less than 1%; the urine-to-feces ratio being about 5 to 1. The lower absorption by the second patient may be ascribed to the fact that he was given a larger total dose (3 g) of Na_2HPO_4, which may have acted as a mild cathartic and hastened the passage of the ingested material through the small intestine.

It is to be noted that in the first patient 26% of the cells were young forms including metamyelocytes, myelocytes, and a few blasts. In the second patient, 33% of the cells were of such forms. Thus we are unable to state that the handling of phosphorus observed in these cases is characteristic of normal white cells, but the red cell findings may be assumed those of normal red cells.§

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Use of Liver Extract in Place of Yeast in Low Fat Diets.*

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During our studies on the B vitamins in liver extract, we found that definite retardation of growth and development of specific symptoms resulted when our synthetic diet was supplemented with liver extract without the addition of fat. The relation of fat to the normal development of the rat has been approached by several methods. Fats have been found to cure the dermatitis produced in rats on highly synthetic diets free of the B complex but containing B_1 and flavin.[1, 2] However, on such diets very little growth is obtained when the fat is added. With rations containing ether-extracted yeast to supply the B complex, symptoms of fat deficiency, namely scaly tail and paws, appear in 4-8 months. Upon addition of fat at

§ The radiophosphorus used in these studies was produced by the cyclotron, through the generous cooperation of the staff of the Radiation Laboratory. We acknowledge with thanks also grants-in-aid from the Josiah Macy, Jr., Foundation. Assistance from the W.P.A. is also acknowledged.

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1 Quackenbush, F. W., Platz, B. R., and Steenbock, H., *J. Nutrition*, 1939, **17**, 115.

2 Birch, T. W., *J. Biol. Chem.*, 1938, **124**, 775.

this stage a small growth response and alleviation of the deficiency symptoms result.[3-5] Both types of deficiency have been cured by purified preparations of linoleic acid. Since there is still a great interest in the nutritional significance of this and other fatty materials, we felt that our experience with liver extract might be of some value to workers interested in the fat problem.

The basal diet, designated J_{35}, used in this work consisted of sucrose 78%, casein 18%, and salts 3† 4%. In addition, each rat received daily supplements of 10 gamma of thiamin, 20 gamma of riboflavin, 1 mg of choline and 500 mg of liver extract powder. Twelve mg of haliver oil were given each week.‡ The casein was purified by washing and reprecipitation followed by 4 hot alcoholic extractions. The liver extract powder was the water soluble fraction (1 part equals 20 parts whole liver) prepared by Wilson and Company.§

Rats from our stock colony were used in all of these experiments. The young rats with their mothers were placed on raised screens during the last week of the suckling period. The young were given access to the experimental diet only and the mothers were removed from the cage each day for feeding. This enabled us to produce rats with a uniformly low storage of fat. This was necessary, for otherwise some litters continued to grow and failed to show the deficiency symptoms to be described below.

On this ration growth is fairly good for the first few weeks. The animals show a growth plateau at weights ranging between 80 and 140 g. Since our ration contained liver extract instead of the usual yeast, we will describe the appearance and condition of the rats in detail.

The deficiency was manifested roughly in both acute and chronic forms. In the acute form growth ceased very abruptly. A scaliness and reddening of the paws then occurred. The animals became very weak and emaciated and showed a marked loss of muscle tonus. There was occasional diarrhea. An extremely rapid loss in weight

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[3] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.

[4] Martin, G. J., *J. Nutrition*, 1939, **17**, 127.

[5] Hume, E. M., Nunn, L. C. A., Smedley-MacLean, I., and Smith, H. H., *Biochem. J.*, 1938, **32**, 2162.

† The salts 3 are the same as salts 1[10] with an additional 1.21 g $MnSO_4 \cdot 4H_2O$ per kilo of salt mixture.

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

‡ The haliver oil was kindly supplied by Dr. C. Nielsen, Abbott Laboratories, North Chicago.

§ The liver extract was generously furnished by Dr. David Klein, Wilson and Company, Chicago.

TABLE I.
Growth Response to Supplements of Various Fats.

Rat No.	Sex	Supplement	Wt when test started	Gain in in wt	Avg daily gain	Duration of test (days)	Type of deficiency
13712	♀	0	103	— 2	—0.2	11*	acute
13716	♂	2 drops corn oil per day	62	98	2.8	35	"
13814	♂	0	112	—	—	—*	"
13845	♀	3 drops coconut oil per day	122	11	0.3	35	chronic
13846	♂	0	90	— 31	—4.8	7	acute
13382	♀	3 drops coconut oil per day	110	— 9	—1.8	5	"
14261	♀	350 gamma of tocopherol per wk.	125	7	0.4	19	chronic
14383	♀	3 dr. butterfat per day	118	— 5	—0.2	20	acute
13726	♀	4 " linseed oil " "	83	75	2.1	35	chronic
13725	♂	2 " " " " "	72	112	3.2	35	"
13721	♀	0	104	—	—	—*	acute
12951	♂	3 dr. corn oil per day	129	63	3.0	21	"
12952	♂	3 " " " " "	129	59	2.8	21	"
12954	♂	3 " butterfat " "	121	35	1.7	21	"
12956	♀	3 " " " "	86	20	1.0	21	"
11841	♀	3 " wheat germ oil per day	118	60	1.8	21	"
11843	♂	3 " " " " " "	122	79	3.8	21	"
11844	♂	3 " "snowdrift" per day	137	65	3.1	21	"
11845	♀	3 " " " "	132	53	2.5	21	"

*Animals died.

then ensued and nasal hemorrhages and cyanosis preceded death. A quick recovery would follow if certain fats were given before the animals became too weak.

In the chronic form the plateau in the rate of growth was delayed or incomplete. A severe scaliness of the tail and paws, typical of that described by Burr and Burr[3] occurred. Also a progressive atrophy of the testes in the male and a delayed opening of the vagina in the female was a common occurrence. A severe eczema of the back and neck and an erosion and erythema about the eyes was seen less frequently. These animals did not show the rapid weight loss and sudden death that was shown in the acute form.

Rats in the above condition could be rapidly restored to normal by the addition of 2 or 3 drops daily of corn oil, linseed oil, or wheat germ oil. The growth response in most cases averaged at least 3 g per day and the deficiency symptoms rapidly disappeared. Muscle tonus and general activity was quickly restored. Although the animals grew rapidly, the scaly tail condition was very slow to heal. Table I gives examples of the responses obtained. Butterfat gave intermediate responses while coconut oil and α-tocopherol|| gave little or no response.

Necropsies of the animals that died of the acute deficiency showed normal lungs, livers, and kidneys. There was impairment of the reproductive organs. The testes, prostate and seminal vesicles were markedly atrophied. Hatai[6] has found atrophy of the testes on low fat diets.

To obtain maximum responses to concentrates of the vitamin B complex, fat must be present in the diet. To demonstrate this, certain concentrates were fed to rats receiving ration J_{30}, described by Oleson, *et al.*,[7] with and without fat. A crude concentrate of factor W (alcohol ether precipitate) was prepared by the method of Elvehjem, *et al.*,[8] and fed with and without the addition of 2 drops of corn oil per day to each rat. Factor 1 (vitamin B_6) and factor 2 (filtrate factor) concentrates[9] were fed on the same diet with and without the inclusion of 10% lard. These concentrates were kindly supplied to us by Dr. Lepkovsky. Table II shows the increased growth responses obtained from these concentrates when fat was present in the diet as lard or corn oil.

|| We are indebted to Dr. R. T. Major, Merck and Company, Rahway, New Jersey, for a sample of α-tocopherol.

6 Hatai, S., *Anat. Res.*, 1915, **15**, 1.

7 Oleson, J. J., Bird, H. R., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1939, **127**, 23.

8 Elvehjem, C. A., Koehn, C. J., and Oleson, J. J., *J. Biol. Chem.*, 1936, **115**, 707.

9 Lepkovsky, S., Jukes, T. H., and Krause, M. E., *J. Biol. Chem.*, 1936, **115**, 557.

TABLE II.

Group	Ration and Supplements	No. of rats	Avg initial wt	Avg wt at 6 weeks
1	Ration J_{30} + ¼ g alcohol-ether ppt./day (factor W)	3	38	96
2	Same as (1) + 2 drops corn oil/day	3	40	153
3	J_{30} + 0.5 cc factor 2 + .05 cc factor 1 (B_6) concentrates	3	42	109
4	Same as (3) + 10% lard	3	44	189

Summary. When our results are compared with those of other workers quoted above, it is evident that the use of liver extract instead of the usual yeast in studies on low fat diets results in an earlier and more acute deficiency and hence a greater growth response when fat is given. Possibly the liver extract is lower than ether-extracted yeast in the essential fatty acids. This fact may be useful in working out better methods for the assay of at least some of the essential fatty acids in fats. Furthermore, it is necessary to have fat in the diet to obtain maximum growth responses to the liver extract concentrates of the vitamin B complex.

10558 P

Attempted Quantitative Estimation of Atabrine Retardation of Schizogony in Avian Malaria.

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Employing female canaries, mosquito infected with *Plasmodium cathemerium* (Hartman) and kept in the dark from 6 P.M. to 6 A.M. and in the light during the other 12 hours, I have found the following features to characterize the peripheral blood picture at 1 P.M.: (a) the majority of the schizonts in singly infected cells are not yet fully segmented though well advanced in size, and they are contained predominantly in mature erythrocytes; (b) pigmentation is pronounced; (c) vacuolation of an apparently degenerative nature is seen occasionally in the larger but not in the smaller forms.

Atabrine,* given repeatedly in adequate therapeutic dosage, alters this picture as follows: The total number of schizonts is reduced;

* Atabrine for this study was kindly supplied by the Winthrop Chemical Company, Inc.