TABLE III.
Effect of Oleic Acid on Growth of *E. terrapinae*. Mean yields of duplicate cultures $\times 10^{-4}$.

	Oleic acid, mg/ml						
Transfer	0.00	0.01	0.02	0.03	0.04	0.05	Mean
1	510	694	765	610	498	293	560
2	438	377	819	430	202	361	438
3	460	387	670	240	169	174	350
4	514	307	515	452	274	235	383
5	404	371	593	370	288	178	368
Mean	464	427	672	420	286	248	420

graded amounts of oleic acid up to 0.05 mg per ml and the media inoculated with 100,000 amoebae as before. The results over successive transfers are given in Table III. The highly significant effects are associated with concentrations of oleic acid ($F = 33.00$) and the comparison between the initial transfer and those following ($F = 45.01$). The slight heterogeneity existing among the second through fifth transfers and their interaction with concentration ($F = 2.64$ and 2.28 respectively) reflects the progressively poorer yields with oleic acid concentrations other than optimum.

Discussion. Our initial failure to substitute cholesterol for serum in LSB medium appears to be due to a quantitative effect. When supplied with less than some undetermined minimum amoebic growth fails. The stimulating effect of oleic acid in optimum concentration (0.02 mg per ml), together with the smaller yields obtained with cholesteryl oleate, suggests that cholesteryl oleate as such is not available to the amoebae but must be hydrolyzed to its constituent molecules.

Summary. Cholesterol in adequate amounts can be substituted for serum in liver infusion media for culture of entozoic amoebae, but the yields will be statistically heterogeneous in contrast with those obtained when cholesteryl oleate is used as a supplement. Oleic acid in optimum concentrations reinforces the action of cholesterol. It is suggested that cholesteryl oleate is not used as such but must be hydrolyzed to its constituent molecules.

Received Sept. 27, 1949. P.S.E.B.M., 1949, v72.

Beneficial Effects of Liver on Growth and Survival of Immature Rats Fed Lactose-Containing Diets. (17530)

BENJAMIN H. ERSHOFF

From the Emory W. Thurston Laboratories, Los Angeles, Calif.

Available data indicate that the deleterious effects of rations containing high levels of lactose are dependent in considerable degree on the composition of the diet employed. Boutwell *et al.*(1) observed that rats fed a purified ration containing 48% lactose and 28% corn oil developed an unthrifty appearance and extensive diarrhea during the first 6 to 12 days of feeding. A similar ration containing 28% butter fat in place of the corn oil resulted in better growth, larger food intake and a good outward appearance. Furthermore, animals fed the butter fat ration had considerably less diarrhea than those on the corn oil diet.(2) A similar difference between animals fed butter fat and corn oil was observed by Ershoff and Deuel(3) in rats fed diets consisting solely of 70% lactose and 30% fat. Animals fed butter fat or margarine fat in conjunction with 70% lactose lived significantly longer than those fed a similar ration containing corn oil or cottonseed oil as the source of dietary fat. On a natural food ration, however, in which lactose was provided in the form of skim milk powder, no significant difference in growth was observed between rats fed butter

1. Boutwell, R. K., Geyer, R. P., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1944, v55, 153.

2. Boutwell, R. K., Geyer, R. P., Elvehjem, C. A., and Hart, E. B., *Arch. Biochem.*, 1945, v7, 143.

3. Ershoff, B. H., and Deuel, H. J., Jr., *Am. J. Physiol.*, 1947, v148, 45.

TABLE I.
Composition of Experimental Diets.

Dietary component	Diet A_1	B_1	A_2	B_2	A_3	B_3	A_4	B_4
Lactose*			30.2	30.2	45.3	45.3	60.5	60.5
Sucrose	70.5	60.5	40.3	30.3	25.2	15.2	10.0	
Casein†	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Salt mixture‡	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Whole liver powder§		10.0		10.0		10.0		10.0

To each kg of the above diets were added the following synthetic vitamins: Thiamine hydrochloride, 72 mg; riboflavin, 9 mg; pyridoxine hydrochloride, 15 mg; calcium pantothenate, 67.2 mg; nicotinic acid, 60 mg; 2-methyl-naphthoquinone, 5 mg; and choline chloride, 1.2 g. Each rat also received 3 times weekly the following supplement: Cottonseed oil (Wesson), 500 mg; α-tocopherol acetate, 1.5 mg; and a vitamin A-D concentrate‖ containing 50 U.S.P. units of vitamin A and 5 U.S.P. units of vitamin D.

* Milk Sugar Merck, Merck and Co., Inc., Rahway, N. J.

† Vitamin Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

‡ Sure's Salt Mixture No. 1.[9]

§ Whole Dried Liver Powder, Armour and Co., Chicago, Ill.

‖ Nopco Fish Oil Concentrate, assaying 800,000 U.S.P. units of vitamin A and 80,000 U.S.P. units of vitamin D per g.

or vegetable fats.(4-6) Findings by Boutwell *et al.*(2) indicate that the superiority of butter fat over vegetable fat on a lactose-containing diet is dependent on the B vitamin content of the diet. Animals fed butter fat gained significantly more weight than those fed vegetable fats when the basal diet was low in B vitamins. When the B vitamin content of the diet was increased, however. no significant differences in growth were observed on any of the diets employed. Boutwell *et al.*(2) suggest that butter fat may be superior to vegetable fats in promoting the intestinal synthesis of B vitamins or other nutrients essential for optimal lactose utilization. In line with the above Cary and Hartman(7) observed that liver contains a water-soluble factor (distinct from any of the known B vitamins with the possible exception of vitamin B_{12}) which significantly increased the growth of immature rats fed a purified ration containing 27% lactose. Liver has similarly been found to exert a protective effect on the growth and length of survival of immature rats fed a diet containing 73.2% β-lactose.(8) The present experiment was undertaken to obtain further data on the effects of liver feeding on growth and length of survival of immature rats fed purified rations of varying lactose content.

Procedure and results. Two basal rations were employed in the present experiment: diet A and diet B. Diet A was a purified ration containing the B vitamin factors in synthetic form only; diet B was similar in composition but contained in addition 10% defatted and desiccated whole liver. Both rations were supplemented with 0.0, 30.2, 45.3 and 60.5% U.S.P. lactose added in place of an equal amount of sucrose. Sixty-four female rats of the Long-Evans strain were selected at 22 to 24 days of age and an average weight of 44.7 g for the present experiment. Animals were placed in metal cages with raised screen bottoms to prevent access to feces and were fed *ad lib* the various diets listed in Table I (8 animals per group). Feeding was continued for 40 days or until death, whichever occurred sooner. All surviving rats were autopsied after 40 days of feeding. Ovarian weights were determined; ovaries were fixed in 10% formol, and sections were prepared and stained with hematoxylin and eosin.

Findings indicate that growth was retarded in all rats fed lactose-containing rations

4. Deuel, H. J., Jr., Movitt, E., Hallman, L. F., and Mattson, F., *J. Nutrition*, 1944, v27, 107.

5. Deuel, H. J., Jr., Movitt, E., and Hallman, L. F., *J. Nutrition*, 1944, v27, 509.

6. Deuel, H. J., Jr., and Movitt, E., *J. Nutrition*, 1945, v29, 237.

7. Cary, C. A., and Hartman, A. M., Science in Farming, *Yearbook of Agriculture*, 1943-1947, U. S. Dept. of Agriculture, p. 779.

8. Ershoff, B. H., and Deuel, H. J., Jr., *J. Nutrition*, 1944, v28, 225.

9. Sure, B., *J. Nutrition*, 1941, v22, 499.

TABLE II.
Effects of Liver Feeding on Body and Ovarian Weight and the Length of Survival of Immature Rats Fed Lactose-containing Diets.

Dietary group	Lactose content, %	No. of animals	Initial body wt, g	Gain in body wt over 40 day period,* g	% surviving†	Ovarian wt,* mg
A_1	0.0	8	44.5	114.6 ± 5.5 (8)	100	39.0 ± 2.9
B_1	0.0	8	44.4	129.0 ± 7.0 (8)	100	48.7 ± 5.3
A_2	30.2	8	45.6	96.5 ± 3.2 (8)	100	23.5 ± 3.7
B_2	30.2	8	44.3	119.2 ± 5.5 (8)	100	41.0 ± 5.2
A_3	45.3	8	44.6	84.3 ± 4.1 (8)	100	16.1 ± 2.7
B_3	45.3	8	44.5	107.4 ± 6.0 (8)	100	36.6 ± 3.9
A_4	60.5	8	44.9	44.0 ± 7.1 (2)	25	12.2 ± 1.4
B_4	60.5	8	44.7	82.6 ± 2.8 (7)	87.5	17.4 ± 1.8

The values in parentheses indicate the number of animals which survived and on which averages are based.

* Including standard error of the mean calculated as follows: $\sqrt{\frac{\Sigma d^2}{n}} / \sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

† Experimental period—40 days.

(Table II). Growth retardation was most marked in animals fed diet A_4 and B_4 (60.5% lactose) and was progressively less marked in animals fed smaller levels of lactose (diet A_3 and B_3, diet A_2 and B_2). At all levels of lactose feeding animals fed the whole liver powder gained significantly more weight than those fed a similar diet with liver omitted. This was particularly true for animals on the 60.5 per cent lactose ration. In the absence of dietary lactose, rats fed the whole liver powder (diet B_1) also gained more weight than animals on the liver free ration (diet A_1). The differences in growth between the latter, however, were not statistically significant. All lactose fed rats developed diarrhea during the first 10 days of the experiment. It occurred earlier and was more extensive in rats fed the higher lactose diets than in animals fed the 30.2% lactose diet. It was particularly marked on diet A_4. Subsequent to this period the diarrhea decreased in severity and gradually disappeared although it persisted in 2 rats on diet B_4 and 1 rat on diet B_3 for the duration of the experiment. In general no significant differences were noted between diet A and diet B in the incidence of diarrhea or its extensiveness for animals fed similar levels of lactose. For the first 10 days of feeding rats fed diets B_2, B_3 and B_4 did not differ in body weight from corresponding animals on diet A, although animals fed diet B_1 gained approximately 20% more weight than rats on diet A_1. Subsequent to this period, however, animals fed diets B_2, B_3 and B_4 consistently gained more weight than did corresponding rats on diet A. Two rats each on diets A_3 and A_4 developed extensive alopecia during the third week of the experiment. Alopecia was not observed in other rats on these diets nor in any of the animals fed liver-containing diets. Subsequent to the 4th week of feeding new hair replaced the areas of alopecia in rats fed diet A_3. The 2 rats with alopecia on diet A_4 succumbed. Six of the 8 rats on diet A_4 died during the experimental period with an average survival time for the decedents of 21.8 days (range 11-26 days). In contrast to the above only 1 rat on diet B_4 succumbed (8th day). All other rats survived. At autopsy a marked distension was observed in the cecum of rats fed diets containing 45.3

or 60.5% lactose. This was observed on both diets A and B. Cecal distension was also present on diets A_2 and B_2 but was less pronounced.

A significant difference in ovarian weight was observed in lactose-fed rats on the various diets (Table II). Ovaries appeared immature both in weight and microscopic appearance in animals fed diets A_2, A_3, A_4 and B_4. No mature follicles or corpora lutea were present in any of these rats with the exception of 1 animal on diet A_2. Six of the 8 rats on diet B_2 and 5 animals on diet B_3, however, had ovaries which were indistinguishable grossly and in histological appearance from normal controls (diets A_1 and B_1). In these animals mature follicles were numerous and corpora lutea were present.

Previous findings(10,11) indicate that the failure of rats to survive on diets containing high levels of lactose was due, at least in part, to an inability to hydrolyze adequately large amounts of this disaccharide. Present findings suggest that the administration of defatted and desiccated whole liver powder increased the rats' ability to digest lactose, although a period of approximately 10 days elapsed before this effect occurred. No data are available concerning the factor or factors in liver responsible for the increased growth, survival and ovarian development on lactose-containing diets. Recent findings by Hartman *et al.* (12) suggest, however, that vitamin B_{12} may be responsible, at least in part, for these effects.

Summary. The oral administration of 10% defatted and desiccated whole liver powder increased significantly the gain in body weight of immature rats fed purified diets containing 30.2, 45.3 and 60.5% lactose. It prolonged the survival time of immature rats fed a purified diet containing 60.5 per cent lactose, and it counteracted the inhibition of ovarian development which occurred on purified rations containing 30.2 and 45.3% lactose.

10. Ershoff, B. H., *Am. J. Physiol.*, 1946, v147, 13.

11. Riggs, L. K., and Beaty, A., *J. Dairy Sci.*, 1947, v30, 939.

12. Hartman, A. M., Dryden, L. P., and Cary, C. A., *J. Am. Diet. Assn.*, 1949, v25, 929.

Received November 9, 1949. P.S.E.B.M., 1949, v72.

Effect of Protein Level and Inanition on Glutamine Content of Rat Tissues.* (17531)

HENRY TIGERMAN AND R. W. MACVICAR (Introduced by Mark R. Everett)

From the Department of Agricultural Chemistry Research, Oklahoma Agricultural Experiment Station, Stillwater, Okla.

A study by Roine(1) suggested the importance of glutamine in protein synthesis in yeast and indicated the need for investigation of the glutamine content of blood and tissues of higher animals. Wiss(2) observed that the blood glutamine content of rats increased when the rats were placed on an inanition routine and decreased when protein was fed. These results implied that blood glutamine concentrations are governed to some extent by the level of protein ingested. This work was undertaken to relate various dietary factors to changes in glutamine concentration in animal tissues.

* Published with the approval of the Director, Oklahoma Agricultural Experiment Station. Supported by a grant from the United States Public Health Service through the National Institutes of Health. Appreciation is expressed to Merck and Company, Rahway, N.J., for supplies of crystalline vitamins and to the Lederle Laboratories Division, American Cyanamid Co., Pearl River, N.Y., for pteroylglutamic acid.

1. Roine, Paavo, *Suomen Kemistilehti,* 1946, v19B, 113.

2. Wiss, O., *Helv. Physiol. et Pharmacol. Acta,* 1948, v6, C35.