mond,[1] Osborne and Mendel,[2] and McCollum[3] and their associates have largely been confined to these phases of study. Comparatively little has been done on reproduction.

The diets employed by us consisted of casein from 18 to 79.3%, salt mixture (185) 3.7%, wheat embryo 12%, cod liver oil (Squibb) daily 5%, and the remainder of the ration was composed of dextrin to 100%. The following levels of casein were studied: 18, 25, 30, 40, 50, 60, 75.3 and 79.3%. Growth was normal on levels of 18 to and including 60% casein but below normal on higher levels. Reproduction was good on all levels of casein studied, but was delayed on the levels above 60%. No young were weaned on the 79.3% level. Young were weaned on the lower levels and most successfully on the 18% level of casein. The young weaned on the 18 and 25% levels were normal in weight but on higher levels of casein growth of the young was stunted.

The experiments employed 7 animals on each lot, 3 males and 4 females. In other words there were 7 animals on each level of protein.

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Effect of Toxins and Venoms upon Protozoa.

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Since the completion of a previous study on the effect of pathogenic bacteria and bacterial toxins upon paramecia,[1] an investigation has been in progress on the effect of snake venoms and of toxins (tetanus, ricin and botulinus) upon protozoa. The recent appearance of Tunnicliff's related study with other toxins,[2] and particularly her consideration of the use of paramecia for determining the strength of antitoxins, has made advisable the recording of a pre-

[1] Drummond, J. C., Crowden, G. P., and Hill, E. L. G., *J. Physiol.*, 1922, lxvi, 413. Reader, V., and Drummond, J. C., *Biochem. J.*, 1926, xx, 1256.

[2] Osborne, T. B., Mendel, L. B., Park, E. A., and Winternitz, M. C., *J. Biol. Chem.*, 1926-27, lxxi, 317.

[3] Polvogt, L. M., McCollum, E. V., and Simmonds, Nina, *Johns Hopkins Bull.*, 1923, xxxiv, 168.

[1] Philpott, C. H., *J. Morph. and Physiol.*, 1928, xlvi, 85.

[2] Tunnicliff, Ruth, PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 213.

liminary report of the present investigation in so far as it is related to her findings.

In the study of the effect of tetanus and botulinus toxins, of ricin and of snake venoms upon protozoa, only the venoms have definitely given positive results. *Crotalus atrox* (Texas rattlesnake) venom,* in dilutions containing 0.00025 gm. per cc, had an immediate lethal effect upon *Paramecium caudatum, Stentor coeruleus, Bursaria truncatella* and *Frontonia leucas*. With *Volvox spermatosphara* and *Oxytrichia fallax* the venom was lethal in effect but very slow in action. The effect on *Chilomonas paramecium* was slight and only temporary while *Coleps hirtus, Podophyra fixa*, and *Dileptus gigas* were not affected.

A race of *Paramecium caudatum* has thus far lent itself well to and has given consistent results in measurements of the potency of snake venoms. The M.L.D. of *Crotalus atrox* venom for the race of *Paramecium caudatum*, in this case the least amount in one cubic centimeter of medium required to kill an animal in 24 hours, was 0.00002 gm. This was one twentieth of the least amount required to kill a 20 gm. mouse. The M.L.D. of *Agkistrodon piscivorous* (Mocassin) venom for *P. caudatum* was 0.0000014 gm., while for *Bothrops atrox* (Fer de lance of Central America) it was 0.0000125 gm. It is evident that the agent in these venoms that is principally responsible for the death of paramecia is thermolabile. It is hoped that further fractionation may make possible the study of the effect of each of the several constituents of venom upon protozoa.

Antivenins neutralize the constituent in *Crotalus* venom that is lethal to paramecia. This makes possible the use of paramecia in the titration of antivenins. Titrations have been made and repeated with consistent results. Further study is being made to determine the reliability of paramecia in these titrations and to compare titers obtained with paramecia and with mice.

A certain degree of resistance to *Crotalus atrox* venom has been developed in races of *P. caudatum* by growing the animals from day to day in media containing increasing amounts of venom. The resistance developed in 17 days enabled animals to grow in the presence of 3 lethal doses of the venom. This resistance is specific for *Crotalus atrox* venom and does not protect from Mocassin or Bothrops venom. The resistance disappeared after the race grew for 13 days in venom-free media. Study is in progress to determine more definitely the mechanism of this resistance.

* The venoms, in desiccated form and the antivenins used in this study were supplied through the courtesy of the Antivenin Institute of America at Glenolden, Pa.