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Inadequacy for Mice of a Synthetic Diet Supplemented with All Known Vitamin B Factors.*

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Since the work of Beard[1] and of Bing and Mendel,[2] the mouse has until recently been largely neglected as a subject for research on

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1 Beard, H. D., *Am. J. Physiology*, 1926, **75**, 645.

2 Bing, F. C., and Mendel, L. B., *J. Nutrition*, 1929, **2**, 49.

the vitamin B complex. A great interest in this animal has arisen suddenly in several quarters.

Norris and Hauschildt[3] reported that the "filtrate factor" would not cure the skin symptoms exhibited by mice receiving pyridoxin, thiamin, nicotinic acid and riboflavin as factors of the B complex. Sandza and Cerecedo[4] and Martin,[5] however, found that these symptoms could be cured by pantothenic acid, a most important constituent of the "filtrate factor." The series of experiments carried out by Woolley[6-9] have demonstrated the importance of inositol, in addition to pantothenic acid, for the growth and hair maintenance of mice, although Sandza and Cerecedo[4] and Martin[5] have been unable to demonstrate that lack of inositol produces an alopecia.

Ansbacher[10] has claimed that p-aminobenzoic acid is a chromotrichia factor for mice as well as for rats.

According to Carruthers,[11] synthetic diets containing a high percent of yeast as a source of the vitamin B complex, are inadequate for mice; fresh flaked wheat germ and aqueous liver extract were found to be most potent in the factor or factors which are missing, or inadequate, in yeast.

Sandza and Cerecedo[4] also reported that the 92% alcohol extract of liver contains an unknown alkali stable factor necessary for the growth of mice; their supplements included only thiamin, riboflavin, pyridoxin, and pantothenic acid, however.

The present experiments have shown that when male mice are fed a synthetic basal diet supplemented with thiamin chloride, riboflavin, pyridoxin hydrochloride, calcium pantothenate, nicotinic acid, choline hydrochloride, ascorbic acid, inositol and p-aminobenzoic acid, the growth of the animals can be definitely improved by feeding them as an additional supplement either fresh beef liver or a concentrate which we have prepared from fresh beef liver. The feeding of the additional supplements, especially the fresh liver, gave the mice in these particular experiments a more consistently clean and good looking coat of fur.

Experimental. Feeding and Handling of Animals. Twenty-six

3 Norris, E. R., and Hauschildt, J., *Science*, 1940, **92**, 316.
4 Sandza, J. G., and Cerecedo, L. R., *J. Nutrition*, 1941, **21**, 609.
5 Martin, G. J., *Science*, 1941, **93**, 422.
6 Woolley, D. W., *J. Biol. Chem.*, 1940, **136**, 113.
7 Woolley, D. W., *Science*, 1940, **92**, 384.
8 Woolley, D. W., *J. Biol. Chem.*, 1941, **139**, 29.
9 Woolley, D. W., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 565.
10 Martin, G. J., and Ansbacher, S., *J. Biol. Chem.*, 1941, **138**, 441.
11 Carruthers, C., *Science*, 1941, **93**, 44.

male mice from 4 litters were placed at the age of 21 days on a basal diet of the following composition: extracted casein[12] 20, sucrose 67, salts† 5, cod liver oil 3, corn oil 2, and butter fat (washed and filtered) 3. The corn oil should furnish more than enough vitamin E for male mice.[14] Beginning on the following day, the animals were given the following basic supplements daily for 6 days each week: thiamin chloride 10 μg, riboflavin 25 μg, pyridoxin hydrochloride 10 μg, calcium pantothenate 100 μg, nicotinic acid 100 μg, choline hydrochloride 4 mg, ascorbic acid 800 μg,[15] inositol 3 mg, and p-aminobenzoic acid 1.5 mg. These supplements were given in 2 cc of a 5% ethyl alcohol solution placed in a special supplement cup; the regular drinking water bottles were withheld until the supplement solutions were quantitatively consumed.

In preliminary experiments, 24 mice were used to determine whether these basal rations used contained enough casein, salts and pure vitamin supplements. Six of the preliminary mice received the same diet as the control group (Group I) of the main experiment, detailed below, and showed an appearance and rate of growth similar to that which will be described for Group I; the condition and growth of the remaining 18 preliminary animals, divided into 3 groups of 6, were no better, even though one group received 30% casein and 7% salts, another double quantities of basal supplements, and the third group both the double supplements and the increased casein and salts.

By the end of 3 weeks on the basal diet and supplements, 3 of the 26 mice had died. The first died at the age of 36 days, showing paralysis of the hind legs, diarrhea, and brownish discoloration around the genitals and anus; a second died at the age of 37 days with a diarrhea; the third died at the age of 42 days, exhibiting paralysis of the hind legs, diarrhea, and brown discoloration around the anus and urethra and extending extensively up underneath the body. All 3 of these animals lost weight markedly before death.

At the age of 37 days, the 23 mice which were steadily gaining weight were divided into 4 groups which were as homogeneous as possible with regard to litters, weights of the animals at the age of 37 days, and weight gains between the ages of 21 and 37 days.

† Hawk and Oser salt mixture,[13] with 1.68 parts of $ZnCl_2$ and 2.00 parts of $CuSO_4 \cdot 5\ H_2O$ substituted for every 3.68 parts of NaF.

[12] Halliday, N., and Evans, H. M., *J. Nutrition*, 1937, **13**, 657.

[13] Hawk, P. P., and Oser, B. L., *Science*, 1931, **74**, 369.

[14] Bryan, W. L., and Mason, K. E., *Am. J. Physiol.*, 1940, **131**, 263.

[15] Kleiner, I. S., and Tauber, H., *Food Research*, 1936, **1**, 399.

Additional supplements were then fed daily on 6 days a week as follows:

Group I, the control group, was given no additional supplement; Group II received 2 mg of pimelic acid, increased to 10 mg when the mice were 56 days old; Group III received about a quarter gram of fresh beef liver, gradually increased until approximately a half gram was being given when the mice were 59 days old; Group IV was given a dried concentrate from an extract of fresh beef liver, to be described below. For about the first 6 weeks this liver concentrate was mixed with the daily solution of basic supplements; about 60 mg was given at first, which was gradually increased to approximately 140 mg when the mice were 57 days old. This method of giving the concentrate was not entirely satisfactory, however, since some animals showed a tendency to leave a dried residue in the supplement cups, and it was then necessary to resuspend this residue in water so that it would be entirely consumed. Accordingly, when the animals were 66 days old, the liver concentrate was finally given as 5%‡ of the basal diet.

All the mice were killed at the age of 116 days.

Preparation of Beef Liver Concentrate. For the preparation of the liver concentrate given to Group IV, 3500 g of fresh beef liver was first freed from all tough tissue and finely ground. With this, in succession, were mixed thoroughly an equal volume of distilled water, about 40 cc of hydrochloric acid, and about 200 cc of toluene. The final pH was 4.5. After this preparation had autolyzed at 37°C for 3 weeks, 5 pounds of butyl alcohol was thoroughly mixed with it, and the entire batch warmed to about 60-70°C on a steam bath. It was then allowed to stand at room temperature over night. In the morning, the process of filtration and concentration of the filtrate *in vacuo* was begun. The residue was thoroughly extracted in a soxlet extractor, first with a mixture of butyl alcohol and distilled water and then with acetone. The acetone was removed from the latter extract by fanning in an open dish, and the combined extracts were then added to the original filtrate.

The filtrate, after having been freed from butyl alcohol and toluene by concentration to a mushy syrup *in vacuo,* was transferred to a large separatory funnel and washed with petroleum ether until the petroleum ether washing was only a light yellow. The remaining syrupy water solution with suspended solids was placed in a large evaporating dish with a little toluene and evaporated under an electric fan to a thick mush; it was then quickly and thoroughly

‡ Five g of concentrate were mixed with every 95 g of the basal diet.

dried in a vacuum desiccator over potassium hydroxide. The dried material was washed with petroleum ether to remove the last traces of toluene and ground to a fine powder, which was somewhat hygroscopic.

Effects on Growth. The growth curves of the 4 groups of mice are shown in Fig. 1. For the first 15 days of the experiments, the average growth rates and the weights did not differ appreciably. However, after the additional supplements were given Groups II, III and IV, as described above, the average growth rates of the mice in Groups III and IV were increased as compared with the growth rates of the animals in I and II, which did not differ from each other to any appreciable extent. The animals fed fresh beef liver grew better than any of the other groups; however, it is, of course, impossible to say whether there was any qualitative difference between the growth-promoting factor or factors of the fresh liver

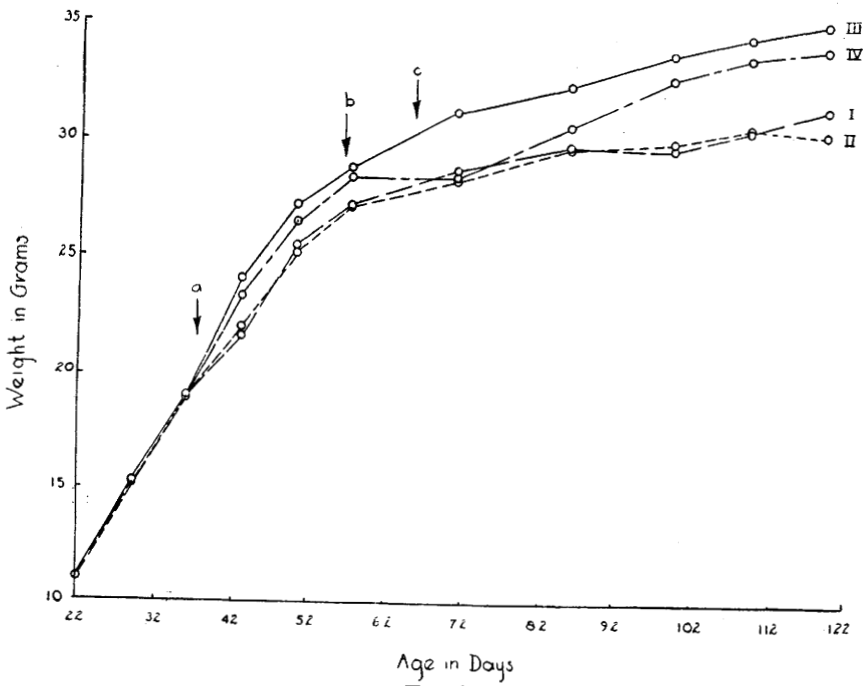

FIG. 1.

Growth curves of mice. I, 6 mice, control group; II, 6 mice, additional supplement 2 mg pimelic acid per day, later increased to 10 mg; III, 5 mice, about 0.25 g fresh liver per day, gradually increased to about 0.5 g; IV, 6 mice, about 60 mg of the liver concentrate per day, gradually increased to 140 mg and finally given as 5% of the basal diet.

a, began to give additional supplements; b, pimelic acid increased to 10 mg per day; c, liver concentrate given as 5% of basal diet.

given Group III and the factor or factors of the liver concentrate which was given to Group IV. The temporary failure of Group IV to gain weight, which was evidenced from the weights of the animals at the age of 71 days, was very probably due to the unsatisfactory method of giving the liver concentrate. Soon after the concentrate was incorporated into the basal diet, Group IV gained weight at an even more rapid rate than Group III. This very rapid weight gain shown by Group IV soon levelled off, however, and substantially paralleled the rate of weight gain shown by Group III. The final difference between the average weights of Groups III and IV may not be significant.

Appearance of Animals. All 5 mice in Group III, receiving fresh liver always possessed good looking fur and a clean appearance, with the exception of a pale yellow staining which appeared on the fur around the genitals of 3 of the animals towards the end of the experimental period. The condition of the pelage of the animals in Group IV, given the liver concentrate, was less consistently satisfactory as to cleanliness and neatness; this may have been at least partly due to the unsatisfactory method initially used for the administration of the liver concentrate. Groups I and II were the least satisfactory in average cleanliness and neatness of the pelage; pimelic acid had definitely no beneficial effect on the fur of the animals in these experiments. In Group I some alopecia developed around the genitals of one mouse; in Group II one animal showed an extensive alopecia on the face and chest, around the legs and in a large area surrounding the genitals, while the fur on the back of a second animal was very thin.

Discussion. The synthetic diet and supplements used for mice in these experiments, which covered the first 4 months of life, were inadequate to promote the most rapid rate of growth. Both fresh beef liver and a liver concentrate increased the growth rate, and the fresh liver especially improved the condition of the fur. While pimelic acid had no significant effect on growth, and certainly no beneficial effect on the fur of the animals, its value as a nutritional factor still remains undetermined, since the addition of a single nutritional factor to a synthetic ration may have no effect without the addition of other factors, or may even have an adverse effect.[16]

Summary. When fed a basal diet containing extracted casein, sucrose, salts, cod liver oil, corn oil, and butter fat, together with supplements consisting of thiamin chloride, riboflavin, pyridoxin

[16] Morgan, A. F., *Science*, 1941, **93**, 261.

hydrochloride, nicotinic acid, calcium pantothenate, choline hydrochloride, ascorbic acid, inositol and p-aminobenzoic acid, young mice grew at a good rate but after some weeks did not present consistently clean and good looking fur. Increasing the casein and salts or doubling the daily quota of the supplements, or both, failed to improve the appearance or growth of the mice. Feeding fresh beef liver increased the growth rate and improved the pelage. A concentrate of a liver extract had similar but not as pronounced effects. Pimelic acid had no significant effect on growth and certainly no beneficial effect on the pelage, for the time range of these experiments.

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Acetylcholine Esterase Content of Brain Tumors.

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The acetylcholine esterase content of 50 brain tumors, and of 13 brain lesions not neoplastic in character, has been determined by the pharmacological method previously described.[1] All material was obtained immediately following removal in the neurosurgical operating room. The tissue to be examined for acetylcholine esterase content was exposed to the action of no drugs. The acetylcholine esterase activity (Q.CHE.) was determined as the number of milligrams of acetylcholine iodide hydrolized by 100 mg of tissue dry weight in one hour at 37°C and pH 7.61.

The histologic or pathologic structure of the tissue resected at operation and examined for Q.CHE. content was established by multiple sections, fixed in Cajal's formalin-ammonium-bromide solution, and after the appropriate time of fixation stained with, (1) Penfield's second modification of Rio-Hortega's silver sodium carbonate stain, (2) Rio-Hortega's silver lithium carbonate stain, and (3) Cajal's gold chloride sublimate method. Other material fixed in Zenker's fixative was stained with hematoxylin and eosin and with phosphotungstic hematoxylin.

As indicated in Table I, 8 tumors of the astroblastic and astrocytic

[1] Youngstrom, K. A., *J. Neurophysiol.*, 1938, **1**, 357.