.7	8.1	1.97
Tn.	28	55	199	5.3	14	28	145	6.6	8.5	6.35
Br.	20	39	134	4.9	10	20	140	8.6	9.9	0.61
Cs.	20	40	149	3.3	10	20	138	5.1	6.1	1.8
Rn.	30	59	146	3.0	11	21	139	4.9	5.7	1.23
Fy.	20	39	148	4.6	10	20	163	12.0	12.8	1.17
Average			157				146			

*SD diff. means is the standard deviation of the difference between the mean or average of the control period and the period of medication. Critical ratio is the difference between these two means divided by the SD diff. means. The critical ratio should be 2 or greater to give a reasonable probability that the differences are greater than would be expected from chance fluctuation.

FIBROGEN

Fibrogen is also an extract of tissue, marketed by Merrell and Company, to whom we are indebted for a supply of the product. Fibrogen was introduced in an extensive series of papers by Mills and his associates, who asserted that its ingestion by mouth could shorten the coagulation time of blood. These claims, which have been reviewed by Gold,[2] need not be considered here. It is worthy of note that the Council on Pharmacy and Chemistry and the Council on Dental Therapeutics have not accepted the claims of the manufacturer that this material is effective by mouth. However, since it is still so used in dental practice, it was included in the present study. Attempts were made to determine whether prolonged administration under our conditions might have effects which do not occur with the single administrations commonly made.

Fibrogen was taken daily in quantities of 15 cc., freshly prepared material being used to eliminate the possibility that deterioration was responsible for negative effects. The product was kept cool from the time of receipt from the distributor. It was taken by mouth and for the same periods as the agents discussed above, with the exception of one case in which it caused so much nausea in the subject that only seven days' administration was possible.

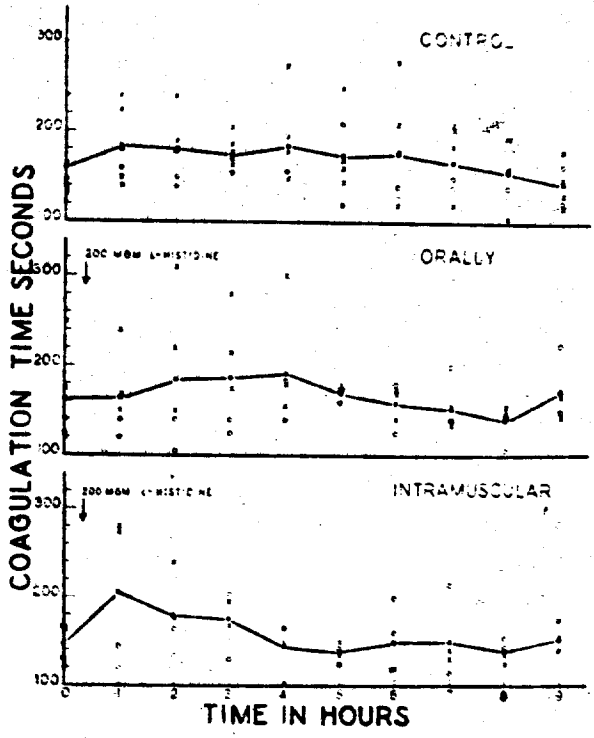

Fig. 2.—Coagulation times of blood in two subjects when 200 mg. of histidine was given by mouth and intramuscularly at 9 a.m., coagulation times being measured at hourly intervals throughout the day; results obtained in five such trials without medication, and in 4 trials, each after oral and intramuscular administration. The curves represent averages of the coagulation times.

The table shows that the average coagulation times for the entire group of subjects were increased ten seconds. In four of the six subjects, the average time was increased, and in only two decreased.

In view of the fact that both the increases and the decreases were relatively small, and within range of spontaneous variation, the fibrogen had no consistent or significant effect on the coagulation time. It can be concluded that administration of fibrogen in maximal doses by mouth for many days at a time did not effectively speed up the coagulation of blood, and therefore it lacks clinical efficiency as an oral hemostatic.

HISTIDINE

Histidine is an amino-acid derived from proteins, and therefore is a basic constituent of some of the agents considered above. Under the name "Larostidin," it has been recently introduced into medical practice for the treatment of gastric ulcer. In connection with studies of its value in gastric ulcer, Burger and Schrade[5] and Kohl[6] asserted that injection of this agent or its administration by mouth shortens the coagulation time of blood about 50 per cent. The same claim has been made by Hicks,[7] but is denied by Bloch *et al.*[8] Because of the contradictory nature of these reports, it was thought desirable to determine whether oral administration could actually decrease the coagulation time.

Histidine was used as the hydrochloride salt of the levo-isomer which is marketed under the name of "Larostidin" by Hoffman La Roche, Inc., to whom we are indebted for the material used in this study. Six subjects took material twice a day from ten to fifteen days. Each dose was 5 cc. of a 4 per cent solution, or 0.2 gm. of histidine taken with a small amount of water. The coagulation time was measured from three to five hours after the administration, when the coagulation time is claimed to be decreased most, according to previous reports. The control observations were made for two weeks before and after the periods of medication.

The table shows that the coagulation time for the entire group of six subjects was decreased an average of only eleven seconds during the histidine medication. In only one subject was there a decrease of sufficient magnitude to give a critical ratio greater than 2, this change being an average shortening of forty-four seconds. Figure 2 shows that in five of the six subjects, the changes were easily within the range of spontaneous variation. Since administration of histidine failed in the majority of subjects used, it was not demonstrably effective by mouth on blood coagulation. Therefore, the previous reports of hemostatic action could not be confirmed.

However, the considerable number of reports asserting that there was a reduction of 50 per cent in blood coagulation time were sufficiently definite, even though inadequately controlled, to make it desirable to test these claims by another procedure. Burger and Schrade's data showed that the maximum effect on coagulation time was obtained at the end of three hours. Although our first coagulation times were measured at the alleged time of maximum effect, there might have been the possibility in our subjects that the effect was delayed, or perhaps missed by an improper selection of the time interval. Therefore, additional tests were made to show the progressive change in the coagulation time after histidine administration, if any. The coagulation time was determined at hourly intervals throughout the entire day, and the histidine was taken after the first control reading in the morning. In this way, it was hoped to detect any shortening in the coagulation time which might have been missed previously. Two subjects were used for repeated observations over a number of days. Experimental days with hourly control observations, but without histidine, were carried through alternately with the days of medication, so that variations in coagulation time for different hours of the day would be determined and allowed for. The results of these observations are presented

graphically in Figure 2. Six complete day-long tests were performed as controls, with the average coagulation times fairly uniform throughout the entire day, at about 150 seconds. When 200 mg. of histidine were taken by mouth, immediately after the first or control observation in the morning, there was no change in the coagulation time in the five tests made beyond that due to spontaneous fluctuation or chance variation. Therefore, there was a complete failure to confirm the 50 per cent reduction in coagulation time claimed abroad.

It was thought that possibly the histidine might be more effective by injection, since this is the usual method of administration in the treatment of gastric ulcer, although this therapeutic use is now discredited. Therefore, four more tests were performed by the same technic except that the histidine was injected intramuscularly. Figure 2 shows that there was again no decrease in the average coagulation time, the greatest change, as a matter of fact, being an apparent prolongation of the time beyond that of the control. This change, however, was not of statistical significance in view of the variability of the individual measurements. At least, the histidine injection did not shorten the coagulation time.

Our results with histidine, therefore, confirm the negative results of Bloch *et al.*[8] Since there was a complete failure to demonstrate a shortening of coagulation time by oral administration and intramuscular injection of histidine under controlled conditions, statements regarding the alleged shortening of coagulation time by others cannot be accepted.

COMMENT

The observations made by us again demonstrate that there is no reliable means of shortening the coagulation time of blood with agents given by mouth. Our trials differ from those commonly made in that the administration of agents has been continued for periods of about two weeks, and during these periods repeated measurements of the coagulation time have been made. In the trials of this report, there have also been made hundreds of measurements of the coagulation times before and after medication, which give reliable average values for the normal times. From these extensive control observations, the significance of variation during the period of medication can be determined with some certainty. No one of the four alleged hemostatic agents tested was found to have any value when administered by mouth. Therefore, there is no rational basis for their further use for this purpose in dentistry.

There are methods of controlling hemorrhage which are effective and which should be employed when the need arises, rather than depending on giving the patient something by mouth which has been clearly demonstrated to be useless. The clinical problem of hemorrhage in dentistry can best be met by local application of hemostatic agents. That is, the bleeding may be controlled, first, by direct pressure, either digital, or pack, or by suturing, which keeps the vessel closed long enough for a clot to form in the lumen, or, second, by direct application of a vasoconstrictor such as epinephrine or kephrine, which will keep the lumen occluded by constriction of the smooth muscle until the clot can form.

If these two methods are not successful, an attempt can be made to speed up blood coagulation by local application of agents which are effective. Fibrogen or thromboplastin-local can be applied on a tampon to the bleeding area, and may speed up the coagulation process. More potent than these agents is the newly introduced venom of the fer-de-lance snake, which is reported to be effective in promoting blood coagulation in purpura hemorrhagica, and even in hemophilia, when applied locally. Since by proper use of these local measures, almost any hemorrhage can be adequately handled, the need for an orally adminis-

ered drug should not arise. In fact, it is quite evident from the negative results of our studies, as well as those of the more critical observations reported by others, that reliance on hemostatic agents administered by mouth is unwarranted, and may not be to the best interests of the patient.

CONCLUSIONS

1. The coagulation times of healthy subjects were measured twice daily and continuously for periods of many months. During this time, two-week intervals of oral medication with some alleged hemostatic agents were alternated with periods without medication. The average coagulation times during the periods without medication, before and after medication, were compared with those when the agents were administered. The average values so obtained were analyzed statistically for their probable reliability to determine whether the changes could be accounted for on the basis of chance fluctuation in the individual measurements.

2. Administration of gelatin by mouth three times daily in doses of 15 gm. did not change the coagulation times in six subjects.

3. Administration of 5 gm. of coagulen daily by mouth also did not shorten the coagulation time in five of seven subjects, and in two produced changes of no real clinical significance.

4. Fibrogen administration in daily doses of 15 cc. by mouth caused no changes in the coagulation time of six subjects.

5. Histidine administration in doses of 0.2 gm. twice daily by mouth caused a shortening of the coagulation time in only one of six subjects. Therefore, these negative results in the majority were not in keeping with the statements made regarding it as an effective hemostatic when given by mouth.

6. In two other subjects, oral or intramuscular administration of the same dose of histidine did not reduce the coagulation time when observations were made at hourly intervals throughout the day. Therefore, no confirmation was made of prior statements that histidine shortens the coagulation time 50 per cent at the end of three hours.

7. The results of this study, taken with those previously reported in THE JOURNAL and from this institution, leave no doubt that the agents commonly advocated for oral administration as hemostatics in dentistry are not effective when so used. The dental practitioner is advised to direct his efforts at controlling hemorrhage to local application of demonstrably effective agents rather than to try to speed up the coagulation process by systemic medication.

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Jour. Lab. and Clin. Med. 32(9): 1115-1120, 1947

INTRAVENOUSLY ADMINISTERED GELATIN—A TOXICITY STUDY

MILTON J. VANDER BROOK, PH.D., STANLEY C. LYSTER, BOYD E. GRAHAM, B.S., NOEL E. POMEROY, AND GEORGE F. CARTLAND, PH.D.
KALAMAZOO, MICH.

IT IS generally recognized that an infusion of blood or plasma is the most effective means of compensating for fluid loss which occurs in hemorrhage and shock of various types. Since blood and plasma are not always immediately at hand, however, it is desirable that a plasma substitute be found which is innocuous, effective, inexpensive, and readily available.

The recent literature records that substitutes somewhat less effective than plasma in restoring circulating fluid volume, but much more effective than crystalloidal solutions, are aqueous solutions of macromolecular substances, such as gelatin, gum arabic, pectin, ovalbumin, polyvinyl alcohol, and methyl cellulose. Gelatin[1-20] appears to possess desirable characteristics to a greater degree than most of the other macromolecular substances mentioned.

Observations in this laboratory on the toxic effects in animals of infused gelatin solutions which were subsequently tested for efficacy by laboratory animal experimentation[11, 18] and clinical investigation [15, 16, 19, 20] confirm the findings of others in many respects. Some of the undesirable properties of gelatin, however, such as impairment of the production of plasma proteins and hemoglobin,[17] and degenerative and proliferative arterial lesions of the sclerosing type reported by Heuper[6] in dogs, are apparently observed only when large amounts of highly concentrated gelatin solution are repeatedly infused into the blood stream of intact animals, thus placing a severe strain on the circulatory system. When a 5 to 8 per cent gelatin solution is infused into dogs or human subjects suffering fluid loss from hemorrhagic, traumatic, or burn shock in amounts approximately equal to the amount of fluid which has escaped, such untoward effects have not been reported.

The experiments herewith presented were designed to test whether or not the circulatory system of dogs under the stress of light anesthesia and severe blood loss could tolerate large quantities of 5 per cent gelatin administered in divided doses over a period of several weeks.

The solutions of gelatin* were prepared by electrodialysis of osseous calcium gelatinate. The 5 per cent solutions preserved with phenyl-mercuric-borate, 1:25,000, were degraded by autoclaving to obtain a mean molecular weight of 20,000 (number average). These solutions were nonantigenic and nonpyrogenic.

EXPERIMENTAL

Nine mongrel dogs supplied with a high protein diet supplemented with raw liver were bled of 29 to 54 per cent of their estimated blood volume at weekly intervals for periods of two to thirteen weeks (average, seven weeks). The blood, aseptically removed from the

From the Research Laboratories, The Upjohn Company.
Received for publication, April 28, 1947.
*Supplied by Dr. S. H. Eppstein, of these laboratories.

exposed jugular veins under light ether anesthesia, was replaced at the same site by infusion of 5 per cent gelatin solution in amounts equal to 80 per cent of the blood removed. Control animals consisted of two dogs bled just as severely as those mentioned, but with no parenteral fluid replacement, and twenty apparently healthy dogs with no treatment whatsoever.

At intervals determinations of plasma protein and gelatin concentration in plasma, urine, and aqueous tissue extracts* were made by the method of Waters[4] which makes use of the fact that gelatin is not precipitated by trichloracetic acid but is precipitated by acid tungstate. Plasma protein nitrogen was thus determined by the usual Kjeldahl technique on the trichloracetic acid precipitate and gelatin nitrogen by difference between nitrogen contents of the acid tungstate and trichloracetic acid precipitates. The usual methods were employed for determination of red cell volume, hemoglobin, red cell count, white cell count, and sedimentation rate.[22]

Extensive pathologic studies on tissues of experimental and control dogs were done.†

On one of the nine dogs, a celiotomy was performed both prior to and one week after a series of six hemorrhages and gelatin infusions. Liver tissue was removed and examined at these times. Seven weeks later this dog was sacrificed and the tissues examined. A second dog was bled and infused with gelatin five times after a celiotomy had been performed for removel of liver tissue. He was sacrificed two weeks after the last hemorrhage and infusion.

RESULTS

Repeated massive hemorrhages and infusions of purified gelatin in dogs under the conditions described produced no notable changes in deportment or body weight (Table I). The only gross disturbance observed was the formation of thrombi at the sites of injection in some dogs which Patek and co-workers[24] have demonstrated to be due to the mercurial preservative employed. Histologic examination of the jugular veins at autopsy revealed no changes in the intima. Considerable scar tissue formed about these veins, presumably due to the surgery involved, and fluid regurgitated into surrounding tissue when the needle was withdrawn. Wound healing was not impaired. There was no indication of the presence of pyrogenic or antigenic material in the gelatin.

TABLE I. SHOWING THE EFFECT OF REPEATED MASSIVE HEMORRHAGES AND GELATIN INFUSIONS ON THE BODY WEIGHTS OF DOGS

DOG	NUMBER OF HEMORRHAGES AND INFUSIONS (WK.)	INITIAL BODY WEIGHT (KG.)	CHANGE IN BODY WEIGHT (KG.)	AMOUNT OF BLOOD REMOVED (ML.)	AMOUNT OF 5% GELATIN INFUSED (ML.)
2	13	12.3	+2.6	5,668	4,534
3	12	12.4	+6.4	7,366	5,893
4	3	19.8	+0.2	2,345	None
5	12	15.6	+4.8	8,531	None
6	7	15.5	+1.3	3,532	2,826
10	7	9.5	+0.8	2,250	1,800
11	7	13.4	+0.1	3,530	2,824
12	2	11.6	+2.4	905	725
13	2	10.2	+0.7	640	520
14	6	19.8	−1.3	3,155	2,524
15	5	19.0	−0.8	2,410	1,928

*Weighed quantities of kidney and liver from both control and gelatin-infused dogs were thoroughly macerated with saline in a Waring blendor. Gelatin in the supernatant fluid was determined by the method used for plasma gelatin.

†By Dr. Walter Schiller and Dr. Bruno Volk.

Examination of blood and plasma during the experimental period revealed that the changes observed in hemoglobin and plasma protein levels could be accounted for by the severe hemorrhage alone and were not markedly affected by the infused gelatin (Tables II and III).

TABLE II. HEMOGLOBIN, RED CELL VOLUME, AND PLASMA PROTEIN NITROGEN LEVELS OF DOGS SUBJECTED TO WEEKLY MASSIVE HEMORRHAGES BOTH WITH AND WITHOUT REPLACEMENT OF THE WITHDRAWN BLOOD BY GELATIN SOLUTION

	TEST GROUP (WITHDRAWN BLOOD REPLACED BY GELATIN)									CONTROL GROUP (NO PARENTERAL FLUID REPLACEMENT)					
	DOG 2			DOG 3			DOG 6			DOG 4			DOG 5		
TIME (WK.)	Hg. (GM. %)	CELL VOL. (%)	PPN (Mg. %)	Hg. (GM. %)	CELL VOL. (%)	PPN (Mg. %)	Hg. (GM. %)	CELL VOL. (%)	PPN (Mg. %)	Hg. (GM. %)	CELL VOL. (%)	PPN (Mg. %)	Hg. (GM. %)	CELL VOL. (%)	PPN (Mg. %)
1		48.1	1,010	14.0	45.6	843	16.5	48.9	978	20.8	58.8	924	11.6	36.4	811
2	13.2	44.0	930	8.9	31.8	825	18.5	55.0	1,025	13.6	44.4	890	8.9	31.3	865
3	12.2	40.8	977	11.0	41.4	840	13.9	44.1	1,022	11.0	36.6	838	9.5	32.1	728
4	11.2	39.6	1,006	11.4	40.8	735		31.3	873				9.9	36.3	903
5	(No hemorrhage or infusion)			14.8	46.8	886	10.4	37.5	914				9.3	32.5	923
6	13.8	46.8	969	12.6	45.6	829	9.3	35.0						27.5	777
7	11.6	40.8	879	14.0	46.8	861		31.3	919				9.9	35.0	855
8	11.0	40.8	904	13.4	45.0	847							10.1	37.5	
9	10.7	39.6	992	14.6	47.5	926								30.0	817
10	11.0	40.8	904	13.4	43.8	840							9.4	35.0	876
11	9.5	35.4	908	11.6	40.6	797							9.1	33.5	
12	9.7	32.9	956	12.8	45.0	792							7.9	30.5	948
13	8.3	31.3	898												
14	9.7	36.0	889												

TABLE III. ILLUSTRATING TYPICAL RATES OF PLASMA PROTEIN REGENERATION AND DISAPPEARANCE OF PLASMA GELATIN IN DOGS (DATA FROM DOG 3 OBTAINED ON TENTH WEEK OF HEMORRHAGE AND INFUSION STUDIES; FOR FURTHER CONDITIONS OF EXPERIMENT, SEE TEXT)

TIME (HR.)	PLASMA PROTEIN NITROGEN (MG. %)	PLASMA GELATIN NITROGEN (MG. %)	SEDIMENTATION RATE (MM/HR.)
Before hemorrhage	840	0	1.0
Blood withdrawn (658 ml. or 3.6% of body weight) and 520 ml. of 5% gelatin infused			
1/4	326	281	70.0
2	435	178	56.0
4	482	139	50.0
7	541	110	37.0
11	566	90	30.0
16	621	65	27.0
22	701	35	28.0
30	730	32	27.0
48	774	18	43.0
168	797	12	1.0

The sedimentation rate of erythrocytes was greatly accelerated by the infused gelatin. In most instances the sedimentation rate decreased approximately at the same rate as the gelatin concentration in the plasma decreased. In all instances, the rate had returned to normal the following week (Table III).

Plasma gelatin determinations revealed the presence of approximately 80 per cent of that infused at one-fourth hour after infusion, 50 per cent at four hours, and 25 per cent at sixteen hours. Urine gelatin determinations revealed that only 37 to 62 per cent of the gelatin infused was excreted unchanged (Table IV). This occurred within twenty-four hours. Gelatin determinations on aqueous extracts of the liver and kidneys of one dog at autopsy eight days after thirteen infusions indicated that there was no free gelatin stored in these organs.

TABLE IV. RATE OF APPEARANCE OF GELATIN IN URINE FOR THREE SUCCESSIVE WEEKS (DATA FROM DOG 3)

FIRST WEEK		SECOND WEEK		THIRD WEEK	
PERIOD AFTER INFUSION (HR.)	AMOUNT OF GELATIN IN URINE (% OF GELATIN INFUSED)	PERIOD AFTER INFUSION (HR.)	AMOUNT OF GELATIN IN URINE (% OF GELATIN INFUSED)	PERIOD AFTER INFUSION (HR.)	AMOUNT OF GELATIN IN URINE (% OF GELATIN INFUSED)
0-2	38.5	0-6	18.8	0-6	36.7
2-16	23.0	6-18	18.3	6-18	20.3
16-40	0.0				
Total excreted	61.5		37.1		57.0

Observations at autopsy revealed no gross pathology, except for scar tissue and thrombi in some instances at the sites of bleeding and gelatin infusion as noted previously.

Histologic examination of the heart, coronary vessels, aorta, carotid arteries, jugular veins, and renal arteries showed, in some instances, changes attributable only to prior disease. There was no vacuolization or subendothelial edema of the arteries and arterioles. Sections of the trachea, lungs, stomach, intestines, spleen, gall bladder, adrenals, striated muscle, brain, meninges, and gonads likewise showed no changes attributable to gelatin.

The parenchyma of the liver was normal. Many of the Kupffer cells, however, in all gelatin-infused dogs contained sudanophile droplets. Those in the areas of the acini around the periportal fields were most affected. The Kupffer cells in nine of twenty-four control sections of liver tissue showed moderate fat staining. The Kupffer cells of human liver, according to Levine,[28] normally contain neutral fat. The Kupffer cells of one of the dogs from which a portion of the liver was removed prior to gelatin infusions were fat-free. Immediately after completion of the series of infusions, the Kupffer cells were loaded with sudanophile droplets. At autopsy, seven weeks later, the same condition obtained. The nuclei of the Kupffer cells appeared normal in all cases.

Histologic examination of the kidneys of gelatin-infused dogs showed, in some instances, slight departures from the normal. The epithelial cells of the tubules of the proximal convolutions were sometimes swollen and occasionally vacuolated. Since gelatin is cleared through the kidneys, it is conceivable that in some animals it may have been responsible for the slight pathology observed.

CONCLUSIONS

A highly purified and highly degraded 6 per cent gelatin solution prepared especially for intravenous therapy in human subjects was well tolerated by dogs when given repeatedly for several weeks in amounts commensurate with the fluid lost by hemorrhage. The only morphological pathology which might be attributable to gelatin in this series of dogs was the presence or increase of sudanophile droplets in the Kupffer cells. The significance of such a change is not known. It appears that the function of the Kupffer cells was not materially altered. None of our dogs developed infection of the respiratory tract or at the sites of bleeding and infusion.

The vascular pathology described by Hueper[6] was not observed in our series. Although Morehead and Little[21] found degenerative and reparative changes in the vascular system of their gelatin-infused dogs, they concluded that the lesions were not due to gelatin since similar changes were present in their control series.

Of the blood constants determined, only the sedimentation rate was altered by gelatin. This phenomenon is common to all macromolecular substances and occurs also when plasma protein concentrations are elevated. Gelatin did not interfere with the formation of hemoglobin or plasma protein, both of which were partially depleted at each hemorrhage, when infused weekly for periods as long as three months.

Gelatin appears not to be stored in the liver or kidneys. A major portion is excreted by the latter. The fate of the balance is unknown.

SUMMARY

1. Gelatin solutions prepared for intravenous therapy proved innocuous to dogs when administered intravenously following repeated massive hemorrhages over a period of several weeks.

2. Vascular pathology was absent. The presence or increase of sudanophile droplets in the Kupffer cells was the only morphologic change attributable to gelatin.

3. Gelatin did not interfere with the formation of hemoglobin or plasma protein and appeared not to be stored in the liver or kidneys. A major portion was excreted by the kidneys.

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PROBLEMY GEMATOLOGII 13:53-62, JUN.'68

The current status of the question of plasma substitutes from gelatin (literature survey).

by T. V. Znamenskaya, Leningrad Institute of Hematology and Blood Transfusion. (Director - A. D. Belyakov).

From the time that Hogan (84) in 1915 first used the injection of a gelatin solution for a blood substitute during fatal blood loss more than half a century has passed. Since that time many plasma substitute from gelatin have been proposed.

Gelatins offer denatured protein separated from a collagen of living tissue. In a chemical analysis gelatin includes a large quantity of glycine (nearly 30%) and does not contain the amino acids tryptophan, tyrosine or cystine, which makes it ineffective for parenteral nourishment without the addition of these amino acids.

Gelatin plasma substitutes were first used abroad at the time of World War II; our Soviet preparations were manufactured somewhat later.

1. Solutions of autoclaved gelatin

Autoclaved of partially hydrolyzed gelatin (4-8% osseous gelatin in an isotonic solution of sodium chloride) was extensively used in the WWII period for a blood plasma substitute. This solution is prepared by autoclaving apurified collagen of bovine bones. The solution thus obtained gels at room temperature; before application it must be heated. Of a sterile, humanly non-toxic solution of such gelatin, nearly 50% is retained in the circulatory system after 24 hours. In a chemical analysis, hydrolyzed gelatin appears as a colloidal solution and consists of a mixture of peptides of various molecular weights. An increase in the period of autoclaving produced decomposition products of protein with small molecular weights. Such a solution remains liquid at room temperature, but its effectiveness is insignificant. By the method of fractionation 3 fractions can be obtained from hydrolyzed gelatin, with molecular weights of 69000, 36000, and 20000. Knoefell and Lehmann (93) determined that the period for retention of these fractions in the vascular system depends on the magnitude of the molecular weight: low molecular weight fractions are quickly dissipated in the circulatory system and excreted in the urine. Arterial blood pressure in animals with normal capacity blood circulation (OTsK) quickly increases and slowly normalizes independently of the magnitude of the molecular weight.

During experiments and clinical studies were demonstrated high effectiveness, complete harmlessness and the absence of antigen qualities of preparations of autoclaved gelatin in doses to 2000 ml (52, 94,95,98,118). The use of gelation solutions in experiments during hemorrhagic shock showed that they quickly raised and stably maintained arterial blood pressure, normalized heart activity and respiration (95, 118),and even increased the amount of plasma circulation (OTsP) (85).

As early as 1896 it was established that gelatin solutions accelerate coagulation of the blood (15). Somewhat later E. I. Renard (33) also discovered the increase in blood coagulation rate after the

infusion of gelatin solutions. However, preparations of autoclaved gelatin do not show a significant effect on the coagulation of the circulatory system (69, 94, 95, 122). After the infusion of gelatin solutions, ROE (sedimentation rate) was significantly accelerated (69, 93, 95, 124, 138). The injection of large quantities of gelatin solutions was accompanied by a dilution of the blood and an increase in its content of residual nitrogen.

Regardless of its deficiency in some irreplaceable amino acids, gelatin can participate in the metabolism of some substances. Brunschwig and coauthors (56) detected a positive balance of nitrogen in dogs which subsisted on a no-protein diet and into which gelatin solutions were intravenously injected. Rhode and coauthors (125) in analogous experiments found an increased volume of urea in the urine as an end product of protein metabolism. The addition of protein hydrolyzates to the gelatin solution significantly increased its assimilibility and produced a positive balance of nitrogen (97).

Gelatin solutions do not cumulate in the organism; they go primarily into the urine, as is apparent in further studies, and may participate in the metabolism of substances within the organism since in all the cells and tissues proteolytic ferments of fissionable gelatin can be found. For this use, the greater preference is for all gelatin preparations in comparison with synthetic plasma substitutes, which can not participate in the metabolism of substances in the organism.

After the injection of gelatin solutions, no variation in the tissues of the liver, kidneys or adrenals could be found (64, 96). We have only isolated reports about the appearance of temporary vacuolization of the renal epithelium after the injection of autoclaved gelatin (80, 122, 134). These events do not always occur, not reoccuring and quickly disappearing in the functional condition of the kidneys.

The relative lenghth of the residence of gelatin preparations in the blood stream has no corresponding data. Experiments on healthy dogs performed by Little and Dameron (100) 30 minutes after the injection of gelatin revealed 50% of the injected solution in the blood stream, but Holt and Knoefell (85) determined that 66% of the injected gelatin disappeared from the blood plasma after 60 minutes. During a decrease in the volume of blood as the result of blood loss, gelatin disappears from the plasma with the same speed as in normal animals (101). It is indubitable that the speed of the disappearance of gelatin preparations from the vascular system is connected to the magnitude of their molecular weights, being larger for the low molecular weight preparations (52, 83, 102). After the injection of gelatin some difference in its quality was found in the vascular system, making it possible to explain that the studies are not exactly identical in relation to the molecular weight of the preparation, as during hydrolytic cleaving. Of compounds with high molecular weights, gelatin, for example, it is impossible that peptides are obtained exactly identical according to the magnitude of the molecular weight. Besides that, gelatin solutions disappear from the circulatory system during the period of injection of the preparation since the low molecular weight peptides resulting from autoclaving of the preparation are rapidly excreted in the urine.

2. Oxypolygelatin (OPG)

In 1951 Pauling suggested oxypolygelatin as a gelatin plasma substitute, visualizing oxidized hydrogen peroxide as a product of the polymerization of gelatin with glyoxal. OPG is retained in the blood

stream of the liver to the extent of 25-50% for 6 hours and does not congeal at room temperature; its liquifaction zone lies in the range of 10 to 13°C; consequently it is more suitable for transfusions than solutions of autoclaved gelatin.

In the process of manufacturing, OPG is derived partially from the hydrolysis of gelatin and, therefore, it also appears to be a preparation of various molecular weights with a median weight of 20000 to 35000 (including peptides with molecular weights of 10000 to 100000) (104).

OPG is non-pyrogenic and non-toxic, and does not cause an antigen reaction in experiments (60, 63, 123) and clinics (63, 79). During subcutaneous injections of such gelatin there were no negative reactions.

The effectiveness af OPG as a plasma substitute was studied in experiments after loss of blood (during hemorrhagic shock). The injection of adequate quantities of the solution quickly raised falling arterial blood pressure and increased the amount of circulating blood (63, 123, 138). The result of the injection of the OPG solution during experimental burn shock was that the duration of life of the animal increased (109). After the injection of the preparation ROE accelerated significantly, increasing the level of general and residual nitrogen in the blood serum. OPG was used in clinics for the treatment of traumatic shock (89). After the injection of 300-500 ml of the preparation arterial pressure quickly increased, improving the condition of the subject.

The preparation was excreted from the organism through the kidneys. Part of it, as during the use of autoclaved gelatin, appeared in the urine in an insignificant amount (63). Over a period of several days after the injection of 500-1000 ml of the solution traces of protein were found in the subjects urine. OPG does not cumulate in the tissues and organs. Harrfeldt (79), during the histological study of the organs of rabbits after the injection of the OPG solution, did not find any change in the liver, kidney, spleen and heart muscle. However, the method of treatment and staining of the tissues were not completely ideal and did not allow the discovery of minor changes in the tissue of the kidney. In 1964 a report by Czole and coauthors (65) appeared about the fact that after the injection of sick rats with a dose of the OPG preparation vacuolization occurred in the cytoplasm of the cells of the convoluted tubules of the kidneys. In the straight tubules and nephrons these changes were not found.

The authors with great persuasiveness demonstrated that these histological changes are reversible and completely disappear within 8-14 days. It is also established that the vacuolization depends upon the quality of the solution injected and the number of injections. The authors connect the changes found in the kidneys with the differing rates of reabsorption of the injected preparations by the epithelium of the tubules. They resemble those changes in the kidneys which set in after the injection of hypertonic solutions of sugar, sodium sulfate or carbamide (127). The function of the kidneys during these changes was not disturbed, but in some cases during the injection of large quantities of solution diuresis lessened. But this effect is also reversible and quickly passes.

The use of plasma substitutes from heterogenic proteins raises a question about the antigenic qualities of these preparations. For many years purified gelatin has been considered non-antigenic and during the use of gelatin solutions for plasma substitutes an anaphylactic reaction has never been observed. In the literature we have many works confirming the lack of this kind of specificity of this

protein. OPG also seems to be a non-antigenic preparation as regards man and animals (60, 79, 123).

With the development of immuno-chemistry and such sensitive methods as precipitation, in the last year it has been demonstrated that gelatin appears to be an antigenic protein, although an extremely weak one. Maurer (105, 106) first showed that in normal human serum and some types of animal serum can be found antibodies caused by the precipitation of gelatin. The first gelatin preparation studied for immunology was OPG (105). Maurer (108) convincingly shows that immunization to gelatin raises the percentage of antibodies in human blood serum. In rabbits not having anti-gelatin bodies in their serum, antibodies appear after immunization. Native gelatin precipitates larger antibodies than OPG. Partially hydrolyzed acids and proteolytic ferments of gelatin never precipitate antibodies (107). The gelatin-antigelatin system is not connected to a complement and does not cause an anaphylactic reaction in man or animals. Therefore we failed to observe the appearance of antigenic reactions during the injection of gelatin plasma substitutes.

In the last year works appeared by Maurer (108), Sela (131), Arnon (48), and others, permitting clarification of the role of individualamino acids and peptides in the intensification of antigenic properties of various proteins. During this native qualities of proteins are completely preserved. Sela and coauthors (131, 132) caused typical anaphylactic shock in guinea pigs sensitized by polytyrosyl gelatin by the injection of this same substance. A mixture of endocardial gelatin and tyrosine did not yield such a reaction.

By adding to gelatin various polypeptide chains from tryptophan, phenylalanine and cystine, Sela and Arnon (131) obtained a derivative of gelatin with intensely expressed antigenic qualities. The addition of polyglutamyl, polyalanyl or polylysyl to such sensitized preparations in animals did not cause anaphylactic reactions. Peptides from methionine, leucine and glutamic acid increased the antigenic properties of gelatin (71).

However, up till now it is not completely clear whether or not the intensification of the antigenic qualities of gelatin appears as a result of the concentration of its aromatic amino acids or whether that activation develops as a consequence of structural changes of the gelatin molecule adding to it various peptides.

3. Solutions of modified fluid gelatin (MFG)

In the beginning of the 50th year, Tourtellotte (135, 136) with the help of chemical modifications and the elimination of fractionation methods significantly improved the quality of gelatin solutions and produced modified fluid gelatin (MFG) - an effective plasma substitute with a gelling zone of 0-4°C. MFG is obtained by means of the degradation and chemical condensation of gelatin with decarbonized acids or their anhydrides (46). In the last year a series of preparations made on the model of MFG appeared abroad under various names - American and German preparations of MFG, the French preparation Plasmatel, the Indian MG-6 and MG-7, and others.

MFG offers a light-yellow, transparent solution with a comparative viscosity of 2.2. At room temperature it does not congeal. For medical purposes a 3% solution of MFG is used which, according to the capacity, can maintain arterial blood pressure similar to blood plasma of serum albumen. A midmolecular weight preparation of 34000 to 40000 has fluctuations of the molecular weight of peptides within the limits of 10000 to 100000. The majority of molecules have low molecular weights, therefore MFG can easily be removed via the capillary wall. Electrophoretic MFG visualizes a single-component homogenic solution (124).

Hemodynamic qualities of MFG solutions were studied in an experiment during massive blood loss, causing disruption of respiration and cardiac activity (49, 68, 90, 99). In all cases of injection of adequate quantities of the solution it caused anti-shock and substituting action; arterial blood pressure swiftly increased and remained stable at a high level, restoring disrupted functions. Thanks to the injection of MFG all of the animals survived. According to its capacity to increase lowered arterial pressure during blood loss, a solution of modified gelatin correspond to the activity of a 5% albumen solution.

In comparative studies of various plasma substitutes (dextran, MFG, OPG, and salt solutions) during hemorrhagic shock with repeated or delayed intravenous injections of the solutions, the animals which were injected with MFG survived; in the remaining cases a 100% loss of the animals was observed (117). Attempts to use modified gelatin for the treatment of burns was not effective. In these cases better reactions occurred with the injection of preparations of dextran(57, 58).

Clinical studies at length confirmed the conclusions obtained in experiments during the study of the MFG preparation. Lassner and Abouse (99) first studied the plasma preparation in a clinic. The solution is easily transferred by the subjects, not causing pyrogenic or toxic reactions, with no change in the patients temperature or pulse. Analogous data was presented by Julliard and coauthors (87), Bardhan (50) and Zabisch (140), who used the preparation on 20, 50, and 68 patients, respectively, at the time of operation. Repeated injections of Plasmagel also proceeded without reactions.

Hittascher and M. Hascher (81) injected 500 patients with doses of 500 ml of Plasmagel with a better therapeutic effect and without reaction. The injection of modified gelatin during the state of shock and hemorrhage removed hemodynamic disturbances and normalized respiratory activity and the activity of the cardio-vascular system. After the injection of 500 ml of Plasmagel Lassner and coauthors (89, 99), and also Goltner and Laug (73) observed an increase of OTsP on the average of 460-400 ml. On a level with the increase of OTsK, dilution of the erythrocytes occurred, depending on the quantity of injected solution. In several cases a decrease in the transfer of erythrocytes in comparison with the initial level was observed unrelated to the loss of blood.

The entrapment of the preparation in the organism depends on the transfer of blood; in people with a low transfer of blood after the blood loss the gelatin solution is retained in the quantity of 91 and 88% in a period of 4 to 24 hours. In volunteers with a normal transfer of blood this retention of the preparation in the blood stream amounts to only 23 and 4% respectively. Elimination of the solution with the urine in both groups occurred in the same manner, but blood plasma protein after blood loss is restored slowly (68). Solutions of MFG are quickly removed from the vascular system. After 12 hours nearly 30% of the preparation is eliminated, after 24 hours 40-60% (68, 81). The main quantity is removed in one day, the remainder within a period of seven days. After the injection of the solution diuresis increases and the specific gravity of the urine decreases (73, 81). However, protein, leukocytes, and erythrocytes do not appear in the urine. MFG preparations cause short-term and considerably less acceleration of the ROE than OPG or autoclaved gelatin (88). They also do not accumulate in the organism, undergoing cleavage by the tissue proteinases - kathepsin and trypsin (65).

With the use of some synthetic colloid plasma substitutes partialaccumulation may occur in the organs of the reticulo-endothelial system (RES), where they undergo cleavage in the long run. Thus, after the injection of polyvinylpyrrilidone (PVP) the weight of the spleen was observed to increase, but after the injection of polyvinyl alcohol (PVA) Z. A. Chaplygina and V. N. Teodorovich (43) found impurities of the preparation in the cellular RES; however, inhibition of phagocytic activity of the reticulo-endothelium during this injection did not occur. Having received results of similar studies with a Plasmagel preparation, H. Hascher and M. Hascher (81) cite conclusive proof of the absence of accumulation of it in the spleen.

After massive injections of plasma gel, Griem and coauthors (77) discovered in the cells of the convoluted tubules of the kidneys vacuolization of the parenchyma analogous to that which was discovered in them after the injection of other gelatin preparations (75,76) and which appeared after the use of hypertonic solutions of sugar, sorbitol and carbamide (127).

The preparation "Blutersatz G," supplied by the Merck firm, belongs to the solutions of the modified gelatin type (52,104). This is a partially hydrolyzed solution of 6% gelatin with methyl-glucosamine and 2% fructose. The molecular weight of the preparation is 18000. "Blutersatz G" is used in clinics for the treatment and prophylaxis of operational, traumatic and hemorrhagic shock, and also during toxemia and barbiturate poisoning. During shock the preparation increased the OTsK and heightened the blood pressure, and normalized respiration and cardiac activity. The transmissibility of the plasma substitutes was very good (52, 103).

The intravenous injection of 500 ml of the preparation for the prophylaxis of shock increased the OTsK by 540 ml; after one hour its increment comprised 300 ml in comparison with the initial level (104). "Blutersatz G" was excreted from the organism somewhat more quickly than the rest of the gelatin preparations: after three hours there remained in the circulatory system only 50% of the injected solution. This solution does not accumulate in the organism, little of it enters into the metabolism of substances, the food value of the preparation is negligible (52).

4. Gemaktsel

Up till now, all suggested preparations of gelatin to be used for the substitution of plasma were heterogenous in structure, that is, consisted for a large part of peptide chains from 10000 to 100000. In 1962 for the first time a report appeared about the creation of a homogeneous gelatin plasma substitute - the preparation "Haemaccel" from the firm Bering (FRG) (129).

Gemaktsel is obtained from purified bovine gelatin, which decomposes into polypeptides and subsequently, using only peptides with a molecular weight of 12000, synthesizes into a polymer. Each molecule of gemaktsel consists of three polypeptide chains, having a molecular weight of 35000 and a lattice structure. The preparation is isotonic with a relative viscosity of 3.7, not congealing at 3°C. It is very transmissible during intravenous and endocutaneous injections, does not cause local macroscopic and microscopic reactions (113), does not accumulate in the organism, and is easily decomposed by tissue proteinase (129). Using the same distinct recent methods,

Schvick and Freund (130) were not able to obtain from gemaktsel antibodies against gelatin. In the course of two years, the preparation has been thoroughly and resourcefully studied clinically. These studies have confirmed its perfect transmissibility during the injection of up to 1500 ml of the solution, and the absence of a reaction (70, 128).

Gemaktsel appears to be an effective substitute for plasma during therapy and prophylaxis of hemorrhagic shock. Bohmert and Munchen (54) cite the results of 520 observations, in which a quick normalization of arterial blood pressure was noticed at the time of operation after the injection of 500-1500 ml of the solution. Analogous results were published by Brinkmann and Kellersman (55), not observing the changes from the point of view of the blood coagulation, quantity of thrombocytes, and time of coagulation of the blood. After the use of gemaktsel, OTsK increases. Thus, during the injection of 500-1000 ml of the solution OTsK increases by 350-500 ml; during the injection of 1000-1500 ml, by 650-750 ml (70).

During the injection of gemaktsel the function of the liver is not disrupted, as certified by the observation of Killian (91) of 100 subjects, and Scherer and Wunsch (128) of 866 patients during various operations. Distinct changes from the point of view of the spectrum of serum proteins after the use of large doses of the preparation also were not discovered (82). Changes from the point of view of the function of the kidneys after the injection of gemaktsel were not found (110, 113). When 26 patients with kidney ailments (pyelonephritis, glomerulonephritis) were injected with the preparation, Moeller and Sykudes (111) did not find changes in the secretions of the tubules, glomerulic filtration or reabsorption of the urine. However, in 1964 Griem and coauthors (76) studying the histological and physiological changes in the kidneys after the injection of such dissimilar gelatin preparations as gemaktsel discovered vacuolization of the cytoplasm of the cells of the convoluted tubules of the kidneys. These deviations from the normal state were also reversible and quickly and completely disappeared, causing only an insignigicant decrease of diuresis. It should be noted that after the injection of rats with a homogeneous serum, within the cells of the convoluted tubules of the kidneys there appeared more pronounced vacuolization than appeared after the injection of gemaktsel.

One of the criteria for effectiveness of the plasma substitutes was the duration of its residence in the circulatory system. The length of the half-life of gemaktsel, that is the time during the course of which 50% of the injected solution disappears from the blood, consists on the average of 5 hours. After 24 hours only traces of the preparation can be detected in the circulating blood. Basically, this quantity is excreted during the course of one day after the injection (82). Nearly 75% of the gemaktsel was excreted in the urine. The preparation does not inhibit the determination of serological tests (74) and can be used for the propagation of serum and the suspension of erythrocytes (92).

In 1963 the gemaktsel of the Bering firm appeared in Italy under the name "Emagel" (53, 126, 133). Many references devoted to studies of Emagel in experiments and clinics clmpletely confirm and supplement the information obtained during the study of gemaktsel.

Emagel is also absolutely harmless and does not accumulate in the organism (51, 119, 121). It does not act on the coagulation of the blood system (72, 78, 133), does not disrupt the functions

of the kidneys and liver (72, 78, 115, 120) and does not hinder the determination of the Rh-factor (114). During the clinical use of Emagel for prophylaxis and treatment of shock, and excellent therapeutic effect was obtained (61, 130, 116, 120, 137). In serious cases, up to 3 l of the preparation was injected. Excellent results were noted not only during the treatment of burns by Emagel (126, 139), but also in obstetrics and gynecology (66).

Daily repeated injected of Emagel for 20 patients up till the time of operation assisted in an increase of blood protein to normal and subnormal quantities and in many cases improved the function of the kidneys (59). The protein fraction of the blood after the injection of massive doses of the preparation was shown to be unchanged, except for an insignificant increase of albumen and general protein of the serum (114). These observations demonstrate that the injected preparation can be assimilated by the organism.

During the study of the duration of the circulation of Emagel in the organism, data were obtained confirming the speed of its excretion from the blood stream. Within four hours after the injection of 500 ml of Emagel, 47% of the preparation was found in the blood serum(62).

To the group of our native plasma substitutes from gelatin belong the preparations ferrofusine, gelatinol, and gelatin solution.

5. Ferrofusine

The preparation ferrofusine, proposed by I. M. Goldberg in 1958 (12), offers a mixture of the Ringer-Lokk salt solution, including colloidal iron and 1% gelatin. The author obtained the colloidal iron by the exposure of pure iron to a quartz lamp.

Ferrofusine was used in experiments for the treatment of traumatic (2, 8), visceral (9, 13), and burn shock (1). During the injection, arterial blood pressure swiftly rose and respiratory and cardiac activity improved, which did not occur if a salt solution with gelatin but without colloidal iron was used. However, in the long run in the majority of cases the animals died. Studying the mechanism of ferrofusine activity , T. T. Tkachev (35) discovered that during the effect on the isolated heart valve the preparation caused a higher amplitude of heart constriction than that same solution without colloidal iron. After the injection of 100 ml ferrofusine into rabbits a hydremia condition set in lasting 48 hours. On the basis of these data the author concluded that the period of residence of the preparation in the blood stream is two days.

Ferrofusine causes an insignificant change in the case of ROE (the speed of this reaction was up to 12 mm per hour), which set in after 5 multiple injections of the solution into rabbits in doses of 12 ml/kg of weight (32).

Under clinical conditions ferrofusine exhibits a fine therapeutic effect (10, 26). In doses of 500 ml the preparation quickly removed the shock effect of the first and second levels, stabilizing blood pressure and not causing complications. The effective activity of the preparation was recorded when it was injected in conjunction with blood during 364 transfusions in obstetrical-gynecological practice (39).

6. Gelatinol

The Leningrad Institute of Hematology and Transfusion of Blood Plasma Substitutes created gelatinol in 1960 from gelatin

(4, 6), offering an 8% solution of partially hydrolyzed alimentary gelatin with 0.9% sodium chloride. Like many gelatin plasma substitutes this preparation seems to be of various molecular weights, its average molecular weight being 10000 (including peptides from 5000 to 100000). General nitrogen contents comprise 1.3-1.5%, the relative viscosity is 2.2-3.0, the KOD 220-240 mm water phase.

The preparation does not congeal at temperatures of 4-6°. During cleavage by electrophoresis in an agar gel it produces a diffusion patch lying in the area of availability of globulin.

Nearly 50% of the composition of the preparation is represented by the fraction with a molecular weight of 10,000, 23-35% by the fraction with molecular weight 20,000 and more, and 15-27% of the composition of the preparation is comprised of peptides with a molecular weight of approximately 5000 (19). Gelatinol is non-toxic, does not cause antigenic or pyrogenic reactions and does not accumulate in the organism during continuous injections (16).

Substitution activity of the preparation was studied in an experiment on bleeding dogs in which 60-70% of the OTsK was replaced by Gelatinol (5). Arterial blood pressure falling after blood loss to 570 mm rt. st. quickly increased and remained stable at the high level. Cardiac activity and respiration normalized; all of the animals lived. Immediately after the injection of the solution the OTsK surpassed the initial level by 10-20%, and within one day amounted to 80% of the initial level. On the fifth day after the experiment the blood serum protein normalized and on the tenth day after the experiment it was on a level with the cases of high initial amounts. The level of the nitrogen remaining in the blood increased immediately after the experiment to 20-30 mg%; within a day the amount of it in the serum decreased to 10-15 mg% and simultaneously the quantity of it in the urine increased.

The injection of large doses of gelatinol causes temporary (to 5 days) acceleration of the ROE with a maximum rate of 20 mm per hour within a day. The preparation does not have a significant effect on the circulatory system; during the substitution of it for 78% of the OTsK, the time of blood circulation remained within the normal limits. In 3 or 4 cases the quantity of fibrinogen lowered by blood loss normalized within a day; the remainder of the cases were shown to change insignificantly. In a morphological sample of peripheral blood the greatest change was noted within 24 hours after the injection of gelatinol; complete normalization occurred on the 21st to 25th day. The number of leukocytes reached the initial level on the 5th day.

I. M. Khlebrikova and T. V. Znamenskaya (41,42), in experiments with carbohydrates studying the lipoid and pigment functions of the liver, noted that after the substitution of gelatinol for up to 78% of the OTsK, changes in the characteristics of the functions studied were within the normal range. In the blood serum the percentage of residual nitrogen temporarily increased and traces of protein appeared in the urine (40). Within one day in the urine residue leukocytes were discovered. But all of these changes were temporary.

Gelatinol is removed quickly from the blood stream. After its substitution for 60-70% of the OTsK, 2-9% of the preparation remains in the blood stream after one day; in the second day gelatinol was not discovered in the blood (17). The basic quantity of the solution (approximately 40%) is excreted in the urine in the period of 1 day. Just like other plasma substitutes from gelatin, gelatinol is not entirely excreted; 40-60% of the solution was discovered in the urine, the remaining part apparently metabolized in the organism.

Our observations on the study of the assimilibility of gelatinol in the experiment showed that parenteral injection of the solution with an accompanying no-protein diet reduced the negative balance of nitrogen and increased the amount of carbamide in the urine (16). G.D. Shushkov and coauthors (44), experimentally using gelatinol for the treatment of traumatic shock observed an increase of the OTsK and the elevation and stabilization of arterial blood pressure in the torpid phase of shock. In the terminal phase of shock the preparation did not exhibit a medicinal effect. Analogous results were obtained by F. I. Kovshikov (23) using gelatinol for the treatment of burn shock and combined trauma Ye. G. Vodokhlebova (11) and Ts. A. Puni (30), using the preparation during serious traumatic shock, and also V. A. Bondina (7), studying the effect of gelatinol under conditions of extended hypotension caused by blood loss.

During the use of gelatinol in the clinic for prophylaxis and treatment of hemorrhagic, operational, and traumatic shock, the preparation recommended itself as an effective plasma substitute which stabilizes the arterial blood pressure and increases the OTsK (14, 22, 27, 31, 44). In a series of cases gelatinol had a more expressed ability to raise the arterial blood pressure than preserved blood (28, 29). After the injection of this preparation in doses of 500-2500 ml there was no disruption from the point of view of general condition, using as indicators of hemodynamics, respiration, diuresis, and temperature. Repeated injections of the preparation did not cause negative reaction (27, 31). There were no essential improvements from the point of view of the coagulation factors of the blood, with the exception of some tendencies to speed the coagulation of the blood within 24 hours after the injection.

In the past year gelatinol has been used with success for filling apparatus for blood circulation research (3, 18). Under conditions of hemodiluted perfusion, gelatinol reduced the viscosity and increased the mass of circulating blood, and also lessened hemolysis of the blood in 2 or 3 cases. They showed that the transfer processes in the tissues undergo significantly less change than during the use of donated blood.

Thanks to its varied molecular weight, the preparation was retained well in the blood stream and yet quickly enough evacuated from it during forced diuresis. All of this made it possible on the basis of gelatinol to create for controlled hemodilution the so-called perfusion cocktail ("PC") - a combined preparation, guaranteeing buffer stability of the blood and normalized transfer processes in the organism (24, 34). Clinical investigations during open heart surgery confirmed the high effectiveness of the "PC" preparation. Biochemical and functional improvements in the organism during this were minimal.

At the present time research is being carried out on the study of disintoxication properties of the gelatinol preparation. D. S. Kuz'min and A. N. Vedenskiy (25) used gelatinol in an experiment for plasma pheresis during acicular atropine poisoning. After the injection of dogs with a fatal dose of atropine the substitution of the gelatinol solution for the blood plasma was carried out with a return of the erythrocytes, cleansed by this same solution. A 100% animal survival was observed. In the control experiments during the use of physiological solutions all of the animals died.

Gelatinol was successfully used to combat intoxication during acute surgical illness of the organs of the abdominal cavity in 50 seriously ill subjects (6). Observations in this area are continuing.

7. Gelatin solution

In 1962 in the Scientific Research Institute of Hematology and

and Blood Transfusion of the Georgian SSR (Tbilisi) another plasma substitute from gelatin was proposed (37). This preparation is also prepared from nutritive gelatin by partial hydrolysis into cephadexes of various sorts with the help of ethyl alcohol (45). The molecular weight of the preparation is 60000 to 70000. According to the author's data it does not gel at room temperatures.

The effect of such a gelatin solution under conditions of acute blood loss in an experiment (approximately 40% OTsK) produced a quick and stable hemodynamic effect. The protein content of the blood during this experiment was restored more quickly than during the injection of no-protein solutions. This preparation of gelatin does not depress the hemogenesis and does not significantly affect the circulatory system (36).

The medical effectiveness of new plasma substitutes was investigated in an experiment in complex treatment of acute radiation sickness. After irradiation by a dose of 600 roentgen in the course of several days, dogs received internal injections of gelatin solution in the quantity of 100 ml/kg body weight in conjunction with a bone marrow transplant. The course of acute radiation sickness in these animals was significantly milder than in the controls, among whom 100% fatality occurred. After the injection of the gelatin solution there was no recorded significant disturbance of the protein content of the blood (20, 21, 38).

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Поступила в редакцию 10.IV 1967 г.

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107 : 2132-2

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Council on Foods

THE COUNCIL ON FOODS HAS AUTHORIZED PUBLICATION OF THE FOLLOWING REPORT.

FRANKLIN C. BING, Secretary.

THE NUTRITIONAL SIGNIFICANCE OF GELATIN

Experimental investigations of the nutritive value of gelatin were begun early in the nineteenth century. A considerable mass of evidence has accumulated since that time. The older experiments, including the contributions of Voit and of Murlin on the so-called protein sparing action of gelatin, have been reviewed by Lusk[1] and others. In recent years much attention has been directed to the amino acid makeup of gelatin. The results of laboratory work have been reflected in advertising copy and in popular articles on nutrition. As might be expected, publicity that is promulgated by biased or scientifically untrained persons has been both favorable and unfavorable and, in the opinion of the Council, frequently has been misleading. Thus it has been stated that because gelatin is biochemically classed as an incomplete protein, lacking in certain essential amino acids, it is a food that should be avoided. And the use of gelatin as a means of increasing the protein content of the diet has been recommended just as enthusiastically as its use as a food has been condemned. Since the discovery of the dramatic success that has been reported to be attendant on the prescribing of aminoacetic acid to patients with certain myopathies, the advertising of one firm in particular has called attention to the relatively high proportion of this amino acid in gelatin and has suggested the use of gelatin in the treatment of muscular dystrophies. Gelatin has been recommended also for inclusion in the diet of infants. In the present report the Council has endeavored to evaluate the nutritional claims made for this food in the light of available evidence.

Preparation and Composition of Gelatin.—Gelatin is a protein derived from the collagen present in white fibrous tissue. It can be obtained from tendon, cartilage, bone and skin by boiling with water. This process is usually referred to as "hydrolysis," although there is some evidence that the changes involved are physical rather than chemical. There are several methods in common use for the commercial preparation of gelatin. Cow skins and trimmings are usually steeped for several weeks in a solution of lime water, then washed and boiled with water. Pig skins and trimmings are subjected to a preliminary treatment with acid. Bones are treated to remove the fat and mineral constituents, and the organic residue is largely converted into gelatin by boiling with water. Fish skins are a minor source of gelatin; they require only treatment with water to alter the collagen. Regardless of the source, there is obtained by appropriate preliminary treatment an aqueous solution containing gelatin. This is filtered, the water is evaporated, and the dried product is broken up and ground.

Gelatin is graded chiefly according to physical characteristics such as viscosity and jelly strength. These properties depend largely on the source of the gelatin and the method of manufacture. Different lots are therefore often tested and blended to make uniform products of the desired grade.

1. Lusk, Graham: The Elements of the Science of Nutrition, ed. 4, Philadelphia, W. B. Saunders Company, 1928, pp. 190, 365-367.

The dried gelatin usually consists of 85 to 90 per cent pure protein. A typical analysis of gelatin is recorded in table 1. The values for a powdered gelatin dessert are also listed because many persons think that the two products are almost identical.[2] It may be noted that the prepared gelatin dessert powders contain only about 10 per cent gelatin. Gelatin desserts can be prepared from edible gelatin as well as from the prepared mixtures; this may be advantageous in the preparation of low carbohydrate diets because of the possibility of omitting sugar from the final product.

Edible gelatin may contain traces of heavy metals which are present in the animal tissues from which the gelatin is derived. In order to guard against contamination during the manufacturing process the federal Food and Drug Administration has established certain limits of tolerance for arsenic, lead and copper in gelatin and other foods. These tolerances have been adopted also by the Council on Foods.

Gelatin as a Source of Protein in the Diet.—Many investigations over a period of years have brought out the significant fact that the nutritive value of proteins is measured largely by their digestibility and their amino acid makeup. It is interesting to note the occasional statement from prejudiced sources that because gelatin is obtained by partial hydrolysis it is partly digested to begin with and therefore is easily digested when eaten. Of course gelatin is readily digested by the proteolytic enzymes of the alimentary tract; but from the practical point of view the ready digestibility of gelatin does not confer on it any superiority over the common proteins.

The most extensive analysis of gelatin for amino acids is that reported by Dakin in 1920. His figures, which were

TABLE 1.—*Typical Analyses of Gelatin and Gelatin Dessert Powder*

	Gelatin, per Cent	Gelatin Dessert Powder, per Cent
Moisture	10.0	0.8
Ash	1.2	0.2
Fat (ether extract)	0.0	0.0
Protein (N × 5.5)	88.5	11.0
Carbohydrates	0.0	86.0
Tartaric or citric acid	none	2.0
Added flavor	none	present
Added color	none	present
Calories per gram (approximate)	3.5	3.9

based largely on the isolation of the individual amino acids, are recorded in table 2, together with additional data obtained by later investigators.[3] It is interesting to note that the sum of the nitrogen in the form of the various amino acids, plus the ammonia nitrogen reported by Dakin, amounts to 91 per cent of the nitrogen of gelatin. From the standpoint of our present knowledge of protein composition, this means that the amino acid makeup of gelatin is well known. The figures show that a number of amino acids have not been found in gelatin or are present in very small concentration. Valine, isoleucine, hydroxyglutamic acid and tryptophane are lacking. Tyrosine, cystine and methionine are present in small amounts. According to the results obtained in the feeding experiments of Rose and his collaborators, the essential amino acid which was later identified as alpha-amino-beta-hydroxybutyric acid[4] (and now called threonine[5]) is present in much smaller amounts in gelatin than in the casein of milk.[6] To date the following amino acids have been shown by feeding experiments with animals to be essential for growth: leucine, isoleucine, lysine,

2. The values of gelatin are from the analysis published in THE JOURNAL Feb. 27, 1932, p. 737, and of the powdered gelatin dessert from the analysis published May 12, 1934, page 1566.

3. The references to the investigators named in table 2 are as follows:
Dakin, H. D.: J. Biol. Chem. **44**: 499 (Nov.) 1920.
Looney, J. M.: J. Biol. Chem. **69**: 519 (Aug.) 1926.
M. T. Hanke (J. Biol. Chem. **66**: 489 [Dec.] 1925) found 0.5 per cent in commercial gelatin.
Folin, Otto, and Looney, J. M.: J. Biol. Chem. **51**: 421 (April) 1922.
Jones, D. B.; Gersdorff, C. E. F., and Moeller, O.: J. Biol. Chem. **62**: 183 (Nov.) 1924.
Baernstein, H. D.: J. Biol. Chem. **97**: 663 (Sept.) 1932.
May, C. E., and Rose, E. R.: J. Biol. Chem. **54**: 213 (Oct.) 1922.
Bergmann, Max: J. Biol. Chem. **110**: 471 (July) 1935.

4. McCoy, R. H.; Meyer, C. E., and Rose, W. C.: J. Biol. Chem. **112**: 283 (Dec.) 1935.

5. Meyer, C. E., and Rose, W. C.: J. Biol. Chem. **115**: 721 (Oct.) 1936.

6. Ellis, R. H., and Rose, W. C.: J. Biol. Chem. **94**: 167 (Nov.) 1931.

dine, lysine, cystine or methionine,[7] phenylalanine, tryptophane and threonine. Future investigation may show that the animal body is incapable of synthesizing other amino acids.[7a] According to available data, gelatin may be classed as an incomplete protein because it lacks or contains too low a concentration of isoleucine, tryptophane and methionine.

It is the opinion of the Council that the amino acid composition of gelatin is of no practical disadvantage unless gelatin is the sole source of protein in the diet. The essential amino acids that are lacking in gelatin can and should be obtained from other sources in the diet. No one would attempt to live on a diet in which gelatin forms the sole source of protein. As a matter of fact, several investigators have attempted this feat as a scientific experiment without success. Kauffmann[8] reported in 1905 some feeding experiments in which he derived nitrogen entirely from gelatin supplemented with tyrosine, cystine and tryptophane and remained in nitrogen balance for a few days. His experiments, however, are inconclusive and are of historic interest only.

Gelatin, because of its swelling properties, cannot be eaten readily in the dry form or sprinkled on cereal. It is usually consumed as a solution which, if the concentration of gelatin is approximately 1 per cent or more, forms a gel on cooling. An ordinary serving of a gelatin dessert contains about 2.5 Gm. of gelatin. Recipes have been suggested in which a concentration of gelatin as high as 10 per cent may be prepared in the form of hot soups. It requires considerable ingenuity, however, to formulate a menu that contains more than about an ounce of dry gelatin per day.

Much information of lasting value has been gained through experimental studies of the nutritive advantages and disadvantages of gelatin. The report by Jackson and his collaborators[9] includes a review of the work to 1929 on the supplementary value of gelatin added to other proteins in the diet of rats. Thus it has been shown by Osborne and Mendel[10] that gelatin, which contains a relatively high concentration of lysine, will supplement gliadin, which contains about one-tenth as much of this essential amino acid. However, the application of these observations to human nutrition is obscure. The human being does not ingest synthetic diets, and an actual deficiency of lysine in the diet has never been demonstrated.

The suggested use of gelatin (containing 25 per cent of aminoacetic acid) in the treatment of the myopathies has already been mentioned. The dosage of aminoacetic acid in the treatment of myasthenia gravis or pseudohypertrophic muscular dystrophy usually is from 20 to 30 Gm. It should be apparent that gelatin can furnish only a small proportion of the proper dosage of this amino acid. In the opinion of the Council it is improper for manufacturers to exploit gelatin in connection with the observations on the use of aminoacetic acid in the treatment of the myopathies. Even if it were possible to secure appreciable quantities of aminoacetic acid by eating large quantities of gelatin, the recommendation to do so is objectionable. Each patient requires a specific amount and, in the opinion of the Council, to try to take aminoacetic acid in the form of gelatin would not only be haphazard but in many cases ineffective.

Other Uses of Gelatin of Interest in Nutrition.—Because of its characteristic physical properties, gelatin has a number of uses in the food industry. For example, it is customary to incorporate about 0.5 per cent of gelatin in many ice cream mixtures.[11] It is added in order to improve the "smooth" consistency of the product by enhancing its ability to be whipped and in other ways. Gelatin may also be an important ingredient of confections such as marshmallows.

Effect of Gelatin on Digestibility of Milk.—Of particular interest to physicians is the evidence regarding the effect of the addition of gelatin to milk. This has been supposed to increase the digestibility of the milk and in the past the use of gelatin in infant feeding was looked on with favor among pediatricians. It has been thought that gelatin as a colloid would coat the newly formed casein curds in the stomach and prevent their coalescence into larger curds. Some evidence has been obtained which indicates that the curd formed when milk plus gelatin is treated with acid tends to be softer than the curd formed with milk alone plus acid.

Clinical studies of the value of gelatin in infant feeding have yielded questionable or conflicting results. Julius Hess and Chamberlain[12] reported an outpatient study on infants fed gelatin. The gelatin was well tolerated but there was a tendency toward the formation of foul smelling, firm stools which were grayish yellow and which at times were passed with difficulty. Other infants receiving egg yolk in place of gelatin passed stools which were more plastic and in general were canary colored. Perlman[13] observed a slightly higher growth rate in infants fed gelatin than in infants fed the same diet without gelatin. From the observations reported by Elterich, Boyd and Neff[14] as a result of a careful study of eleven infants under hospital supervision, it may be concluded that gelatin does not specifically accelerate growth but may increase the caloric intake.

The use of gelatin in the form of an isotonic solution which also contained dextrose and sodium chloride has been suggested by Kugelmass[15] and by Halpern[16] for the prevention of weight losses in the first few days of life of new-born babies. Senn,[17] however, while confirming the fact that loss in weight is not so great when this solution is used, concluded that it is not a safe therapeutic procedure because it occasionally gives rise to edema formation and fails to prevent dehydration fever.

TABLE 2.—*Amino Acids in Gelatin*

	Dakin	Other Investigators
Aminoacetic acid	25.5	25.7 (Bergmann)
Alanine	8.7	
Valine	0	
Leucine	7.1	
Isoleucine	0	
Serine	0.4	
Phenylalanine	1.4	
Tyrosine	0.01	0 (Looney; Hanke)
Proline	9.5	19.7 (Bergmann)
Hydroxyproline	14.1	14.4 (Bergmann)
Aspartic acid	3.4	
Glutamic acid	5.8	
Hydroxyglutamic acid	0	
Histidine	0.9	
Arginine	8.2	
Lysine	5.9	
Cystine		0.16 (Looney; Folin and Looney; Jones, Gersdorff and Moeller)
Methionine		0.97 (Baernstein)
Tryptophane		0 (Looney; Folin and Looney; Jones, Gersdorff and Moeller; May and Rose)

Allowable Claims for Gelatin.—In view of the available evidence the Council believes that gelatin properly made is a wholesome food, that it has special usefulness when one desires to add variety to the diet by incorporating gelatin in nutritious soups or pleasant desserts which appeal to the appetite of many persons, and that for these reasons gelatin is often a useful food for inclusion in the diet of healthy persons or of sick or convalescent patients. Gelatin appears to be well tolerated. The claim that gelatin is an aid in the digestion of milk, however, is in the opinion of the Council not established. The claim that gelatin is of value as a source of aminoacetic acid in the treatment of some of the myopathies cannot be recognized; in the light of present evidence, gelatin has no special significance as a source of amino acids in the diet. Indeed, it is notoriously deficient in certain essential amino acids.

7. There is conclusive evidence that methionine can replace cystine; at the present time it is doubtful whether cystine can replace methionine.

7a. W. C. Rose and his collaborators have reported before the 1936 meeting of the Society of Biological Chemists that valine, an amino acid not present in gelatin, is indispensable in the diet.

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