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A Bioassay of Certain Protein Supplements When Fed to Baby Chicks.*

S. F. COOK AND K. G. SCOTT.

From the Division of Physiology, University of California Medical School, Berkeley.

Several investigators have reported the occurrence of a syndrome in chicks which is similar to scurvy in appearance.[1, 2, 3] Since several different diets were used in those studies employing different protein sources, it seemed useful to compare the effects produced by various proteins when fed with the same basal ration in order to observe if other proteins allowed the same symptoms to occur as did fish meal, as reported by Holst.

The syndrome has been well described independently by Holst, McFarlane, *et al.*, by Dam, and by Schönheyder.[4] In using a ration similar to the one employed by Holst we were able to produce this syndrome which is characterized by (1) the production of hemorrhages without respect to location, (2) an anemia, and (3) a prolonged blood clotting time.

Feeding experiments. Two groups of experiments were carried on. The diets used and results were as follows:

Diet I—Control. Of 100 parts, corn 55, wheat 20, skim milk 20, NaCl 0.5, cod liver oil (Patch) 1, $Ca_3(PO_4)_2$ 1, $CaCO_3$ 1, dried yeast 1. When 21 chicks were placed upon this ration none died after 10 weeks of feeding. No hemorrhages were observed.

Diet II—Control. Essentially the same as No. I. A commercial casein was used as a protein supplement. Fifteen chicks were placed upon this diet and no hemorrhages were observed at the end of 10 weeks. The mortality was zero.

Diet III—Experimental. Of 100 parts, corn 55.5, wheat 20, fish meal (from 2 samples sold commercially) 20, NaCl 5, cod liver oil 1, $Ca_3(PO_4)_2$ 1, $CaCO_3$ 1, and dried yeast 1. When 17 chicks were placed upon this diet, the mortality was 35% in 10 weeks; all

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1 Holst, W. F., and Halbrook, E. R., *Science*, N. S., 1933, **77**, 354.

2 McFarlane, W. D., Graham, W. R., and Hall, G. E., *J. Nutrition*, 1931, **4**, 331.

3 Dam, H., *Biochem. Inst.*, Copenhagen, May, 1934.

4 Schönheyder, F., *Nature*, 1935, **135**, 652.

of the birds had hemorrhages from time to time which were often fatal. When the fish meal concentration was reduced to 10% the same effect was observed.

We concluded from these experiments that the effects of feeding such fish meals were essentially different from other protein supplements here reported. The syndrome was much reduced when fish meal which had been washed with water and allowed to dry at 65°C. was used as a protein supplement in diet No. III.

A second series of experiment was planned upon a much larger scale and the protein supplements fed were commercial fish meals, types 1, 2, and 3; Commercial casein, type 4; and commercial meat scrap, type 5. The diets used in these experiments are to be found in Table I with analyses of the calcium, phosphorus, and protein content after the diets were mixed.

TABLE I.
Feed Formulas.
All diets (1-17 inclusive) contained sardine oil 1% and salt 0.5%.

Diet No.	Corn	Wheat	Casein	Calcium	Phosphorus	Yeast	— Protein Supplements — Type 1	Type 2	Type 3	Type 5	Protein
1	76.5	31	5.8	1.15	.67	1.25	6.25				16.4
2	75.9	31		1.13	.67	1.25	13.4				16.4
3	69.8	31		1.25	.72	1.25	20.0				19.3
4	76.8	31	5.4	1.17	.68	1.25		6.25			16.3
5	76.8	31		1.19	.66	1.25		12.5			16.5
6	71.1	31		1.13	.66	1.25		18.8			19.0
7	76.6	31	5.8	1.15	.68	1.25			6.25		16.4
8	76	31		1.11	.69	1.25			13.6		16.3
9	69.4	31		1.33	.77	1.25			20.4		18.9
10	77.1	31	11	1.25	.71	1.25					16.7
11			Same as No. 10								
12	72.2	31	16	1.19	.69	1.25					19.4
13	67.8	31		1.41	.84	1.25				22.3	19.1
14	69.8	31		1.21	.76	3.75	20.0				19.4
15	71.1	31		1.15	.69	3.75		18.75			19.0
16	69.4	31		1.29	.79	3.75			20.4		18.5
17A	69.8	31		1.17	.67	0	20.0				18.9
17B	69.8	31		1.17	.69	0		20.0			18.0

No. 18 contained Alfalfa 5, Bran 15, Wheat 27, Corn 35, Fish Meal 10, Skim Milk 5, Salt 0.5, Sardine Oil 0.5, Calcium 1.35%, Phosphorus 0.85%, and protein 17.5%.

Calcium, Phosphorus and Protein are percentages present in total mixture by analysis. Other figures are parts in 125.

Parallel experiments were carried on at 2 different laboratories for a period of 10 weeks. Fifteen chicks were placed in each group. Since the results of the 2 sets of experiments were very comparable, they are incorporated in the same table (Table II).

Results—Mortality. The mortality of the birds placed upon the diets containing fish meal types 1 and 2 (Table II) is excessively

TABLE II.
(Second Series of Experiments.)

Group No.	Protein used	% Mortality	% Hemorrhage	% Hemoglobin			Clotting Time	
				Aver.	High	Low	High min.	Low min.
1	Type 1, 5 %	3	17	48	67	25	*	1
2	" 1, 10 "	30	67	44	67	15	*	2
3	" 1, 15 "	37	80	47	69	15	*	2
4	" 2, 5 "	13	10	57	75	22	30	1
5	" 2, 10 "	43	80	49	67	20	*	1
6	" 2, 15 "	33	60	49	76	13	*	3
7	" 3, 5 "	3	3	61	84	49	12	1
8	" 3, 10 "	10	0	62	87	15	9	1
9	" 3, 15 "	0	7	57	75	13	7	1
10	" 4, 8.7 %	7	0	60	76	18	19	1
11	" " 8.7 "	7	0	62	71	47	21	1
12	" " 13 "	10	3	64	72	55	15	1
13	" 5, 18 "	3	3	55	84	30	62	1
14	" 1	20	53	50	65	20	*	2
15	" 2	24	64	50	69	9	*	2
16	" 3	3	0	63	79	49	4	1
17A	" 1	20	47	57	68	45	15	1
17B	" 2	53	46	36	52	26	33	2
18	" 1 and 2	0	0	58	75	14	4	1

* Clotting times longer than 100 min.

high with the exception of diet No. 18, and not at all comparable with the other protein supplements used.

Hemorrhages. The occurrence of hemorrhages was quantitatively much greater upon the fish meal types 1 and 2. Hemorrhages did not occur upon the other groups, including group 18. Some doubtful cases appeared upon supplements 3 and 4, but none upon supplement 5.

Hemoglobin level. When the protein level was the same, the groups fed fish meal types 1 and 2 were on the average distinctly anemic with respect to those fed other protein supplements (Sahli's method). Furthermore, in those groups fed fish meal types 1 and 2 with the same protein levels the average hemoglobin per cent was inversely proportional to the fish meal concentration (see groups 1 and 2, 4 and 5, Table II).

Blood Clotting Time. When fish meal types 1 and 2 were fed, a profound increase in blood clotting time was observed. For quantitative evidence see Table II.

Growth. The growth in all groups was comparable except in those to which no yeast was added, and was apparently normal for the stock used in the experiment.

Summary. Experimental evidence is offered to show that certain diets containing some types of fish meal induce the condition in

chicks which has been described in the text. Apparently the syndrome can be ascribed to the fish meals used in that they contained objectionable materials and/or lacked some accessory factor. It was not possible to produce this syndrome quantitatively by the use of other protein supplements.

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Metabolism of Benzene, Anthracene and Phenanthrene in Adult and Growing Dogs.

JAKOB A. STEKOL.

From the Department of Chemistry, Fordham University, New York City.

In view of similarity in the metabolism of bromobenzene and naphthalene in rabbits[1] and in adult and growing dogs,[2] it seemed of interest to extend the comparison of metabolism of aromatic hydrocarbons in dogs to benzene, anthracene and phenanthrene. Each substance was fed on 4 occasions to each of the 4 growing and 2 adult dogs in 1.0 gm. doses. The pups and dogs were maintained on a diet of constant composition and the urine collected every 24 hours. The diet, the general experimental procedure, methods of analysis of urine were the same as described by us elsewhere.[3] The pups were fed benzene, anthracene and phenanthrene at the age of 2, 3, 4, and 7 months at sufficiently large intervals (7-8 days) to allow the animals to return to the original nitrogen and sulfur balance. The analysis of urine collected after feeding the hydrocarbons to pups and dogs indicated the formation of ethereal sulfates from benzene and anthracene; benzene, in addition, and especially phenanthrene, raised the output of neutral sulfur. All 3 hydrocarbons increased the output of glycuronates in the urine.

Hele[4] on the basis of experiments with benzene, similar to those described here, suggested that benzene probably yields a mercapturic acid, although the latter could not be isolated by Baumann-Preusse method.[5] Pending the isolation of detoxication products of benzene, anthracene and phenanthrene from the urine of dogs and pups, now

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2 Stekol, J. A., *J. Biol. Chem.*, 1935, **110**, 463.

3 Stekol, J. A., *J. Biol. Chem.*, 1934, **107**, 641; 1935, **109**, 147.

4 Callow, E. H., and Hele, T. S., 1926, **20**, 598.

5 Baumann, E., and Preusse, C., *Ber. deutsch. chem. Ges.*, 1879, **12**, 806.