Iowa Section

State University of Iowa, February 19, 1935.

7948 C

Effect of Heat and Alcohol Extraction on the Nutritive Value of Casein.

H. W. SCHULTZ, W. H. SEEGERS AND H. A. MATTILL.

From the Biochemical Laboratory, State University of Iowa, Iowa City.

The methods employed for the purification of casein for experimental diets require either the use of solvents to extract extraneous materials or the application of heat to destroy the unwanted accessories, or both solvents and heat may be employed. Casein treated in either or both of these ways does not permit optimum growth in experimental animals. The experiments described below, which are, in many respects, similar to those reported from other laboratories, can readily be explained on the basis of the removal of a nutritional factor, with or without the coincident impairment of the protein itself.

An attempt was first made to demonstrate what effect, if any, alcohol extraction of casein might have on the value of a diet in supporting lactation in rats in the first and second generations. By the method of Kozlowska, McCay and Maynard,[1] lactation was studied on stock females which were fed the following diet beginning 2 days after parturition: protein, 18%; hydrogenated cottonseed oil (crisco), 22%; corn starch, 41.5%; salts,[2] 4.5%; agar agar, 2%; yeast,* 10%; cod liver oil, 2%. The protein was commercial casein (diet A) or casein which had been extracted in a percolator for 4 days with boiling 95% alcohol (diet B). This same method of extraction, first described by Sperry, was used for all preparations. At the end of the 40-day lactation period the young

[1] Kozlowska, M., McCay, C. M., and Maynard, L. A., *J. Nutr.*, 1932, **5**, 61.

[2] Hawk, P. B., and Oser, B. L., *Science*, 1931, **74**, 369.

* Kindly supplied by Northwestern Yeast Company.

from these litters were continued on the same diet and their growth rate (Fig. 1) and lactation capacity were also studied.

From Table I it can be seen that lactation was much better on diet A than on diet B, but this difference was only slightly accentuated in the second generation. The growth rate of the second generation males was inferior on the extracted casein but the females did not show this difference (Fig. 1). Such sex differences are not uncommon in nutrition studies and their cause is obscure. Additional male rats from inbred stock ("standard" animals) again revealed the same difference in the growth-promoting capacity of the 2 rations (Fig. 1).

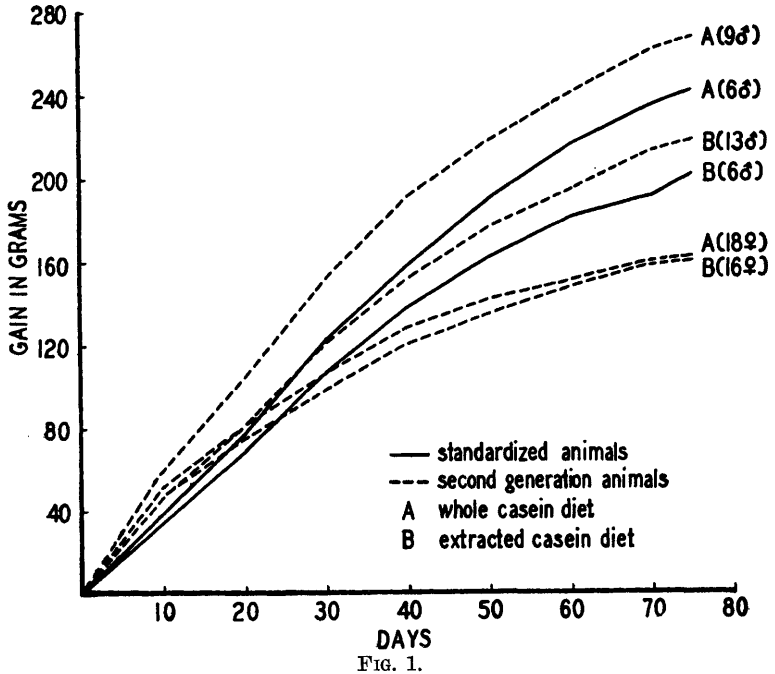

FIG. 1.
Effect of Alcohol Extraction of Casein on its Nutritive Value for Growth.

TABLE I.
Effect of Extraction of Casein on Lactation.

Diet	No. of litters	Litters reared	Whole litters reared	Surviving young %
		First generation (40 days)		
A	8	6 (75%)	5 (63%)	73
B	13	6 (46%)	4 (31%)	40
		Second generation (15 days)		
A	12	8 (67%)	6 (50%)	64
B	11	4 (36%)	2 (18%)	24

For studies on the effect of heat and on the corrective influence of various extracts the animals used were equally divided as to sex and weighed from 130 to 140 gm. The yeast and cod liver oil of the basal ration were replaced by starch and daily supplements of 0.5 gm. yeast and 5 drops of cod liver oil were fed. Casein extracted for 4 days and heated for 2 hours at 120°C. gave the least satisfactory growth of any of the preparations (Table II); prolonged heating seems to destroy or alter more effectively than short heating, even at a higher temperature.

TABLE II.
Effect of Alcohol Extract of Wheat Germ as Supplement to a Diet Containing Extracted and Heated Casein on the Growth of Rats.

No. of lot	No. of animals	Treatment of casein	Addition	Gain in wt. gm.	Food intake gm.	Gain per gm. of protein gm.
1	6	Extracted, heated 150°C., 30 min.	None	41.9	168.0	1.386
2	6	Same	Hot alcohol extract of wheat germ	42.1	159.2	1.469
3	5	Same, 120°C., 2 hrs.	None	27.6	159.0	0.964
4	6	Same	Hot alcohol extract of wheat germ	40.6	149.4	1.510

This poorly utilized ration was improved by supplementing with a hot alcohol extract of wheat germ. (Compare lots 3 and 4, Table II.) The supplementary effect of such an extract was practically without significance when fed with a diet containing extracted casein heated for 30 minutes at 150°C. (Lots 1 and 2.)

Ether extracts of wheat germ were ineffective. The improved growth which Coward and her coworkers[3] obtained by supplementing their basal diet with similar extracts may have been due to traces of vitamin A in the oil, since the animals were on an A-deficient diet. Their favorable results with ether extracts of wheat germ and alcohol extracts of wheat germ and casein are therefore not conclusive proof of a new dietary factor. The foregoing experiments, however, show that the presence of such a factor in casein and wheat germ can be demonstrated even when all other known dietary essentials are amply supplied.

The favorable effect of added extracts in increasing growth rate

[3] Coward, K. H., Key, K. M., and Morgan, B. G. E., *Biochem. J.*, 1929, **23**, 695.

does not preclude the possibility of protein damage. Morgan[4] and Fixsen and Jackson[5] have demonstrated that heating lowers the biological value of casein, but the nature and extent of such damage, under given conditions, are not clear. The possibility of a change in the digestibility of the protein, similar to that found with animal tissues (Seegers[6]), is not excluded. The supplementary value of alcohol extracts of wheat germ is less well explained by such considerations than on the generally accepted grounds that such extracts provide a labile factor (B_4?)[7, 8] which is removed from casein by alcohol extraction and which is present in only small amounts if at all in yeast.

7949 P

Tyrosinase in Ontogenesis (Orthoptera)

J. H. BODINE AND E. J. BOELL.
From the Zoological Laboratories, State University of Iowa.

Variations in concentration of tyrosinase throughout the entire embryonic development of the grasshopper, *Melanoplus differentialis,* have been determined by measuring the oxygen uptake of the tyrosinase-tyrosine reaction with the Barcroft-Warburg apparatus. Tyrosinase activity of eggs at different developmental stages has thus been expressed as the amount of O_2 consumed per 100-minute interval at 25°C. in the oxidation of a given amount of tyrosine by the enzyme extracted (in phosphate buffer pH 8.0) from 20 eggs.

The growth curve for tyrosinase in the whole egg is sigmoid during the first 3 weeks. Maximum enzyme concentration is reached on the 20th day and is maintained at this level throughout a period of suspended embryonic development (diapause) which occurs then. The post-diapause developmental period, during which the embryo pigments and hatches, is characterized by a decrease in concentration of tyrosinase.

The largest part of the tyrosinase content of the egg is found in the yolk and in the serosa cells and fluids surrounding the embryo. The amount of tyrosinase in the embryo alone is low but increases

[4] Morgan, A. F., *J. Biol. Chem.*, 1931, **90**, 771.
[5] Fixsen, M. A. B., and Jackson, H. M., *Biochem. J.*, 1932, **26**, 1923.
[6] Seegers, W. H., in press.
[7] Reader, V., *Biochem. J.*, 1929, **23**, 689.
[8] Halliday, N., *J. Biol. Chem.*, 1934, **106**, 29.