

## DIETARY EFFECTS ON ANEMIA PLUS HYPOPROTEINEMIA IN DOGS

### II. THE FINDINGS WITH MILK PRODUCTS, WHEAT, AND PEANUT FLOURS AS COMPARED WITH LIVER\*

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When we compare the effect of the proteins in milk products, wheat and peanut flour, and liver with that of the proteins tabulated in Paper I (1), there are significant differences. *Casein* purified or as a commercial powder is a superior protein as promoting new hemoglobin and plasma protein output. *Casein* in fact compares favorably with liver and meat under the experimental conditions. *Lactalbumin* by contrast is not as effective in promoting blood protein output and tends to favor plasma protein production, in this respect resembling egg albumin. *Peanut flour* is definitely inadequate to promote satisfactory new hemoglobin and plasma protein output. *Wheat gluten* is neither superior nor inferior to many proteins but is difficult to feed over many weeks and distaste limits food intake, causes weight loss, and complicates the observations. *Liver* serves as a control as its capacity to promote hemoglobin and plasma protein production is well established.

The details of method and the experimental histories are given in Paper I.

#### EXPERIMENTAL OBSERVATIONS

*Casein* purified for the Bureau of Biological Research of Rutgers University was tested in three adequate experiments, given in Table 21. The casein was precipitated by acid and washed with suitable acid water solutions at the proper pH. No solvents were used. Drying was done in warm air (60–70°C.) and material was ground in a hammer mill. All experiments show complete food consumption and essential weight balance during 4 and 5 week diet periods. The blood protein output was very high, 45 to 70 gm. per week, which corresponds to beef muscle feeding. The ratio of hemoglobin production was high (3 or even 4 times the plasma protein) and the ratio of blood protein output to food protein intake was also high (26 to 30 per cent).

*Commercial casein* purchased in the open market was also tested (Table 22). The response was much like that noted with pure casein (Table 21). The total

\* We are indebted to Eli Lilly and Company for aid in conducting this work. We are indebted to Dr. James B. Allison, Dr. William H. Cole, and Dr. R. H. Barnes of Rutgers University who initiated this cooperative investigation on evaluating protein foods.

TABLE 21  
Casein (Rutgers University)

Weight	Protein intake		Food consumption	Protein output				Production ratio plasma protein to hemoglobin
	Type	Weekly		Hemoglobin		Plasma protein		
				Level	Output per wk.	Level	Output per wk.	
kg.		gm.	per cent	gm. per cent	gm.	gm. per cent	gm.	per cent
14.9	<i>Dog 45-2</i> Basal	19	100	8.6	61.5	4.6	24.4	
14.9	Casein	208	100	9.3	35.4	4.5	16.5	
14.9	Casein	237	100	10.7	47.4	4.9	19.6	
14.4	Casein	237	100	10.5	53.7	5.2	21.9	
14.8	Casein	237	100	10.4	44.9	4.9	19.8	
14.6	Casein	237	100	11.0	45.3	4.9	18.5	
-0.3	Totals . . . . .	1156		(255.1)	226.7	(95.9)	96.3	38
11.8	<i>Dog 45-6</i> Whole egg	166	96	6.6	20.5	5.0	15.3	
11.9	Casein	208	100	9.7	1.9	5.0	1.6	
11.8	Casein	208	100	10.2	48.1	4.7	16.9	
11.7	Casein	208	100	11.2	45.0	4.8	17.1	
11.7	Casein	208	100	10.7	42.4	4.7	15.4	
11.7	Casein	208	100	12.0	43.0	4.9	16.4	
-0.1	Totals . . . . .	1040		(248.6)	180.4	(64.7)	67.4	26
17.0	<i>Dog 42-1</i> Lactalbumin	173	100	5.9	19.9	5.2	15.0	
17.2	Casein	178	100	8.0	1.5	5.6	1.0	
17.1	Casein	178	100	8.1	28.8	5.4	17.5	
16.9	Casein	173	97	8.6	37.7	5.2	21.5	
17.0	Casein	160	90	10.5	21.1	4.8	11.2	
0	Totals . . . . .	689		(143.3)	89.1	(39.5)	51.2	28

In the tables figures in parentheses indicate "net corrected total output."

weekly output of hemoglobin and plasma protein was 53 and 67 gm. One dog lost 0.7 kilo in 5 weeks but this was a large animal (22 kilos). This dog showed a higher ratio of plasma protein to hemoglobin than the others on a casein diet. Both dogs ate all their diet and were in perfect condition.

*Lactalbumin* (Table 23) showed significant differences when compared with

casein. This was a commercial product. One dog had a slight weight gain, one a slight weight loss, and a third considerable weight loss in a 5 week experiment. The total weekly blood protein output was lower than that with casein—31 to 57 gm. and the ratio of protein output to food protein intake was also lower. The most striking difference was in the ratio of plasma protein to

TABLE 22  
*Casein (Commercial)*

Weight	Protein intake		Food consumption	Protein output				Production ratio plasma protein to hemoglobin
	Type	Weekly		Hemoglobin		Plasma protein		
				Level	Output per wk.	Level	Output per wk.	
kg.		gm.	per cent	gm. per cent	gm.	gm. per cent	gm.	per cent
22.7	<i>Dog 40-32</i> Fibrin	245	100	8.3	2.5	5.8	1.4	
22.8	Casein	222	100	7.8	27.6	5.5	17.0	
22.4	Casein	222	100	9.4	39.7	6.3	26.2	
22.1	Casein	222	100	7.5	42.9	6.4	28.1	
22.3	Casein	222	100	9.2	26.4	6.2	20.3	
22.0	Casein	222	100	9.5	45.6	6.6	33.3	
-0.7	Totals . . . . .	1110		(199.7)	182.2	(133.8)	124.9	67
15.3	<i>Dog 44-16</i> Basal	18	97	10.9	25.5	4.3	9.8	
14.9	Casein	222	100	10.7	27.3	4.1	9.5	
14.4	Casein	222	100	12.0	26.9	4.2	8.5	
14.3	Casein	222	100	13.2	31.4	4.4	10.9	
14.7	Casein	222	100	11.4	47.0	4.6	16.0	
15.2	Casein	222	100	13.6	34.4	4.7	11.2	
-0.1	Totals . . . . .	1110		(205.5)	167.0	(57.6)	56.1	28

hemoglobin which was high—72, 90, and 94 per cent. One notes that in all experiments the hemoglobin levels fell and the plasma protein levels rose as was noted in the experiments with whole egg and egg albumin. *Lactalbumin* seems to favor plasma protein production in contrast to casein and liver (Table 27).

*Peanut flour*, purified or commercial, (Tables 24 and 25) did not support much new blood protein production. Peanut flour was prepared for the Bureau of Biological Research of Rutgers University using the McMath-Howard process. This gave a partially defatted flour; analyses will be reported elsewhere. The findings with processed flour differed in no significant

TABLE 23  
*Lactalbumin (Commercial)*

Weight	Protein intake		Food consumption	Protein output				Production ratio plasma protein to hemoglobin
	Type	Weekly		Hemoglobin		Plasma protein		
				Level	Output per wk.	Level	Output per wk.	
kg.		gm.	per cent	gm. per cent	gm.	gm. per cent	gm.	per cent
14.6	<i>Dog 45-2</i> Casein	237	100	11.0	45.3	4.9	18.5	
14.8	Lactalbumin	239	100	9.0	63.9	4.9	24.3	
15.3	Lactalbumin	239	100	6.9	49.9	5.1	28.8	
15.0	Lactalbumin	225	94	7.3	22.5	5.3	14.6	
15.3	Lactalbumin	239	100	7.4	25.0	5.6	14.9	
15.4	Lactalbumin	239	100	7.7	38.9	5.6	25.0	
+0.8	Totals . . . . .	1181		(166.7)	200.2	(119.7)	107.6	72
11.7	<i>Dog 45-6</i> Casein	208	100	12.0	43.0	4.9	16.4	
11.5	Lactalbumin	148	73	8.3	60.4	4.8	22.3	
11.5	Lactalbumin	124	61	8.3	21.0	4.8	12.1	
11.2	Lactalbumin	144	71	5.7	28.2	5.1	19.2	
11.6	Lactalbumin	195	96	7.9	1.7	5.3	1.3	
11.4	Lactalbumin	173	85	8.1	26.3	5.8	16.3	
-0.3	Totals . . . . .	784		(80.6)	137.6	(75.5)	71.2	94
14.1	<i>Dog 46-4</i> Fibrin	228	93	8.3	27.8	5.5	16.4	
14.0	Lactalbumin	207	85	7.7	35.5	5.2	22.6	
13.7	Lactalbumin	173	71	8.6	21.9	5.4	15.3	
12.9	Lactalbumin	110	45	6.8	20.8	5.7	16.0	
12.6	Lactalbumin	150	80	7.7	19.9	6.0	14.1	
11.7	Lactalbumin	147	83	6.0	28.6	5.4	21.4	
-2.4	Totals . . . . .	787		(95.7)	126.7	(86.2)	89.4	90

manner from those with the commercial product when it was incorporated in these diets.

The peanut flour was not well eaten and distaste for the food mixture terminated some experiments not reported here, and shortened others. Peanut flour was incorporated in baked biscuit with no increase in palatability. All dogs

lost weight—1 to 2 kilos in 3 to 5 weeks. Only one dog tolerated the diet for 5 weeks and this dog, 47-25, (Table 24) gave the highest output of blood protein per week—33 gm. A glance at the table however shows that the peanut flour experiment followed a casein experiment with no basal diet period intervening. There was obviously some “carry-over” from the very favorable casein diet,

TABLE 24  
*Peanut Flour (Rutgers University)*

Weight	Protein intake		Food consumption	Protein output				Production ratio plasma protein to hemoglobin
	Type	Weekly		Hemoglobin		Plasma protein		
				Level	Output per wk.	Level	Output per wk.	
				gm. per cent	gm.	gm. per cent	gm.	
<i>kg.</i>		<i>gm.</i>	<i>per cent</i>	<i>gm. per cent</i>	<i>gm.</i>	<i>gm. per cent</i>	<i>gm.</i>	<i>per cent</i>
11.8	<i>Dog 47-25</i> Casein	242	100	9.3	34.0	5.0	18.0	
11.8	Peanut flour	239	100	8.8	41.4	5.2	21.9	
11.5	Peanut flour	220	92	10.2	24.3	4.7	11.4	
11.0	Peanut flour	174	78	9.7	23.6	4.8	9.7	
10.7	Peanut flour	123	67	10.9	14.5	4.3	5.5	
10.7	Peanut flour	147	80	11.1	2.4	4.5	0.8	
-1.1	Totals . . . . .	903		(122.5)	106.2	(44.5)	49.3	36
11.6	<i>Dog 46-5</i> Basal	19	100	8.7	40.2	4.4	17.4	
11.1	Peanut flour	134	91	8.7	19.6	4.2	8.8	
10.5	Peanut flour	113	77	11.2	2.0	4.3	1.2	
10.2	Peanut flour	100	90	11.8	2.0	4.7	1.0	
-1.4	Totals . . . . .	347		(40.3)	23.6	(7.4)	11.0	18

and the 1st week showed very high output figures for hemoglobin and plasma protein—a surplus of about 30 gm. due to the casein carry-over. This would reduce the *true weekly output* from 33.7 gm. to approximately 28 gm. The total blood protein output in all experiments did not amount to 50 per cent of the blood protein output due to casein feeding. The ratio of blood protein production to food protein intake was very low. The ratio of plasma protein to hemoglobin was low in all experiments except dog 41-53 (Table 25). Here egg feeding preceded the peanut flour diet, and as there was no basal diet interval there probably was some “carry-over” with more plasma protein produced

(egg favors plasma protein over hemoglobin). This may in part explain the high plasma protein to hemoglobin ratio.

TABLE 25  
*Peanut Flour (Commercial)*

Weight	Protein intake		Food consumption	Protein output				Production ratio plasma protein to hemoglobin
	Type	Weekly		Hemoglobin		Plasma protein		
				Level	Output per wk.	Level	Output per wk.	
kg.		gm.	per cent	gm. per cent	gm.	gm. per cent	gm.	per cent
19.3	<i>Dog 41-53</i> Basal	23	90	5.9	24.0	5.0	15.6	
18.7	Peanut flour	198	84	6.3	1.4	5.4	1.0	
18.4	Peanut flour	221	94	6.6	42.6	5.0	23.2	
17.6	Peanut flour	182	77	6.6	1.9	4.5	1.2	
-1.7	Totals . . . . .	601		(49.3)	45.9	(16.9)	25.4	34
16.5	<i>Dog 41-53</i> Egg	160	76	8.0	21.3	5.4	13.2	
16.2	Peanut flour biscuit*	181	75	5.6	32.3	5.0	18.2	
15.2	Peanut flour biscuit*	253	83	5.6	7.6	4.4	5.1	
14.6	Peanut flour biscuit*	276	91	6.9	22.5	4.8	12.4	
-1.9	Totals . . . . .	710		(43.5)	62.4	(29.0)	35.7	67

\* Baked into biscuit.

Wheat gluten (Table 26) was not well suited to this type of experiment and the dogs usually ate the mixture with reluctance. Wheat gluten as prepared for the Bureau of Biological Research of Rutgers University represented wheat flour treated with cold water to hydrate the gluten. Water was added repeatedly in further treatment to remove the starch and the washings were at a temperature of 90—170°F. Finally the gummy matrix was dried *in vacuo* in trays at 130°F. No defatting was attempted.

The experiments with *wheat gluten* show differences which we believe are in large part explained by weight loss. One dog (45-3, Table 26) ate the diet for 4 weeks and actually gained 0.1 kilo. This dog showed a low average figure (34 gm.) for total net weekly blood protein output. The two other dogs of Table 26 showed rapid weight loss (3.0 and 2.8 kilos) in 4 weeks and a lower wheat gluten intake because of distaste for this food. This weight loss we assume explains some of the increase in net figures for whole blood protein

TABLE 26  
*Wheat Gluten (Rutgers University)*

Weight	Protein intake		Food consumption	Protein output				Production ratio plasma protein to hemoglobin
	Type	Weekly		Hemoglobin		Plasma protein		
				Level	Output per wk.	Level	Output per wk.	
kg.		gm.	per cent	gm. per cent	gm.	gm. per cent	gm.	per cent
21.7	<i>Dog 42-1</i> Basal	19	100	9.8	39.9	4.9	20.1	
20.5	Wheat gluten	173	84	10.1	41.2	4.6	18.7	
19.8	Wheat gluten	182	95	8.7	54.4	5.0	27.3	
19.0	Wheat gluten	155	72	8.7	27.1	4.9	14.0	
18.7	Wheat gluten	188	85	9.6	26.8	4.5	11.8	
-3.0	Totals . . . . .	698		(125.6)	149.5	(60.2)	71.8	48
12.6	<i>Dog 45-3</i> Basal	31	100	11.0	30.0	4.2	10.7	
12.9	Wheat gluten	244	100	12.8	2.0	4.1	1.0	
12.9	Wheat gluten	207	98	12.3	31.0	4.4	8.4	
13.1	Wheat gluten	202	95	12.0	33.3	4.5	9.9	
12.7	Wheat gluten	201	94	10.1	42.7	4.4	15.7	
+0.1	Totals . . . . .	854		(96.8)	109.0	(37.4)	35.0	39
21.9	<i>Dog 40-32</i> Amino acid, casein	211	100	9.3	36.4	4.6	17.1	
20.7	Wheat gluten	165	76	8.2	59.1	4.9	26.8	
20.0	Wheat gluten	115	67	9.8	43.4	5.2	20.8	
19.7	Wheat gluten	168	84	7.8	45.9	5.2	23.9	
19.1	Wheat gluten	212	98	8.8	36.0	4.7	19.3	
-2.8	Totals . . . . .	660		(153.7)	184.4	(85.6)	90.8	55

output (60 and 47 gm.) as the body conserves material under conditions of weight loss which contribute to new blood proteins (2). The same explanation holds for the high ratios of protein output to intake (Summary Table 28).

*Pig liver* (Table 27) is a standard diet factor and we have completed many such experiments. The three given are fair examples. There were trivial weight fluctuations in the first two experiments and gain of 1.0 kilo in the third experiment. Production of hemoglobin was conspicuous and exceeded the plasma protein output three to one. The hemoglobin level in the circulating

blood rose in spite of heavy bleeding. The weekly net output of hemoglobin and plasma protein was 45, 48, and 53 gm.

TABLE 27  
*Pig Liver*

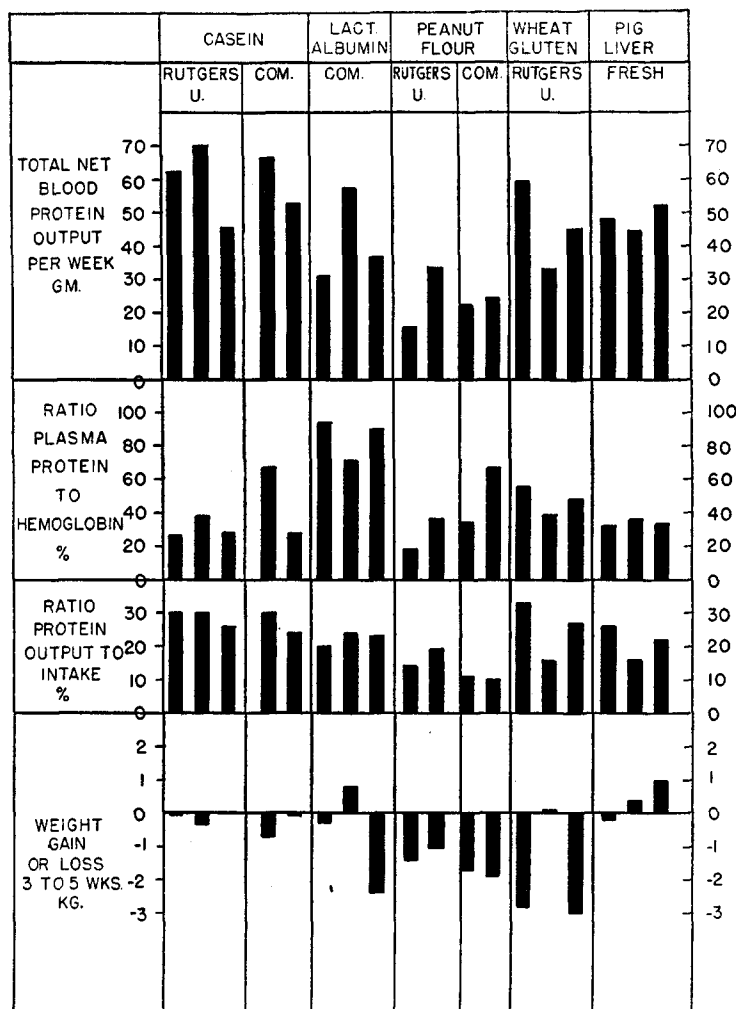
Weight	Protein intake		Food consumption	Protein output				Production ratio plasma protein to hemoglobin
	Type	Weekly		Hemoglobin		Plasma protein		
				Level	Output per wk.	Level	Output per wk.	
kg.		gm.	per cent	gm. per cent	gm.	gm. per cent	gm.	per cent
11.0	<i>Dog 45-3</i> Amino acids	169	68	7.7	22.4	4.6	10.6	
11.1	Liver	261	98	9.7	13.8	4.2	6.4	
11.2	Liver	281	100	8.3	44.0	4.7	20.3	
11.4	Liver	281	100	9.4	24.3	4.4	11.0	
+0.4	Totals . . . . .	823		(98.4)	82.1	(35.3)	37.7	36
15.5	<i>Dog 46-22</i> Amino acids	155	48	10.3	29.6	4.4	12.5	
15.9	Liver	239	98	10.1	24.2	4.5	10.1	
15.8	Liver	234	96	9.2	43.5	4.7	16.8	
15.6	Liver	102	65	9.7	27.5	4.4	10.8	
15.1	Liver	153	85	10.3	28.2	4.5	10.6	
15.3	Liver	202	100	11.9	32.4	4.4	11.5	
-0.2	Totals . . . . .	930		(182.0)	155.8	(58.2)	59.8	32
16.3	<i>Dog 46-9</i> Basal	19	100	8.9	67.5	4.6	22.5	
16.3	Liver	247	100	10.6	31.2	4.5	12.4	
16.4	Liver	247	100	8.3	49.2	4.7	20.2	
16.6	Liver	247	100	9.5	26.9	4.8	12.1	
16.7	Liver	240	100	10.7	30.6	4.5	11.8	
17.3	Liver	239	100	10.4	30.2	4.4	10.8	
+1.0	Totals . . . . .	1220		(196.6)	168.1	(66.6)	67.3	34

Summary Table 28 gives comparative values for these various proteins. *Casein* and *liver* stand out as most efficient. Both give maximal amounts of new formed blood protein (hemoglobin and plasma protein), if anything casein somewhat more than whole liver. The ratio of hemoglobin to plasma protein is about 3 to 1 with both pure and commercial casein and whole liver. The ratio



of blood protein output to food protein intake is correspondingly high. Weight is constant, food consumption adequate, and condition of the dogs excellent.

SUMMARY TABLE 28



*Peanut flour*, purified or commercial, stands in striking contrast to casein. The new blood protein produced is about one-half that following casein feeding. Plasma protein to hemoglobin ratio ranges from 20 to 67 per cent. The ratio of blood protein output to food protein intake is low. There is some weight loss in all experiments and the food mixtures are distasteful to dogs.

*Lactalbumin* is not as favorable for new blood protein production as is casein but the lactalbumin definitely favors plasma protein production and the ratio of plasma protein to hemoglobin is high (72, 90, and 94 per cent).

*Wheat gluten* is better than peanut flour but is not in the class with casein. The ratio of plasma protein to hemoglobin runs 40 to 55 per cent. There is conspicuous loss of weight (food distasteful) which in part is responsible for the apparently high levels of total blood protein production in two experiments.

#### SUMMARY

Casein (purified or commercial) in this type of experiment falls in the top bracket as a protein consistently favorable for maximal new hemoglobin and plasma protein production in doubly depleted dogs (anemic and hypoproteinemic).

Lactalbumin is less favorable for total blood protein production and the ratio of plasma protein to hemoglobin is high—that is lactalbumin favors plasma protein production as compared with casein, or is less favorable for hemoglobin production.

Peanut flour (purified or commercial) is less than half as effective as casein in promoting new blood protein production. The ratio of plasma protein to hemoglobin is about the same as casein.

Wheat gluten as tested is distasteful to dogs. It is neither very good nor very poor for blood protein production when it is eaten. There is nothing unusual about the response. Weight loss usually confuses the picture.

Liver stands as a control base line for the above experiments. Its capacity to further hemoglobin and plasma protein production is well established. The production of hemoglobin was about 3 times that of plasma protein in the experiments.

#### BIBLIOGRAPHY

1. Robscheit-Robbins, F. S., and Whipple, G. H., *J. Exp. Med.*, 1949, **89**, 339.
2. Whipple, G. H., Miller, L. L., and Robscheit-Robbins, F. S., *J. Exp. Med.*, 1947, **85**, 277.