tion† of the toxic O antigens of *Salmonella* and *Shigella*, noted by Morgan and Partridge,[9] is an ability to combine with carbohydrate to form highly antigenic complexes whose specificity is determined by the particular carbohydrate used. For instance, blood group A polysaccharide obtained from hog gastric mucin was coupled with shigella protein to form an artificial antigen.[10] This antigen could be used in the production of high-titer A typing sera. The occurrence of a comparable toxic carrier-protein in snake venom could account for the cross-protection observed despite the lack of serological cross-reaction.‡

Summary. Mice immunized with moccasin venom were protected against otherwise lethal doses of the endotoxin of *Salmonella typhimurium;* and, inversely, salmonella-immunized mice were protected against the venom. This cross-protection may be due to the presence in gram-negative organisms and moccasin venom of a common factor characterized by hemorrhagic action, antigenicity, and a lack of serological specificity.

† In an earlier paper of this series[3] we referred to this portion of the toxic O antigen as a polypeptide rather than as a protein. In this usage we followed the early nomenclature of Morgan and Partridge, who have since considered this portion of the antigen to be protein rather than polypeptide.

[9] Morgan, W. T. J., and Partridge, S. M., *Brit. J. Exp. Path.*, 1942, **23**, 151.

[10] Morgan, W. T. J., *Brit. J. Exp. Path.*, 1943, **24**, 41.

‡ The cross-protection as well as the reported hemorrhagic activity of moccasin venom could conceivably have resulted from an inapparent contamination of the venom preparations by a gram-negative bacterium. In an effort to rule out the possibility of contamination, solutions of the venom were tested for agglutination against an O-typing antiserum for *Salmonella typhimurium*. There was no reaction, indicating that the type of cross-protection reported in this paper is not due to carbohydrate-specific antigens held in common by the materials tested. Also, concentrated dispersions of the venom were stained heavily with Loeffler's methylene blue. No evidence of contamination was found.

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Significance of the Protein Level in Synthetic Diets.

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McIntire *et al.*[1] suggest that to achieve optimal growth in rats (males), one or more factors are needed which are deficient in currently used synthetic diets with pure B factors. This deficiency is made up by liver extract. Their basal diet contains 18% purified casein, salts, sugar, fat, fat-soluble vitamins, thiamine, pyridoxine, riboflavin, pantothenic acid, nicotinic acid, and choline. In looking for the missing factor they apparently restrict consideration to the B complex on the assumption that the diet furnishes proper amounts of all other factors which should be fed and because growth is increased when liver extract is given.

We have published observations on dietary protein and growth[2] in which the assumption is made (and substantiated) that the sources of B complex in certain seed meals plus white flour are adequate for optimal growth. A preliminary report[3] dealing with casein levels was based on a similar assumption regarding adequacy of a mixture of rice bran extract, riboflavin, and yeast extract. On the basis of these assumptions both reports point to the

[1] McIntire, J. M., Henderson, L. M., Schweigert, B. S., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 98.

[2] Zucker, T. F., and Zucker, L., *Ind. Eng. Chem.*, 1943, **35**, 868.

[3] Zucker, T. F., and Zucker, L., Abstracts, 105th ACS Meeting (Detroit), April, 1943, Div. Biol. Chem., p. 5B.

fact that the more rapidly growing male requires more protein as expressed in percent protein in the diet than the more slowly growing female; the male level should in the case of casein be put at 24% actual casein, or when weighed out as crude casein, at 27.9% in the diet. A similar conclusion as regards sex difference can be drawn for the chick from the work of Irwin and Kempster.[4]

In the casein level experiments we used crude casein, which is perfectly permissible if our assumption is correct that 5 g rice bran extract, 0.5 g yeast extract, and 100 γ riboflavin per 100 g diet supply all the B factors necessary. Since McIntire's assumption and ours are mutually exclusive, the answer must either be that raising the casein level in the McIntire diet will produce optimal growth, thus making the postulated new factor unnecessary, or the unknown factor is furnished in adequate amounts in crude casein as an impurity when this is raised from 20.9%, which is suboptimal, to 27.9% (N × 6.25 = 18 and 24% respectively). This would demonstrate a fallacy in our conclusion that the male requirement for protein is greater than that of the female.

We have recently observed the growth of females on a diet containing 27.9% Labco casein (N × 6.24 = 24%), otherwise similar to that of McIntire. It was intended for both males and females, but male experiments have not yet been completed. The data (see Fig. 1) indicate that females grow optimally on a diet of 26.7% Labco casein (N × 6.25 = 24%), 67.7% cerelose, 3.6% modified Wesson salts with phosphorus adjusted for casein level, 0.1% carotene in oil, 0.25% wheat germ oil, 1.65% Wesson oil, and the following supplements fed daily: 40 γ thiamine hydrochloride, 50 γ pyridoxine hydrochloride, 80 γ riboflavin, 200 γ calcium pantothenate, 1 mg nicotinic acid, and 10 mg choline hydrochloride. This result fits in very nicely with our assumptions. In terms of McIntire's assumptions, it must be concluded that females do not require the postulated new factor.

It is also seen in Fig. 1 that in male rats on crude casein diets the relation between the growth curve for 20.9% casein and optimal growth (which is achieved on 27.9% casein) is of the same form as that for McIntire's male rats without and with liver. It is also of the same form as the growth of our females on inadequate (N × 6.25 = 15% and below) and adequate protein levels. This demonstrates two other facts. The factor under consideration, if it is not one or more amino acids contained in casein, must be present as an impurity to about the same extent in 7 g crude casein as in 2 g liver extract. Furthermore such a factor has an important nutritional property in common with protein, namely, that the fractional growth deficit decreases as time goes on. McIntire's deficient rats after 3 weeks on experiment have 73% of the weight of those growing optimally with liver supplement. The deficit decreases until after 6 weeks on experiment they show 94% of the weight of the controls. For a discussion of protein and spontaneous realimentation see Zucker *et al.*[5,7]

McIntire *et al.* have shown that the factor is present in yeast in only moderate amounts. It can be only minimally present in rice bran extract, since in our 20.9% crude casein diet on which the growth deficit for males appears, rice bran extract is included in addition to yeast extract. Further, on diets made up of oil seed meals and white flour which support optimal growth in females but result in the same form of male growth deficit already described, the addition of rice bran extract has no effect on the growth, while the addition of fibrin which has been extracted with hot alcohol or of cottonseed flour, restores the male growth to optimal.[2] Therefore the factor is present in cottonseed flour and in fibrin which has been extracted with hot alcohol.

Schneider and Steenbock[8] fed 18% of

[4] Irwin, M. R., and Kempster, H. L., *Mo. Agr. Exp. Sta. Bull.* No. 441, 1942, 3.

[5] Zucker, T. F., Hall, L., Young, M., and Zucker, L., *J. Nutrition*, 1941, **22**, 123.

[6] Zucker, L., and Zucker, T. F., *J. Gen. Physiol.*, 1942, **25**, 445, see esp. graph p. 452.

[7] Zucker, L., Hall, L., Young, M., and Zucker, T. F., *Growth*, 1941, **5**, 399, see esp. p. 409.

[8] Schneider, H. H., and Steenbock, H., *J. Biol. Chem.*, 1939, **128**, 159.

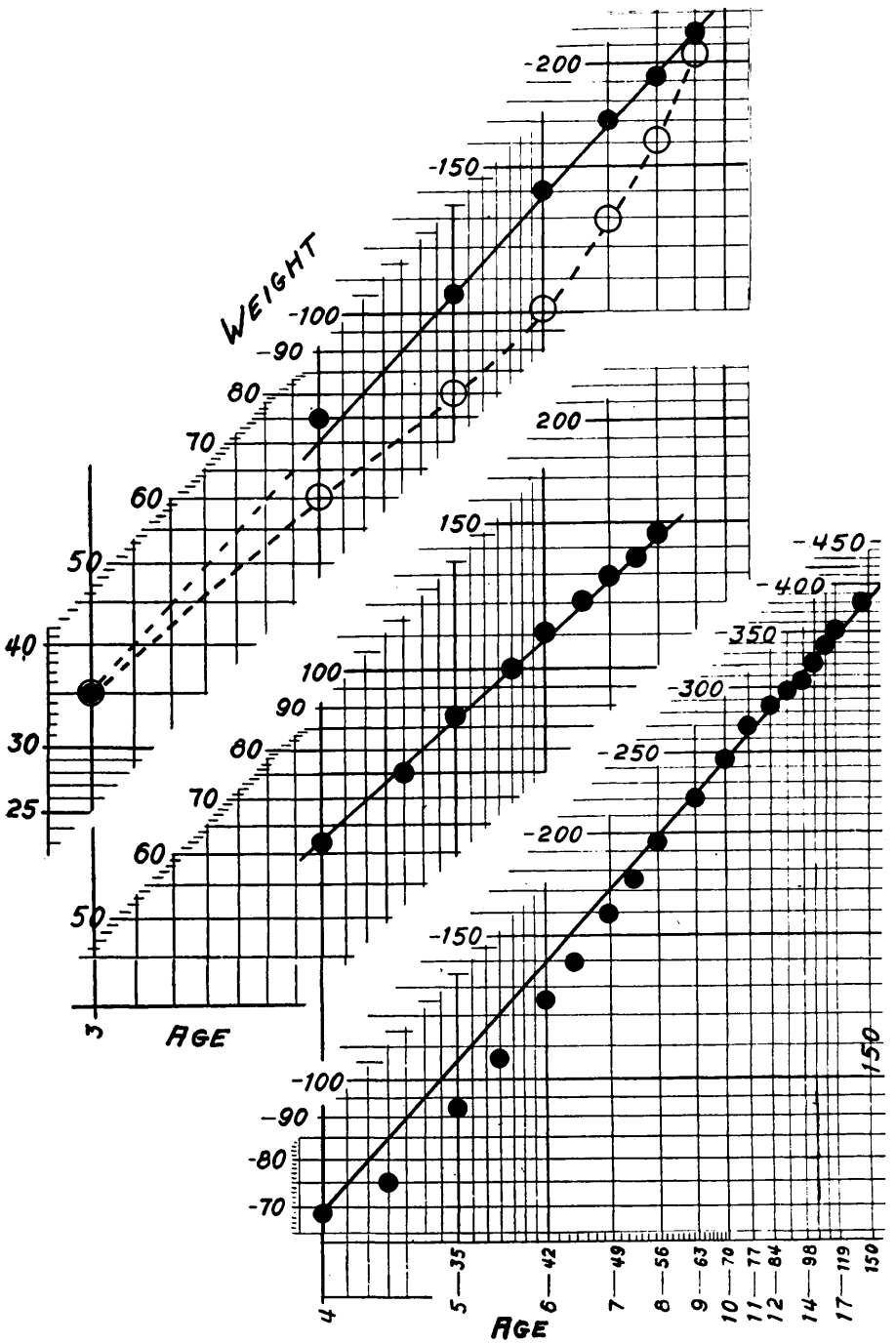

FIG. 1.

Optimal postweaning growth of rats gives a straight line when log weight is plotted against the reciprocal of age.[5,6] The heavy straight lines represent the slopes for male and female growth. The top section shows the McIntire male growth data from 3 weeks on without liver (open circles) and with liver (filled circles). The growth curve with liver agrees with the line for optimal growth. The middle section shows the growth of our females from 4 weeks on, together with the female growth standard. The diet is comparable to McIntire's diet without liver. Females do not appear to need any unknown factor from liver. The bottom

section shows the growth of our males on a suboptimal level of crude casein compared with optimal growth. The typical form of protein (amino acid) deficiency curve, *i.e.*, immediate reduction in growth rate with smooth return towards normal, is common to both top and bottom sections, but the difference in starting age and the difference in the exact protein levels makes the extent of deviation of the experimental growth from the control not strictly comparable.

heated egg albumen. The B factors were derived from rice bran extract and the egg albumen. Their male growth gives an excellent fit to the same line as shown for McIntire's data with 18% casein plus liver extract. Since rice bran extract does not contain the factor in question, egg albumen must either contain more of it than casein, or casein has a less favorable amino acid composition than egg albumen. Both feeding trials and amino acid composition (especially as regards the sulfur amino acids) point to a superiority of egg albumen over casein.

We have thus outlined an experimental basis for judging protein requirement for optimal growth in male and female rats. Experiments now in progress reveal that for 4 weeks there is no significant difference between two groups of male rats on diets with 30% Labco casein (otherwise identical with that of McIntire *et al.*), one with and the other without a supplement of 2% of liver extract. Judging both by our experience and McIntire's results, any existing difference should have reached its maximum by this time. The results will be published in detail when comparable experiments dealing with amino acid supplements to 18% casein have been completed.

Summary. It has been reported by McIntire *et al.* that a diet containing 18% purified casein and 6 pure B factors does not support optimal growth in male rats, and that the missing factor is present in liver extract. Evidence is presented to show that the factor, whatever it is, may not easily be found in the usual concentrated sources of B complex, but rather that it is associated, whether as an impurity or an essential amino acid, with protein of good quality.

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Effect of Massive Doses of Thiamine on Fertility and Lactation in the Albino Mouse.*

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The experiments of Perla[1] showed that feeding rats with large amounts of thiamine resulted after one generation in interference with lactation, loss of maternal instinct, cannibalism, and progressive loss of fertility. Continuing this study, Perla and Sandberg[2] found that these symptoms were prevented by giving manganese chloride daily to the animals. If the diet was supplemented with manganese chloride alone, interference with lactation resulted, particularly marked after one generation. The observations of Perla were confirmed by Sure.[3] In contrast to the findings of Perla, and of Sure, Williams and Spies[4] reported normal reproduction in rats kept on stock diets supplemented with large amounts of thiamine through 3 generations. The

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1 Perla, D., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 169.

2 Perla, D., and Sandberg, M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 522.

3 Sure, B., *J. Nutrition*, 1939, **18**, 187.

4 Williams, R. R., and Spies, T. D., *Vitamin B_1 and Its Use in Medicine*, Macmillan Co., New York, 1938, p. 286.