

Northrup and Kunitz definitely appreciate the fact that isoelectric gelatin must have a diffusible anion present (and it should be added "or cation" if the gelatin is, by any chance, of acid conditioned stock). It would seem, however, that they, as well as Hitchcock,⁴ who uses their method of purification, render their gelatin free of a part of the acetic acid necessary to maintain it at its isoelectric point when they precipitate it and wash it with alcohol and ether. At any rate, the pH for the new isoelectric point which Hitchcock recently gave for ash free gelatin is more nearly that of pure gelatin, free from diffusible ionic impurities, and the conductivity of this gelatin is also that of Cooper's purified, electrolyte-free gelatin which was recently purified electrolytically by the method described by the writer some years ago.

This new concept of isoelectric gelatin and its relation to the physical properties of gelatin in no way diminishes the validity or importance of Loeb's work or of that of many other investigators who have helped to develop the concept that proteins are amphoteric electrolytes which react with acids and bases in a perfectly normal manner. It helps rather to emphasize this point of view and to clarify the confusion which now exists because of the variable pH of different proteins and of different concentrations of proteins. This critical point of hydrogen ion concentration, where proteins have these "minimum physical properties," previously referred to as the isoelectric point, now becomes the point where the protein in its purest form exists as an acid or a base, and has a pH which is a function of the ionization constants of the protein in question.

Data and references in support of some of the contentions which have been made will be given in detail later.

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Effect of Feeding Whale Oil on the Depot Fat of the White Rat.

J. B. BROWN AND A. L. RAWLINS.

From the Department of Physiological Chemistry, College of Medicine, Ohio State University, Columbus, Ohio.

Forty-three female white rats of average weight 154 gm. were fed for 6 weeks on a diet of 62.5% whole wheat flour, 15% casein, 15% skim milk powder, 1.5% precipitated chalk, 1% NaCl, 5%

⁴ Hitchcock, David, *J. Gen. Physiol.*, 1929, xii, 495.

cotton seed oil and 0.3% cod liver oil. Following this control diet, the animals were changed to one containing 10% whale oil and 90% control mixture for 2 weeks, and from then on to a diet of 20% whale oil and 80% control. After 10 weeks of whale oil feeding the control diet was resumed. At intervals during the experiment groups of 6 rats were killed and the depot fat was removed by dissection and rendered. The results of the analysis of the specimens of fat are given in Table I.

TABLE I.

Time Weeks	Fat				Fatty Acids	
	Iodine No.	Sapon. No.	Ref. Index	Polybro- mide No.	Polybro- mide No.	% Br. in Bromides
			20°			
0*	86.06	200.8	1.4677	0.	— —	— —
2	88.46	197.2	1.4694	3.53	— —	— —
4	96.73	193.7	1.4702	6.70	— —	— —
6	97.26	193.1	1.4702	13.08	14.08	67.53
8	97.88	193.1	1.4703	14.10	14.98	67.61
10	97.90	193.2	1.4703	14.15	15.12	67.63
13†	94.27	195.2	1.4700	10.03	10.82	67.58
Whale oil	127.32	189.1	—	24.20	25.24	67.58

*After 6 weeks on control diet.

†After resumption of control diet for 3 weeks.

From the above data it is concluded that equilibrium between the food fat and the depot fat is established within 4 to 6 weeks on a given diet. The characteristic highly unsaturated fatty acids of whale oil, including acids of the C_{18} , C_{20} and C_{22} series with 4, 5 and 6 double bonds, are apparently deposited as such since the bromine content of the polybromides is practically the same as that of the original whale oil. (Chemically whale oil is classed with the fish oils.) A maximum polybromide number of 15.12 containing 67.63% bromine indicates a storage of at least 4.9% of unsaturated acids on a 20% whale oil diet. On the other hand, these acids give a polybromide number only about 1/3 of the theoretical,¹ from which a storage of nearly 15% may be calculated. Resumption of the control diet for 3 weeks still showed a considerable quantity of highly unsaturated acids to be present. When once stored, they are apparently removed only slowly.

¹ Brown, J. B., and Beal, G. D., *J. Am. Chem. Soc.*, 1923, **xlv**, 1289.