alloxan. These speculations require further investigation.

*Conclusion.* Alloxan diabetes can be produced in a high percentage of dogs by the simple expedient of starving the animals for 3 days prior to the injection of alloxan.

## 16411

## Effect of Liver Extract on Growth of Rabbits.*

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The increasing use of rabbits in biochemical and nutritional investigations has led to further study in preparation of simplified diets for these animals. Wooley and Sebrell[1] described a purified diet supplemented only with pure vitamin preparations. Nearly normal growth was obtained for a limited period of time when this diet was fed.

In the present investigation we have studied the adequacy of diets for rabbits in which the vitamins were supplied in a purified form and also the effect of the addition of a 1:20 liver concentrate powder† to this diet.

*Experimental.* Rabbits of the New Zealand White breed were used in all experiments. The rabbits were weaned at 5 to 6 weeks of age at a weight of about 950 g and placed on the experimental diets. The change from the stock diet to the simplified diets was effected gradually over a period of three days after which time the new diet was accepted by all animals.

Preliminary observations were obtained with several diets to determine the best diet to use as a basis for comparison in a series of investigations on the nutritional requirements of rabbits. Results obtained with 3 of these diets, 2 of which contained 4% of the liver concentrate (rations 11 and 12), indicated that when liver concentrate was added to the diet an improved growth response was obtained. The average rate of growth of the rabbits which received the liver concentrate was significantly higher as determined by "analysis of variance" than for the rabbits fed ration 1, without the liver concentrate (P equals 0.02). The average rate of growth, during a 6 week experimental period, of the animals fed ration 1 was 175 g per week, while the average gains of those fed rations 11 and 12 containing liver concentrate were 226 and 209 g per week for the respective diets.

Rations 11 and 12 differed essentially only in the cellulose content. Variation in rate of growth was not as great on the 6% level of cellulose (ration 11) as on the 12% level (ration 12). The average gains, however, were not significantly different and in the statistical analysis the data obtained from rabbits on theses diets were treated as a single group.

To extend these observations weanling rabbits were fed a purified basal diet (ration 14) which was composed of casein 22%, peanut oil 8%, cerelose 53.5%, cellulose 12%, Salts IV[2] 4%, Vitamin A and D Concentrate (Nopco XX) 0.5%, mixed tocopherols 50 mg, choline 200 mg, niacin 20 mg, inositol 10 mg, pyridoxine 0.7 mg, thiamine 0.7 mg, riboflavin 0.7 mg, calcium pantothenate 1.5

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1 Wooley, J. G., and Sebrell, W. H., *J. Nutrition*, 1945, **29**, 191.

† Generously supplied by the Wilson Laboratories, Wilson and Co., Inc., Chicago, Ill.

2 Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 1935, **109**, 657.

TABLE I.
Growth of Rabbits Fed Purified Diets and Diets Containing Liver Extract.

| Experimental series No. | Diet No. | Diet | No. of animals | Growth in g per week |
|---|---|---|---|---|
| I | 1 | Purified diet | 12 | 175 |
| | 11 | " " + liver concentrate | 6 | 226 |
| | 12 | " " + " " | 6 | 209 |
| II | 14 | Purified diet | 5* | 107 |
| | 16 | " " + liver concentrate | 5 | 168 |
| III | 14 | Purified diet† | 9 | 107 |
| | 14A | " " + folic acid, biotin, *p*-aminobenzoic acid, and cystine | 9 | 114 |
| | 14B | Diet 14A + liver concentrate | 9 | 152 |

* One rabbit died after 4 weeks.
† In this series purified casein (Labco) was used and in Experiments I and II crude casein was used.

mg, and 0.075 mg of 2-methyl, 1, 4 naphthoquinone per 100 g of ration. The liver concentrate was added to diet 16 at a 4% level at the expense of the cerelose.

The average gain per week, over a 6 week period, of rabbits fed ration 14 was 107 g while that of the rabbits fed ration 16 which contained the liver concentrate was 168 g (Table I). This confirmed the preliminary observations that the addition of liver concentrate gives an increased growth response.

Three rabbits fed ration 14 had one or more onsets of severe diarrhea during the course of the experiment and one rabbit died from this cause. Post mortem examination revealed only a small amount of food in the gastrointestinal tract, liquefaction of fecal material and gaseous distention of the intestines and cecum. Microscopic examination of the fecal material from rabbits with diarrhea failed to reveal any significant presence of pathogenic organisms. Similar cases of diarrhea and death occurred in other experiments where the diet did not contain the liver concentrate.

Several of the rabbits kept on simplified diets which did not contain liver concentrate for 6 or more weeks suffered an abnormal loss of hair. It was doubtful in some cases whether or not this loss of hair was entirely spontaneous for it appeared to be due in some cases to the animals pulling and eating the hair from each other. A pronounced symmetrical alopecia occurred on both the dorsal and ventral aspects of the body and on the face and limbs of the animals fed ration 14. The hair coat of rabbits fed the liver concentrate diets appeared normal and healthy at all times.

Although recent studies have demonstrated that the rabbit synthesizes large amounts of pantothenic acid, riboflavin, folic acid and biotin,[3] it seemed desirable to test the effect on the rate of growth of supplementing the basal ration with folic acid and biotin since these two vitamins were not included in basal ration 14. *P*-aminobenzoic acid was included in the ration along with folic acid and biotin because of the possibility of synthesis of folic acid from this compound, and cystine was included because of the possibility of a combined deficiency of methionine and cystine as a consequence of using casein as the only protein in the ration. Three groups with 9 rabbits in each group were used in this experiment. One group received the basal ration (No. 14), the second group the basal ration plus the following supplements per 100 g of ration: folic acid (pteroylglutamic acid) 0.2 mg, biotin 10 $\mu$g, *p*-aminobenzoic acid 0.2 mg, and cystine 200 mg (ration No. 14A). The third group received ration No. 14A plus 4% of liver extract added at the expense of the cerelose. The animals were fed these diets for 7 weeks and the average rates of gain per week are shown in Table I. It can readily be seen that the addition of liver extract again resulted in an improved

[3] Oleese, Orlando, Pearson, P. B., and Schweigert, B. S., *J. Nutrition*, 1948, **38**, 577.

performance and that the addition of pteroylglutamic acid, biotin, *p*-aminobenzoic acid and cystine did not significantly improve the rate of growth. An analysis of variance showed that the addition of liver extract significantly increased the rate of growth as compared to when the basal diet was fed ($P = <.01$) and also as compared to when the supplemental diet (No. 14A) was fed ($P = <.05$).

Hemoglobin determinations were run after the animals had been on experiment for 5 weeks and no difference in the hemoglobin levels was observed for the groups receiving diets with or without liver extract.

*Discussion.* The results of these experiments demonstrate an improved performance in the rate of growth and general condition of health when rabbits are fed a diet containing a 1:20 liver concentrate. The data suggest that a water soluble concentrated liver extract, such as that used in this study, contains an unidentified factor or factors necessary for optimum growth and the general well being of the rabbit. It is also concluded that *p*-aminobenzoic acid, pteroylglutamic acid and biotin are not dietary essentials for the rabbit.

It has been reported that the ingestion of liver and various liver extracts results in improved performance with other species when the diets used were apparently adequate in all known nutrients. Schweigert *et al.*[4] reported increased growth rates with the cotton rat when fed liver extract. Recent work with the cotton rat[5] indicates that pteroylglutamic acid and/or biotin do not give the response given by the liver concentrate. Improved lactation in rats has been reported when pork or beef liver was added to a ration supplemented with minerals, fatty acids, and all known vitamins.[6] An increase in the percentage of young surviving the lactation period and small increases in the rate of growth were obtained when liver or liver concentrates were added to diets containing 10 B vitamins.[7] Various liver concentrates have been shown to markedly improve the performance of monkeys fed purified diets over long periods, particularly when the animals have been subjected to other nutritional deficiencies.[8,9] Similarly, liver concentrates contain factors not identical with known nutrients which are essential for the mink and fox.[10,11] These examples, as well as the observations reported here, suggest that liver and liver concentrates contain factors which are necessary for several species and the need for these factors becomes evident with the use of a variety of experimental criteria. The similarity and identity of these factors remain to be determined.

It is rather unlikely that the liver concentrate would contribute factors which would be supplementary to the protein portion of the diet. The 22 per cent level of casein and additional cystine is generally considered adequate for most species of animals.

*Summary.* The addition of 4% 1:20 liver concentrate to purified rations increased the rate of growth and aided in the maintenance of a general healthy appearance of rabbits. The improved performance noted when liver extract was added is apparently not attributable to known nutrients including folic acid, biotin, *p*-aminobenzoic acid and cystine. The results indicate that liver contains an unidentified factor essential for the maximum rate of growth of the rabbit.

[4] Schweigert, B. S., Shaw, J. H., Phillips, P. H., and Elvehjem, C. A., *J. Nutrition*, 1945, **29**, 405.

[5] Schweigert, B. S., unpublished data.

[6] Spitzer, R. R., and Phillips, P. H., *Fed. Proc.*, 1947, **6**, 422.

[7] Sporn, E. M., Ruegamer, W. R., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 5.

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

[9] Ruegamer, W. R., Cooperman, J. M., Sporn, E. M., Snell, E. E., and Elvehjem, C. A., *J. Biol. Chem.*, 1947, **167**, 861.

[10] Schaefer, A. E., Whitehair, C. K., and Elvehjem, C. A., *J. Nutrition*, 1948, **35**, 147.

[11] Schaefer, A. E., Tove, S. B., Whitehair, C. K., and Elvehjem, C. A., *J. Nutrition*, 1948, **35**, 157; *Z. f. Vitaminforschung*, 1947, **19**, 12.