or accidental spraying with 2,4-D until the role of 2,4-D in the accumulation of toxic quantities of nitrates in these species has been more fully determined.

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An Analysis of the Enzyme Activity of the Conditioned Salivary Response in Human Subjects

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In the early work on conditioning, it was generally assumed that the conditioned response was identical with the original or unconditioned response, both in a qualitative and a quantitative sense. Later, many workers, including Pavlov, found that the conditioned response did differ in a quantitative way from the original response. As a rule, it was found to be less vigorous (motor responses) or associated with a decreased amount of saliva (glandular response). The aim of the present experiment was to determine whether there is any chemical difference in the saliva of human subjects between the original and the conditioned response.

Eleven female college students were used in the experiment. Four specimens of saliva were taken from each subject: previous to the experiment, during the response to a bell before conditioning, during the presentation of food, and after the process of conditioning had been completed. The saliva was collected from the sublingual spaces by means of a glass pipette, so that the secretion from all the salivary glands would be represented.

The tests were made in an isolated room, with the blinds drawn to eliminate gross nonexperimental stimuli. Each subject was tested individually during a single session of about 3 hr. The unconditioned stimulus was a Cryst-O-Mint candy wafer, which induced a free flow of saliva and was neutral with respect to the chemical techniques later applied. The conditioned stimulus was an electric bell. The subjects were instructed not to eat or smoke for 4 hr previous to the series of tests and reported that they had adhered to this schedule.

The bell preceded the wafer by 10 sec, and the latter was held on the tongue for 20 sec during each trial, following the suggestion by Razran (*1*). The paired stimuli (bell–wafer) were given at short, irregular intervals, so that the time interval itself could not operate as a conditioned stimulus. The time interval between presentations of the pair ranged from 30 to 90 sec, and the series of presentations was randomized. The conditioning phase consisted of 150 paired presentations.

At ½-hr intervals, each subject was asked to report any change in the amount of the salivary secretion noticed. The 11 subjects utilized in this experiment reported a definite increase in saliva from the first interval onward.

The amount of amylase in the saliva was measured for each condition. This substance was chosen because it is the starch-hydrolyzing enzyme and the most active component of saliva (*2*). The results are reported in units of amylase activity per ml of saliva. One unit of amylase may be regarded as the amount required to digest 5 ml of 1% soluble starch to the achromic point in 10 min under the conditions of the standard analysis as presented by Hawk, Oser, and Summerson (*3*). The analytical reagents employed were: (1) light-yellow aqueous iodine solution, (2) 1% aqueous solution of soluble starch, (3) 1% aqueous solution sodium chloride, and (4) phosphate buffer ($K_2HPO_4 + KH_2PO_4$) adjusted to pH 6.6.

The results of this experiment are: (1) With respect to amylase activity, there is a statistically significant quantitative difference between the salivary secretion in response to a conditioned stimulus and the reflex response. (2) There is more amylase activity in the salivary component during the conditioned response phase than in the unconditioned response. There is a mean gain of 31.7 in units of amylase activity in the salivary conditioned response over that of the unconditioned response. This difference is significant at the 0.01 level of confidence. (3) The amylase activity in the determination of experimental conditions (1) and (2) is consistently close.

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Spectrophotometric Assay of Ascorbic Acid with Peri-Naphthindanetrione Hydrate

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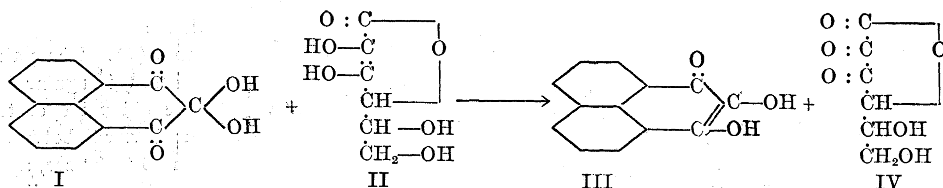
When peri-naphthindanetrione hydrate (I) (*1, 2*) is allowed to react with ascorbic acid (II), the reaction is of a reddish color owing to the formation of dihydroxy-peri-naphthindone (III) (*3*). This reaction is an oxidation reduction system in which the stage of oxidation stops at the point of the formation of dehydroascorbic acid (IV).



The proportionality of the color obtained in this reaction is in good agreement with Beer's law.

We have used this reaction in the estimation of ascorbic acid through spectrographic determination of dihydroxy-peri-naphthindone produced by reduction of the reagent by ascorbic acid in pure solution. The amount of the reduction product thus produced is estimated by the intensity of absorption at 475 mμ; the excess of the reagent, which should always be present, does not absorb at this region of the spectrum. This is not true if estimation is carried out at 345 mμ, and higher values are obtained. However, if just enough reagent is added to react completely with the known amount of ascorbic acid present, good results have been obtained.

The nature of this reaction is illustrated by the following experiment. Eleven milligrams of pure ascorbic acid is weighed, dissolved, and completed to 100 ml with ethyl alcohol. To 5 ml of this solution is added one ml of peri-naphthindanetrione hydrate in alcohol (2 mg/one ml). These are mixed thoroughly and stoppered at room temperature. The color gradually develops and reaches its maximum intensity after 10 min, when it is ready for estimation. The stability of the color permits its measurement with ease at any time from 10 min to 24 hr after the reagents are mixed. A cell of 5-mm thickness is filled with this solution; the control cell is filled with pure ethyl alcohol and is estimated, using a Hilger Barfit quartz spectrograph.

For pure dihydroxy-peri-naphthindone the two bands, one being in the ultraviolet and the other in the visible part of the spectrum, have intensities which can be expressed as $E^{1\%}_{1\,cm}$ $345 = 525$ and $E^{1\%}_{1\,cm}$ $475 = 150$.

This reagent, apart from the fact that it is easily prepared, inexpensive, and stable, is specific. To a solution of peri-naphthindanetrione hydrate a solution of the possible interfering substances in plant, animal, and biological media was added under the same experimental conditions used in the case of ascorbic acid, and no color was developed. The following substances were tested: glucose, fructose, alanine, leucine, isoleucine, phenylalanine, lactic acid, aceto-acetic acid, pyruvic acid, urea uric acid, acetone, and dehydroascorbic acid.

Details of this method will be published elsewhere.

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A Method for Evaluating the Relation of Glycogen to Inorganic Salt Deposition in Surviving Cartilage Slices *in Vitro*[1]

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Glycogen has been known to be present in the hypertrophic cartilage cell for almost a century (*1*), and its relation to the ensuing ossification of the cartilage was suggested by Creighton (*2*) as early as 1896. The evidence that has accumulated since then has been, for the most part, favorable to the concept that a close relationship exists between the region of glycogen accumulation in cartilage prior to calcification and the area of subsequent lime salt deposition (*3, 4*).

Harris (*5*) suggested that the role of glycogen was to furnish phosphate ester substrate, possibly for the phosphatase found to be present in cartilage by Robison (*6*). More specific evidence of the relation of glycogen to calcification was provided by Gutman and his associates, who demonstrated phosphorylase activity in cartilage (*7*), as well as the role of phosphorylative mechanisms in the deposition of calcium in cartilage *in vitro* (*8*). This latter observation has been confirmed in our laboratory and found to apply to strontium as well (*9*). Interest in strontium grew out of experiments in man which suggest that strontium can supplement calcium to cause a more rapid remineralization of the skeleton in osteoporosis than usually occurs with calcium alone (*10*).

Previous histochemical studies dealing with the relation between glycogen and calcification of cartilage have utilized techniques which involved a comparison of the glycogen-staining areas in one piece of cartilage with the calcified portion in another (*4*). It is the purpose of this note to describe a method by which a direct correlation can be made in the same cartilage slice between the glycogen zone and the area of subsequent lime salt deposition. The procedures involved are simpler than those required for the staining of glycogen in fixed sections, and the method is particularly applicable to the analysis, in surviving cartilage slices, of the enzymatic reactions involved in the process of calcification.

The details are as follows: The proximal ends of the tibiae and the distal ends of the femurs of mildly rachitic

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