Production of a Nutritional Deficiency in Mice Fed a Low Fat Diet. (18320)

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Variations in dietary composition with regard to fat, carbohydrate, or protein markedly influence an animal's requirements for certain of the vitamins. It has been shown, for example, that the vit. B_{12} requirement for growth of the mouse is influenced by the fat and protein levels in the diet(1). Other factors may influence dietary vitamin requirements. Among these are stresses, such as the feeding of thyroid active materials, or the administration of intestinal bacteriostatic agents that suppress the synthesis of essential nutrients by the intestinal flora. During the course of investigations involving possible interrelationships between dietary composition, stress factors, and the administration of intestinal bacteriostatic agents it was found that the addition of succinylsulfathiazole (SST) to a diet low in fat but otherwise complete with regard to all other known essential nutrients caused a growth retardation in mice.

This study was undertaken to investigate the nature of this SST-induced deficiency.

Experimental and results. The basal ration employed in these studies had the composition shown in Table I. Although this diet is low in fat (0.5%), sufficient vitamin B_{12} to assure optimal growth was given(1). All substitutions in this diet were made at the expense of equivalent weights of white dextrin. The various diets were fed *ad libitum* to groups of male weanling mice (Sharp and Dohme, Swiss Webster strain) for 2-week periods.

Good growth was obtained with the basal diet. However, when 2% SST was incorporated in the diet a marked retardation in growth rate resulted. Doubling the dietary levels of all the known vitamins had no effect on the SST-induced growth retardation. Growth rates were improved, however, when fat or certain vegetable materials such as defatted cottonseed meal, rolled oats, or dried grass were added to the basal diet. All liver fractions that were tested showed a marked toxicity. The deleterious effect of liver could be prevented by the addition to the diet of fat or cottonseed meal. The results of this portion of the study are shown in Table II.

TABLE I. Composition of Basal Diet.

Corn oil*	0.5
Casein†	20
Glucose‡	20
White dextrin	53.5
Salt mixture(2)	4
Cellu flour	2
Each 100 g of diet supplemented with:	
Vit. A	900 U.S.P. units
Vit. D	180 U.S.P. units
α-tocopherol	4 mg
2-methyl-1,4-naphthoquinone diacetate	1 "
Thiamine hydrochloride	0.8 "
Riboflavin	1.6 "
Pyridoxine hydrochloride	0.8 "
Niacin	4.0 "
Ca pantothenate	4.4 "
Para-aminobenzoic acid	4.0 "
Choline chloride	200.0 "
Inositol	21 "
Folic acid	0.2 "
Biotin	0.02"

* Mazola, Corn Products Refining Co., Argo, Ill.

† Vitamin test casein, General Biochemicals, Inc., Chagrin Falls, O.

‡ Cerelose, Corn Products Refining Co., Argo, Ill. Jones and Foster(2).

Each mouse received 0.1 μg of vit. B_{12} per day by weekly intraper. inj.

A second study was made to determine the effect of increasing the SST level in the diet from 2% to 4%. The effect of varying levels of cottonseed meal and of fat also was determined with the higher SST level. The results, shown in Table III, indicate that diets containing 4% of SST caused a greater growth retardation than did those containing 2% of SST. Both cottonseed meal and fat were effective in restoring growth rates to the control values but higher levels of cottonseed meal were necessary when the diet contained

1. Bosshardt, D. K., Paul, Winifred J., and Barnes, R. H., *J. Nutrition,* 1950, v40, 595.

2. Jones, J. H., and Foster, Claire, *J. Nutrition,* 1942, v24, 245.

TABLE II. Effect of Succinylsulfathiazole (SST), Fat, and Natural Products on Growth of Mice.

Dietary supplement	No. of mice	Avg gain in body wt, 14 day period (g)
None	64	8.5 (59)
2% SST	64	5.0 (57)
" " + 3% partially defatted cottonseed meal*	56	7.4 (52)
" " + 3% petroleum ether extracted Proflo	8	7.3 (8)
" " + 3% Cerophyll powder†	8	6.2 (6)
" " + 3% rolled oats	8	7.5 (7)
" " + 3% distiller's dried solubles	8	2.3 (6)
" " + 3% Bacto yeast extract	8	3.9 (2)
" " + 3% wheat germ	8	4.5 (8)
" " + 3% dried yeast‡	8	4.7 (8)
" " + 3% fish solubles	8	4.5 (4)
" " + 3% dried beef muscle	8	3.6 (8)
" " + 3% olive oil	8	6.5 (7)
" " + 25% olive oil	8	7.1 (8)
" " + 3% lard	8	5.4 (7)
" " + 25% lard	8	8.5 (8)
" " + 3% corn oil§	8	6.7 (7)
" " + 25% corn oil	8	8.0 (8)
" " + 3% butterfat	8	5.7 (8)
" " + 25% butterfat	8	7.6 (8)
" " + 3% cottonseed oil	8	5.3 (8)
" " + 25% cottonseed oil	8	8.4 (7)
" " + 25% hydrogenated cottonseed oil‖	8	9.3 (8)
" " + 25% wheat germ oil	8	9.5 (8)
" " + 25% peanut oil	8	8.6 (8)
" " + 3% lyophilized fresh liver	8	1.3 (6)
" " + 3% Wilson's liver fraction L	8	—1.1 (4)
" " + 3% Wilson's liver fraction S	8	—0.5 (5)
" " + 3% defatted, desiccated liver¶	8	2.9 (3)
" " + 3% defatted, desiccated liver¶ + 25% hydrogenated cottonseed oil‖	8	10.3 (8)
" " + 3% Wilson's 1:20 liver powder	8	0.5 (2)
" " + 1% Wilson's 1:20 liver powder	8	1.8 (6)
" " + 1% Wilson's 1:20 liver + 3% cottonseed meal*	8	7.9 (8)

The figures in parentheses are the number of survivors and numbers on which average weight gains are based.

* Proflo, Traders Oil Mill Co., Fort Worth, Texas.
† Cerophyll Laboratories, Inc., Kansas City, Mo.
‡ Strain G, Anheuser Busch Co., St. Louis, Mo.
§ Mazola.
‖ Primex.
¶ VioBin.

4% of SST.

Discussion. It has long been recognized that fat has a beneficial effect on growth. Since the findings of Burr and Burr(3) were published, it has been recognized that one of the factors involved is the content of the "essential" unsaturated fatty acids in the dietary fat. White, Foy, and Cerecedo(4) noted that mice grew better when fed a diet containing 10% lard than when fed a fat-free diet. Furthermore, the mice fed the 10% lard diet did not develop the dermatitis typical of an essential fatty acid deficiency that was noted with the mice fed a fat-free diet. Although the dermatitis appeared on the 30-40th day, a growth difference was noted from the start of the experiment. This would suggest a secondary deficiency; it has been stated by Burr(5) with reference to his work with rats, "If the rats do not grow rapidly at first some other deficiency should be suspected". It has been shown by Deuel and coworkers(6) that fat *per se* has a beneficial effect on the growth of rats. This effect was not affected by the incorporation of methyl linolate in the basal fat-free diet.

3. Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, v82, 345; 1930, v86, 587.

4. White, E. A., Foy, J. R., and Cerecedo, L. R., PROC. SOC. EXP. BIOL. AND MED., 1943, v54, 301.

5. Burr, G. O., *Fed. Proc.*, 1942, v1, 224.

6. Deuel, H. J., Jr., Mesere, E. R., Straub, E., Hendrick, C., and Scheer, B. T., *J. Nutrition*, 1947, v33, 569.

TABLE III. Effect of Succinylsulfathiazole (SST), and Cottonseed Meal on Growth of Mice.

Supplement	No. of mice	Avg gain in body wt, 13-day period (g)
None	72	7.6 ± .35*(70)
2% SST	32	5.7 ± .54 (29)
" " + 3% cottonseed meal	32	8.0 ± .50 (29)
4% SST	40	4.1 ± .47 (39)
" " + 3% cottonseed meal	8	6.9 ± .76 (7)
" " + 4% cottonseed meal	8	7.1 ± .97 (8)
" " + 5% cottonseed meal	40	7.8 ± .38 (39)
" " + 10% cottonseed meal	8	8.3 ± .91 (7)
" " + 20% Primex	8	7.2 ± .70 (7)

The figures in parentheses are the numbers of survivors and numbers on which average weight gains are based.

$$^{*}\text{S.E.} = \sqrt{\frac{\Sigma d^2}{n\,(n-1)}}.$$

The results obtained in this study show that the addition of SST to an apparently complete diet caused a marked growth retardation. Fat and several natural products of vegetable origin counteracted this effect. It seems unlikely that the fat effect is due to the intake of linoleic acid. The basal diet, containing 0.5% corn oil, contains 200 mg of linoleic acid per 100 g of diet. Regardless of the fat source, better growth was obtained with diets containing 25% of fat than with those containing 3% of fat. However, as shown in Table IV, 3% of fats such as cottonseed oil, peanut oil, corn oil, or wheat germ oil contributed as much or more linoleic acid to the diet as did 25% of butterfat, lard or olive oil. It would appear from these data that the fat effect is not due to the essential fatty acids present but rather to the fact that fat *per se* is an essential nutrient.

If lipids are essential nutrients for animal growth they must either be supplied in the diet or synthesized by the animal from other foodstuffs. It is known that animals are able to synthesize lipids from intermediary metabolites of carbohydrate and certain amino acids. If it can be assumed that the sole role of SST is that of an intestinal bacteriostatic agent the results obtained in this study would suggest that a factor essential for lipid synthesis by the animal is formed by the intestinal flora. The results obtained with cottonseed, oats, and grass would indicate that these materials contain this factor.

The inhibitory effects of the liver preparations on the growth of mice fed low fat diets containing SST cannot be explained at present. Since the liver effect can be counteracted by the addition to the diet of fat or cottonseed meal it is possible that liver contains an antagonist or an anti-metabolite for the postulated factor or factors. If such is the case it is possible that some of the materials shown to be inactive (Table II) may have contained the factor, or factors, but that a beneficial effect was masked by the presence of an antagonist.

It appears improbable that the postulated factor/s, is the vitamin B_{13} of Novak, Hauge, and Carrick(8) although the basal diets were quite similar. Several sources of vitamin B_{13}

TABLE IV. Dietary Levels of Linoleic Acid.*

Supplement	Fat level in diet* 3% mg/100 g diet	25% mg/100 g diet
Butterfat	120	1000
Lard	183	1525
Olive oil	210	1750
Cottonseed oil	1500	12500
Peanut oil	750	6250
Corn oil	1200	10000
Wheat germ oil	1470	12250

* Linoleic acid values are those reported by Hilditch(7).

† In addition each diet contained 200 mg linoleic acid per 100 g diet contributed by the 0.5% corn oil in the basal diet.

7. Hilditch, T. P., 1949, The Chemical Constitution of Natural Fats. John Wiley and Sons, New York.

8. Novak, A. F., Hauge, S. M., and Carrick, C. W., *Poultry Sci.*, 1947, v26, 604.

such as fish solubles, liver, and distillers' dried solubles were found to be inactive. However, it has been impossible to confirm the results of Novak *et al.* using mice. Likewise it is not known whether or not the postulated factor/s, is identical with that proposed by Ruegamer(9) or by Zucker and Zucker(10).

Summary. The addition of succinylsulfathiazole to low fat diets caused a growth retardation in mice although adequate quantities of all known accessory growth factors were fed. Normal growth resulted when fat, cottonseed meal, or rolled oats were added to the basal diet containing succinylsulfathiazole.

9. Ruegamer, W. R., *Arch. Biochem.*, 1949, v23, 236.

10. Zucker, T. F., and Zucker, L. N., *Arch. Biochem.*, 1948, v19, 323.

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Thromboplastic Potency Changes. A Result of Bacterial Action. (18321)

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Recent work in this laboratory has shown a significant factor in the potency deterioration of tissue extracts currently used as sources of thromboplastin to be associated with the activity of a bacterial contaminant, *B. subtilis*, in the thromboplastic suspensions.

It is the purpose of this paper to describe this effect and some properties of thromboplastic suspensions so altered.

Materials and methods. Rabbit brain and rabbit lung were used as sources of thromboplastic powder. Details of preparation of thromboplastic powders and their extraction to yield the final suspensions used, have been described elsewhere(1). Diluent for the thromboplastic suspension was 1:1 NaCl-$CaCl_2$ mixture (see below). Normal human oxalated plasma was employed (1 vol. of 0.1 M sodium oxalate to 9 vol. of freshly drawn whole blood). Ten per cent prothrombin plasma was obtained by diluting 100% prothrombin plasma with prothrombin-free plasma critically adsorbed(2) with $Ca_3(PO_4)_2$. All plasma was kept in an ice-water bath when not in use. Saline 0.85% NaCl and calcium 0.025 M $CaCl_2$ were used. Clotting time determinations: Our modification (1) of the Quick one-stage method was used, employing chemically clean glassware throughout. *B. subtilis* was cultured from a thromboplastic suspension which had undergone alteration upon incubation (see below). Routine bacteriological technic was employed. Difco brain-heart infusion with 2% agar was used as the plating medium. During the course of a day's routine clinical prothrombin determinations it was observed that the potency of the thromboplastic suspension decreased sharply during the period of the determinations. Observations made during a controlled study of the nature of the change in this batch of thromboplastic material was subsequently found to apply to all batches of brain and lung powders investigated.

Results. Brain thromboplastic suspension was incubated at 37.5°C for a period of 9 hours in a test tube open to air. During this time, oxalated plasma was kept in an ice-water bath and 0.1 cc portions removed as needed. Little change in clotting time occurred for the first 5 hours (Fig. 1). Between 5½ and 6 hours, however, there was a sudden, rapid prolongation of clotting time. The plasma, when tested at this time with a fresh thromboplastic suspension clotted in 12.4 seconds. The sudden prolongation in clotting time was therefore attributed to the loss in potency of the incubated brain thromboplastic suspension. A similar loss in potency was shown to occur with lung thromboplastic sus-

1. Gollub, S., Kaplan, F. E., and Meranze, D. R., *Am. J. Physiol.*, 1950, v162, 293.

2. Gollub, S., Kaplan, F. E., Meranze, D. R., and Tuft, H., *Am. J. Clin. Path.*, 1949, v19, 1021.