(3) The loop is drawn snugly about the bundle (Fig. 1 B). If the thread is drawn too tightly, the meristems will flare apart. The free ends of this loop are prevented from projecting forward and interfering with subsequent sectioning by being tied back with a second loop placed proximal to the first (Fig. 1 C).

(4) As many as three such bundles are placed in a vial and covered with 1 ml of 15% alcohol.

(5) A hole about 1 mm in diameter is punctured with a dissecting needle in a paper disc about 3 mm from the margin.

(6) The disc is centered, with the rough edges of the hole turned up, over the top of the vial so that the edges of the paper will project uniformly about 1 mm beyond the circumference of the vial (Fig. 2 A).

(7) With a cylindrical plunger (e.g. a No. 6 or No. 5 cork borer) which just fits easily into the vial, the disc, the edges of which are turned up in the process, is forced part way down into the vial (Fig. 2 A and B).

(8) The vial is then tilted (Fig. 2 C) so that the hole in the disc is uppermost as the disc is pushed the rest of the way downward into contact with the liquid. Air displacement through the hole is usually complete if this step is adroitly executed, but small bubbles are not objectionable. Highly absorbent papers are not recommended for the discs, because they are less likely to maintain their shape when wet and their absorbency makes complete air displacement more difficult.

(9) Four ml of 97.5% alcohol is added above the disc. No special care is necessary, since the paper quite effectively prevents the two concentrations from mixing immediately. For demonstration purposes, one edge of the disc may be drawn up very slowly with a curved pair of forceps or a hooked needle and may thus be removed completely, revealing the interface between the two concentrations. In practice it is not necessary to remove the disc, because its freely permeable nature does not interfere appreciably with the diffusion process.

(10) The vial is then stoppered and held at approximately 40° C for a minimum of 24 hrs. A bacteriological incubator set at 37.5° C is satisfactory, but a period of somewhat longer than 24 hrs may be necessary before a complete breakdown of the interface is accomplished and a uniform mixture of the two concentrations is established. It has not yet been determined whether higher temperatures for shorter periods would be equally effective without injury to the tissues.

(11) The tissues may now be left indefinitely in the resultant 80% alcoholic concentration, or the portion above the disc may be replaced with 4 ml of 100% alcohol. After another 12–24-hr period the dehydration is practically complete, but removal of the disc, followed by one or two changes of 100% alcohol at intervals of 2–4 hrs, is advised in the interest of safety.

(12) To the dehydrated tissues in 1 ml of 100% alcohol is added 4 ml of melted paraffin which has cooled to the point where it will partly solidify upon coming into contact with the alcohol at room temperature. There seems to be no injury to root tips occasioned by failure to pass them through pure normal butyl alcohol to eliminate the balance of the ethyl alcohol, and it has been determined experimentally that small amounts of the latter are soluble in paraffin at the usual oven temperatures.

(13) The unstoppered vial is now placed in the paraffin oven for a 24-hr period. After two or more changes of pure paraffin at intervals of 4–24 hrs, the tissues are embedded in the conventional manner in fresh, filtered paraffin containing no traces of alcoholic impurities.

The method just described has been used very successfully for classroom work in the preparation of slides for chromosome studies on a number of different plant species. If the sides of the paraffin block are shaved closely to the root tip bundle, it is usually possible to mount the entire meristematic portion under a 22-mm by 50-mm coverslip, provided the sections are not thinner than 10 μ. Sections thinner than 10 μ are usually undesirable because too many division figures are destroyed by cutting. The initial steps of diffusion-dehydration will doubtless find a wide application in biological microtomy.

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Effect of Acetone and Alcohol Fixation and Paraffin Embedding on Activity of Acid and Alkaline Phosphatases in Rat Tissues[1]

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In the course of research involving studies of phosphatase activity in animal tissues, it became of interest to determine quantitatively how much of the enzyme activity present in fresh tissue is preserved in the technical processes of Gomori's histochemical methods (*3*, *4*) for acid and alkaline phosphatases.

It is known that it is more difficult to demonstrate acid phosphatase (AcP-ase) histochemically than alkaline phosphatase (AlP-ase) and that acetone fixation gives better preservation of AcP-ase activity than alcohol. The effect of fixation and embedding on the cytological localization of AlP-ase has been studied in tissue sections by Danielli (*1*) and Emmel (*2*). The only quantitative data on the effect of fixation are those of Gomori (*4*), who showed that alcohol destroys AcP-ase but causes only 20% inactivation of AlP-ase, and those of Danielli (*1*), who estimated that paraffin embedding causes a 75% loss of activity of AlP-ase. To obtain more complete quantitative data on the effects of fixatives and embedding on

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both AcP-ase and AlP-ase, the experiments described below were performed.

Enzyme activity was measured in (a) pieces of tissue freshly removed from the animals, (b) pieces fixed for varying times in acetone and alcohol, and (c) pieces subjected to fixation, embedding in paraffin, sectioning, and deparaffinizing. The method for determination of phosphatase activity was that of Huggins and Talalay (*5*), modified as reported previously (*6*).

The tissues on which data were obtained were liver, kidney cortex, and duodenum of albino rats. An attempt was made to study uterus as well, but values obtained were too small to be of significance. The tissues were removed as rapidly as possible after the animals had been killed; small pieces of the selected tissues were weighed to the nearest milligram and dropped into chilled absolute acetone and 80% ethyl alcohol. One unfixed piece of each tissue was transferred directly to a glass homogenizing tube for the fresh tissue determination. Phosphatase determinations were made on these unfixed tissues within 1 hr after removal from the animals. Values for the fixed tissues were determined after 7, 28, 50, and 72 hrs of refrigerated storage in the fixative.

TABLE 1

Tissue and fixative	Percentage of original activity remaining								
	AcP-ase					AlP-ase			
	7 hrs	28 hrs	50 hrs	72 hrs	Embedded	28 hrs	50 hrs	72 hrs	Embedded
Alcohol									
Kidney	38	22	23	37	12	63	95	92	31
Liver	20	14	17	16	2	..	..	..	..
Duodenum	..	..	..	..	..	86	82	90	8
Mean	29	18	20	26	7	74	88	91	20
Acetone									
Kidney	35	24	20	43	3	52	66	77	34
Liver	22	17	25	37	6	..	..	..	..
Duodenum	..	..	..	..	..	90	64	50	24
Mean	28	20	22	40	4	71	65	64	29

After 48 hrs of storage, one piece of each tissue was removed from the fixative. The alcohol-fixed tissues were dehydrated with 95% and absolute alcohol, then transferred to xylene; the acetone-fixed tissues were transferred directly to xylene. They were then subjected to the following treatment: xylene, 30 min; xylene, 30 min; paraffin, 60 min at 56° C; paraffin, 60 min at 56° C; paraffin embedding; sectioning at 30 μ. The sections were transferred to xylene, the xylene decanted, washed once with xylene, twice with absolute alcohol, and once with distilled water.

The tissues, fixed and/or embedded as described above, were then homogenized and run through the Huggins-Talalay determinations in the same manner as the fresh tissues. AcP-ase and AlP-ase unitage was calculated, and the values, expressed as units[4]/100 mg of the fresh weight of the tissue, were converted to percentages of the fresh tissue values.

Since the AlP-ase of liver was of too small concentration to give interpretable values, the values reported here are for the AlP-ase of kidney and duodenum and the AcP-ase of liver and kidney. The tissues of two animals were used in each series of determinations. The data obtained are presented in Table 1 and summarized graphically in Fig. 1. No values are reported for 7 hrs fixation of AlP-ase because the values obtained for this short period were so variable as to be utterly without significance. Apparently the variation in degree of penetration of the fixative, diffusion of ions, and other unknown factors was great enough at this stage of the process to cause these extremely divergent results.

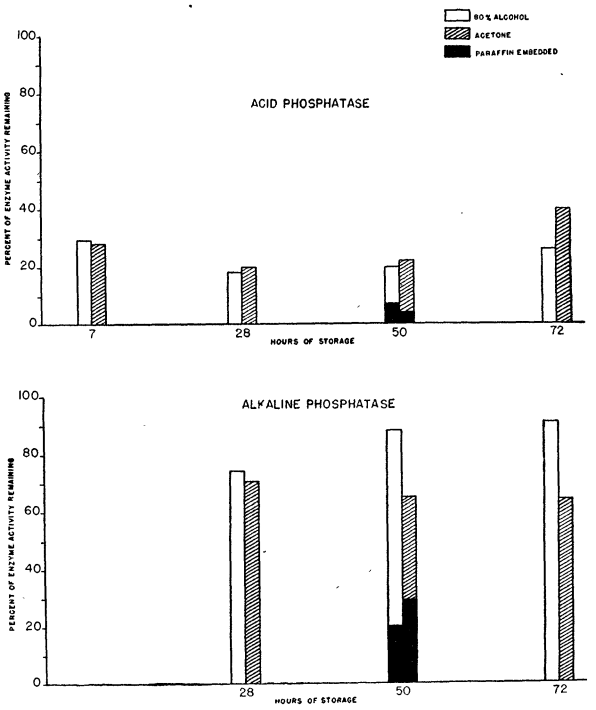

FIG. 1

The data for AcP-ase show why histochemical demonstration of this enzyme is frequently difficult. The fixation destroys 70–80% of the original enzyme activity of the tissue in 80% alcohol and 60–80% of the activity in absolute acetone. The process of clearing, embedding, and sectioning destroys most of the activity remaining in the fixed tissue, so that by the time the tissues are ready for incubation with the substrate the activity is reduced to a mean of about 5% of the original value. It should be noted that in most cases AcP-ase survived acetone fixation to a somewhat greater extent than alcohol fixation. The great reduction inherent in the embedding and associated procedures seems to be about equally destructive of either acetone-fixed or alcohol-fixed enzyme.

The data for AlP-ase demonstrate that the inactivation

[4] Ten phosphatase units is that amount of enzyme which will liberate the colorimetric equivalent of 1 mg of phenolphthalein from an excess of sodium phenolphthalein phosphate in 1 hr at 37° C at pH 5.4 or 9.7.

by the fixatives is not nearly so great as that of AcP-ase, and again a differential between the two fixatives exists. Whereas acetone fixation inactivates the original enzyme by 30–35%, alcohol destroys only 10–25% of the activity. In so far as alcohol is concerned, this confirms the findings of Gomori mentioned above (*3*). Here, as in the case of the AcP-ase, the bulk of the loss of enzyme occurs in the processes attendant upon embedding and sectioning, since an over-all loss of 70–80% is found after these processes, confirming Danielli's observation (*1*).

Since this investigation was undertaken with purely technical objectives, no attempt has been made to determine the cause of the behavior of these tissue enzymes during microtechnical procedures. Undoubtedly, a study of such factors as magnesium ion diffusion in relation to the type of fixative and to the size of the block of tissue, physical structures of the different tissues, and length of exposure to high temperature in the paraffin oven would result in data that might contribute to the explanation of the mechanisms involved, but such studies are beyond the scope of this paper.

From these data it may be concluded that (1) AcP-ase is inactivated by both acetone and alcohol fixation to a far greater extent than AlP-ase; (2) alcohol fixation preserves a somewhat greater amount of AlP-ase activity than does acetone fixation; and (3) the enzyme activity remaining in paraffin-embedded tissue sections is approximately 5% in the case of AcP-ase and 20–30% in the case of AlP-ase.

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A Simple Stereoscope for Viewing Double-Lens Camera Stereographs Without Transposition

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A single-film stereograph taken with a double-lens stereoscopic camera may be viewed qualitatively in correct perspective through the use of the stereoscope described herein, which accomplishes the necessary transposition of the two images by optical means. Therefore, the stereoscopic images do not have to be transposed physically, as would be necessary if they were viewed with the usual stereoscope, and the original spatial relationship between the images is preserved for any subsequent quantitative distance-measurements that might be made from the stereograph. Viewing such a stereograph with an ordinary stereoscope is to be avoided, since it results in a pseudoscopic view that is confusing under most circumstances (*1*).

A simple prism-stereoscope which has been built by the author for viewing untransposed stereographic transparencies is shown in schematic plan view in Fig. 1. A suitable light source (A) and a diffusing screen of translucent glass (B) provide uniform illumination for the untransposed stereograph (C). This is viewed by the observer (G) through the lenses (D) and the prisms (F). In the prism-stereoscope built for viewing stereographs taken on 35-mm color film with $2\frac{1}{2}''$ separation, the lenses are planoconvex, and each has a diameter of 3″ and a focal length of $4\frac{1}{2}''$. The lenses are cut and placed together about 1″ in front of the stereograph so that they provide complete enlarged virtual images of both views.

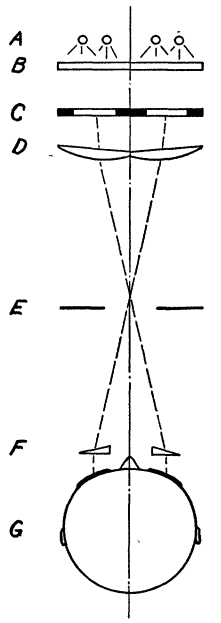

Fig. 1

To effect the necessary image transposition, by making the right eye of the observer see the left picture of the stereoscopic pair and vice versa, two 10-diopter deviation prisms are mounted about 12″ from the transparency. A mask (E) is interposed so that the right transparency is blocked from view by the right eye and the left transparency is not visible to the left eye. Since the glass-mounted stereograph is flat and each picture is viewed at an angle with this arrangement, a small amount of keystone distortion is present on the upper and lower margins of the pictures. This can be corrected by tilting the joined sections of the two lenses slightly away from the stereograph, as indicated in Fig. 1. The inside of the housing box (not shown) should be painted white in the region of A and B and dull black elsewhere. The stereograph (C) is inserted through a slot that provides for proper positioning of the images.

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