Titrations were carried out on filtrates from each passage.

In assaying the virus content of the embryos from the 5th passage, eggs were separated on the basis of the routes by which the inoculations were made and different portions of the embryos in each group were titrated separately for virus content. These portions included pools of the chorioallantois, pools of the heads of embryos, and pools of the abdominal viscera (kidneys, liver, spleen, stomach and intestine). In those inoculated by the intraembryonic method, pooled brains only were titrated for virus.

After the 5th passage the intraembryonic method was abandoned, and the passages made by the 2 remaining routes were carried independently of each other. In the passages which were made by the chorioallantoic route, the material for the next inoculation was always obtained from the pooled heads of embryos harvested from the previous passage; in those made by the yolk sac route, the material inoculated was always harvested from the pooled viscera of embryos previously infected by the yolk sac route. The purpose of these 2 separate lines of passage was to determine whether the relative difference in the amount of nervous tissue represented would influence appreciably the amount of virus produced.

It has been stated that a high per cent (about 80%) of the embryos died between the 2nd and 5th days. These deaths occurred from the first passage onward. No distinctive gross or microscopic lesions in either the chorioallantois or the embryos have thus far been identified, but this is under further study. The incidence of deaths in embryos in eggs incubated at 37°C was lower (40%) than that in eggs incubated at 35°C.

Our observations are summarized in Table I. Results on certain of the passages have been omitted to save space. These are in agreement with those given. All of the titrations were carried out by inoculating the individual dilutions of the passage material intracranially into groups of 3 to 5 white mice, each with 0.025 ml of the dilution, and 50% infection in the individual groups was counted as the end point.

Neutralization tests against anti-SK serum, prepared before these studies were initiated, were carried out with virus from the 10th egg passage. The filtrate used in the tests titered 10^{-5}, based on 50% infection. It was employed in a dilution of 10^{-4} and 0.5 ml of this dilution was treated with an equal volume of the individual serum dilutions. Complete neutralization was obtained in serum dilutions up to and including 1:512, the highest serum dilution tested. Theiler's GD-VII strain of mouse encephalomyelitis virus was not neutralized by the serum.

Summary. The murine SK strain of poliomyelitis virus was carried through 14 passages on developing eggs without evident diminution of virus content. Its multiplication was associated with a high incidence of deaths in the embryos. The incidence of deaths was influenced by the temperature at which the eggs were incubated after inoculation.†

† Since this manuscript was submitted, we have made observations which indicate that the Lansing strain also, may be cultivated under these conditions.

15472

Alopecia in Rats Fed Certain Soybean Oil Meal Rations.*

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It is generally believed that the rat does not require dietary sources of inositol and biotin when purified rations are fed. However, certain dietary constituents may in some

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TABLE I.
Effect of Biotin and Inositol on Hair Loss (Experiment I).

Lot No.	Ration	Total No. of rats	No. of rats showing hair loss	Avg wt in g after 6 wks (initial wt, 40 g)
1	Soybean oil meal basal	20	13	135
2	S.O.M. basal + 0.1% inositol	32	23	141
3	S.O.M. basal + 0.1% inositol + 4 μg biotin daily (oral)	32	0*	141
4	S.O.M. basal + 0.1% inositol + 4 μg biotin daily (inj)	24	0*	144
5	S.O.M. basal + 0.3% inositol	6	0	132
6	Raw soybean oil meal basal	22	15	94
7	Raw S.O.M. basal + 1 μg biotin daily (oral)	14	2	104
8	Raw S.O.M. basal + 0.3% inositol	8	0	91

* In each of these groups there was one rat which showed early signs of hair loss but spontaneous cure followed.

manner bring out a need for one or both of these vitamins. It is well known that the inclusion of a sulfonamide drug in a purified ration may give rise to biotin and inositol deficiencies.[1,2] The drug probably acts by increasing the demands of the organism beyond its synthetic capacities for the factors or by inhibiting intestinal organisms which ordinarily act as a source of these factors.[3,4] An inositol deficiency has also been produced by feeding a ration composed largely of corn, soybean oil meal and alfalfa.[5] It was suggested that the need for inositol when this natural ration was fed was the result of a vitamin imbalance or of some change in the intestinal flora. A biotin deficiency will also result if a ration containing raw egg white is fed.[6,7] The avidin of the egg white combines with the biotin in the intestinal tract thus forming a complex which is unavailable to the rat.[8]

We have observed an alopecia in rats fed certain soybean oil meal rations which was prevented by supplements of biotin and/or inositol. The condition did not develop when the rations were supplemented with cystine or methionine.

Experimental. Weanling male and female rats of Sprague-Dawley breeding were used in these studies. The soybean oil meal basal ration was composed of soybean oil meal (expeller processed, commercial) 34.0 g, sucrose 57.5 g, salts IV 4.0 g,[9] corn oil 4.0 g, choline chloride 0.3 g, niacin 2.5 mg, calcium pantothenate 1.0 mg, pyridoxine 0.3 mg, thiamine hydrochloride 0.3 mg, riboflavin 0.3 mg, and 2-methyl-1, 4-naphthoquinone 0.1 mg, per 100 g ration. The raw soybean oil meal basal ration differed from this ration in that unheated solvent extracted flakes replaced the soybean oil meal. Two drops of halibut oil were given weekly. Oral biotin supplements were given by dropper, and injected biotin supplements were injected intradermally in saline solution.

Rations were mixed every 2 weeks and were stored at refrigerator temperatures to avoid possible development of rancidity. Rations and distilled water were given daily to insure *ad libitum* consumption.

Results. Experiment I. Table I summarizes growth data and the incidence of alopecia in rats fed the soybean oil meal rations. Only small differences in growth rate

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TABLE II.
Effect of Cystine and Methionine on Growth and Hair Loss (Experiment II).

Lot No.	Ration	Total No. of rats	No. of rats showing hair loss	Avg wt in g after 6 wk (initial wt, 40 g)
1	Soybean oil meal basal + 0.1% inositol	8	7	132
2	S.O.M. basal + 0.1% inositol + 0.2% *l*-cystine	8	0*	143
3	Raw soybean oil meal basal	16	10	87
4	Raw S.O.M. basal + 0.4% *l*-cystine	8	0*	126
5	Raw S.O.M. basal + 0.4% *dl*-methionine	8	0*	134

* In each of these groups there was one rat which showed early signs of hair loss but spontaneous cure followed.

were observed in the rats receiving the soybean oil meal basal ration and supplemented rations (lots 1-5)). However, 13 of 20 rats receiving the soybean oil meal basal ration (lot 1) developed a marked alopecia. The condition was first observed during the 4th week of the experiment and in all cases was still evident at the end of the 12-week experimental period. The hair loss started in the dorsal region of the head and proceeded bilaterally along the sides to the tail region. All the hair loss reported herein followed this general pattern and persisted throughout the experimental period unless otherwise indicated. The alopecia was not prevented by supplementation with 0.1% inositol (lot 2). The condition was prevented when the basal ration was supplemented with 0.3% inositol (lot 5) or a combination of 0.1% inositol and a daily biotin supplement of 4μg. Oral administration or intradermal injections of biotin were equally effective (lots 3 and 4).

The growth rate of rats fed the raw soybean oil meal basal ration (lot 6) was less than the growth rate of rats receiving the heated commercial soybean oil meal basal ration (lot 1). A slight stimulatory effect on growth was observed when biotin was added to the raw soybean oil meal basal ration (lots 6 and 7). Hair loss similar to that observed in lots 1 and 2 was also noticed in lot 6 when the raw soybean oil meal basal ration was fed. Fifteen of 22 rats in the later group exhibited the characteristic symptoms. Supplementation of this ration with biotin (1 μg per rat per day, lot 7) reduced the incidence of hair loss. Only 2 rats in a group of 14 showed any signs of alopecia. When the raw soybean oil meal basal ration was supplemented with 0.3% inositol (lot 8) no hair loss occurred.

Experiment II. The effects of adding cystine and methionine to the soybean oil meal rations are summarized in Table II. The characteristic hair loss was again observed when the soybean oil meal basal ration was supplemented with 0.1% inositol (lot 1). It also developed when the raw soybean oil meal ration (lot 3) was fed. However, when these rations were supplemented with cystine or methionine no permanent alopecia was noticed. In each of these supplemented groups (lots 2, 4, 5) there was one rat that showed early signs of hair loss which cleared up spontaneously.

Supplementation of the rations with cystine and methionine also resulted in a marked growth stimulus, especially in the case of the raw soybean oil meal rations.

Discussion. Apparently, soybean oil meal in some manner increases the dietary requirement of biotin and/or inositol. This effect may be due to alteration in the intestinal flora, to some absorption disturbance, to a vitamin imbalance or to the action of antivitamins which may be present in soybean oil meal. It is possible that only one of these vitamins is the limiting factor and that the second factor acts only in the formation of the first. It is also possible that both of these factors function in the formation of a third factor which is really the necessary factor. It is interesting that the condition does not develop when the soybean oil meal rations were supplemented with cystine or methionine. It seems possible that inositol

and/or biotin may be closely related to cystine and methionine in normal hair formation.

Another type of hair loss, referred to as the "spectacle eye" condition, has been associated with a biotin deficiency by Neilsen and Elvehjem[10] and to an inositol deficiency by Pavcek and Baum.[11] It seems possible that these two factors may be closely related.

The commercial soybean oil meal rations supported better growth than did the raw soybean oil meal rations. Cystine and methionine supplements stimulated growth especially when added to the raw soybean oil meal rations. These findings support the observations reported by earlier investigators.[12,13] Biotin supplements had a slight stimulatory effect on growth when added to the raw soybean oil meal basal ration. Cunha[14] reported earlier that biotin supplements stimulated growth in rats fed a diet consisting largely of corn, soybean oil meal and alfalfa.

Further investigations now in progress suggest that the situation may become more complicated under certain conditions. While low levels (1-4 μg) of biotin have been shown to prevent the loss of hair, higher levels (12 μg) of this vitamin may actually accentuate the condition. The hair loss resulting from feeding such a high level of biotin was prevented by supplementation with adequate inositol.

Summary. Rats fed certain soybean oil meal rations developed a characteristic hair loss which was prevented by supplementation of inositol and/or biotin. The condition did not develop if the rations contained added cystine or methionine. Inositol supplementation resulted in no marked changes in rate of growth. However, supplements of cystine and methionine markedly increased the rate of growth when added to the raw soybean oil meal basal ration. Biotin supplements had a slight stimulatory effect on growth when added to the same ration. It is believed that soybean oil meal in some manner increases the dietary requirement for biotin and/or inositol. Possible mechanisms for such action may be an alteration of the intestinal flora, some absorptive disturbance, a vitamin imbalance or the presence of antivitamins in the soybean oil meal.

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15473

Effect of Low Protein Diets upon Creatine Excretion of the Rat.

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In a previous study[1] qualitative tests upon the urine of rats after a period on a low protein and low choline intake suggested an increased excretion of creatine by these animals. Roberts and Eckstein[2] have shown that the creatine content of the gastrocnemius muscle of young rats is not lowered when a diet deficient in choline and methionine is fed for 3 weeks. A similar finding in chicks was reported by Almquist and coworkers[3] who found a deficiency in dietary choline did not appreciably diminish the muscle creatine.

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