

sistent with the view that *Maetra* glycogen consists entirely of D-glucose polymerized into chains by glucoside bonds.

Table 1. ANALYSES OF GLYCOGEN FROM *M. fragilis*

	Muscle	Viscera
Yield (gm. per 100 gm. wet tissue)	2.4	2.0
Ash (per cent)	3.1	1.7
Reducing substances (as diglucoside) (per cent)	94.8	96.9
Glucose by glucose oxidase (as diglucoside) (per cent)	108.5	93.3
Average chain-length	13.3	7.3

The result of the average chain-length determination on muscle glycogen is similar to that obtained by Fales² with oyster glycogen, and is in the range reported by other investigators who used somewhat different methods of periodate oxidation on glycogen from a variety of species^{1,4}. The viscera glycogen of this animal has a considerably shorter average chain-length. The observations do not provide any information about the location in the glucose units of the glucoside bonds, but it may be assumed that they are the customary α -1,4-glycoside bonds with α -1,6-bonds at the branch points.

It is evident that more data on the structure of glycogens from a variety of species must be assembled before any generalizations can be made. Particular emphasis should probably be placed on the examination of material from the most primitive forms available.

These investigations were supported in part by grants from the Association for the Aid to Crippled Children, the Charles A. and Marjorie King Fund, and the National Institutes of Health, U.S. Public Health Service.

DWAIN D. HAGERMAN

Department of Biological Chemistry,
Harvard Medical School,
Research Laboratories of the
Boston Lying-in Hospital,
Boston, Mass., and
Marine Biological Laboratory,
Woods Hole, Mass.

¹ Manners, D. J., *Adv. Carbohydrate Chem.*, **12**, 261 (1957).

² Fales, F. W., *Anal. Chem.*, **31**, 1898 (1959).

³ Baldwin, E., and Bell, D. J., *Biochem. J.*, **34**, 139 (1940).

⁴ Liddle, A. M., and Manners, D. J., *J. Chem. Soc.*, 3432 (1957).

Absence of Chitinase in *Limnoria*

THE question of the presence or absence of a chitinase in *Limnoria* is of interest for two reasons. First, if a chitinase were present, chitin could serve as a principal nitrogen source. Ray and Julian¹ and Ray² have shown that *Limnoria*, which eats wood, can break down cellulose to glucose, and can therefore utilize wood as a source of energy. However, it is not yet known what source of nitrogen *Limnoria* utilizes because wood contains so little. One possible nitrogen source is chitin, present in cell walls of fungi growing in wood and often eaten with the wood³, and present in the animal's exuviae, which it may eat.

This question is also of interest for another reason. Since both cellulose and chitin are B-glucosides, it is possible that the same enzyme could hydrolyse both; that is, the cellulase found by Ray in *Limnoria* could be a general B-glucosidase capable of splitting chitin also.

In an attempt to answer this question, 20, 30, 40 and 60 mgm. of powdered chitin prepared from *Loligo* pens

were incubated at 25° and 30° C. for 2–12 days with 0.2 ml. of *Limnoria* enzyme solution and 0–1.0 ml. of McIlvaine's buffer, pH 5.6. The enzyme solution was prepared by the method described by Ray⁴. The midgut diverticula of approximately 60 animals were used to make each 0.2 ml. of enzyme solution. At the end of the incubation periods the solutions were tested by paper partition chromatography to determine whether acetylglucosamine or any other reducing sugar had been obtained from the chitin. The tests were all negative, although control solutions of carboxymethyl-cellulose plus enzyme incubated at the same times under the same conditions did contain reducing sugars (mostly glucose) at the end of the incubation periods.

In another experiment, suspensions of chitosan, suspensions of partially hydrolysed chitin, and solutions of carboxymethyl-cellulose were incubated at 30° C. for 7 days with 0.2 ml. of enzyme solution. Each tube contained 20 mgm. of substrate suspended or dissolved in 1 ml. of McIlvaine's buffer at pH 5.6. The chitosan was prepared from *Loligo* chitin by treating with hot saturated potassium hydroxide, washing, dissolving in acetic acid, and dialysing against buffer. At the end of the incubation period the solutions were treated as above. No reducing sugar was found, although the control solution of carboxymethyl-cellulose plus enzyme did contain reducing sugar.

Apparently the cellulase present in the midgut diverticula of *Limnoria* is not a general B-glucosidase capable of hydrolysing chitin. It is still possible that *Limnoria* produces a chitinase inactive under the conditions tested or that some other portion of the gut produces a chitinase. However, this seems unlikely. Probably, *Limnoria* is not able to utilize chitin as a source of nitrogen.

This work was carried out under contract with the Office of Naval Research and in co-operation with Dr. D. L. Ray.

J. ROSS STEVENSON

Department of Biology,
Kent State University,
Kent, Ohio.

¹ Ray, D. L., and Julian, J. R., *Nature*, **169**, 32 (1952).

² Ray, D. L., *Amer. Wood Pres. Assoc., Proc.*, **54**, 120 (1958).

³ Becker, G., *Proc. Friday Harbor Symp.*, **62** (1959).

⁴ Ray, D. L., *Proc. Friday Harbor Symp.*, **372** (1959).

Comparative Chemistry and the Relationship of the Hamamelidaceae

IN many of the more recent systems of classification, the Hamamelidaceae have been given a central position, several authors believing that they have been derived from rosalian or magnolialian ancestors, and that they, in turn, have given rise to some of the amentiferous families. Although the family is usually included in the Rosales, a number of systematists have assigned it to the order Hamamelidales.

In investigating the relationships of the Hamamelidaceae and those families which have been included in an order Hamamelidales, as well as those of the Rosaceae and some of the amentiferous families, chemical characters have been used.

The chemical characters of the Hamamelidaceae, based on testing twenty-five species representing twelve genera, are the following¹: (a) they are positive to the hydrochloric acid methanol test; (b) they are positive to the leuco-anthocyanin test;