IN THE LABORATORY

A Method for Reclaiming Dried Zoological Specimens

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In most zoological laboratories alcoholic specimens which have dried out are discarded, regardless of their original significance. Material of distinct taxonomic value and critical specimens on which distribution records are based are often included in the losses to which laboratory collections are exposed, in spite of improved types of containers and systematic replacement of preservatives. Various methods have in the past been suggested as a means of reclaiming and at least partially restoring accidentally dried specimens. Potassium hydroxide and lactic acid are two reagents which have been used with some success on certain kinds of dried specimens, especially on some arthropods and nematodes. Both of these chemicals have definite limits to their usefulness, however. Caustic potash has the very real disadvantage of destroying tissues.

In this laboratory it was discovered a few years ago that commercial grade of trisodium phosphate in aqueous solution alters the permeability of membranes of preserved specimens of various invertebrates. This chemical has been of great value in treating specimens of parasitic worms to ensure free passage of fluids through the body wall when permanent whole mounts are being prepared. It has been observed that specimens which have become hardened and somewhat shriveled in the preserving medium become softened and pliable in trisodium phosphate. The specimens also become somewhat plumped but show no evidence of unnatural swelling. Furthermore, treatment with this chemical does not destroy the internal tissues. It thus has a very distinct advantage over caustic potash.

The highly satisfactory results obtained from the use of trisodium phosphate on small objects suggested the possibility of using this detergent for recovery of larger specimens which had become useless through drying. In numerous preliminary tests various kinds of dried animals have been subjected to the action of trisodium phosphate, and in most instances the results have been extremely gratifying. The results are not uniform for all kinds of dried specimens treated, but in every instance the reclaimed specimens showed restoration of general body form and revealed details of diagnostic characters which had been wholly unavailable in the dried specimens and in those treated with water or alcohol alone.

Trisodium phosphate becomes effective on dried specimens at low concentrations. A very dilute solution working for a long time seemed to be as satisfactory as a stronger solution for a shorter period. In routine procedure, quantities of the chemical were not measured, but it was later found that 0.25 to 0.5 per cent of commercial trisodium phosphate in distilled water was effective in restoring the appearance of specimens treated. Warmed solutions were more rapid in their action than cold. In many instances, with small or originally delicate objects, the maximum effect was obtained in about one hour, although longer treatment continued to soften tissues and make the specimens more pliable. Some leeches that had been dry for several years were placed in trisodium phosphate in an oven at 35°C. After about an hour most of the external features had been restored to normal appearance, and after two days the leeches were relatively soft and pliable without showing any evidence of dissociation of the tissues.

Insect nymphs and delicate crustaceans which had been completely dried out in storage vials were restored to conditions wherein all characters needed for classification were observable. Dried centipedes and millipedes had all the shriveled appendages restored to condition fully available for identification. A preserved sample of fresh-water plankton which had been dry in a bottle for more than two years was covered with warmed trisodium phosphate. Less than one hour thereafter five species of cladocerans, two species of copepods, and several species of shelled rhizopods were all readily determinable.

A small minnow infected by a large liguloid tapeworm had dried to badly shriveled condition and chalky whiteness. Treatment with trisodium phosphate restored general external appearance of the fish, and the coils of the cestode were readily observable through its body wall.

Many other discarded objects have been reclaimed by this simple process. Perhaps the most striking example is a collection of relatively large nematodes which had been dry for many years. Some of these worms were reclaimed while the others were left dry for comparison. Samples of the reclaimed specimens were stained and mounted by the usual procedure for making microscopic mounts. These show details of buccal armature, form and structure of the esophagus and pharynx, and details of the uterine coils.

Vacuum Infiltration as a Method for Determining Enzymic Activity *in Vivo*

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The usual method for the study of enzymes is to extract them from macerated tissues and observe their activity in a controlled medium outside living cells. Shortly before the war there appeared in Russian literature a description of a method for the determination of the activity of enzymes inside living cells. This was based on the vacuum infiltration of living cells with an enzymic substrate and on subsequent determination of what is left of it after a period of time. In the original

method living tissues were infiltrated with a solution of either invert sugars or sucrose, and the rates of sucrose synthesis or hydrolysis were determined (*1*). Later on this was extended for the determination of the activity of phosphatases (*6*) and also for the synthetic activity of proteases (*3*). In the hands of Russian investigators these methods, and particularly the original one, have proved to be useful tools in several fields of physiological investigations. It was thought, therefore, that a brief description of the method itself, together with a few results obtained so far, might be of interest to workers on this continent.

The technique of the original method is as follows: Three comparable samples of leaves are placed separately in 150-cc. beakers. One sample is covered with distilled water and serves as a control. The second sample is covered with a 0.1 M solution of sucrose, and the third with a 0.2 M solution of invert sugars. The beakers are placed in a desiccator and evacuated down to 20–40 mm. of pressure. When the stream of air bubbles from the leaves has ceased, the air pressure is slowly brought to normal, and the leaves become infiltrated with the surrounding solution. They are then removed from the solution, dried, left for a few hours at room temperature, and at the end of this period analyzed for their sugar content. From the difference between the sucrose contents of leaves infiltrated with invert sugars and of those infiltrated with distilled water, one obtains the amounts of sucrose synthetized in leaves during this period of time. In a similar manner, the difference between the invert sugar contents of leaves infiltrated with sucrose and of those infiltrated with distilled water gives the value for the amounts of sucrose hydrolyzed.

A sample of calculations follows. Leaf samples were infiltrated with distilled water, invert sugars, and sucrose and, three hours after infiltration, were analyzed for various forms of sugar. The results of the analysis are given in Table 1.

TABLE 1

SUGAR CONTENT OF LEAF SAMPLES EXPRESSED AS MG. OF GLUCOSE/1 GRAM OF LEAF DRY WEIGHT

	Invert sugars	Sucrose	Total sugars
(1) Control	32.8	18.4	51.2
(2) Infiltrated with 0.2 M invert sugars	125.0	35.6	160.6
(3) Infiltrated with 0 1 M sucrose	67.2	97.3	164.5

Invert sugars in the amount of 109.4 mg. were infiltrated into Sample 2, and 113.3 mg. of sucrose into Sample 3. In Sample 2, 17.2 mg. (35.6 − 18.4) of sucrose, or 15.7 per cent of the amounts introduced, were synthetized. In Sample 3, 34.4 mg. (67.2 − 32.8) of sucrose, or 30.5 per cent of the amounts introduced, were hydrolyzed. Calculated per hour per gram dry weight of leaf tissues, these rates become 5.7 mg. of sucrose synthesis and 11.5 mg. of sucrose hydrolysis. The synthesis: hydrolysis ratio of 5.7: 11.5 indicates a considerable excess of hydrolysis over synthesis.

It was found that in a given plant organ the synthesis: hydrolysis ratio does not remain constant but undergoes a series of diurnal and ontogenetic changes. Thus, it was reported that in the leaves of sugar beets (*12*) and in *Bromus* (*9*) this ratio increases in the forenoon, comes to a peak at noon, and then declines. This ratio is high in a young leaf and declines in an old one (*14*). Vernalization decreases the ratio in the leaves of both wheat and cotton and causes it to approach that of plants in more advanced stages of their development (*3*). In ripening fruits there is at first a short rise in the ratio, followed by a decline as a consequence of a considerable increase in hydrolysis and a drop in synthesis. These changes are accelerated by the ethylene treatment (*5*). In late varieties of plants the synthesis:hydrolysis ratio is higher than in the early ones (*3, 10, 11, 13*). When in different varieties of onion bulbs, beet roots, and watermelon fruits the ratio is high, a greater proportion of their sugars is present in the form of sucrose (*10*).

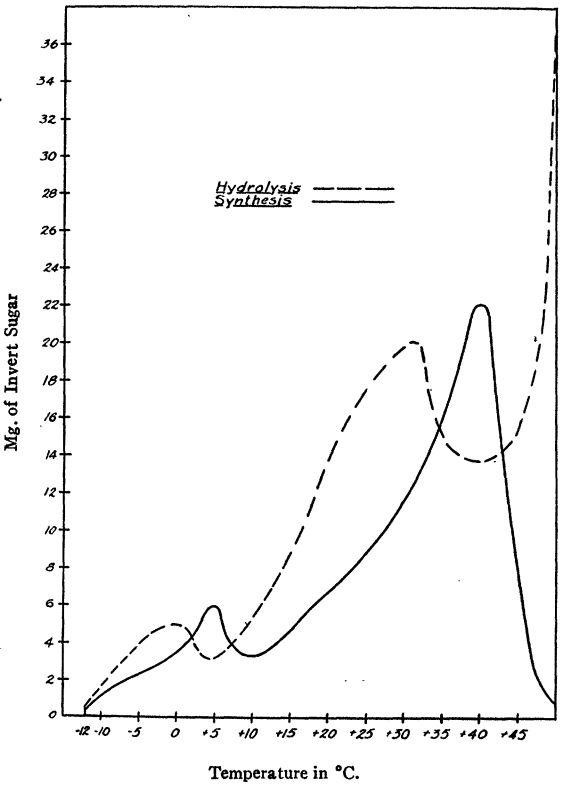

FIG. 1. Effect of temperature on the synthesis and hydrolysis in 25-day-old seedlings of winter rye. (Figure from paper by A. Kursanov, N. Krjukova, and A. Morozov, *8*.)

The effects of inorganic constituents on the enzymic activity within the living cells can be studied by either infiltration of tissues with such constituents or by using plants grown on deficient media. It was observed that the deficiency of phosphorus decreases the synthetic capacity of sugar beets (*14*) and chicory leaves (*16*). Simultaneously, the per cent of reducing sugars in the total sugar fraction is increased. The presence of potash was reported to stimulate synthesis in a number of plants (*4, 18*). On the other hand, NaCl and $CaCl_2$ depress synthesis (*18*).

By keeping the leaf samples after their infiltration at different temperatures, the effects of this last factor were studied (*8*). The results obtained are shown in Fig. 1. From an examination of the figure it is seen that at the middle range of

temperatures there is a direct relation between temperature and either synthesis or hydrolysis. At both high and low temperature extremes there are, however, two short ranges of temperature, when the rates of synthesis are above those of hydrolysis. When a hardier and a frost-susceptible variety of Cinchona tree were placed for a short time at 0° C., there was a decrease in the absolute rates of both synthesis and hydrolysis in the leaves of both varieties. On return to a higher temperature with a hardy variety, this decline was found to be a reversible one, and this variety recovered. With a frost-susceptible variety the decline was found to be an irreversible one, and the plant died (*7*). On the strength of such experiments it was suggested that a low-temperature injury or even death in a plant may be a result of interference with the normal functioning of its enzymic mechanism. That a low-temperature injury may occur above 0° C. supports this contention.

The effects of decreasing water content of leaves on synthesis and hydrolysis were determined in wilting tea leaves (*2*).

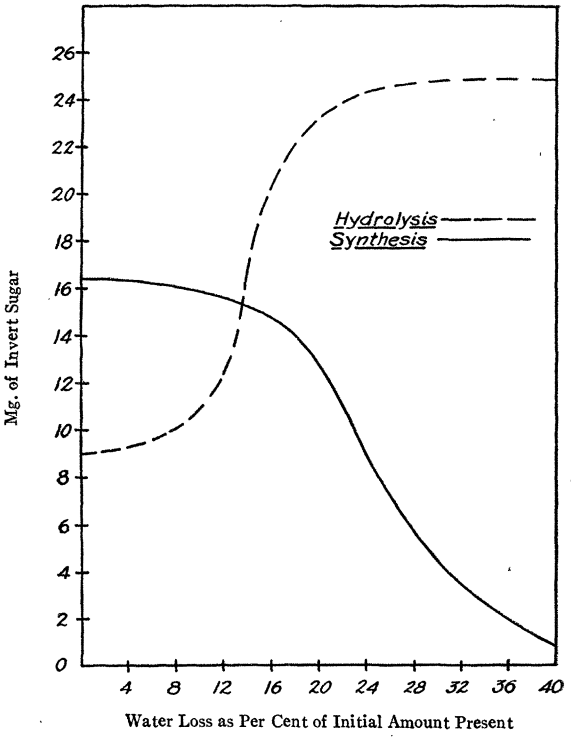

FIG. 2. Effect of water deficit on the synthesis and hydrolysis in tea leaves. (Figure from paper by A. Kursanov, *2*.)

Fig. 2 gives the data obtained. It is seen that a decrease in the water content of leaves results in a decrease in synthesis and an increase in hydrolysis. The two graphs are approximately mirror images of one another. Though the initial synthesis: hydrolysis ratio is higher in the leaves of drought-susceptible than in those of the resistant varieties of wheat, under the conditions of wilting this ratio drops off more rapidly in the former varieties than in the latter (*15*). The same was reported for the synthetic and hydrolytic action of proteases (*17*). It was suggested that the absolute value of this ratio, and the rate of its fall under the conditions of wilting, may be used in diagnosis of drought-resistant and nonresistant varieties of plants. This could be done even on plants which are in their seedling stage.

References

1. KURSANOW, A. L. *Biokhimiia*, 1936, **1**, 269-294.
2. KURSANOV, A. A collection of papers dedicated to K. A. Timiriazev. *Acad. Sci. U. R. S. S.*, 1941, 131-169.
3. KURSSANOV, A., and BRIUSHKOVA, K. *Biokhimiia*, 1938, **3**, 569-582.
4. KURSSANOV, A., and KRIUKOVA, N. *Biokhimiia*, 1937, **2**, 674-686.
5. KURSSANOV, A., and KRIUKOVA, N. *Biokhimiia*, 1938, **3**, 202-217.
6. KURSSANOV, A., and KRIUKOVA, N. *Biokhimiia*, 1938, **3**, 529-539.
7. KURSANOV, A., and KRYUKOVA, N. *Biokhimiia*, 1939, **4**, 562-565.
8. KURSANOV, A., KRJUKOVA, N., and MOROZOV, A. *Bull. Acad. Sci. U. R. S. S.*, 1938, **1**, 51-66.
9. MOROZOV, A. S. *C. R. Acad. Sci. U. R. S. S.*, 1940, **26**, 175-176.
10. RUBIN, B. A. *Biokhimiia*, 1936, **1**, 467-478.
11. RUBIN, B. A. *Bull. Acad. Sci. U. R. S. S.*, 1937, **6**, 1755-1770.
12. RUBIN, B. A., and LUTIKOWA, O. T. *Biokhimiia*, 1937, **2**, 423-436.
13. RUBIN, B. A., and LUTIKOVA, O. T. *C. R. Acad. Sci. U. R. S. S.*, 1937, **17**, 255-258.
14. SSYSSAKJAN, N. M. *Biokhimiia*, 1936, **1**, 301-320.
15. SYSSAKYAN, N. M. *Bull. Acad. Sci. U. R. S. S.*, 1937, **6**, 1771-1780; *Biokhimiia*, 1938, **2**, 687-699.
16. SYSSAKYAN, N. M. *Biokhimiia*, 1938, **3**, 95-101.
17. SYSSAKYAN, N., and KOBIAKOVA, A. *Biokhimiia*, 1938, **3**, 796-803.
18. SISSAKIAN, N., and RUBIN, B. *C. R. Acad. Sci. U. R. S. S.*, 1939, **25**, 298-301.

Action of Subtilin in Reducing Infection by a Seed-borne Pathogen

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Previous studies (*2*) at this laboratory have indicated that infection by certain seed-borne pathogens may be reduced by the antibiotic action of the natural microflora of the soil. More recently attention has been concentrated on individual antagonists from the soil, especially on those showing evidence of producing antibiotic substances capable of preventing seed-borne pathogens from producing infection. A survey for such organisms in Alberta soils, conducted in collaboration with A. W. Jackson, resulted in the selection of *Bacillus subtilis* Cohn, emend. Prazmowski, as a promising antagonist. It proved to be particularly active in culture against *Xanthomonas translucens* (J. J. and R.) Dowson, a seed-borne bacterial pathogen which we had under observation. Moreover, subtilin, a known antibiotic substance (*3*) produced by *B. subtilis*, was found in plate tests to be active against *X. translucens*. Hence, this substance was chosen for the special study reported here, which has to do with the effects of subtilin on *X. translucens* and, in turn, on the disease which it produces in barley.[1]

The sample of subtilin used was kindly supplied by the Western Regional Research Laboratory of Albany, California, at the request of the National Research Council of Canada. A pathogenic strain of *X. translucens f. sp. cerealis* (*1*) was provided by W. A. F. Hagborg, of the Dominion Laboratory of Plant Pathology, Winnipeg. O.A.C. 21 barley was employed

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