trol ration was fed with and without ascorbic acid to 2 groups of 3 rats (21 days old) both groups grew equally well for a 5-week period and appeared normal in all respects.

Until we know the mechanism of action of ascorbic acid and other compounds that stimulate growth when fed to chicks receiving such rations as used in these experiments they should be regarded as growth stimulants and not necessarily as specific growth factors. Further studies on these compounds and their mode of action are in progress at our laboratory.

*Summary.* Ascorbic acid, when fed to chicks receiving various *purified* rations consistently stimulates the growth rate to a small but definite extent. The exact mechanism of action is unknown at present. The relationship of growth effects obtained with ascorbic acid to those obtained with *p*-aminobenzoic acid is compared and discussed.

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## A Cross-Protective Reaction Between Moccasin Venom and the Endotoxin of *Salmonella typhimurium*.

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*Introduction.* We have reported elsewhere[1,2] that mice immunized against the endotoxin of *Salmonella typhimurium* are to a limited extent protected against both lethal and tumor-hemorrhage doses of the endotoxin of *Rhodospirillum rubrum* and *Shigella paradysenteriæ* Flexner, and that a similar cross-protection in respect to the other two organisms is found when the immunization is performed with *Rhodospirillum* or *Shigella*. Further, a characteristic of the endotoxin of gram-negative bacteria generally has been shown to be the ability to induce hemorrhage in transplanted mouse tumors.[3,4] Analogous hemorrhage-induction has been described for venom of the water-moccasin.[5]

The present study was undertaken to determine whether a cross-protection occurs in mice immunized with the endotoxin of a gram-negative bacterium and mice immunized with the ostensibly unrelated hemorrhage-inducing snake venom.

As a representative gram-negative organism, *Salmonella typhimurium* was selected for the source of endotoxin to be compared with the lyophilized venom of the moccasin *Agkistrodon piscivorus*.* The antigenicity of this venom is recognized.[6,7,8]

*Experimental.* For 10 days several groups of white Rockland male mice were given daily sublethal intraperitoneal injections (about 0.5 $LD_{50}$) of an aqueous dispersion of the venom. At the end of this period the animals had developed sufficient immunity to survive about 2 $LD_{50}$ amounts of venom. Other groups of mice were immunized with the endotoxin of *Salmonella*. The preparation of this bacterial endotoxin and the dosage schedule employed for the immunization are described elsewhere.[2]

1 Zahl, Paul A., Hutner, S. H., and Cooper, F. S., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 187.

2 Zahl, Paul A., and Hutner, S. H., *Am. J. Hyg.*, in press.

3 Zahl, Paul A., Hutner, S. H., Spitz, S., Sugiura, K., and Cooper, F. S., *Am. J. Hyg.*, 1942, **36**, 224.

4 Hutner, S. H., and Zahl, Paul A., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 364.

5 Shimkin, M. B., and Zon, L., *J. Nat. Canc. Inst.*, 1943, **3**, 379.

* Purchased from the Ross Allen Reptile Institute, Ocala, Florida.

6 Githens, T. S., and Wolff, N., *J. Immunol.*, 1939, **37**, 33; 1939, **37**, 47; 1941, **42**, 149.

7 Peck, S. M., and Sobotka, H., *J. Exp. Med.*, 1931, **54**, 407; Peck, S. M., *J. Immunol.*, 1933, **25**, 447; 1934, **27**, 89.

8 Taylor, J., and Mallick, S. M. K., *Indian J. Med. Res.*, 1936, **24**, 272.

TABLE I.

Survival at the end of 48 hours of normal and immunized mice following the intraperitoneal injection of various dosages of salmonella endotoxin-containing material and moccasin venom. Numerator = No. of animals surviving. Denominator = total No. of animals used. In each instance the % survival is indicated.

| Condition of mice | Amt of salmonella material inj. | | Amount of venom injected | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 4 mg | 8 mg | .1 mg | .15 mg | .2 mg | .25 mg | .3 mg | .35 mg |
| Normal | 7/22 (31%) | 1/24 (4%) | 7/12 (58%) | 4/18 (22%) | 0/15 (0%) | 0/13 (0%) | 0/14 (0%) | |
| Salmonella-immunized | 18/18 (100%) | 23/26 (88%) | | 20/24 (83%) | 12/17 (70%) | 7/16 (43%) | 5/22 (23%) | 0/10 (0%) |
| Venom-immunized | 16/23 (70%) | 9/17 (53%) | | | 14/19 (73%) | 18/24 (75%) | 6/17 (35%) | 0/10 (0%) |

The degree of cross-protection was determined by observing survival following injection of varying amounts of venom into mice previously immunized against *Salmonella typhimurium*. Inversely, salmonella endotoxin was similarly injected into the venom-immunized mice.

*Results and Discussion.* The data listed in Table I indicate that: (1) venom-immunized mice were protected against about 4 $LD_{50}$ of the salmonella endotoxin, and (2) salmonella-immunized mice were protected against about 2 $LD_{50}$ of venom.

Following immunization with the venom, only a limited degree of homologous immunity was developed (Table I). On the other hand, it has been repeatedly demonstrated that injection of the endotoxins of many gram-negative bacteria may confer a resistance to as much as 10-20 $LD_{50}$ of the homologous endotoxin. It may be assumed either that the venom is a poor antigen or that a large proportion of the toxicity of the venom resides in components other than the hemorrhagic. Support of the latter view is furnished by Shimkin and Zon[5] who found it necessary to inject as much as one-half the lethal dose of moccasin venom in order to induce hemorrhage in transplanted tumors. Taylor and Mallick,[8] working with a number of snake venoms, including that of *Agkistrodon piscivorus*, found that while the hemorrhage component of these venoms was intergenerically protective, the neurotoxic components were species-specific. Githens and Wolff[6] also reported a species-specificity of the neurotoxin component and a relative non-specificity for the local necrotic (hemorrhagic) factor in snake venoms, including that of *Agkistrodon*. The proteolytic factor of the venom was not neutralized by antivenin.

The existence of an antigenic relation between moccasin venom and salmonella endotoxin could perhaps have been surmised from the finding of Peck[7] that rabbits injected with the venom of *Agkistrodon piscivorus* became refractory to preparations of gram-negative bacteria which otherwise would have elicited the Sanarelli-Shwartzman response.

A unique property of the free protein por-

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.