ponent is compared with the skin activity of each fraction. The ratio of the concentration in grams per 100 ml (Table I) of each component was calculated by using the amount of each component in the primary extract as the reference standard for that component. If the ratios of concentration of the components are compared with the ratios of the areas of reactivity in skin tests (Table II) it will be noted that the concentration of component 1 parallels results of skin tests on the fractions. The ratios of the concentrations of components 2 and 3 and of skin activity are not proportional. These calculations indicate a stability of component 1 and suggest that the allergen is associated with this component.

Summary. A saline-soluble portion of larvae of *Trichinella spiralis* has been compared as to its electrophoretic characteristics and production of allergic response in sensitized animals with other fractions, prepared by acidification and by heating. Each of the 3 fractions separated into 3 electrophoretic components in addition to an incompletely separated portion associated with component 2 and a fast moving heterogeneous mixture. In the primary extract the heterogeneous mass represented more than 60% of the total concentration. Either acid or heat treatment removed practically all of this mixture. More material was precipitated by heating than by acidification. The 3 fractions were diluted on the basis of the original dry weight of larvae and intradermal reactions compared. There was no apparent difference in the area of edema produced by the primary extract and the acid-soluble fraction. The heat-treated allergen caused slightly less reaction than the other 2. There is an indication that the allergen is associated with component 1.

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Purified Rations and the Importance of Folic Acid in Mink Nutrition.*

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Domestication of mink has introduced many practical nutritional problems. The use of purified rations which has elucidated practical feeding problems in many other species of animals appears to offer a useful tool in the study of these problems in mink. Establishment of nutritional requirements of an animal so different from those previously studied would also be of considerable academic interest. In this paper we wish to present data on the use of purified rations and the requirement of folic acid for the mink.

Experimental. Previous to being placed on experiment the mink were housed in individual outdoor wire pens. They were fed a ration containing 72% horsemeat (including bone), 10% fresh liver, 12% cereal mixture, and 6% tomato puree. When the animals were placed on experiment the cages were moved into an unheated room and the nest boxes removed. The composition of the purified rations employed is given in Table I. The basal ration was similar to that used for dogs by Schaefer *et al.*[1] All rations were weighed into pound butter crocks, fitted with inverted conical shields in order to reduce spillage. Weekly weight and daily food consumption data were recorded. Blood samples were taken by severing the metatarsal vein according to the technic described by Kennedy.[2] White blood cell counts were determined by the

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1 Schaefer, A. E., McKibbin, J. M., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 365.

2 Kennedy, A. H., *Ontario Gov't. Exp. Fur Farm, Bul. No. 6*, 1933.

TABLE I.
Synthetic Rations.

	Basal, %	High protein, %	Basal + gelatin, %
Sucrose	66	55	56
Casein—alcohol extracted	19	30	19
Gelatin	--	--	10
Cottonseed oil	8	8	8
Cod liver oil	3	3	3
Salts No. IV	4	4	4

Vitamin Supplements

	Mg per 100 g of ration
Thiamine	0.2
Riboflavin	0.4
Pyridoxine	0.2
Calcium pantothenate	1.5
Niacin	4.0
Choline	100

Vitamins Per Animal Per Day, Added Where Indicated
(Mixed in ration daily)

Folic acid	50	μg
Biotin	50	"
Inositol	20	mg
Para-aminobenzoic acid	50	"
Alpha-tocopherol	2	"
2-methyl-1,4-naphthoquinone	.5	"

usual methods and hemoglobin by the method of Evelyn.[3]

Results. Approximately one-half of the mink began eating the purified ration immediately whereas the remainder were given in addition to the purified ration 20 g of stock meat ration for the first 7 to 10 days. In general 2 weeks were necessary for the mink to become accustomed to the change in ration and the frequent handling.

Growth curves of Mink 9 and 10 placed on the basal ration are shown in Fig. 1. The animals lost weight at first and then showed a rather constant weight. After 10 to 13 weeks weight loss was again severe. This weight loss was accompanied by extreme weakness, bloody, watery feces and irritability. In the case of Mink 9 there was marked anorexia. At this time 250 μg folic acid was fed orally and 50 μg added to the ration daily thereafter. There was an immediate marked improvement in the condition of the animal. In the following 24 hours the entire allotment of the ration was consumed. The diarrhea, weakness and general irritability completely disappeared in a few days. After 14 weeks on this ration Mink 10 died before supplementation was started. Normal food consumption had been maintained until the time of death.

The basal ration was modified by increasing the casein level to 30% at the expense of sucrose (Table I) and 4 mink were placed on this regimen. Growth curves are shown in Fig. 2. At about the ninth week the 4 mink began to lose weight markedly and Mink 16 was fed 100 μg of biotin orally and 50 μg was added to the ration daily. The loss in weight continued and after the biotin supplement had been continued for 2 weeks the previously described syndrome was very evident. At this time folic acid therapy (1.0 mg orally and 50 μg added to the ration daily) was started. An immediate response to this therapy was noted. At the beginning of the 12th week Mink 18 was given biotin at the levels described for Mink 16. Loss of body weight continued. After one week of such therapy the animal appeared normal at 8 A.M. but large quantities of bloody, watery fecal discharge were noted. An hour later the animal was found in a flaccid, morbid condition. Blood samples were taken and the animal autopsied. Mink 15 and 17 exhibited the severe deficiency symptoms in 10 to 11 weeks. Folic acid therapy as described for Mink 16 was started at this time. Symptoms such as weakness, irritability, bloody watery fecal discharge and anorexia disappeared rapidly, with resultant increase in food consumption and body weight. Hemoglobin and white cell count determinations prior to and at the time of critical deficiency and after therapy indicates that the decrease in the white cell count and slight drop in hemoglobin was corrected by folic acid (Table II).

The basal ration was modified by adding 10% gelatin at the expense of sucrose (Table I), and 4 animals placed on this regimen. Weight curves are shown in Fig. 2. All animals began losing weight after 4 to 8 weeks. After receiving this ration for periods of 10 to 13 weeks Mink 1, 3 and 6 were fed orally an initial dose of biotin at levels at 100 μg, 750 μg and 250 μg respectively in addition to starting the daily supplement of 50 μg

[3] Evelyn, K. A., *J. Biol. Chem.*, 1936, **115**, 63.

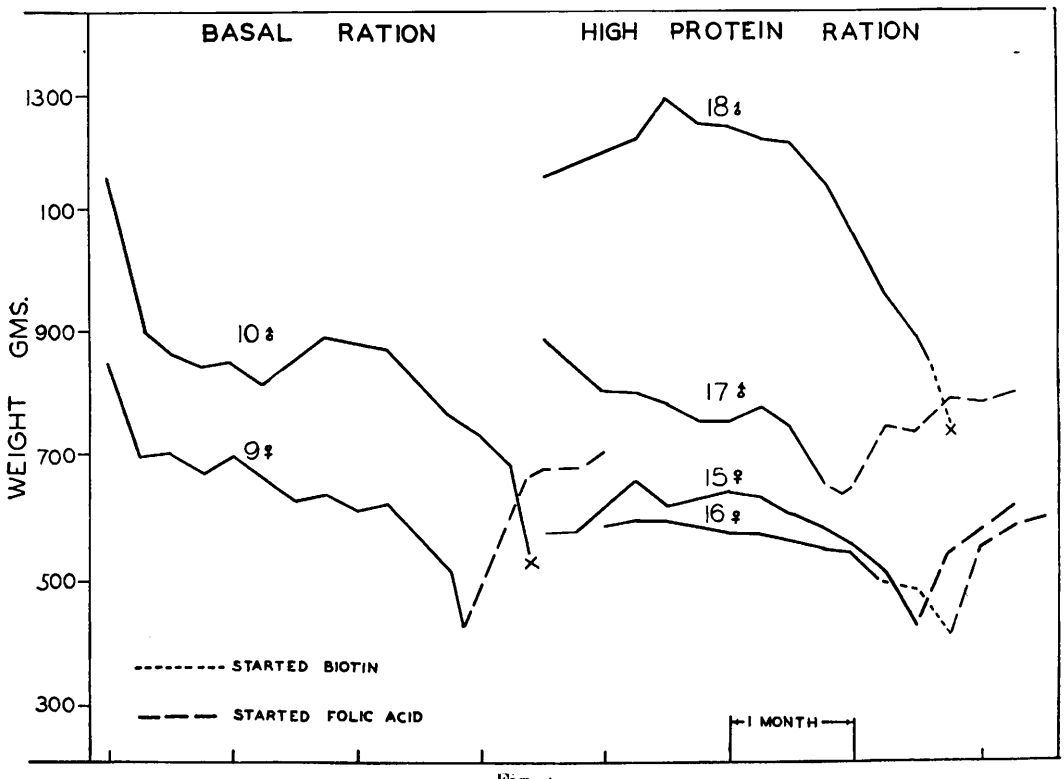

Fig. 1.

Body weight curves of Minks 9 and 10 receiving the basal ration and Minks 15, 16, 17, 18 receiving the high protein ration.

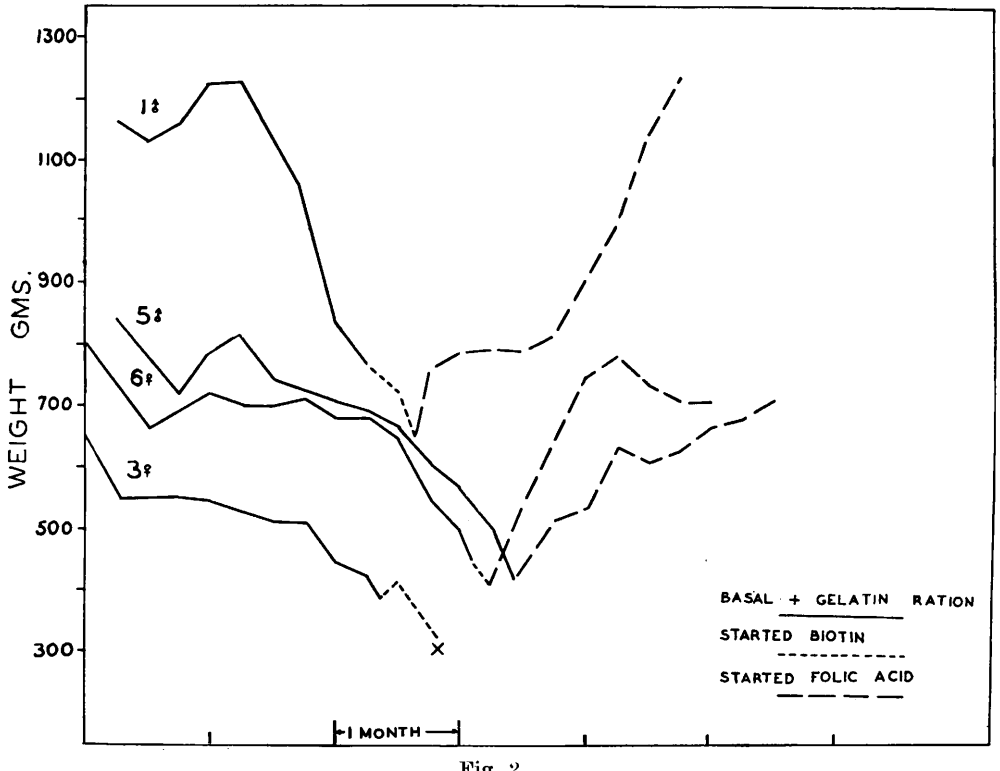

Fig. 2.

Body weight curves of Minks 1, 3, 5, and 6 receiving the basal plus gelatin ration.

TABLE II.
Blood Analysis.

Mink No.	Ration	Total days on exp.	Body wt in g	Hb g%	WBC per comm.	Remarks
4	Basal + gel + 10% fresh liver	120	750	18.7	17,400	
7	Basal + gel + 2% liver fraction "L"	130	500	16.3	14,400	
5	Basal + gel	30	750	19.3	—	
	"	84	420	14.7	7,300	Started folic acid
	"	133	680	19.2	16,000	
6	"	84	410	15.3	—	" " "
	"	135	715	18.0	15,000	
19	"	79	605	16.8	8,800	
		88	505	15.9	6,600	" " "
		97	660	14.3	15,100	
15	High protein	63	540	15.0	9,000	
		77	430	14.3	8,700	" " "
		91	615	18.3	15,300	
16	"	63	500	17.2	8,800	
		77	420	15.7	8,760	" " "
		91	585	18.9	13,400	
17	"	79	655	15.8	8,100	" " "
		103	800	18.7	21,000	
18	"	76	900	17.4	10,000	
		87	700	13.5	6,160	Fatal deficiency

biotin. Loss of body weight continued and severe deficiency symptoms characterized by approximately 50% loss in body weight accompanied by bloody fecal discharge; irritability, general weakness and anorexia developed. At this time Mink 3 died and Mink 1 and 6 were fed 250 μg folic acid orally in addition to starting the daily supplement of 50 μg per day. Biotin was not fed to Mink 5, and after being on this ration for 10 weeks the typical syndrome was noted. 550 μg folic acid was administered orally and the daily supplement started. Immediate remission of deficiency symptoms occurred in the 3 animals receiving folic acid. Blood analysis (Table II) of Mink 5 and 6 indicates that the number of white blood cells was markedly increased and hemoglobin slightly increased after folic acid therapy.

Later in the experiment Mink 19 was started on this same ration. Failure to maintain body weight was noted at the end of the 11th week. At this time para-aminobenzoic acid, inositol, α-tocopherol and 2-methyl-1,4-naphthoquinone were added daily to the ration. Loss of body weight continued and 10 days later the animal exhibited the typical deficiency symptoms noted in the previously described mink. At this time folic acid therapy of 1 mg orally plus the daily supplement was started. Dramatic recovery was noted. The 35% loss of total body weight was regained in a period of 18 days.

Autopsies were performed on Mink 3, 10 and 18. Ulcerative hemorrhagic gastritis with large volumes of blood in the stomach and intestines were noted in all cases. Macroscopically the liver appeared congested; kidney, spleen, and other organs appeared normal.

In conjunction with studies on the purified rations supplemented only with the 6 synthetic vitamins, 5 mink were fed additional supplements of whole liver and liver extracts. Weight curves are shown in Fig. 3. Mink 2, 4, 7 and 8 were started on the basal plus gelatin ration supplemented with various liver fractions. Mink 2 received 2% 1:20 liver

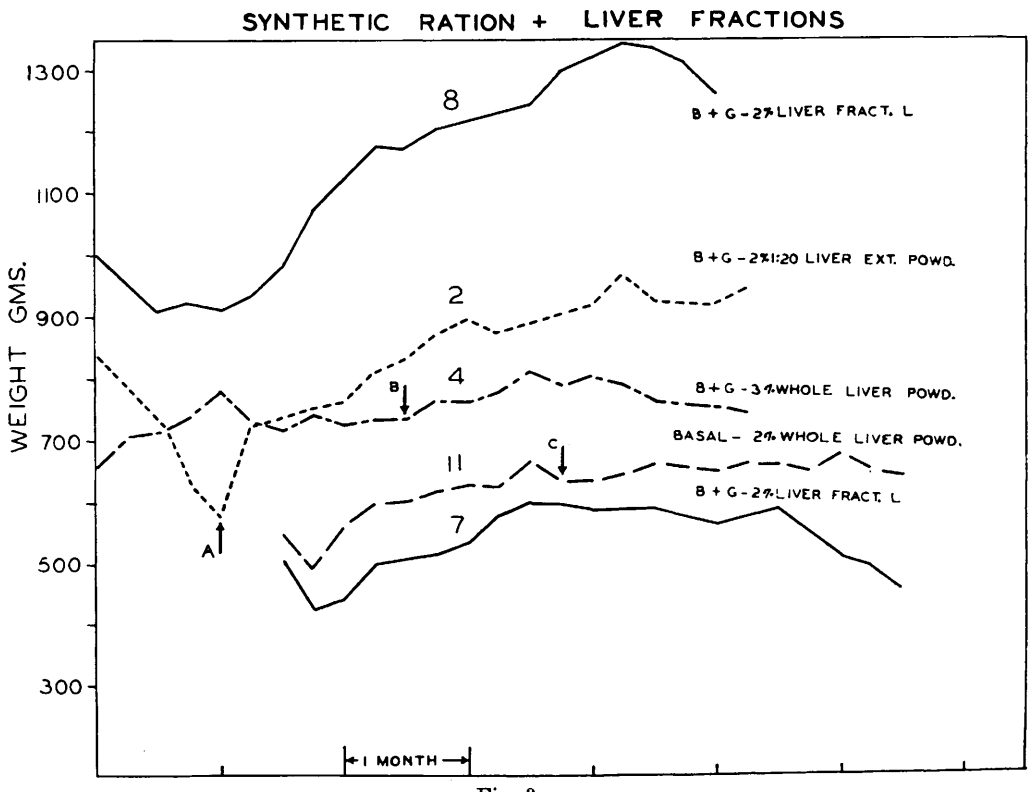

Fig. 3.

Body weight curves of 5 mink receiving purified rations supplemented with various liver concentrates.

B + G—Basal + gelatin ration.

A—Started 50 μg of biotin per day.

B—Changed supplement from 3% whole liver powder to 10% fresh calves liver.

C—Increased whole liver powder from 2% to 4%.

extract powder and lost 30% body weight in 27 days. At this time 50 μg of biotin daily was added to the ration. An immediate response in body weight was noted during the first week. However, remission was slow after this initial gain. In an effort to determine whether fresh liver would increase body weight above that obtained with liver extracts the supplement of 3% dried whole liver substance for Mink 4 was changed to 10% fresh calves liver. The plateau in body weight continued. Although liver concentrates as fraction "L" and dried whole liver powder maintained body weight and produced some additional growth during 5 months a general decline in weight in Mink 7 is now being observed.

Discussion. The use of purified rations for mink introduces another species of animal for experimental nutritional studies.

Eleven mink placed on purified rations containing varying levels of protein supplemented with thiamine, riboflavin, pyridoxine, pantothenic acid, niacin and choline developed critical deficiency symptoms in 9 to 13 weeks. White blood cell counts were reduced to 6,160-8,760. The hemoglobin level was slightly lowered. The administration of folic acid to 8 of the 11 mink resulted in a dramatic recovery from the syndrome characterized by irritability, weakness, bloody fecal discharge, anorexia and severe weight loss. White blood cell counts rose to 13,400-21,000; also a slight increase in hemoglobin was noted. This increase in white blood cells upon administration of folic acid is similar to the response

noted in monkeys as reported by Cooperman *et al.*[4]

Preliminary observation in Mink 1 at the present time indicates that an additional factor in fresh liver is required. After the response in body weight to folic acid therapy this animal lost 40% of the total body weight in a period of 3 weeks. Inositol, *p*-aminobenzoic acid, vitamin E and K at levels given in Table I were added to the ration with no response in body weight. The feeding of 10% fresh liver for 18 days resulted in a regain of 63% of the body weight loss; hemoglobin rose from 10.7 at the time of deficiency to 17.3 g per 100 cc of blood.

The possible role of biotin in the nutrition of the mink is still obscure. A response to biotin in Mink 2 was obtained. However, further efforts to produce an uncomplicated biotin deficiency has been unsuccessful to date.

The role of *p*-aminobenzoic acid, inositol, α-tocopherol and 2-methyl-1,4-naphthoquinone has not been established in our preliminary work.

The practical aspect of these studies indicates the importance of considering the folic acid content of mink rations. Further studies as to the requirement of the known members of the vitamin B complex and additional factors in relation to growth, maintenance, reproduction, and development of fur are necessary.

Summary. 1. Purified rations have been successfully employed in studying nutritional requirements of adult mink. 2. Feeding highly purified vitamin B complex free rations of varying protein levels supplemented with thiamine, riboflavin, niacin, pyridoxine, pantothenic acid and choline produced a severe deficiency in 10 to 13 weeks. The characteristic symptoms are given. 3. The administration of synthetic folic acid to 8 mink at the time of severe deficiency symptoms resulted in an immediate recovery, whereas 3 mink not receiving folic acid died. 4. Preliminary observation indicates the existence of another factor present in liver distinct from the known vitamins which is seemingly necessary for maintenance of body weight and hemoglobin regeneration.

We wish to acknowledge our indebtedness to Merck and Co., Rahway, N. J., for the crystalline vitamins; to Wilson Laboratories, Chicago, Ill., for the various liver preparations; to Dr. B. L. Hutchings of Lederle Laboratories, Inc., Pearl River, N. Y., for the synthetic folic acid.

[4] Cooperman, J. M., Elvehjem, C. A., McCall, K. B., and Ruegamer, W. R., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 92.

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Toxicity of Choline in the Diet of Growing Chickens.

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Choline has a wide potential use in rations for poultry. Because of the probability of this substance's being fed in amounts greater than needed for maximum benefits, it is desirable that information on the toxicity of the compound for poultry be obtained.

One of the authors (P.B.P.) found that an oral dose of 5 g was fatal to 5 kg rabbits within 30 minutes, whereas with rats, Hodge and Goldstein[1] found that 6.7 g per kg body weight given by stomach tube to rats starved for several hours "represented the LD_{50} for albino rats."

Hodge[2] later reports the results of supply-

[1] Hodge, H. C., and Goldstein, Max R., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 281.

[2] Hodge, H. C., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 212.