ANEMIA PLUS HYPOPROTEINEMIA IN DOGS

Various Proteins in Diet Show Various Patterns in Blood Protein Production

Beef Muscle, Egg, Lactalbumin, Fibrin, Viscera, and Supplements*

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Proteins may be tested biologically in various ways according to their growth pattern, weight maintenance, or nitrogen balance. Proteins may be tested in combined anemia and hypoproteinemia. This is a severe test as the protein reserve stores are largely depleted and the need to form new hemoglobin and various plasma proteins forces the body to use toward this end every bit of available protein to best advantage. In fact it has been shown that the dog will raid its own body proteins to supply its acute need when the protein intake is inadequate (6).

We have already reported experiments to show the general pattern of protein use in doubly depleted dogs (anemia plus hypoproteinemia) (3, 5). In general the canine body favors hemoglobin above plasma proteins with a production ratio of about 2 gm. hemoglobin to 1 gm. of plasma proteins, and this reaction is maintained for weeks as the same dietary intake continues. There are some exceptions which interest us particularly. Striated *muscle* (meat) diet favors hemoglobin, so that as a rule 3 to 4 gm. of new hemoglobin to 1 gm. of plasma protein are produced. In contrast, *egg protein* diet favors plasma protein production and one may observe equal amounts of new plasma protein and hemoglobin. Sometimes more plasma protein than hemoglobin is produced (Table 1). *Lactalbumin* diet favors plasma protein production but not quite as strongly as does egg protein.

The amino acid make-up of the proteins is not yet completely established but one suspects that differences in percentage composition of the amino acids in the given proteins must be largely responsible. Supplements of certain amino acids to these proteins are tested in the work which follows. *Histidine* is rather

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low in egg protein and, as a supplement to egg feeding, usually causes a fall in the plasma protein to hemoglobin ratio from 90 to 100 per cent toward the common protein ratio of 50 per cent. Supplementing egg protein and lactalbumin by digests of other proteins causes a fall in the plasma protein–hemoglobin ratio—that is, a return to the usual pattern of 2 gm. hemoglobin to 1 gm. plasma protein or a 50 per cent ratio.

We have tested hemoglobin given intraperitoneally or by mouth in the doubly depleted dogs, expecting to find that hemoglobin production would be favored over that of plasma protein. No such reaction was observed, but the usual 50 per cent ratio of plasma protein to hemoglobin was repeatedly noted (3). Plasma protein was tested similarly, given by vein as plasma or by mouth as plasma or as a digest. There is no evidence that this intake favors the production of plasma protein. The body accepts these widely different proteins and in the emergency of anemia plus hypoproteinemia can use them to produce new hemoglobin and plasma protein in about the usual ratio.

Finding that hemoglobin and plasma protein did not favor the production of these proteins in doubly depleted dogs we were surprised to find that *fibrin by mouth* did favor fibrinogen production (Tables 6 and 7). The albumin-globulin ratio is not changed significantly, but there is a definite and sustained rise in the blood fibrinogen levels during the 5 week periods of fibrin feeding. Fibronogen is a very labile protein (1) which in the dog influenced by infection, liver disturbance, and many other factors, some unknown, may swing from 250 to 1000 mg. per 100 cc. in a period of hours. Fibrinogen is produced wholly in the liver, and its sustained high level on fibrin feeding is therefore of significance. In view of these findings the feeding of albumin or globulins (other than fibrin) should be tested over considerable periods to determine whether the newly produced albumin or globulin will be increased in doubly depleted dogs.

The albumin–globulin ratio has been followed in the experiments here described and no significant variation observed due to these diet factors. The doubly depleted dog has an A/G ratio close to unity, which may indicate a somewhat more rapid production of globulins (2).

Methods

The dogs used in the experiments are raised in the laboratory kennels and are of mixed strain (white bull terrier and coach dog). The animals are maintained under optimum dietary conditions and kept under constant observation. They are protected from infections by proper housing, isolation, vaccination, and handling. Depletion of their blood proteins is produced as rapidly as is consistent with their well being. A non-protein diet plus frequent blood removal accomplishes depletion of both hemoglobin and plasma proteins within a 3 to 4 week period. During this depletion period the dogs lose considerable weight, making it desirable to produce the depletion in as short a time as possible. The desired "double depletion" represents a hemoglobin level of about 6.5 gm. per cent and a plasma protein level of approximately 4.5 gm. per cent. Below these levels the dogs' health and appetite suffer.

The basal non-protein diet consists of a biscuit containing adequate carbohydrates, fats, and minerals, including an excess of iron and choline chloride. 100 gm. biscuit contains 73 gm. carbohydrate, 13 gm. fat, 78 mg. iron, 70 mg. choline. At times canned vegetables such as carrots or onions (low protein content) are added to maintain appetite during the depletion period. Vitamin additions to the diet consist of either a synthetic liquid vitamin mixture containing all known essentials (Lilly), or vitamin pills of like make-up or a dried yeast powder (Standard Brands type 200 B) and a liver powder (Lilly) prepared from pig liver. The small amount of nitrogen these powders contain is added to the daily protein intake indicated in the tables.

Peanut flour, wheat gluten, whole egg powder (designated Rutgers University in the tables) represent protein materials prepared for the "Bureau of Biological Research of Rutgers University for Comparative Studies on Methods of Evaluating Protein Foods." These tests for potency concerning blood protein production are part of a cooperative investigation. Commercial casein and peanut flour are used for comparison experiments. Lactalbumin, casein, soy bean, and fibrin are commercial products. "Somagen" represents a protein mixture composed of milk protein with added concentrates of yeast and liver. The mixture is supplemented by crystalline vitamins. The protein content is 70 per cent. Casein digest (Lilly) is an enzymatic digest of casein using papain containing 12.6 gm. per cent nitrogen. Folic acid was fed as folvite. B_{12} is "cobione" (Merck) administered subcutaneously (1 cc. = 15 μg.). "Fresh beef muscle" is ground lean round steak.

Visceral products were freed of fat, boiled, and then put through a meat grinder (pig kidney, pig pancreas, pig stomach, calf thymus, and beef brains). In some experiments the pig stomach was fed as an uncooked, hashed mixture. Fresh egg albumin is separated and coagulated in a double boiler. Whole fresh egg is beaten thoroughly and coagulated in a double boiler. "Canned salmon muscle" is a commercial product, designated "pink Alaska salmon."

The experimental dogs are kept in metabolism cages in a separate room during the entire experimental period for each test as indicated in the tables. The dogs are weighed daily and their daily protein intake is accurately calculated. General technical procedures concerning the animals and experiments proper have been described elsewhere (3).

Fibrin is determined according to the method of Cullen and Van Slyke. Fibrinogen is clotted with calcium chloride, dried, and nitrogen is determined by micro-Kjeldahl analysis.

In the following tables periods for any given dog run consecutively unless otherwise noted. Hemoglobin levels are those obtained by sampling 48 hours after blood removal, in case of a single bleeding. In case of repeated bleedings during the course of a week the hemoglobin level is obtained during the blood volume determination at the end of the week. Plasma protein levels represent the average of samples of each bleeding during the week. "Output per week" is the total hemoglobin or plasma protein removed. The values indicating the "net total output" are the amounts of blood protein actually removed by bleeding, plus or minus the calculated amounts related to differences in the circulating levels of hemoglobin and plasma proteins, as determined by blood volumes at the start and at the end of the test period.

The experimental histories are not detailed because the dogs with a single exception (Table I) seemed normal at all times. The chief experimental difficulty related to diets which were sometimes refused after a period of 2 to 3 weeks and this terminates many experiments not recorded here. The double depletion experiments cannot be tolerated indefinitely and rest periods of months may be necessary after experiments lasting 30 to 60 weeks. Regular blood plasma volume determinations and cell counts are done each week. Urinary nitrogen is determined on each week's output. Albumin-globulin ratios are recorded each week. Many of these A/G figures have been recorded in preceding experimental histories (4). Tables show the weight curve; in summary tables the initial weight and the gain or loss during each 5 week experiment are recorded. Each test diet period runs for 5 weeks unless otherwise noted.

EXPERIMENTAL OBSERVATIONS

The first three tables show that whole egg or egg albumin favors the production of plasma protein in doubly depleted dogs. The plasma protein to hemoglobin ratio is very high in Table 1 but the total blood protein output is definitely below normal. The high ratio in favor of plasma protein in this and other dogs is due largely to a low hemoglobin output rather than a high plasma protein production. Tyrosine values in whole egg are given by some analysts as below those for many other food proteins. Daily tyrosine supplements show a sustained response in Table 1, Experiment 3—a rise in total protein, a rise in hemoglobin, a slight fall in plasma protein, and a fall in the plasma protein to hemoglobin ratio from 115 to 67 per cent.

Table 1, dog 40-32, gives observations on the only abnormal dog in this colony. This dog has been under experimental observation for 10 years; it was a year old when depletion was established. For the last 3 years there has been some albumin and casts in the urine, but its condition remained excellent and continued so during the experiments listed in Table 1. About 10 weeks after these experiments some ascites and edema developed yet the dog was active and appeared well. Ascitic fluid was withdrawn several times. Plasma proteins remained at a low normal level, 5.9 to 6.7 gm. per cent. Within 3 weeks the ascites cleared and the edema vanished. At present the dog is quite well and we cannot report on the actual cause for the edema and ascites.

Table 2 shows a clean-cut series of experiments which shows the usual pattern for whole beef diet—a large total output of blood protein with great excess of hemoglobin, a production ratio, plasma protein to hemoglobin, of 27 per cent. When some of the beef is replaced by whole egg the ratio of plasma protein to hemoglobin rises owing to decrease in new hemoglobin. In the third period the diet was largely egg supplemented by beef. The new hemoglobin output fell and the plasma protein-hemoglobin ratio rose. This Table 2 illustrates how the output of new hemoglobin and plasma protein can be modified at will in these depleted dogs.

Table 3 shows satisfactory experiments with *egg albumin*. The total blood protein output is low and the plasma protein–hemoglobin ratio is high (79 per cent). It is accepted that egg albumin is low in *histidine* and this amino acid, as a daily supplement, gives a larger output of hemoglobin and a fall in the plasma protein–hemoglobin ratio. It should be noted that the second experimental period in this dog (Table 3) is only 4 weeks instead of 5. The total blood protein for a 5 week period would stand as about 215 gm. (instead of 170 recorded for 4 weeks in the table).

TABLE 1

Whole Egg, Egg Powder, Tyrosine*
Chronic Nephritis

Dog 40-32.

Weight	Protein intake: Type	Protein intake: Week	Food consumption	Protein output: Hemoglobin Level	Protein output: Hemoglobin Week	Protein output: Plasma protein Level	Protein output: Plasma protein Week	Total	Production ratio plasma protein to hemoglobin	Urinary nitrogen output week
kg.	*gm. per day*	*gm.*	*per cent*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*gm.*	*per cent*	*gm.*
18.7	Egg 210	180	90	6.1	37.3	4.9	20.5			10.4
18.6	Egg 210	168	84	7.4	13.4	5.0	9.0			13.0
18.6	Egg 210	200	100	6.2	22.9	5.4	17.6			10.2
18.2	Egg 210	194	97	6.3	10.2	5.6	7.8			10.2
−0.9	Net total.................	742			46.6		65.8	112	141	43.8
19.9	Egg powder 40	94	45	6.5	40.0	5.2	26.1			8.1
19.8	Egg powder 40	115	55	5.7	1.3	5.1	1.2			8.6
20.4	Egg powder 40	210	100	7.6	1.1	5.3	1.3			—
20.1	Egg powder 40	185	88	7.2	28.6	5.9	20.4			9.7
19.7	Egg powder 40	93	44	6.9	13.9	5.8	12.3			8.5
−0.4	Net total.................	697			59.5		68.3	128	115	34.9
19.6	Egg powder 40, *dl*-tyrosine 2	195	91	6.2	13.2	5.4	11.0			7.7
19.8	Egg 200, *dl*-tyrosine 2	214	100	6.8	11.0	6.1	9.8			7.6
19.1	Egg 200, *dl*-tyrosine 2	214	100	7.9	13.3	5.4	9.0			8.3
18.8	Egg 200, *dl*-tyrosine 2	195	91	7.1	27.7	5.3	18.9			8.9
19.0	Egg 200, *dl*-tyrosine 2	180	84	7.4	23.3	5.7	16.8			8.7
−0.7	Net total.................	998			87.8		58.4	146	67	41.2

Last two experiments are continuous.

* Bureau Biological Research of Rutgers University.

When, in the third experiment of Table 3, the histidine is replaced by *valine*, the plasma protein–hemoglobin ratio swings back toward the egg albumin figure, owing to a fall in new hemoglobin output and an increase in plasma protein output during the 5 week period. Obviously, histidine supplements

TABLE 2

Whole Egg and Beef Muscle

Dog 47-30.

Weight	Protein intake		Food consumption	Protein output					Production ratio, plasma protein to hemoglobin	Urinary nitrogen output week
	Type	Week		Hemoglobin		Plasma protein		Total		
				Level	Week	Level	Week			
kg.	*gm. per day*	*gm.*	*per cent*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*gm.*	*per cent*	*gm.*
16.3	Beef 150	221	100	8.3	26.1	5.3	16.1			11.4
16.5	Beef 150	221	100	9.4	32.6	4.8	14.7			10.8
16.5	Beef 150	221	100	10.6	30.1	4.9	12.1			11.8
16.4	Beef 150	217	98	10.2	52.1	4.7	19.7			10.2
16.6	Beef 150	208	94	11.3	31.6	4.6	11.9			10.6
+0.9	Net total...........	1088			244.4		66.8	312	27	54.8
16.7	Beef 120, egg 60	223	100	9.7	55.8	4.8	18.2			10.9
16.8	Beef 120, egg 60	201	90	11.9	31.5	4.7	10.6			10.0
16.9	Beef 120, egg 60	223	100	9.8	37.5	4.7	12.5			10.1
16.9	Beef 120, egg 60	223	100	11.4	44.5	4.7	16.1			10.4
17.3	Beef 120, egg 60	223	100	11.4	32.3	4.6	11.3			9.5
+0.7	Net total...........	1093			200.9		70.7	272	34	50.9
17.1	Egg 183, beef 40	224	100	10.9	29.6	4.6	9.8			9.2
17.1	Egg 183, beef 40	224	100	9.7	45.2	4.7	15.1			9.3
17.3	Egg 183, beef 40	224	100	8.3	29.3	4.8	12.9			10.4
17.1	Egg 183, beef 40	224	100	9.2	30.4	4.9	12.4			10.1
17.4	Egg 183, beef 40	224	100	9.0	27.9	4.8	12.7			10.8
+0.1	Net total...........	1120			127.1		65.9	193	52	49.8

Experiments are continuous.

egg albumin effectively to improve its capacity to produce new hemoglobin and plasma protein in these depleted dogs. Other similar experiments are found in Summary Tables 4 and 5.

Tables 4 and 5 give a number of 5 week experiments in various dogs fed whole egg alone or supplemented by various amino acids. Because egg albu-

TABLE 3

Fresh Egg Albumin Plus Histidine or Valine

Dog 46-9.

Weight	Protein intake		Food consumption	Protein output					Production ratio plasma protein to hemoglobin	Urinary nitrogen output week
	Type	Week		Hemoglobin		Plasma protein		Total		
				Level	Week	Level	Week			
kg.	*gm. per day*	*gm.*	*per cent*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*gm.*	*per cent*	*gm.*
15.3	Egg albumin 227	162	100	8.6	11.9	4.6	6.6			9.0
15.1	Egg albumin 227	172	100	6.4	38.2	5.4	22.2			12.5
15.3	Egg albumin 227	179	100	6.9	13.0	5.1	8.3			10.4
15.4	Egg albumin 227	187	100	7.5	21.3	5.5	15.3			10.1
15.3	Egg albumin 227	187	100	7.1	26.0	5.5	18.1			11.3
−0.3	Net total................	887			96.2		76.1	172	79	53.3
15.0	Egg albumin 260, *l*+histidine 1	199	100	8.7	19.2	5.2	13.5			13.4
14.8	Egg albumin 260, *l*+histidine 1	199	100	8.7	33.7	5.0	18.1			10.9
14.5	Egg albumin 260, *l*+histidine 1	199	100	8.1	41.0	5.0	22.0			8.9
14.0	Egg albumin 260, *l*+histidine 1	175	88	7.8	21.9	4.7	12.7			10.0
−1.3	Net total................	772			115.2		54.8	170	48	43.2
14.1	Egg albumin 250, *dl*-valine 4.7	196	96	7.7	23.2	5.0	13.3			12.6
14.2	Egg albumin 250, *dl*-valine 4.7	204	100	7.7	26.1	5.4	14.7			11.3
13.9	Egg albumin 250, *dl*-valine 4.7	196	96	7.5	26.5	4.5	15.1			12.6
14.3	Egg albumin 250, *dl*-valine 4.7	204	100	7.2	24.7	5.4	17.2			12.0
14.2	Egg albumin 250, *dl*-valine 4.7	204	100	6.8	20.8	5.3	9.0			11.8
+0.2	Net total................	1004			117.8		75	193	64	60.3

Experiments are continuous.

min is low in *histidine,* this amino acid was tested in many experiments alone with egg or with other amino acids, especially *tyrosine.* These Tables 1, 4, and 5 support the thesis that histidine alone and/or tyrosine (Table 1) are effective as supplements to egg protein. These supplements cause an increase

SUMMARY TABLE 4

Whole Egg with Amino Acid Supplements

Weight gain or loss	Protein intake: Type	Protein intake: Week	Food consumption	Protein output: Hemoglobin: Level	Protein output: Hemoglobin: Week	Protein output: Plasma protein: Level	Protein output: Plasma protein: Week	Total 5 weeks	Production ratio plasma protein to hemoglobin	Urinary nitrogen output week
kg.	*gm. per day*	*gm.*	*per cent*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*gm.*	*per cent*	*gm.*
15.6	Egg 200, *l*-tyrosine 1, *l* + valine 1, *l*-histidine 1	205	98	8.4	39.3	5.3	21.1			10.6
−0.7	Dog 45-3 Total.............				196.4		105.3	302	54	
14.9	Egg 200, *l*-tyrosine 1, *l*-histidine 1	192	92	9.0	39.1	5.0	16.1			8.7
−0.3	Dog 45-3 Total.............				195.7		80.5	276	41	
14.6	Egg 225, *l*-histidine 1	195	90	8.9	34.9	5.0	19.2			9.6
+0.1	Dog 45-3 Total.............				174.7		96.1	271	55	
14.7	Egg 255	190	90	7.1	18.9	5.3	12.9			10.2
+0.4	Dog 45-3 Total.............				94.5		64.5	159	68	
21.9	Egg 225	211	98	7.5	28.9	5.5	18.2			8.9
0	Dog 46-9 Total.............				144.5		91.2	236	63	
17.3	Egg 225, *l*-tyrosine 1, *l* + valine 1, *l*-histidine 1	209	93	8.4	38.4	5.8	11.2			12.5
+0.3	Dog 46-9 Total.............				191.8		56.0	248	29	
17.6	Egg 225, *l*-histidine 1	201	92	9.2	39.7	4.8	16.5			10.5
−0.6	Dog 40-9 Total.............				198.7		82.3	281	41	
14.2	Egg 200, *l*-tyrosine 1, *l*-histidine 1, *dl*-tryptophane 1, *d*-glutamic acid 1	217	98	9.4	19.6	6.0	14.6			11.0
−0.4	Dog 47-27 Total............				197.8		72.9	271	37	
13.9	Egg 200, glutamic acid 1	198	99	7.8	26.2	5.6	11.2			10.7
+0.1	Dog 47-27 Total............				131.2		75.8	207	58	
14.0	Egg 210	177	87	7.8	22.8	5.8	13.8			7.0
−0.6	Dog 47-27 Total............				114.9		69.3	184	61	
13.4	Egg 225, *l*-histidine 1	214	92	8.3	26.9	6.0	21.9			10.5
−0.2	Dog 47-27 Total............				134.4		109.5	244	82	
13.2	Egg 225, *dl*-tryptophane 2	208	94	7.9	23.2	6.2	18.4			10.4
+0.5	Dog 47-27 Total............				116.2		91.8	208	79	

SUMMARY TABLE 5

Whole Egg with Amino Acid Supplements

Weight gain or loss	Protein intake: Type	Protein intake: Week	Food consumption	Protein output: Hemoglobin: Level	Protein output: Hemoglobin: Week	Protein output: Plasma protein: Level	Protein output: Plasma protein: Week	Protein output: Total 5 weeks	Production ratio plasma protein to hemoglobin	Urinary nitrogen output week
kg.	*gm. per day*	*gm.*	*per cent*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*gm.*	*per cent*	*gm.*
20.4	Egg 225	208	100	9.4	17.8	4.6	16.6			9.4
+0.4	Dog 45-2 Total..............				89.1		82.9	172	93	
20.8	Egg 225, *dl*-phenylal. 2	216	100	7.2	20.0	5.5	12.0			11.4
−0.4	Dog 45-2 Total..............				99.8		60.0	160	60	
20.4	Egg 225, *dl*-valine 2	218	100	7.1	19.7	5.3	15.0			13.0
−0.7	Dog 45-2 Total..............				98.4		75.0	173	76	
15.7	Egg 225	208	100	6.6	13.9	5.1	11.5			8.6
+0.2	Dog 45-2 Total..............				69.7		57.3	127	82	
15.9	Egg 225, *dl*-phenylal. 2 glutamic acid 1	220	100	6.8	11.4	5.9	8.5			9.6
+0.8	Dog 45-2 Total..............				57.1		42.4	100	74	
16.7	Egg 225, *dl*-threonine 2, *dl*-tyrosine 2	225	100	6.8	15.6	5.8	10.4			10.3
0	Dog 45-2 Total..............				78.1		52.0	130	67	
19.9	Egg 225	208	100	10.6	21.7	4.7	15.8			9.7
−0.9	Dog 47-26 Total.............				108.3		79.2	188	73	
19.0	Egg 225, *l* + lysine 1	214	100	7.3	25.0	5.1	15.4			10.9
+0.1	Dog 47-26 Total.............				125.2		76.8	202	61	
19.1	Egg 225, *l* + lysine 1 *dl*-leucine 2	223	100	8.1	23.7	5.2	13.0			13.0
−0.3	Dog 47-26 Total.............				118.6		64.8	183	54	
18.8	Egg 225	208	100	7.0	18.1	5.3	17.6			11.0
+0.5	Dog 47-26 Total.............				90.7		88.1	179	97	
16.9	Egg 225, *dl*-tyrosine 2	215	100	7.1	16.4	5.8	12.6			10.2
+0.3	Dog 47-26 Total.............				82.0		62.8	145	77	
17.2	Egg 225, *dl*-threonine 2	218	100	7.0	19.5	6.1	15.0			12.1
−0.2	Dog 47-26 Total.............				97.7		74.9	173	77	

in total blood protein output, more especially in the production of hemoglobin which causes a fall in the plasma protein–hemoglobin ratio. The blood protein production response then approaches the usual food protein effect

SUMMARY TABLE 6

Beef Fibrin with Valine and Casein Digest Supplements

Weight gain or loss	Protein intake		Food consumption	Protein output					Production ratio plasma protein to hemoglobin	Urinary nitrogen output week
	Type	Week		Hemoglobin		Plasma protein		Total 5 weeks		
				Level	Week	Level	Week			
kg.	*gm. per day*	*gm.*	*per cent*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*gm.*	*per cent*	*gm.*
17.7	Fibrin 39, *dl*-valine 4.7	261	100	9.1	41.4	4.9	19.6			16.0
−0.3	Dog 45-2 Total............				207.2		97.9	305.1	46	
17.4	Fibrin 43	263	100	9.4	45.0	4.8	19.6			14.1
−0.2	Dog 45-2 Total............				224.9		98.1	323.0	44	
14.0	Fibrin 30, casein digest 10	236	100	10.6	23.1	4.5	11.7			11.7
+1.6	Dog 47-26 Total...........				115.3		58.5	173.8	51	
21.9	Fibrin 40	245	100	8.4	32.8	5.6	22.4			14.8
+0.8	Dog 40-32 Total...........				163.8		112.3	276.1	68	
13.6	Fibrin 40	242	99	8.9	26.3	4.9	15.2			9.7
+0.5	Dog 46-4 Total............				131.4		75.8	207.2	58	
13.7	Fibrin 40	212	89	7.5	20.8	5.5	20.8			9.1
+0.5	Dog 45-3 Total............				103.9		75.1	179.0	72	
16.9	Fibrin 35	214	100	9.2	27.7	4.5	13.7			9.4
−0.9	Dog 47-20 Total...........				138.3		68.5	207	49	
15.6	Fibrin 40	245	100	8.3	30.1	4.7	18.2			12.7
+0.5	Dog 46-12 Total...........				180.4		109.3	290	61	

(for example that of casein)—a ratio of 2 gm. hemoglobin to 1 gm. plasma protein.

Table 5 shows the effects of supplementing egg protein with phenylalanine, valine, glutamic acid, threonine, and lysine alone or in various mixtures as noted. These amino acids do not have the striking effect of histidine, but in general there is some evidence that they may cause a slight increase in new blood protein and a slight decrease in the ratio of plasma protein to hemoglobin.

Fibrin turns out to be a good diet protein to produce hemoglobin and plasma protein in these depleted dogs (Table 6). The total output is not quite as high as with a casein diet and the usual ratio of hemoglobin to plasma protein can be noted. Fibrin is a peculiar protein on several counts. Its amino acid make-up is not completely known but it contains more than average amounts of arginine, threonine, and tyrosine, when compared with casein for example. As concerns the ingredients to form new hemoglobin and plasma protein, *fibrin* as a diet factor is not deficient under the conditions of these experiments.

TABLE 7

Beef Fibrin Diet—Fibrinogen Levels and Albumin-Globulin Ratios

Week of experiment	Dog 47-20		Dog 46-12		Dog 45-3		
	Fibrinogen	Albumin-Globulin ratio	Fibrinogen	Albumin-Globulin ratio	Fibrinogen	Electrophoretic pattern*	Albumin-Globulin ratio
Before	475	0.75	396	0.80	372	720	1.2
1st	676	1.1	541	0.74	460	800	1.3
2nd	633	1.1	517	1.3	641	820	1.2
3rd	712	1.2	—	1.2	649	820	1.1
4th	700	1.3	596	1.2	454	760	1.1
5th	666	1.0	493	1.3	451	940	1.2
7th	285‡						

Blood fibrinogen levels in milligrams per cent.

* We are indebted to Dr. Eric L. Alling, Associate in Radiation Biology and Medicine, for the determination of the electrophoretic pattern of Dog 45-3 with the calculated amounts of fibrin.

‡ Dog on kennel diet for one and one-half weeks.

In one respect *diet fibrin* is unusual—in these long experiments it favors slight overproduction of fibrinogen and sustained blood levels of approximately 50 per cent above normal (Table 7). It is also interesting that diet fibrin may cause a slight increase in the albumin-globulin ratio, meaning that if anything more albumin than globulin is produced week by week. The fibrinogen of the blood, as calculated from the electrophoretic pattern, is always higher than the coagulated fibrinogen (Table 7, dog 45-3). It is not known precisely what globulin besides fibrinogen is actually included in this area.

Lactalbumin in some respects resembles egg protein as it is fed in these double depletion experiments. There is a tendency for the plasma protein to hemoglobin ratio to rise above 50 per cent and may in fact reach 100 per cent (Table 8). There is decrease in hemoglobin production and often some increase in plasma protein production.

Table 8 shows a wide swing from the usual plasma protein to hemoglobin ratio of 31 per cent with the beef diet to a ratio of 99 per cent when the diet is largely lactalbumin (Experiment 3, dog 46-10). The total blood protein output is well sustained but the change in amount of new hemoglobin is significant.

TABLE 8

Lactalbumin and Beef Muscle

Dog 46-10.

Weight	Protein intake		Food consumption	Protein output					Production ratio plasma protein to hemoglobin	Urinary nitrogen output week
	Type	Week		Hemoglobin		Plasma protein		Total		
				Level	Week	Level	Week			
kg.	*gm. per day*	*gm.*	*per cent*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*gm.*	*per cent*	*gm.*
19.1	Beef 150	221	100	8.3	28.1	5.6	17.4			9.5
19.2	Beef 150	221	100	8.5	47.9	4.8	21.7			10.6
19.6	Beef 150	221	100	8.7	29.9	4.5	11.5			12.3
19.4	Beef 150	221	100	11.1	1.8	4.4	0.9			11.4
19.4	Beef 150	221	100	11.1	48.0	4.7	16.5			11.8
+1.1	Net total................	1105			179.3		56.0	235	31	55.6
19.9	Beef 125, lactalbumin 10	225	100	10.5	39.2	4.6	12.9			9.6
20.4	Beef 125, lactalbumin 10	225	100	10.5	35.9	4.5	12.9			9.0
20.8	Beef 125, lactalbumin 10	225	100	9.7	36.6	4.9	13.2			10.4
21.1	Beef 125, lactalbumin 10	225	100	10.4	48.7	4.7	20.1			10.5
21.4	Beef 125, lactalbumin 10	225	100	9.5	41.2	4.6	17.7			10.3
+2.0	Net total................	1125			211.6		80.8	292	38	49.8
21.2	Lactalbumin 34, beef 40	228	100	9.3	28.6	4.9	12.3			8.8
21.1	Lactalbumin 34, beef 40	228	100	6.9	42.4	5.0	19.4			10.2
21.5	Lactalbumin 34, beef 40	228	100	7.0	23.3	5.4	15.5			10.2
21.0	Lactalbumin 34, beef 40	228	100	9.0	14.4	4.6	8.5			10.6
21.0	Lactalbumin 34, beef 40	228	100	6.4	42.6	5.8	31.7			12.0
−0.4	Net total................	1140			110.2		108.8	219	99	51.8

Experiments are continuous.

Table 9 gives other experiments with lactalbumin feeding to show that the total blood protein production is satisfactory but not as high as with a casein diet. Supplements of various amino acids and casein digests in the amounts fed cause no large increase in blood protein output.

Casein as a diet factor in these double depletion experiments stands high

SUMMARY TABLE 9

Lactalbumin with Amino Acid, Casein and Casein Digest Supplements

Weight gain or loss	Protein intake: Type	Protein intake: Week	Food consumption	Protein output: Hemoglobin Level	Protein output: Hemoglobin Week	Protein output: Plasma protein Level	Protein output: Plasma protein Week	Total 5 weeks	Production ratio plasma protein to hemoglobin	Urinary nitrogen output week
kg.	*gm. per day*	*gm.*	*per cent*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*gm.*	*per cent*	*gm.*
17.6	Lactalbumin 45	227	100	9.2	23.6	4.7	16.2			11.0
+0.6	Dog 47-20 Total............				118.3		80.9	199	68	
18.2	Lactalbumin 45, *dl*-valine 2	239	100	7.5	19.7	5.2	11.5			15.2
+0.7	Dog 47-20 Total............				98.3		57.5	156	59	
18.9	Lactalbumin 45	229	100	7.4	28.5	5.1	15.5			13.9
−0.9	Dog 47-20 Total............				142.5		77.5	220	54	
15.1	Lactalbumin 45	200	92	7.3	13.9	5.7	11.3			10.7
−0.1	Dog 45-3 Total.............				6[illegible]7		56.7	126	81	
15.0	Lactalbumin 45, *l* + arginine 1	224	97	7.3	18.7	6.2	15.5			13.5
−0.1	Dog 45-3 Total.............				93.7		77.6	171	83	
18.0	Lactalbumin 45, *l* + arginine 1	241	100	7.4	24.4	5.3	17.4			13.5
+0.8	Dog 47-20 Total............				121.5		87.0	199	72	
18.8	Lactalbumin 45, *dl*-methionine 2	237	100	7.4	19.5	5.3	10.1			13.1
+0.6	Dog 47-20 Total............				97.6		50.6	148	52	
19.4	Lactalbumin 45, *l* + arginine 1, *dl*-valine 2	252	100	7.8	22.9	5.2	12.1			14.4
−0.4	Dog 47-20 Total............				114.5		60.4	175	53	
15.9	Lactalbumin 45, *dl*-valine 2, *l* + histidine 1	249	100	10.0	24.8	4.6	13.2			8.6
−0.6	Dog 47-20 Total............				123.3		65.8	189	53	
15.3	Lactalbumin 37, casein 10	248	100	7.3	21.1	5.1	14.9			10.2
+1.2	Dog 47-20 Total............				105.3		74.4	180	72	
14.2	Lactalbumin 41, casein 5	234	97	7.0	23.5	6.1	14.4			13.4
−0.7	Dog 47-27 Total............				117.5		72.1	190	61	
13.5	Lactalbumin 41, casein digest 5.4	240	100	7.7	28.6	6.3	20.0			15.0
+0.4	Dog 47-27 Total............				143.0		100.0	243	70	

and is responsible for a large total blood protein production and a ratio of plasma protein to hemoglobin of 40 to 50 per cent (5). Various preparations of casein and casein digests have been tested with similar results (Table 10). "Somagen" is a mixture of milk protein supplemented by various concentrates

SUMMARY TABLE 10

Casein and Somagen

Weight gain or loss	Protein intake		Food consumption	Protein output					Production ratio plasma protein to hemoglobin	Urinary nitrogen output week
	Type	Week		Hemoglobin		Plasma protein		Total 5 weeks		
				Level	Week	Level	Week			
kg.	*gm. per day*	*gm.*	*per cent*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*gm.*	*per cent*	*gm.*
16.2	Casein 40	204	86	10.8	40.6	4.8	17.7			14.0
−2.0	Dog 47-30 Total............				203		88.3	291	44	
14.2	Casein 38, *l*-cystine 2	215	91	9.5	39.2	4.8	18.9			13.1
−0.4	Dog 47-30 Total............				195.8		94.4	290	48	
13.2	Somagen 45	219	100	10.0	39.8	4.8	21.1			12.0
+1.0	Dog 47-42 Total............				199.0		105.4	304	53	
14.9	Somagen 50	225	96	8.9	39.6	5.0	26.0			9.6
+0.3	Dog 46-4 Total.............				197.9		130.0	328	65	
19.9	Somagen 45	208	95	8.7	33.8	5.3	18.9			10.5
+0.2	Dog 40-32 Total............				168.8		94.4	263	55	
15.7	Somagen 40	177	89	8.3	32.2	4.8	21.2			11.1
+1.4	Dog 40-36 Total............				161.0		106.2	267	66	
14.8	Somagen 45	219	100	9.1	40.7	4.8	20.9			8.7
+0.5	Dog 47-33 Total............				203.6		104.5	308	51	
12.1	Somagen 45	195	100	7.7	32.8	6.4	21.7			13.1
+0.9	Dog 47-27 Total............				164.1		108.7	273	66	

and vitamins much used in the diets of young children. When compared with casein it shows abundant total blood protein production and a plasma protein to hemoglobin ratio of 50 to 60 (Table 10). It resembles casein and casein digests as tested.

Folic acid was tested in these doubly depleted dogs in conjunction with various diets. It was suspected that it might stimulate the production of extra hemoglobin but there is no evidence to indicate any such response. In fact the natural stimulus for hemoglobin production is probably maximal in these doubly depleted dogs and they are using every bit of material available to form new hemoglobin and plasma protein. Table 11, dog 46-5, did suggest that larger doses of folic acid might inhibit new blood protein production. Subsequent experiments with other diet proteins gave no support and we must record the response to folic acid in the doses given under these conditions as negative.

We have reported earlier (5) experiments with wheat gluten and peanut flour. The experiments in Table 12 show that wheat gluten gives a good response when fed to these doubly depleted dogs. Small supplements of casein or lean meat improve the response but do not change significantly the plasma protein to hemoglobin ratio. The wheat gluten diet does not appeal to the dogs and it is difficult to carry through 5 week experiments (Table 12).

Peanut flour was tested earlier (5) and the response to this diet is poor. It will be noted in Table 12 that small supplements of casein or beef improve markedly the total blood protein response.

Soy bean flour lies between the wheat gluten and peanut flour as to its capacity to form new blood protein in these depleted dogs. It does not compare with casein. A small addition of casein digest appears to improve the hemoglobin output somewhat.

Visceral products have long held our interest in experiments on dogs with simple anemia or combined anemia and hypoproteinemia (double depletion). In general we can say that visceral proteins are well used and compare favorably with other good food proteins but there are some interesting differences. *Beef heart* (4) is distinctly different from *skeletal muscle*. Perhaps skeletal muscle is able to store material more readily than heart muscle, but whatever the fact it produces more total blood protein and hemoglobin in these experiments (Tables 8 and 11). Beef heart is no better than salmon muscle. *Pig stomach* (Table 13) is less potent than beef heart and we must assume that the *smooth muscle* of the stomach is not as potent as beef heart although the mucosa is a confusing factor. Probably gastric mucosa might be assumed to resemble the pancreas in which case the value for smooth muscle would be even lower.

The responses to *beef spleen* varied a good deal, which perhaps is not surprising, but it is a good dietary protein with a response not unlike that of liver diet. A single experiment with kidney, and two experiments with pancreas yielded results of the same order.

Thymus tissue is rather different in its make-up yet in the diet it resembles beef heart. Obviously, to judge from the findings, nuclear material is very abundant in thymus tissue.

SUMMARY

Dogs with sustained anemia plus hypoproteinemia due to bleeding and a continuing low protein or protein-free diet containing abundant iron have been used in the present work to test food proteins and supplements as to their

SUMMARY TABLE 11

Folic Acid, Salmon, Beef, Lactalbumin

Weight gain or loss	Protein intake: Type	Protein intake: Week	Food consumption	Protein output: Hemoglobin Level	Protein output: Hemoglobin Week	Protein output: Plasma protein Level	Protein output: Plasma protein Week	Protein output: Total 5 weeks	Production ratio plasma protein to hemoglobin	Urinary nitrogen output week
kg.	*gm. per day*	*gm.*	*per cent*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*gm.*	*per cent*	*gm.*
11.0	Salmon 125	208	82	7.0	20.7	4.7	13.8			6.7
+0.1	Dog 46-5 Total.............				103.6		69.0	173	60	
11.1	Salmon 135, folic acid 5 mg.	255	93	7.4	19.2	5.1	10.9			8.6
+0.6	Dog 46-5 Total.............				95.9		54.6	151	57	
11.7	Salmon 125, folic acid 30 mg.	232	94	6.8	15.5	5.4	11.2			10.3
+0.2	Dog 46-5 Total.............				77.7		56.1	134	71	
12.6	Lean beef 165	249	100	11.5	37.0	4.5	12.2			13.6
+0.5	Dog 44-16 Total............				184.0		61.2	245	33	
13.1	Lean beef 165, folic acid 30 mg.	248	100	10.7	33.1	4.8	18.9			16.3
+0.7	Dog 44-16 Total............				165.7		94.5	260	57	
11.7	Lean beef 170	247	99	9.4	38.6	5.0	15.5			9.4
+1.8	Dog 46-4 Total.............				192.9		77.7	271	40	
13.5	Lean beef 165, folic acid 20 mg.	241	97	9.6	38.6	4.9	17.7			9.8
+0.9	Dog 46-4 Total.............				192.9		88.5	281	46	
14.4	Lean beef 165, folic acid 20 mg.	244	100	7.9	30.4	4.9	18.6			10.6
+0.9	Dog 46-4 Total.............				152.2		92.9	245	61	
13.8	Lean beef 150, folic acid 10 mg., B_{12}-6 μg.	267	97	8.6	24.6	5.0	10.7			—
0	Dog 46-4 Total.............				146.4		63.9	210	43	
13.7	Lactalbumin 50, folic acid 5 mg.	244	100	8.4	25.4	4.9	17.7			10.6
+0.9	Dog 46-10 Total............				127.0		88.7	216	70	
15.5	Lactalbumin 45, folic acid 20 mg.	205	90	7.0	19.1	5.1	13.8			11.0
−0.8	Dog 46-10 Total............				95.5		69.0	165	72	

SUMMARY TABLE 11—*Concluded*

Weight gain or loss	Protein intake: Type	Protein intake: Week	Food consumption	Protein output: Hemoglobin: Level	Protein output: Hemoglobin: Week	Protein output: Plasma protein: Level	Protein output: Plasma protein: Week	Protein output: Total 5 weeks	Production ratio plasma protein to hemoglobin	Urinary nitrogen output week
kg.	*gm. per day*	*gm.*	*per cent*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*gm.*	*per cent*	*gm.*
14.7	Lactalbumin 45	143	76	6.7	16.5	5.5	12.0			9.0
−1.6	Dog 46-10 Total............				82.4		60.2	143	73	
10.2	Somagen 40, folic acid 40 mg.	205	93	8.5	41.0	5.0	20.1			9.3
+1.3	Dog 47-33 Total............				205.2		100.8	306	49	

capacity to produce new hemoglobin and plasma proteins. The reserve stores of blood protein–producing materials are thus largely depleted in such animals and sustained levels of 6 to 8 gm. per cent hemoglobin and 4 to 5 gm. per cent plasma protein can be maintained for considerable periods of time. The stimulus of double depletion drives the body to use all protein building materials with the utmost conservation. This represents a severe biological test for food and body proteins and its assay value must have significance.

Measured by this biological test in these experiments, casein stands well up among the best food proteins. The ratio of plasma protein to hemoglobin is about 40 to 50 per cent, which emphasizes the fact that these dogs produce on most diets about 2 gm. hemoglobin to 1 gm. plasma protein. The reason for this preference for hemoglobin production is obscure. The mass of circulating hemoglobin is greater even in this degree of anemia and the life cycle of hemoglobin is much longer than that of the plasma protein.

Egg protein, egg albumin, and lactalbumin all favor the production of more plasma protein and less hemoglobin as compared with casein. The plasma protein to hemoglobin ratio is increased, sometimes above 100 per cent. Supplements to the above proteins of casein digests or several amino acids may return the response toward that which is standard for casein. Histidine as a supplement to egg protein increases the total blood protein output and brings the ratio of plasma protein to hemoglobin toward that of casein.

Beef muscle goes to the other extreme and favors new hemoglobin production up to 4 gm. hemoglobin to 1 gm. plasma protein—a ratio of 25 per cent. The total amounts of new blood proteins are high.

Lactalbumin as compared with casein shows a lower total blood protein output and a plasma protein to hemoglobin ratio of 70 to 90 per cent. Amino acid supplements are less effective.

SUMMARY TABLE 12

Wheat Gluten, Soy Bean and Peanut Flour*

Weight gain or loss	Protein intake: Type	Protein intake: Week	Food consumption	Protein output: Hemoglobin Level	Protein output: Hemoglobin Week	Protein output: Plasma protein Level	Protein output: Plasma protein Week	Protein output: Total 5 weeks	Production ratio plasma protein to hemoglobin	Urinary nitrogen output week
kg.	*gm. per day*	*gm.*	*per cent*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*gm.*	*per cent*	*gm.*
19.7	Wheat gluten 30, casein 10	216	100	8.8	42.6	5.5	19.4			18.9
−2.1	Dog 45-2 Total.............				213.2		97.0	310	45	
16.9	Wheat gluten 40	209	100	8.2	26.7	4.6	15.5			19.4
−1.2	Dog 45-2 Total.............				133.4		77.5	211	58	
24.1	Wheat gluten 40, casein 5	232	97	8.3	42.4	5.5	29.4			19.2
−0.7	Dog 40-32 Total............				212.1		146.9	359	69	
23.4	Wheat gluten 45	180	79	7.9	35.1	5.4	22.1			18.8
−1.4	Dog 40-32 Total............				140.5		88.3	229	63	
18.8	Wheat gluten 30, casein 10	216	100	8.7	37.8	5.5	20.5			19.2
−1.8	Dog 47-26 Total............				189.2		102.3	292	54	
17.0	Wheat gluten 30, lean meat 40	217	100	9.2	43.2	5.0	21.1			17.0
−0.6	Dog 47-26 Total............				216.0		105.6	322	49	
14.6	Soy bean 54, casein digest 5	192	86	8.6	31.0	4.5	11.9			15.4
−0.5	Dog 47-36 Total............				155.0		59.7	215	39	
14.3	Soy bean 72	189	79	9.3	26.3	4.8	9.8			10.5
−1.5	Dog 47-36 Total............				131.3		49.2	181	38	
13.2	Soy bean 60	204	86	7.4	18.7	5.1	14.3			12.2
−0.5	Dog 47-25 Total............				93.4		71.7	165	77	
19.0	Peanut 40, casein 10	225	100	9.1	36.2	5.1	17.9			17.7
−1.9	Dog 47-20 Total............				181.0		89.3	270	49	
17.1	Peanut 50	215	100	10.6	16.4	4.3	4.7			16.4
−1.2	Dog 47-20 Total............				81.9		23.4	105	29	
13.8	Peanut 40, casein 10	216	96	10.5	42.8	4.7	15.0			14.3
−1.0	Dog 49-40 Total............				214.2		81.6	296	38	
12.8	Peanut 30, lean beef 40	174	95	10.7	34.7	4.6	13.0			15.2
−0.4	Dog 49-40 Total............				173.4		64.9	238	37	
12.4	Peanut 43	150	83	12.0	26.8	4.3	6.9			12.9
−1.4	Dog 49-40 Total............				134.2		34.6	169	26	

* Bureau Biological Research of Rutgers University

SUMMARY TABLE 13

Visceral Products

Pig Kidney, Stomach and Pancreas, Beef Spleen, Beef Brain, Calf Thymus

Weight gain or loss	Protein intake: Type	Protein intake: Week	Food consumption	Protein output: Hemoglobin Level	Protein output: Hemoglobin Week	Protein output: Plasma protein Level	Protein output: Plasma protein Week	Total 5 weeks	Production ratio plasma protein to hemoglobin	Urinary nitrogen output week
kg.	*gm. per day*	*gm.*	*per cent*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*gm.*	*per cent*	*gm.*
16.5	Pig kidney 120	242	100	8.5	35.0	5.3	20.1			11.9
+0.4	Dog 47-20 Total............				174.8		100.7	276	58	
12.5	Pig stomach* 300	206	98	12.0	20.5	4.3	6.6			18.0
+0.4	Dog 44-16 Total............				102.3		33.3	136	33	
15.3	Pig stomach* 300	224	100	8.3	27.2	4.7	14.1			16.5
+0.3	Dog 46-9 Total.............				136.1		70.3	206	52	
16.9	Beef spleen 110	207	100	8.8	43.8	4.6	13.4			10.3
0	Dog 47-20 Total............				218.8		66.9	286	30	
19.1	Beef spleen 110	207	100	8.2	29.6	4.6	16.3			10.4
−0.1	Dog 47-33 Total............				148.1		81.6	230	55	
17.0	Beef spleen 110	207	100	7.2	26.5	5.5	17.5			13.2
−0.4	Dog 47-26 Total............				132.4		87.7	220	66	
15.7	Beef brain 222	216	100	7.4	21.9	4.7	11.3			12.4
−0.1	Dog 46-12 Total............				109.7		56.3	166	51	
11.6	Beef brain 185	210	100	8.1	29.3	5.0	17.1			9.3
−1.2	Dog 49-43 Total............				146.4		85.3	232	58	
16.6	Pig pancreas 115	228	100	7.6	24.9	5.6	14.6			12.2
−1.1	Dog 47-26 Total............				149.4		88.2	238	59	
16.8	Pig pancreas* 124	216	97	8.8	30.2	4.7	14.7			10.5
−0.5	Dog 46-9 Total.............				150.9		73.5	224	46	
14.1	Calf thymus 120	220	97	11.0	29.6	4.6	10.8			17.7
−0.7	Dog 45-6 Total.............				147.8		53.9	202	36	
14.2	Calf thymus* 150	212	96	8.6	29.8	5.3	13.5			16.0
−1.0	Dog 45-3 Total.............				149.1		67.6	217	45	
12.7	Calf thymus* 150	207	98	12.1	19.0	4.5	7.8			17.3
+0.1	Dog 45-6 Total.............				94.9		38.8	134	41	

* Fed raw—all other material is cooked.

Fibrin is a good food protein in these experiments—much like casein. When fed over these 5 week periods it causes a *sustained increase in blood fibrinogen*.

Folic acid in the doses given has no effect on the expected response to various diets.

Peanut flour is a very poor diet for the production of new hemoglobin and plasma proteins. Small supplements of casein and beef show a significant response with improved output of blood proteins.

Soy bean flour gives a poor response and wheat gluten a good response with adequate output of blood proteins.

Visceral products show some variety. Beef heart is not as effective as beef muscle. Beef spleen, kidney, and pancreas give good responses but not up to casein. Pig stomach, beef brain, and calf thymus are below average. The plasma protein to hemoglobin ratio shows a narrow range (40 to 60 per cent) in experiments with visceral products.

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