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Precipitin Tests with Glycogen from various Species of Animals.*

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Recent studies[1] indicate that polysaccharides from various parasitic helminths are immunologically active, and that their chemical properties are similar to ordinary preparations of glycogen. The present study was undertaken to ascertain whether precipitins could be produced against glycogen from livers of the guinea pig, chicken and frog, and from fresh water clams.

Preparations of glycogen were obtained as follows: finely ground tissue was suspended in 3 volumes of hot distilled water and kept in a boiling-water bath for 30 minutes. The solution, after the water-insoluble material had been discarded, was acidified to approximately pH 5, and left for several hours at room-temperature in 2 volumes of 95% ethyl alcohol. The precipitate which formed was resuspended in 1.0% acetic acid, and after 0.2 volume of 95% alcohol had been added, was chilled for several hours in the refrigerator. All insoluble material was then discarded, and the soluble glycogen precipitated with one volume of alcohol and rapidly dried with absolute alcohol and ether. Such preparations were essentially free of nitrogen and gave no protein reactions.

Rabbits were immunized by 10 intraäbdominal injections of 0.1 gm. of finely ground fresh material of one of the following: guinea pig, chicken or frog livers or fresh water clams. The "ring" method of precipitin-test was used. Serums were diluted 1:2 with 0.9% NaCl and antigens tested in a series of dilutions from 1:100 to 1:51,200.

The rabbit antiserums did not react with glycogen from guinea pig, chicken or frog livers, but anticlam serums did contain precipitin against the glycogen from fresh water clams (titre as dilution of antigen, 1:12,800). There were no cross-reactions between clam-antigens and antigens from various parasitic helminths. Liver-glycogen, therefore, appears to be either immunologically inactive or else the glycogens present in the 4 vertebrates studied (guinea pig, chicken, frog and rabbit) are antigenically identical, whereas glycogens obtained from clams and helminths are immunologically active and specific.

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[1] Campbell, D. H., *J. Infect. Dis.*, 1936, **59**, 266, and *J. Parasitol.*, (in press).

These results are in part contradictory to those obtained by Ikeda,[2] who found that glycogen from mammalian tissues induced organ-specific antibodies when injected into rabbits.

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Rôle of Thyroid in Increased Protein Metabolism of Phlorhizin Diabetes.*

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The mechanism of phlorhizin diabetes has been the subject of much investigation and controversy. Two distinct views exist, one postulating that the action of phlorhizin is exclusively renal[1, 2] and the other that some primary extrarenal factor also is involved.[3, 4] These views arise from the fact that although no apparent effect can be observed when phlorhizin is administered to dogs without renal function,[2] a definite hyperglycemia,[3] and abnormalities in the glucose tolerance curve[4] may occur in such animals if the phlorhizination is completed before the kidneys are incapacitated.

The observations of Dann, Chambers and Lusk[5] indicate the involvement of extrarenal factors in the genesis of phlorhizin diabetes. They demonstrated that the administration of phlorhizin to the thyroidectomized dog does not result in the increased basal metabolic rate and the increased nitrogen excretion which occur in the intact phlorhizinized dog. During the course of a series of studies on nitrogen metabolism, we had occasion to make some observations which confirm and extend those of Dann, Chambers, and Lusk.

Three groups of animals were studied. The first group consisted of normal dogs which had been fasted for 3 to 4 days; the second, of normal dogs which had received a daily subcutaneous injection

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[2] Ikeda, G., *Jap. J. Exp. Med. Sci. Trans.*, 1932, **7**, 231.

* Aided by the David May Fund.

[1] Minkowski, O., *Arch. f. exp. Path. u. Pharm.*, 1892, **31**, 85.

[2] Deuel, H. J., Jr., Wilson, H. E. C., and Milhorat, A. T., *J. Biol. Chem.*, 1927, **74**, 265.

[3] Underhill, F. P., *J. Biol. Chem.*, 1912, **13**, 15.

[4] Goldstein, L. A., Tatelbaum, A. J., Ehre, S., and Murlin, J. R., *Am. J. Physiol.*, 1932, **101**, 166.

[5] Dann, M., Chambers, W. H., and Lusk, G., *J. Biol. Chem.*, 1931, **94**, 511.