caused a decrease in yield with X1612 approximately as follows:

5 ml sulfite/L depression = 12–20 per cent.
15 ml sulfite/L depression = 20–35 per cent.
30 ml sulfite/L depression = 35–50 per cent.

but in nineteen of the combinations, addition of sulfite waste improved the yields with 1984–A. The range of increases with this strain was from 8 to 73 per cent. The mean increase was 37 per cent.

These experiments emphasized again the inherent differences in the two strains of mold for synthesis of penicillin and point to the necessity of furnishing 1984–A with more complex and partially linked precursors of the penicillin molecule.

The potencies reported in this paper were determined by standard cylinder plate assays using *Staphylococcus aureus,* NRRL 313 (F.D.A. strain 209P) as the test organism and a standard of calcium penicillin G.

ROBERTSON PRATT[1, 2]
JEAN DUFRENOY[1]
DIVISION OF MICROBIOLOGICAL RESEARCH,
THE CUTTER LABORATORIES,
BERKELEY, CALIF.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE HISTOCHEMICAL LOCALIZATION OF ADENOSINETRIPHOSPHATASE IN PLANT AND ANIMAL TISSUES

ADENOSINETRIPHOSPHATASE (ATPase) has become a focus of interest to research workers in widely diverse fields of biological and medical sciences because of its critical role in intermediary metabolism. The principle of Gomori's technique for the localization of alkaline[1] and acid,[2] glycerophosphatases in animal tissues by histochemical reactions has been adapted to the demonstration of ATPase; the various modifications that the glycerophosphatase procedures have undergone have been summarized in a recent review.[3] The principle of Gomori's technique is the precipitation *in situ* of the phosphate, liberated enzymatically, by calcium in alkaline media, and lead in acid media, and the conversion of the insoluble phosphate to the more easily visualized black lead sulfide.

The pH-optimum for ATPase in animal tissues[4, 5] occurs at about 9.0. Mouse heart and wheat were chosen to represent the two types of tissue, and since Booth[6] showed that other phosphatases in wheat have optimum activity at a pH of 5.1–5.2, this range of hydrogen ion concentration was tried for the ATPase and it was found to be suitable.

One innate difficulty with the method is the fact that the presence of free phosphate at a given histological or cytological location may obscure the phosphatase activity at this site. The difficulty is appreciably reduced when paraffin, rather than frozen, sections are employed, since much of the free phosphate is removed in the preparation of the paraffin sections. Of course, the tissue infiltrated with paraffin has undergone some loss in enzyme activity; however, this may be compensated by employing longer digestion periods.

The limited availability of ATP made it imperative that some modification of the usual procedure, employing a staining dish or Coplin jar full of substrate solution, be introduced. Accordingly a simple hanging-drop technique was devised that requires only one drop of the substrate solution for each tissue section. This technique is generally applicable to all cases where it is desirable to use a minimum of substrate or other reagent in histochemical tests.

The acid and alkaline substrate media are prepared in the following manner:

Substrate solution: Dissolve 5 mg of the Ba salt of ATP in 0.5 cc of 0.1 M HCl, add 0.1 cc of a Na_2SO_4 soln. to precipitate the Ba (1.11 per cent. Na_2SO_4 was required by our sample of ATP[7]), centrifuge and neutralize the supernatant liquid with 0.1 M NaOH.
Acid substrate medium: Combine in the following order: 0.5 cc of 0.1 M acetic acid, 0.25 cc of 0.1 M Pb $(NO_3)_2$, 0.25 cc of neutral substrate soln., 0.10 cc of 0.1 M $CaCl_2$, and 1.5 cc of 0.1 M sodium acetate. Centrifuge before use to remove turbidity.
Alkaline substrate medium: Combine the following: 0.75 cc of neutral substrate soln., 0.5 cc of pH 9.0 buffer (9.36 cc 0.1 M sodium barbital + 0.64 cc 0.1 M HCl), and 0.05 cc of 0.1 M $CaCl_2$. Centrifuge before use.
Control media: Replace the neutral substrate soln. by distilled water.

The enzyme activity is demonstrated as follows: Fix heart tissue in acetone for the preparation of both frozen and paraffin sections. Soften the wheat kernels by soaking in water for 4 to 6 hours before sectioning on the freezing microtome, or for 7 hours before fixing in absolute alcohol for the preparation of paraffin sections. Cover a frozen section (15 μ

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[1] With the assistance of Ruth Birch, Marjorie Decker, Iola Dunkle and Patricia Streator.
[2] Currently on leave from the University of California College of Pharmacy.
[7] The ATP and mouse hearts were obtained through the courtesy of Professor M. B. Visscher, Dr. H. G. Wood and Dr. M. F. Utter, of the Physiology Department of the University of Minnesota Medical School.

thick) or a deparaffinized section (10 μ thick), which has been fixed to a slide with a collodion film, with a small drop of substrate medium. Place a hanging-drop slide over it so that the drop is enclosed in the chamber formed by the depression. The drop should not touch the chamber walls at any point. Invert the slides, leaving the section covered by the hanging drop. For digestion periods longer than 4 hours it is necessary to seal the edges of the two slides with vaseline to prevent appreciable evaporation. Place in a 37° oven for the time indicated below:

Wheat kernel, frozen sections: embryo, 15–30 min.; aleurone cell region, 5–10 min.
Wheat kernel, paraffin sections: embryo, 2 hrs.; aleurone cell region, 1 hr.
Heart tissue, frozen sections: 2 hrs.
Heart tissue, paraffin sections: 18–24 hrs.

If vaseline was used, remove with benzol, and wash sections with 3 changes of distilled water. (In the case of alkaline ATPase, place in 1 per cent. $Pb(NO_3)_2$ for 15 minutes at this point, to convert the calcium phosphate to lead phosphate, and wash well in distilled water.) Dip into 2 per cent. acetic acid and rinse thoroughly with distilled water. Place in 2 per cent. ammonium sulfide for 2–3 minutes, wash with several changes of distilled water, dehydrate in 95 per cent. alcohol for 2–3 minutes followed by absolute alcohol for 5 minutes, clear in oil of thyme for 3–4 minutes, treat briefly with 3 changes of xylol, and finally mount in balsam.

Essentially the same procedure has been successfully adapted to the demonstration of glycerophosphatase and thiamine pyrophosphatase in sections of wheat kernels and sprouts. These studies, in addition to those on ATPase, as well as similar ones on other phosphatases in wheat, will be reported in detail elsewhere.

SUMMARY

A method has been described for the histochemical localization of acid and alkaline ATPase in plant and animal tissues respectively. A hanging-drop technique has been developed that permits the use of a minimum of substrate, *i.e.*, one small drop for each tissue section. ATPase in wheat was demonstrated for the first time.

DAVID GLICK
ERNA E. FISCHER

RESEARCH LABORATORIES,
RUSSELL-MILLER MILLING COMPANY,
MINNEAPOLIS, MINN.

DISCUSSION

THE EFFECT OF MOTION PICTURES ON BODY TEMPERATURE

IN SCIENCE for September 7, 1945, Dr. R. Barrington Brock, of Croydon, England, offered two items of criticism concerning my note on "The Effect of Motion Pictures on Body Temperature."[1]

(1) He wonders why "the figures show a similar rise in body temperature for all types of film," but the figures show nothing of the kind. I definitely stated that the oral temperatures on "movie" days varied from 99 to over 100° F. It was because the rise in temperature seemed related to the degree of excitement produced by the film that I suggested that the collective change in body temperature of a preview audience might be used to predict the box office success of a film. Evidently that conclusion escaped Dr. Brock's attention.

(2) This criticism rests on more solid physiological grounds—whether we are not dealing here with a "rise in body temperature occasioned by close contact with masses of other people in a confined space." That this is not the case is shown by the fall in body temperature of students who sit close together at regular university lectures. If the subject-matter of the lecture is not very interesting, and especially if the room is darkened for showing slides or scientific motion pictures, the degree of muscular relaxation and fall in temperature are often great enough to produce drowsiness and even sleep. Furthermore, in this country motion picture theatres are usually air-conditioned, and in the summer the air in them may feel unpleasantly chilly. We have found that sitting down and relaxing under such conditions produces an even greater lowering of the body temperature than occurs in a warm lecture room. However, a comparison of "movie" temperature figures for summer and winter showed no difference. This suggests that the temperature raising effect of motion pictures was sufficient to overcome the downward tendency resulting from air conditioning.

Thus, the rise in body temperature was related to the degree of excitement produced by the film and was in no way due to close contact with other people in a confined space. Indeed, I venture to predict that a comparable rise in body temperature will be found in persons who, in the privacy of their homes, have been listening for some time to a "hair-raising" melodrama over the radio or have been reading an unusually exciting book. The fact that some books act as soporifics, while others keep one widely awake long beyond the customary going-to-bed hour, is prob-

[1] SCIENCE, May 18, 1945.