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13 May 1957

Amylase in Electrophoretic and Ultracentrifugal Patterns of Human Parotid Saliva

The action of human saliva on starch was reported for the first time by Leuchs in 1831, but the active material, an α-amylase, was not crystallized from saliva until 1948, by Kurt Meyer and associates (*1*). The crystallization was confirmed in 1953 by Muus (*2*), who reported electrophoretic and ultracentrifugal analyses of her crystalline preparation. Kinersly (*3*) demonstrated the presence of amylase on an electropherogram of whole saliva on paper but did not relate it to the three protein components which he observed.

The present work describes the location of amylase in ultracentrifugal and electrophoretic patterns of the secretion of the human parotid gland. It is a part of a more extensive investigation of the composition of human parotid and submaxillary gland secretions by electrophoretic and ultracentrifugal methods (*4*). The parotid gland secretion was found (*4*) to contain a maximum of 12 electrophoretically separable components and three or four ultracentrifugally separable components. The electrophoretic components have been numbered, with the most positively charged at *p*H 8.6 as component 1 (*4*).

The parotid gland secretion, stimulated by chewing paraffin, was collected by means of a parotid cup (*5*) similar to that described originally by Carlson and Crittenden and by Lashley. The tubes from the cup emptied directly into receivers supported in an ice bath. The saliva was concentrated to approximately one-fourth of its original volume by dialysis overnight at 4°C, against a 25 percent solution of polyvinylpyrrolidone. It was then dialyzed in the cold for from 65 to 70 hours against the buffer to be used for electrophoresis. The Miller-Golder buffer (*6*) of 0.1 ionic strength and *p*H 8.5 was employed. It contained 0.02*M* Veronal and 0.08*M* sodium chloride. After dialysis, the sample was centrifuged in the ordinary laboratory centrifuge at 2° to 4° for 20 minutes. Electrophoretic, ultracentrifugal, and amylase determinations were performed on the supernatant liquid.

Electrophoresis was performed in a 6-ml cell in the Perkin-Elmer model 38 Tiselius electrophoresis apparatus. Ultracentrifugal analyses were performed in the Spinco model E ultracentrifuge at about 26°C. Amylase activity was determined by the method of Myers, Free, and Rosinski, as adapted for work with saliva by Schneyer (*7*).

In order to locate the position of the amylase boundary in the electrophoretic pattern, both ascending and descending limbs of the cell were sampled at the time of maximum resolution. Fine glass capillaries were inserted into each limb to the center of each successive boundary, as seen by the Longsworth optical system. Five samples of about 0.1 ml were usually taken from each limb, beginning with the uppermost boundary. At the center of each boundary, the concentration of the component forming the boundary should be one-half of the original concentration. In succeeding samples taken from the same limb, the concentration of the first component should be greater than 50 percent of the initial concentration.

Amylase activity was found at the boundary of either component 6 or 7, in both ascending and descending limbs, in about 50 percent of the initial concentration. Since no amylase occurred in the descending limb in either components 4 or 5, all of the components which had appeared up to that point (1, 2, 3, 4, and 5) were eliminated. By similar reasoning, since no amylase appeared in component 8 in the ascending limb, all of the components which had appeared up to that point (12, 11, 10, 9, and 8) were eliminated. Component 6 had an average mobility (± standard deviation) of $-1.1 \pm 0.20 \times 10^{-5}$ cm²/volt-sec, whereas component 7 had an average mobility of $-1.7 \pm 0.05 \times 10^{-5}$ cm²/volt-sec. The closeness of the mobilities of these two components made further identification impossible, particularly since both components showed variation in individual samples and were not always distinguishable. The average mobility of the component containing the amylolytic activity was $-1.5 \pm 0.25 \times 10^{-5}$ cm²/volt-sec. in the Miller-Golder buffer of 0.1 ionic strength and *p*H 8.5. In some samples this activity was contained in component 6 and in others, in component 7. No estimate of the homogeneity of the component which contains the amylase is available. The amylase component is marked with vertical arrows on typical patterns in Fig. 1.

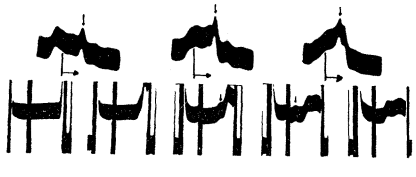

Fig. 1. (Upper patterns) Electrophoretic patterns of the descending limb of parotid gland secretion from three individuals. The horizontal arrows mark the starting boundary. Field strength, 4.4 v/cm; time, 192 minutes. (Lower patterns) Ultracentrifugal patterns of a parotid gland secretion at 0, 24, 48, 96, and 136 minutes. Centrifugal force, about 250,000*g*. The components with amylolytic activity are marked. The experiments were performed in the Miller-Golder, barbital-NaCl buffer, *p*H, 8.5; Γ/2, 0.1.

The crystalline human amylase studied by Muus (*2*) had a somewhat different mobility. From the relationship of mobility to *p*H, as given by Muus (*2*), the mobility of her component A, under the conditions used in this study, should be -2.3×10^{-5} cm²/volt-sec. This value is considerably higher than the mobility, -1.4×10^{-5} cm²/volt-sec, found in the present work for the amylase in the parotid gland secretion. Presumably this difference in mobility arises from the presence of other components in the parotid gland secretion.

A partition cell was used to help locate the ultracentrifugally separable component which contained the amylase. In the general investigation (*4*), parotid saliva was shown to contain two fast moving components and either one or two slowly moving ones. In alternate experiments the ultracentrifuge was allowed to run with a force of about 250,000*g* until either the fastest or the two fastest components had sedimented past the partition. Samples were withdrawn from both the top and bottom chambers of the cell after the rotor had stopped. The amylase content of the samples was then determined.

The amylase in the parotid gland secretion sediments with an average rate of 4.1 Svedberg units (S). This rate agrees well with the average found in the general study (*4*) for one of the components, 4.2 S (*4*). When some of the 4.1 S component remained in the top chamber of the cell, appreciable amylase activity (27 to 61 percent of the original activity) appeared in the sample withdrawn from the top chamber of the cell. In the experiments in which the 4.1 S component was allowed to sediment completely to the bottom chamber, the sample withdrawn from the top chamber contained little or no amylase activity (0–8 percent). It seems definitely established, therefore, that the 4.1 S component contains the amylase. This component is marked with an arrow in Fig. 1, which shows a typical sedimentation pattern of the parotid gland secretion. As with the electrophoretic components, the proportion of the component which is pure amylase has not been determined.

This sedimentation rate is in accord with that reported by Muus (*2*) for her crystalline human salivary amylase. She reported a sedimentation rate (S_{20}) of 4.6 Svedberg units in a preliminary experiment. Neither of the afore-mentioned values has been corrected to zero concentration.

In this work, α-amylase has been located in both the electrophoretic and ultracentrifugal patterns of human parotid gland secretion. It has an average electrophoretic mobility of -1.4×10^{-5} cm²/volt-sec and a sedimentation rate ($S_{20,\,w}$) of 4.1 Svedberg units in the Miller-Golder buffer of 0.1 ionic strength and *p*H 8.5.

JANE REID PATTON*
WARD PIGMAN
Department of Biochemistry, University of Alabama Medical Center, Birmingham

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Possible Interrelationship between Release of Brain Norepinephrine and Serotonin by Reserpine

The finding of a solvent system for the extraction of catechol amines has permitted the development of a simple fluorimetric method for the rapid determination of norepinephrine in various body tissues (*1*). The catechol amine extracted from rabbit brain was predominantly norepinephrine, with a small proportion of epinephrine, as shown by activation and fluorescence spectra (*2*), by rate of oxidation at various *p*H values, and by paper chromatography. Its distribution in brain was found to be in general like that of serotonin; that is, it is high in brain stem and low in cortical areas and cerebellum, as noted by Vogt, who used a bioassay procedure (*3*).

In previous studies, we have shown that reserpine impairs the capacity of body tissues to maintain serotonin in a bound form (*4*), thereby causing its liberation from brain and other tissues

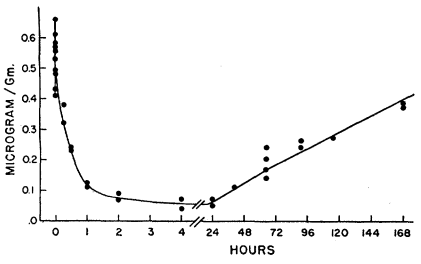

Fig. 1. Norepinephrine content of rabbit brain stem at various times after intravenous administration of reserpine (5 mg/kg).

(*5*). Recent observations that reserpine also lowers the content of catechol amines in various body tissues (*6*) prompted an investigation concerning the nature of this phenomenon and its possible relationship to the release of serotonin.

Rabbits received reserpine (5 mg/kg) by intravenous injection and were sacrificed at various times thereafter. The brains were removed immediately, and the brain stems were analyzed for norepinephrine. The level of norepinephrine declined rapidly, a definite drop occurring within 15 minutes and about 90 percent disappearing within 4 hours. The level remained low for about 48 hours and then increased slowly, attaining the normal value after about 7 days (Fig. 1). The shape and duration of the curve is practically identical with that depicting the effect of reserpine on serotonin in brain (*7*).

The effect of various doses of reserpine on the norepinephrine content of the brain was determined. Doses as low as 0.1 mg/kg had a definite effect, and 1.0 mg/kg was as effective as 5 mg/kg (Fig. 2). The dosage response curve was almost identical with that found for release of serotonin.

It has been shown that reserpine appears to exert an irreversible action, disappearing rapidly from brain and other tissues and not appearing to act through a metabolic product (*5*). Accordingly, the depletion of norepinephrine as well as of serotonin persists long after reserpine has virtually disappeared. The almost identical patterns of effect on serotonin and norepinephrine both with regard to duration and with regard to response to various doses of reserpine suggest that the release of the biogenic substances in brain are closely linked phenomena. It is possible that norepinephrine and serotonin are normally bound in brain tissue by similar mechanisms which are impaired by reserpine. Another possibility is that the serotonin released by reserpine in turn releases norepinephrine (or vice versa).

The release of catechol amines from the adrenal glands was also measured. Depletion of the amines occurred over a period of 16 hours after administration of reserpine (5 mg/kg), and was virtually complete for a period of several hours. The content of the adrenal amines then gradually rose and achieved the normal value after about 7 days, a time when brain norepinephrine and serotonin were again normal. The loss of medullary amines did not result from a direct action of reserpine, since it was prevented by spinal transection at T_1, indicating, as do the findings of Holzbauer and Vogt (*6*), that the depletion was the result of stimulation of sympathetic centers in brain; this suggests that the depletion might be related to norepinephrine (or serotonin) changes in the central nervous system. It is pertinent that reserpine, which produces a generalized parasympathetic predominance, induces a prolonged stimulation of the sympathetic nerve innervating the adrenal gland.

Peripheral norepinephrine was also depleted following administration of reserpine, doses as small as 0.1 mg/kg lowering the content of amine in the heart by about 85 percent within a period of 4 hours. Reserpine also induced the depletion of norepinephrine from spleen. Section of the cord at C_7 or at C_2 did not prevent the release of norepinephrine from the heart following doses of 5 mg/kg of reserpine, indicating that with these doses, the liberation resulted mainly from the action of reserpine directly on the peripheral storage depots.

The ability of reserpine to release norepinephrine and serotonin throughout the body indicates that changes in the amines centrally as well as peripherally must be considered in explaining the over-all action of the *Rauwolfia* alkaloid. Accordingly, the extent to which changes in autonomic balance following administration of reserpine may be attributed to alterations of peripheral and brain norepinephrine is now under investigation. The emerging picture of reserpine as a releaser of serotonin, norepinephrine,

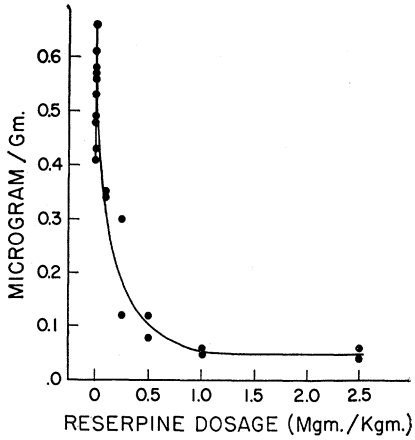

Fig. 2. Norepinephrine content of rabbit brain stem 4 hours after administration of various intravenous doses of reserpine.