

we obtained the A form apparently in a pure condition. Upon recrystallization from dilute ethyl alcohol, the B form was obtained.

As a rule the clusters of needles are obtained when a solution of the pure crystals is freed from solvent by distillation, whereas the plate form appears upon crystallization from hot dilute aqueous acetone or ethyl alcohol or from butyl alcohol. While we have not studied the point in detail, our records indicate that either form may be converted into the other, depending upon the experimental procedure.

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Influence of Ingestion of Butter Fat on Body Fat of the White Rat.

HENRY C. ECKSTEIN.

From the Laboratory of Physiological Chemistry, School of Medicine, University of Michigan, Ann Arbor.

In previous communications^{1, 2} the writer presented evidence indicating that the ingestion of derivatives of butyric and caproic acids by the white rat exerted an effect on the body fat of such animals quite different from that usually observed when fats are fed. The general effect consists of an alteration of body fat accompanied by an incorporation in that fat of derivatives of the ingested fat. While it was apparent, from the investigations referred to, that the tissue fat of rats had been altered as a result of the ingestion of derivatives of the above mentioned acids, no evidence of the presence of the ingested fats in the fat of the rats could be obtained. The results secured when tricaproin was fed were quite satisfactory, since the rats appeared to be normal and grew at the usual rate. On the other hand those on a diet containing the butyryl radical in the form of sodium butyrate appeared to be abnormal throughout the whole experimental period.

This communication is a report of experiments in which the butyryl radical was incorporated in the diet in another manner, namely by feeding considerable amounts of butter fat to the rats. The diet fed consisted of the following mixture: vegex 2%, salt mixture 5%, casein 20%, butter fat 30%, and cornstarch 43%. Each 100 gm. of food contained non-saponifiable matter from 7 gm.

¹ Eckstein, H. C., *J. Biol. Chem.*, 1929, lxxxii, 613.

² Eckstein, H. C., *J. Biol. Chem.*, 1929, lxxxiv, 353.

of cod liver oil. As in previous experiments the animals were kept on the diet for 8 weeks. At the end of that time they were sacrificed by exposure to illuminating gas. The total lipids were then extracted in a manner previously described.¹ Throughout the whole experimental period no evidence of any abnormality was observed.

TABLE I.

*Influence of the Ingestion of Butter Fat and Tricaproin on the Fatty Substances of White Rats.**

Number of rats on diet	Diet	Weight of Animal†	Total lipids	Iodine Number	Saponification Number	Reichert-Meissl Number	Polensky Number
8	Fat Free	140	13.4	68	194	1.28	0.59
7	Tricaproin	159	14.7	59	191	1.28	0.62
10	Butter Fat	149	11.9	63	193	1.16	0.72

* The results presented in the table are the averages for each group.

† Less gastro-intestinal tract.

In Table I are presented the results, together with a summary of data previously obtained. It is quite clear that the amount of fatty acids of low molecular weight is no greater in the fatty substances isolated from the group ingesting butter fat or tricaproin than in the lipids synthesized by rats from fat free precursors. This follows from the great similarity of the Reichert-Meissl and Polensky numbers of the 3 types of lipids. While they are unquestionably alike in this respect they differ somewhat with regard to their degree of unsaturation. The differences between the iodine numbers of the 3 types are not marked, but these are, in the opinion of the writer, true variations and not merely due to the limitations of the method employed. Similar differences were likewise found between the iodine numbers of the fatty acids present in the phosphatides isolated from the 3 types of fat. Those secured from the lipids of animals on the fat free diet had an iodine number of 107, as compared with 101 for those obtained from the lipids secured from rats ingesting butter and 97 for those isolated from the lipids extracted from those on the tricaproin diet.

These results are, therefore, in accordance with those previously reported and again suggest that fatty acids of low molecular weight are, to some extent at least, utilized to build up saturated fatty acids of a higher molecular weight. The difference between the iodine numbers of the control group and that fed butter fat are not as great as those noted between the control group and the tricaproin group. This is to be expected since the percentage of fatty acids of low molecular weight is less in the butter fat diet than in the case of the

diet containing tricaproin. For this reason an attempt is now being made to feed a diet containing much more butter fat with the hope that more striking differences can be secured. Until further work is done the writer wishes to withhold any definite conclusion regarding the effect of the ingestion of butter fat on the chemical composition of body fat.

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Quantitative Studies on Precipitins.

JOSEPH G. BAIER, JR.* (Introduced by T. C. Nelson.)

From the Department of Zoology, Rutgers University, New Brunswick, N. J.

Since the quantitative precipitin method of Boyden and Baier¹ has proved to be fairly simple, rapid, and reliable, it was thought advisable to investigate further some properties of this reaction itself. The volume of precipitate obtained in the reaction was studied as affected by (1) quantity of antigen, (2) temperature of the reaction mixture, (3) time and rate of centrifugation, and (4) length of incubation.

Only simple protein antigens have been used in this study (crystalline egg albumin).² All protein concentrations have been found by making modified Kjeldahls³ on samples. The antisera were obtained from rabbits by intravenous injections of the antigen. They were bled from the heart 10 days after the last injection. Calibrated instruments only were used in making all dilutions of antigen and antiserum in buffered saline.

The technique in performing the reaction consisted in preliminary titer determinations (ring test) to ascertain the strength of the antiserum. Following this the quantitative nature of the reaction was studied, using van Allen thrombocytocrits as stated¹ with suitable modifications for particular experiments. The experimental results are shown graphically.

The quantitative technique employed has verified some of the conclusions of earlier workers with regard to antigen-antibody

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¹ Boyden, A. A., and Baier, J. G., *J. Immunol.*, 1929, xvii, 29.

² Hopkins and Pinkus, *Physiol.*, 1898, xxiii, 130.

³ Folin, O., and Wright, L. E., *J. Biol. Chem.*, 1919, xxxviii, 461.