Oral administration of 2 gm. magnesium chloride 4 times per day for 3 months to 10 patients did not lessen the frequency of attacks, nor did 2 gm. potassium chloride 3 times a day for 3 months to 14 other patients increase the frequency.

8242 P

Cellulase from the Slug, Limax flavus Linnaeus.

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Seillière[1] and Billard[2] and others demonstrated the presence of cellulase in *Helix pomatia* by estimating the amount of glucose produced after action of the cellulase upon suitable substrates. Boynton and Miller[3] showed saccharification of wood by extracts from the ship-worm, *Bankia setacea.* Trager[4] found a cellulase from symbiotic intestinal flagellates of termites. He estimated the amount of glucose produced, both qualitatively (by means of Benedict's reagent) and quantitatively, after action of the enzyme upon filter paper which was dissolved and precipitated.

We also made use of the fact that reducing substances (carbohydrates) were produced, as proof for the presence of slug cellulase. Two substrates were employed: (1) A finely divided suspension of cellulose was prepared by dissolving 1 gm. of Whatman's No. 40 filter paper in 100 cc. of Cross and Bevan's $ZnCl_2$-HCl reagent. This solution was filtered by suction through cotton and poured into about 800 cc. of distilled water. The resulting precipitate was suspended in 400 cc. of distilled water and centrifugalized. This washing process was repeated ten times and the precipitate was finally allowed to settle out. (2) The outer coverings of date seeds were removed and discarded. Filings from the inner, pearly-white endosperm were put through a 100-mesh sieve and were either suspended in distilled water or weighed in the dry state for use as a substrate.

1 Seillière, G., *Compt. Rend. Soc. de Biol.*, 1910, **68**, 107.

2 Billard, G., *Compt. Rend. Soc. de Biol.*, 1914, **76**, 566.

3 Boynton, L. C., and Miller, R. C., *J. Biol. Chem.*, 1927, **75**, 613.

4 Trager, W., *Biochem. J.*, 1932, **26**, 1762.

The slugs were killed with ether. Fifty animals were used. The gastrointestinal tracts and livers were dissected away from the bodies. Three different preparations were studied for cellulase activity: (1) The contents of the stomachs and anterior portions of the intestines. (2) The gut tracts, including the stomachs and intestines. (3) The livers. The second and third preparations were ground with 40-mesh sea sand and extracted with water or 0.7% NaCl for 72 hours. Cellophane was used as a membrane for dialyzing the gastrointestinal contents and extracts of the livers, to remove reducing substances that were originally present. Extracts of the gut tracts showed no reduction with Benedict's qualitative reagent.

The total volume of each reaction mixture was 3 cc. and consisted of 1 cc. of the solution to be tested for cellulase activity, 1 cc. of cellulose suspension or a weighed amount of cellulose and, either 1 or 2 cc. of buffer solution or water.

Both the fluid present in the gastrointestinal tracts and physiological saline (0.7% NaCl) or aqueous extracts of the livers gave positive reactions with Benedict's qualitative reagent after 2 to 8 hours, depending upon the concentration of the enzyme solution and upon the substrate used. With the precipitated filter paper, action did not proceed as rapidly as it did with the date endosperm cellulose. All reaction mixtures were kept in a dark place at room temperature and covered with toluene. No action was demonstrated by extracts from the walls of the stomachs and intestines.

Preliminary experiments indicated that the optimum pH for this cellulase is about pH 5.0 with acetate buffers.

Reaction mixtures containing 10 mg. of date endosperm in 3 cc. of solution (1 cc. of saline extract of liver, 1 cc. of acetate buffer pH 5.093 and 1 cc. of 0.7% NaCl) were run on 3 different liver preparations. The amount of sugar was determined by the Folin and Wu method; the reaction mixtures being treated the same as protein-free filtrates. Table I gives the per cent of sugar in the mixtures after 24, 48 and 72 hours.

TABLE I.

No. of Preparation	% sugar 24 hr.	% sugar 48 hr.	% sugar 72 hr.
1	.112	.143	.130
2	.070	.091	.110
3	.065	.082	.110

The extract used in preparation No. 3 was also allowed to act upon 20, 30, and 40 mg. quantities of substrate; the other constit-

uents of the mixtures being the same as described above. The results are expressed in Table II.

TABLE II.

Amount of substrate	% sugar 24 hr.	% sugar 72 hr.
20 mg.	.135	.144
30 mg.	.202	.234
40 mg.	.270	.340

8243

Protective Action of Concentrated Antityphus Serum (Murine Type) Against European Typhus Virus.

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Two of the writers[1] have described a method of producing concentrated suspensions of Rickettsiae from peritoneal washings of X-ray radiated rats infected with typhus virus of the murine (Mexican-American) type. Such vaccines have since been used for human vaccination and for the production of antityphus serum by horse immunization. The reactions in the horse, the Weil-Felix reactions and the prophylactic and experimental therapeutic action of the immune horse sera have been elsewhere described.[2] Therapeutic test in man, which is giving encouraging results in Mexico, was justified by preliminary experiments in guinea pigs. In these experiments, moderate doses of the serum appeared to confer complete protection against the murine virus upon guinea pigs infected after, together with, or even 3 or 4 days before the administration of the serum. In the case of similar protection experiments in which the classical European virus was employed,[3] protection was not absolute, but sufficiently definite to encourage further efforts. Our observations of the close antigenic overlapping of the 2 principal varieties of human typhus Rickettsiae led us to believe that our difficulties, in regard to protecting against the European "humanized"

1 Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1930, **52**, 649; and PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 840.

2 Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1933, **57**, 391.

3 Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1934, **59**, 471.