Comments and Communications

Multienzyme Systems

The recent book by Malcolm Dixon (*Multi-Enzyme Systems.* New York: Cambridge Univ. Press, 1949) places in print a logical framework of a rationalization for the existence of the phosphagens. The framework is Dixon's definition of phosphate potential and phosphate couples, with their corresponding rP scale, which is analogous to the rH scale for oxidation-reduction couples. Of course, since creatine phosphate exists in appreciable concentrations, it is a reservoir of high-energy phosphate. But why is it necessary to have another compound, in addition to the adenosine phosphate system, for the storage of high-energy phosphate?

In a medium as complex as protoplasm, it may be difficult to define experimentally the thermodynamic phosphate potential, even in a specific ultramicroscopic region of the protoplasm. Yet it seems clear that a low ratio of the concentration of adenosine diphosphate (ADP) to that of adenosine triphosphate (ATP), or simply a high concentration of ATP, is indicative of a high phosphate potential, and conversely.

If all the phosphate present in resting muscle as creatine phosphate were present as ATP, the phosphate potential would be much higher than actually exists. Furthermore, if the performance of other engines is analogous, the fully charged, high-potential system found in the moderately metabolizing tissue would be nearest to a state of equilibrium and would be the most efficient. Stress would not only lower the reserve of high-energy phosphate in the hypothetical system, but would also reduce the potential below its efficient level, and therefore the stress would compound itself.

Evolution has settled on concentrations of ADP and ATP that are quite low and of the same order of magnitude for moderately metabolizing systems. Whereas ATP reacts with a large variety of metabolites, creatine phosphate apparently only reacts with the adenosine phosphate system. Since the phosphate bond energy of creatine phosphate is somewhat lower than that of ATP, the ratio of ATP to ADP must be considerably lower than the ratio of creatine phosphate to creatine. This statement is in keeping with the knowledge that both ADP and ATP are intimately involved in the details of metabolism, whereas creatine apparently only stores energy in its phosphorylated derivative. In conditions of stress, much of the phosphate of creatine phosphate can be fed through the adenosine phosphate system without greatly altering the concentrations of its components. Thus the existence of phosphagen permits the maintenance of the most efficient levels of the active phosphorylated metabolites under conditions of both rest and stress. It is known that the ATP level of highly stimulated muscle does not seriously decrease until the muscle is exhausted, whereas the phosphagen decreases steadily. Thus it seems reasonable to infer that phosphagen is not only a storage depot for high-energy phosphate, but also is a buffer for the maintenance of the most effective levels of the components of the adenosine phosphate system under a wide variety of metabolic conditions. It is even possible that as phosphocreatine surrenders its phosphate to ADP, the free creatine is converted to creatinine, which process would conserve the ratio of phosphocreatine to creatine. This process would be extremely efficient as a buffering agent. The possible inability of organisms using phosphoarginine to destroy the arginine formed after transfer of its phosphate may be a measure of their lower state of development.

It may be that there are other metabolic double-couples, one active couple and one inactive, the inactive couple existing only to increase the span of conditions under which the most effective concentrations of the active couple can be maintained.

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Persistence of 2,4-D in Plant Tissues[1, 2]

Tullis and Davis discuss in a recent issue of SCIENCE, (**111**, 90, [1950]) the effect of supposedly persistent 2,4-D in plant tissues. They cite the effect described by Pridham (*6*) upon bean seedlings grown from seeds of plants sprayed with 2,4-D while the pods were maturing and that described by Dunlap (*3*) upon cotton seedlings grown from seed borne by plants that were injured the previous season by 2,4-D. They note, however, that Brown, Holdeman, and Hagood (*2*) report no evidence of injury on cotton plants grown from seed collected in "fields affected by 2,4-D."

The appearance of injury and of lack of injury to the new growth of two woody plants, Chinese tallow trees and chinaberry trees, respectively, the year following spraying with 2,4-D is also described. The authors state that "no other reports, to the writers' knowledge, have been published that would indicate any persistence of 2,4-D in plant tissues from one growing season to the next other than in seeds." They conclude that in the Chinese tallow trees "the 2,4-D had persisted[3] in the buds and other vegetative tissues of this plant from the time of injury" the previous season and that in the chinaberry trees it did

[1] This paper is based on work done for the Biological Department, Chemical Corps, Camp Detrick, Frederick, Md., under Contracts Nos. W-18-035-CM-168 and W-18-064-CM-237.

[2] Since this paper was written, H. B. Tukey (*Science,* **112**, 282 [1950], has discussed the same subject from a somewhat similar point of view.

[3] This use of the term "persisted" is different from that of publications of the Biological Department, Chemical Corps, Camp Detrick.

not "persist." Their evidence for the presence of the 2,4-D is in the "injury . . . on the earliest growth."

The 2,4-D may have been present in both the seeds and buds of these plants, but recent studies and observations indicate that, even though the 2,4-D be present, the observed effects on some seedlings and the Chinese tallow trees, and the absence of effect on the cotton seedlings grown by Brown, Holdeman, and Hagood and on the chinaberry trees may have another explanation.

Studies by Watson (*8*) on the bean plant, by the author on *Cyperus* (*4*), by Tukey on *Prunus* (*7*), together with observations of the effects of spray or dust treatment (sometimes accidental) on the spring growth of many woody plants, such as privet, rose, grape, and lilac, indicate that injury is done to the developing buds at the time of treatment but that the effect is evident only later when the buds develop. Whether the 2,4-D is still present—that is, "persists" or "is stored"—in the buds for a long time, even into the next growing season, has not as yet been determined so far as the writer knows. But the evidence from anatomy and ontogeny is that, whether or not the 2,4-D is still present, the injury is done at the time of the treatment and is brief, not continuing.

It has been known from the earliest anatomical studies of the effect of growth-regulating substances that the injury or stimulus is restricted to maturing tissues and to those mature tissues that readily awaken into meristematic activity, chiefly the endodermis and pericycle; and that the degree of injury depends upon the degree of maturity of the tissue or organ. In a developing bud, with leaves at various stages of maturity, there is a series in degree of injury to the immature leaves that is directly related to the stage of development of the leaves at the time of treatment. These degrees of injury are not evident at once in a bud that soon becomes dormant, except cytologically, but they become conspicuous when the bud resumes growth after the dormant period. The position of the series of injuries in the new growth can be controlled by the time of treatment of the mother plant (*8*).

The explanation of the apparently conflicting reports of injury and absence of injury after treatment lies in the relation of time of treatment to stage of bud development. This explanation covers not only the reports concerning the injury of the new growth of woody plants after dormancy following treatment, but also those of seedling studies in which the bud in the embryo, the plumule, is or is not affected, dependent upon its stage of development when treated. To understand the results of the treatment of any plant (position and type of injury), it is necessary to know the story of its bud development: number of leaves present in the bud at all stages (this may vary considerably); uniformity of stages in the series of leaves (in the bean plumule, for example, the series is not uniform because the two primary leaves are advanced at all stages far beyond the succeeding trifoliolate leaves); the time of plumule development in the seed or of bud development in the growing season—for example, June, or September–October.

The examples of apparent persistence of 2,4-D cited by Tullis and Davis can probably be explained as follows:

Pridham sprayed bean plants "during the ripening of pods"—that is, while the seeds were maturing and the embryos developing. The plumular leaves were affected, but the injuries did not appear until seedlings were grown from the seeds. Muratti (*5*) repeated Pridham's experiment and obtained results similar to those of Pridham. He showed that the extent and location of the injury varied with the size of the pod at time of treatment—that is, with stage of plumule development.

The conflicting reports concerning the effect upon cotton seedlings can be similarly explained, at least in part. Where no effect was noted, the treatment was probably given at a time when no bolls were developing (embryos would be injured only when bolls were developing), or was so severe that all immature bolls were abscised. Bolls developing from flowers present at time of treatment, or formed after time of treatment (even on severely injured plants), would not be expected to produce injured seeds, if the theory of brief effect is sound. Dunlap reports such injury from seeds "which were picked in September from bolls that were formed several weeks after the original damage occurred." Experiments to check this should be made. Some bolls in early stages may have been present at time of injury to the plant, or some later injury may have occurred.

The injury to the spring growth of the Chinese tallow tree is an example of the commonly seen injury to woody plants affected by 2,4-D during the previous growing season. The time of development of the winter buds of this tree and the date of spray treatment doubtless coincided. In the example of the chinaberry trees, probably the winter buds were mature, or nearly so, before the treatment or were very young; or perhaps the twigs bearing these buds were killed by the treatment. Adventitious buds developing the next spring would not show injury unless they were already partly grown at time of treatment.

Tullis and Davis state that the fact that "no symptoms of 2,4-D injury to chinaberry trees were found in 1949" on trees that were severely injured in this way in 1948 "indicates that 2,4-D does not persist in the vegetative tissues of this plant." This absence of injury indicates, in the writer's opinion, merely that there were no developing buds on the trees when they were sprayed and that, if there were mature buds present, they were killed by the severe treatment given the trees.

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