Comments and Communications

Denaturation of Urease Without Inactivation

It appears to be generally believed that the denaturation of an enzyme always coincides with its inactivation. This has been found to be true even when the enzyme could be partly reactivated later. It appeared of interest, therefore, to bring to attention one of our early papers (J. B. Sumner and V. A. Graham. *Proc. Soc. exp. Biol. Med.*, 1925, 22, 504), in which we describe the preparation of an insoluble, although still highly active, urease. This material was obtained by allowing partially purified urease in 30% alcohol and sodium chloride to stand at room temperature for 1 or 2 days.

Recently I have prepared insoluble urease from once-recrystallized urease. The material is quite insoluble in water or dilute phosphate buffer and is highly active, although considerably less active than soluble urease. It gives a strong nitroprusside test for sulfhydryl groups and is much more resistant to digestion by commercial trypsin at pH 7.0 and 30° C than the same material after boiling. It is evident, therefore, that the reactions which occur when urease is denatured in this manner do not destroy all of the active groups.

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Human Saliva as a Germination Inhibitor

The inhibiting effect of saliva on the germination of wheat seeds, of which D. Yardeni (*Science*, July 16, pp. 62–63) presents a series of interesting examples, is attributed by her to the occurrence of bacteriostatic substances in human saliva.

According to the results of Yardeni's experiments, the inhibiting factor is heat resistant, nonvolatile, removable by dialysis, and affects the growth of the radicles more than that of the coleoptile. One may well wonder whether all these effects could not be easily explained by the eventual growth-hormone content of saliva, which is not even mentioned. As early as 1925 E. Seubert (*Z. Bot.*, 1925, 17, 49) produced curvatures of *Avena* coleoptile by unilateral application of saliva. A. J. Haagen-Smit, in his general review on this subject (*Ergebn. Vitamin Hormonforsch.*, 1944, 2, 355), refers to that paper. H. Lane (*Amer. J. Bot.*, 1936, 23, 532–535), K. V. Thimann (*Amer. J. Bot.*, 1936, 23, 561–569), Th. Solacolu and D. Constantinesco (*C. R. Soc. Biol. Paris*, 1937, 124, 492–494), and many other authors have demonstrated the inhibiting effect of growth hormones on the root growth of germinating seeds. That urine and other animal body liquids contain growth hormones has been proved principally by F. Kögl, A. J. Haagen-Smit, and H. Erxleben (*Z. phys. Chemie*, 1933, 220, 137–161) and confirmed by many other authors—for instance, F. Ubatuba (*Rev. Bras. Biol.*, 1946, 6, 23–62). Thus, it seems to us that the possibility of growth hormones, present in human saliva, being responsible for its germination-inhibiting effect should be taken into consideration.

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Absence of Protective Action of Rutin in Anaphylactic and Histamine Shock

In a recent study of the influence of rutin on anaphylactic and histamine shock, Raiman, Later, and Necheles (*Science*, October 17, 1947, p. 368) reported that significant protection was obtained when sensitized guinea pigs were pretreated with 1 or 2 mg (total dose) of rutin 30–45 min prior to shocking with the sensitizing antigen, but they found no protection by rutin against histamine shock. Wilson, Mortarotti, and DeEds (*J. Pharm. exp. Therap.*, 1947, 90, 120) earlier had reported a significant reduction in mortality from histamine shock, using a higher dose of rutin, but briefly mentioned their inability to demonstrate any protection against anaphylactic shock.

Because of these conflicting results, we repeated such experiments on several series of animals. All rutin injections were intraperitoneal, and all injections of antigen or histamine were via the dorsal penile vein, a route which eliminates the complications of cardiac damage or false shock symptoms which may attend piercing the pleural cavity.

The first series of guinea pigs, sensitized with horse serum, was divided; one group was carried through procedures duplicating in all details those of Raiman, *et al.* except for the route of intravenous injections. The other group received larger doses of rutin, and in some the time interval was shortened. Table 1A shows that the preponderant results were severe or fatal anaphylaxis, with only 3 of 25 animals showing no symptoms. More than half of the remainder succumbed, with typical post-mortem findings of anaphylactic shock. The low incidence of mortality in the control group (50%) was regarded as unsatisfactory, and another series was started.

In the second series, the guinea pigs were sensitized by a multiple-site intramuscular injection of 0.4 cc of a suspension of Freund adjuvant in fresh egg white (J. Freund and K. McDermott. *Proc. Soc. exp. Biol. Med.*, 1942, 49, 548). This technique has been found by previous experience to produce a high degree of sensitization in all animals. The shocking dose of 0.4 cc of a 1:8 dilution of fresh egg white, given 21–26 days later, has been found to be the LD_{100} dose for our animals, in agreement with the data of Marcus (*Proc. Soc. exp. Biol. Med.*, 1947, 66, 181). In this series, all 20 control animals died, and the same dose was used for the pretreated animals. The amount of rutin was increased to 10 mg (total dose) and was given as a 10% solution of the methyl-glucamine salt, having a pH of approximately 8.0. Methyl glucamine *per se* has been found to be pharmacologically inert, while rutin thus prepared was shown