concentrations used. Also, no injury was observed following urea applications to young apple and cherry trees that had just begun growth. This observation is of interest in connection with the data for the hydrolysis of urea given in Table 1 showing greater hydrolysis following bark application to branches with leaves than to branches without leaves.

The surface area of trunk, branches, and twigs of dormant trees and the quantity of nutrients that were retained from bark applications were found to be appreciable. Thus the surface area of a 3-year-old McIntosh tree was found to be 551 cm^2, and that of a 25-year-old McIntosh tree, 86 m^2. The quantity of material in the form of urea, for example, which was retained when applied at 32% concentration was determined at .79 g for a 3-year-old apple tree and 1364.0 g for a 25-year-old apple tree.

It would appear from these data that so-called "foliage feeding" must take into consideration other portions of the plant such as trunk, branches, and shoots as well as foliage. It may be recalled that among the first field applications of commercial nitrogenous fertilizers to fruit trees were sprays to dormant trees (*9*) and that old horticultural practices included coating trunks and branches of fruit trees and vines with various manurial and mineral substances (*10*).

References

1. HAMILTON, J. M., PALMITER, D. H., and ANDERSON, L. C. *Proc. Am. Soc. Hort. Sci.*, **42**, 123 (1943).
2. HAMILTON, J. M., PALMITER, D. H., and WEAVER, L. O. *Phytopathology*, **33**, 5 (1943).
3. WENT, F. W., and CARTER, M. *Am. J. Botany*, **35**, 95 (1948).
4. BOULD, C., and TOLHURST, J. *Ann. Rept. Agr. and Hort. Research Sta., Long Ashton, Bristol*, **1948**, 51 (1949).
5. JONES, R. J., and ROGERS, H. T. *Advances in Agron.*, **1**, 39 (1949).
6. FISHER, E. G., and COOK, J. E. *Proc. Am. Soc. Hort. Sci.*, **55**, 35 (1950).
7. SILBERSTEIN, O., and WITTWER, S. H. *Ibid.*, **58**, 179 (1951).
8. HINSVARK, O. N., WITTWER, S. H., and TUKEY, H. B. *Plant Physiol.* (in press).
9. LEWIS, C. I., and ALLEN, R. W. In *Rept. (1914–15) Hood River (Oregon) Branch Expt. Sta.*, 5 (1916).
10. MILLER, P. *Gardener's Dictionary*. London (1754).

Manuscript received May 29, 1952.

The Activity of α-Amylase as Determined by Adsorption Indicators

Benjamin Carroll and John W. Van Dyk[1]

Newark Colleges of Rutgers University, Newark, New Jersey

It has been previously shown that the activity of a proteolytic enzyme may be indicated by the loss of binding capacity of the protein substrate for simple dyestuffs (*1*). This note presents initial results to show that dextrinogenic activity may be determined in a similar manner. The method for determining the activity of hydrolytic enzymes may be considered a general one. In brief, the method consists of selecting a dye which undergoes a spectral change when it is adsorbed by the substrate. As the substrate is hydrolyzed, its affinity for the bound dye is altered. Usually the fragments of hydrolysis have a smaller binding capacity for the dye, and thus the system reverts to the absorption spectrum for the dye in the absence of the substrate.

The familiar starch-iodine color used in the determination of dextrinogenic activity may be viewed as an application of an adsorption indicator. The use of dyestuffs in place of iodine, however, offers an additional approach to the study of the α-amylases.

It is generally accepted that a chain length of about 10 glucose units is required for a red color with iodine, whereas a chain length of 30 units or more yields a blue color. Chains of 4–6 glucose units give no color with iodine (*2*). We have found that a dye such as Congo Red is adsorbed by dextrins that fail to give a color response with iodine. The minimal size of the polysaccharide which will cause a spectral change with Congo Red is about 5 glucose units in length. The size is comparable with the distance between the two amino groups in Congo Red, these groups probably being the foci of attachment to the starch, via hydrogen bonding (*3*). It would appear, then, that in the case of dextrins of low molecular weight, the use of Congo Red would have a distinct advantage over the iodine method.

The adsorption of Congo Red by starch at pH 7 is accompanied by a 20% loss of intensity of the spectral minimum which occurs at a wavelength of 402 mμ. The spectral peak, however, is enhanced about 5%, the peak being shifted slightly toward the red. The adsorption of dye is rapid and reversible. The same may be said for the desorption process, which occurs during the hydrolysis of the starch. During this process the shape of the spectral curve in the vicinity of the minimum is unchanged.

The effect of a sample of filtered saliva on a solution containing 0.05% Lintner's starch and 3×10^{-5} *M* Congo Red was investigated. The saliva had been diluted by factors of 600–9000. The solution also contained 0.01 *M* NaCl and 0.01 μ phosphate buffer at pH 7. The reaction mixture was observed in a Beckman DU quartz spectrophotometer, the temperature of the cuvettes being regulated to ± 0.1° C by means of cooling blocks. Fig. 1 shows that the addition of the starch causes an appreciable decrease in the spectral intensity, but upon the addition of the enzyme to the mixture, the starch gradually loses its combining capacity for the dye until the optical density for dye alone is attained.

A measure of the activity of the amylase is determined by the reciprocal of the time required to effect a given change in optical density. No knowledge of the kinetics of the reaction is necessary. Here one assumes that initially identical starch solutions, which subsequently have the same optical density, contain the substrate in the same state of hydrolysis. This as-

[1] The authors wish to express their gratitude to the Research Corporation and to the Rutgers Research Council for the financial support that made this work possible.

sumption is verified by the results in Fig. 1, which yield a linear relationship between the relative concentration of the salivary amylase and the reciprocal of the time required to attain given optical density.

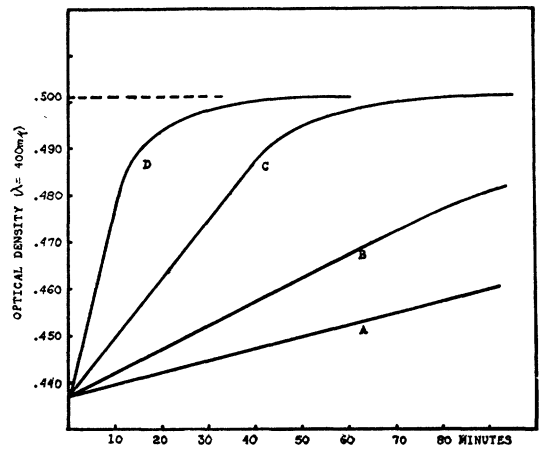

FIG. 1. Effect of salivary amylase on the optical density of 0.05% (Lintner's) starch solution containing 3×10^{-5} *M* Congo Red. The relative concentrations of enzyme are 1, 2, 5, and 15 for curves *A*, *B*, *C*, and *D*, respectively. Broken line is the optical density for the dye in the absence of starch. Solution contained 0.01 *M* NaCl and phosphate buffer at pH = 7.0; temperature, 24° C.

Unlike iodine, which will immediately arrest enzymatic action, the above results indicate that the assay of an α-amylase may be carried out in the presence of the dye. The extent of inhibition by Congo Red appears to be small and is considered in further detail elsewhere (*4*). It may be said here that with the exception of native serum albumin, most proteins bind anionic dyes rather poorly (*5*). In the enzymatic hydrolysis of the starch the concentration of dye is kept at 10^{-5} *M*; thus adsorption of the dye by the protein α-amylase is probably extremely small. The substrate, on the other hand, binds the dye, possibly preventing the accessibility of the substrate to the enzyme. But when one considers that the molecular ratio of dye to glucose units is about 1:100, the inhibiting effect of the dye should not be appreciable. However, for those enzymes where the pH optimum is quite removed from the pH required to give the greatest spectral change, the precision may be improved by following the dextrinogenic activity of the amylase by the addition of the dye to successive samples of the hydrolysate, in a manner similar to that used in the iodine method.

It is interesting to compare the behavior of an adsorption indicator in the hydrolysis of starch with that in the hydrolysis of the protein substrate, native bovine albumin (*1*). Unpublished results from this laboratory indicate that few of the approximately 600 peptide bonds have to be broken before the protein molecule completely loses its binding capacity for the indicator ion. The indicator ion used here lacks such specificity for the starch substrate. One may speculate hopefully that additional work may lead to a series of dyestuffs that may be used to probe the starch substrate and cast additional light upon the mechanism of the starch-amylase reaction.

References

1. CARROLL, B. *Science*, **111**, 387 (1950).
2. SWANSON, M. A. *J. Biol. Chem.*, **172**, 825 (1948).
3. VICKERSTAFF, T. *The Physical Chemistry of Dyeing*. New York: Interscience (1950).
4. CARROLL, B., and VAN DYK, J. W. *J. Amer. Chem. Soc.* (in press).
5. KOLTZ, I. M., and URQUHART, J. M. *Ibid.*, **71**, 1597 (1949).

Manuscript received March 11, 1952.

Systemic Stress as an Inhibitor of Experimental Tumors in Swiss Mice

Harold A. Rashkis[1]

Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, and Wistar Institute of Anatomy and Biology, Philadelphia

The role of systemic stress has been investigated in a wide variety of human ills; yet its relationship to neoplastic disease has remained obscure. Since the known effects of systemic stress are largely catabolic (*1*), it might be expected, because of this, that the growth of tumors might be hindered rather than abetted. Indeed, when one considers the many agents that have been effective experimentally against neoplasms, it seems quite likely that, in addition to the special effects of each, many or all of these agents share in a general action as a systemic stressor. We have accordingly tested the hypothesis that organisms under stress will show less tendency to develop experimentally produced tumors than will their normal controls. This hypothesis has been tested in the Swiss albino mouse, employing forced swimming as a stressor and the ascites tumor and the methylcholanthrene-induced sarcoma as experimental neoplasms.

Fifty young adult male Swiss albino mice were inoculated intraperitoneally with 0.15 ml of a fluid ascites tumor in a 1:4 dilution with normal saline. At the time of inoculation 25 of these mice (the experimental group) had been subjected to forced swimming in glass jars (approx 18 cm^2), containing water at room temperature, with 6–8 mice in each jar. Over a period of 17 days this group had swum a total of 48¾ hr in daily sessions increasing in duration from 1½ to 4¼ hr. Following inoculation, the forced swimming was continued for 34 additional hr over a period of 14 days, in daily sessions the duration of which decreased from 4¼ to ¼ hr. Discontinuance of swimming was necessitated by the growth of tumors in the experimental group, a circumstance that increased the danger of death by drowning.

One member of the control group died with a large fluid tumor on the seventh day following inoculation, and 2 days later one of the experimental group died. By the fourteenth day, when forced swimming was

[1] On leave of absence, Department of Psychology, University of Pennsylvania. The author wishes to thank Margaret Reed Lewis, of the Wistar Institute, for her considerable assistance in this investigation.