GELATIN—ITS USEFULNESS AND TOXICITY

Blood Protein Production Impaired by Continued Gelatin by Vein* ‡

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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

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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: 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 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 7.6 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	Hemoglobin: Output per wk.	Plasma protein: Level	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		Protein output				Production ratio plasma protein to hemoglobin	Ratio protein output to intake	Total nitrogen	
		Type	Weekly	Hemoglobin		Plasma protein				In-take	Output urine
				Level	Output per wk.	Level	Output per wk.				
Dog 41-50		**Gelatin P3-20 plus ½ standard dose amino acids (Vaa Table A)**									
	kg.	*gm.*	*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*
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 1¾ 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
				Hemoglobin		Plasma protein			
		Type	Weekly	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		Protein output				Production ratio plasma protein to hemoglobin	Ratio protein output to intake	Total nitrogen	
		Type	Weekly	Hemoglobin		Plasma protein				In-take	Out-put urine
				Level	Out-put per wk.	Level	Out-put per wk.				
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.

TABLE 6

Gelatin by Mouth and by Vein Plus Amino Acids

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 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	5.4	1.4	—	—	—	—

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.

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 conspicuous 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 150	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		Protein output				Production ratio plasma protein to hemoglobin	Ratio protein output to intake	Total nitrogen	
		Type	Weekly	Hemoglobin		Plasma protein					
				Level	Output per wk.	Level	Output per wk.			Intake	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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