higher plants and fungi respectively. Laboratory methods for the purification of each of these amylases have been described in previous papers. The present experiments were performed with enzyme preparations which had been purified in accordance with these methods. The experiments establish for each of the three amylases the limits of hydrogen ion concentration within which any enzymic activity is shown, and the form of the curve representing the activities at all concentrations of hydrogen ion between these limits. The investigation was carried out with the aid of a grant from the Carnegie Institution of Washington.

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Studies on the amylolytic activity of human saliva with a new method.

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Saliva is the one glandular secretion which can be readily obtained in the human subject under relatively constant conditions, and its amylolytic activity has therefore been a time-honored topic of investigation. Nevertheless the methods used for estimating this activity have been rather crude or tedious. A method is described below which is simple, delicate, and, we believe, very accurate. With it a large series of comparable figures may readily be obtained. The method is similar to that which has been employed here in estimating the diastatic activity of the blood.[1]

The technique is as follows: A specimen of mixed saliva, obtained by the stimulation of paraffin chewing, is filtered and a small portion accurately diluted 1 to 100 with distilled water, and also another portion with 0.3 per cent. sodium chloride as an activating solution. After thorough mixing 1 c.c. of the diluted saliva is pipetted into a test-tube and the tube placed in a water bath at 40°. After 5 minutes 1 c.c. of 1 per cent. soluble starch solution is added and the mixture allowed to incubate for 30 minutes. At the end of this time 3 c.c. of saturated picric solution and 1 c.c.

[1] Myers and Killian, *Jour. Biol. Chem.*, 1917, xxix., 179.

of 20 per cent. sodium carbonate are added and the tube placed in boiling water for 15 to 20 minutes. It is then allowed to cool and diluted with distilled water in an accurately graduated cylinder until the intensity of the color approximates that of the standard (glucose in picric acid treated with sodium carbonate and heated), after which it is compared with the standard in the colorimeter. After correcting for the reducing power of the soluble starch, the activity is recorded in terms of the percentage of starch converted to reducing sugar.

Utilizing the principles outlined above it has been found possible to obtain a demonstrable amylolytic activity at a dilution of 1 to 400 when water was used as the diluent, and at 1 to 2,000 when dilution was made with 0.3 per cent. sodium chloride. For purposes of comparison a dilution of 1 to 100 (actually 1 to 200 allowing for the starch solution) appears to be the most suitable, with distilled water as the diluent. Although 0.3 per cent. sodium chloride is an excellent activator and represents the approximate content of sodium chloride in saliva, nevertheless with the handicap of a low chloride content, it appears possible to bring out greater individual variations than is possible otherwise. It may be noted that, with the dilutions employed, the variations do not appear to be influenced by the native content of sodium chloride in the saliva.

The method has been applied to a considerable number of normal individuals, the activity in the majority of cases falling between 30 and 45 when water was used as the diluent. With sodium chloride the variations were small, most of the figures falling between 46 and 50, suggesting the possibility that in some individuals a considerable part of the ferment is secreted in the zymogen form. Figures obtained on the same individuals at the same time of day agree very closely. The activity has been tested on representatives of a number of different nationalities and found to vary within essentially the same range as above. The same was true of a number of pathological cases including such conditions as diabetes, nephritis, gastric ulcer, etc. Several individuals were encountered, however, who for periods showed low activities, figures 10 to 20. Some of these had persistently suffered from gastric distress, others were suffering from acute in-

fections. An entirely satisfactory explanation for these low values, however, is not apparent. This is a topic to which we plan to give further study.

With the method it is possible to demonstrate a considerable decline in the activity of the saliva as a result of the glandular fatigue produced by the continuous secretion of saliva during paraffin chewing. Our results likewise indicate that the method is a very suitable one to employ in demonstrating the diurnal variation.

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Parathyroids and calcium metabolism.

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Larvæ of the salamanders *Amblystoma maculatum*, *Amblystoma opacum* and *Eurycea bislineata* when fed on thymus exhibit severe tetanic convulsions. In contradiction to other amphibian larvæ which do not show this reaction, they possess no parathyroids; the tetanic convulsions of the thymus-fed salamanders are identical with a true parathyreoprival tetany.

The tetanic action of the thymus is due to the presence in the organ of the tetany toxin which can be antagonized only by the parathyroids, but not by the substances contained in normal food. The addition to the thymus diet of normal diet in amounts sufficiently large to keep a salamander larva in a perfectly normal condition does not prevent the tetanic convulsions. Tetany is caused by a toxic substance contained and excreted by the thymus.

Calcium salts, when dissolved in the water in which the thymus-fed larvæ live, do suppress, to some extent, the tetanic convulsions. Magnesium salts, however, assert the same action and are still more effective than calcium salts. The suppression of the tetanic convulsions by the salts of calcium is not a specific action of the calcium.

Neither calcium nor magnesium is able to prevent the development in the thymus-fed larvæ of severe and permanent lesions of the muscular system, lesions which are caused probably by