(placenta, seeds, jelly and juice). Both salt and non-salt extracts were made. The salt extracts were prepared by dissolving 5 g of sodium chloride in 100 g of the blended tissue, holding for an hour at room temperature, and centrifuging out the solids. Non-salt extracts were prepared similarly.

In a separate set of salt extractions of the pericarp the activity of an aliquot held in boiling water for 5 min was compared with a non-boiled portion.

The salt extracts of the pericarp reduced the drain time during a 20-h period an average of 37·0 per cent, while the non-salt extracts produced essentially no change (Table 1). However, salt had little effect on the extraction from the locular material, which was higher in activity than the pericarp. The small difference due to salt with the locular material is probably due to the high juice content of this portion, which would probably contain the enzyme in solution from the breakdown of the cells around the seeds. The extracts held in boiling water were found to be inactive.

Table 1. Percentage Decrease in Drain Time of Sodium Carboxymethylcellulose during a 20-h Reaction Period with Tomato Fruit Extracts

Extract	Loss in drain time (%)
Pericarp	0·5
Pericarp plus NaCl	37·0
Locular	54·0
Locular plus NaCl	56·7
Pericarp plus NaCl	38·7
Pericarp plus NaCl, boiled	0·0

These results explain why Tracy[2] was unable to detect cellulase in tomato fruits although he did find the enzyme in many portions of the tobacco plant and in several other plants. He used expressed sap, which if taken from the pericarp only is shown here to be extremely low in activity. In addition, he used a short reaction time of 15 min, which was much too short a period to determine the very low activity.

The fact that a cellulolytic enzyme is present in tomato fruits is extremely important in studies on fruit softening since the effect of the cellulolytic enzyme must be assessed as well as the effect of the pectolytic enzymes.

This work was supported in part by a grant (*EF*–169) from the U.S. Public Health Service, National Institutes of Health.

Chesley B. Hall

Department of Food Technology and Nutrition,
Florida Agricultural Experiment Station,
University of Florida, Gainesville.

[1] Bell, T. A., Etchells, J. L., and Jones, I. D., *U.S. Agric. ARS*-72-5 (1955).
[2] Tracy, M. V., *Biochem. J.*, **47**, 431 (1950).

Detection of Phosphorothioate Pesticides

The use of Gibbs's reagent[1] (2 : 6-dichlor- or 2 : 6-dibrom-*p*-benzoquinone-4-chlorimine (DBQC)) for locating certain organic thiophosphate pesticides on paper chromatograms was reported by Menn, Erwin and Gordon[2]. The reagent forms coloured derivatives with a variety of compounds in aqueous alkaline solution, but the development of a red colour in neutral or slightly acid conditions appears to be quite specific for the P→S group, certainly when linked with solubility of the test substance in petroleum solvents. The application of thin-layer chromatography with such a positive identification system offers the possibility of a rapid screening test for these pesticides in toxicological samples.

Using a commercially available silica gel/plaster of Paris mixture having a *p*H 6·5 (determined as a 10 per cent suspension in distilled water) only a weak colour reaction was given with quite large quantities of various thiophosphate pesticides on spraying the developed place with 0·5 per cent DBQC in *cyclo*hexane and heating at temperatures up to 120° C. However, if hydrochloric acid is added to the mixture used to prepare the plate, to give about *p*H 4, very intense red spots are obtained after spraying and heating to 100° C for 5–10 min; the background remains virtually colourless at this temperature. The colour of the spots slowly changes to orange but is then stable for several days; the red colour may be restored by lightly spraying the plate with water.

So far as can be ascertained, the reaction is given by all organic phosphorothioates and phosphorodithioates: 1 μg produces a very distinct spot with the limit of detection about 0·1–0·2 μg. Typical R_F values in benzene-1 : 2-dichlorethane (1+1) are methyl parathion 0·78, 'Delnav' (2 spots) 0·60 and 0·53, 'Asuntol' 0·37, and 'Diazinon' 0·25.

Some variation in the quantity of hydrochloric acid required (which can be determined by titration to *p*H 4) may probably be needed with different silica gels, although a two-fold excess does not seem to cause any adverse effect. Normally 10-g silica gel *G* (containing 13 per cent plaster of Paris) is stirred with 10 ml. water and 10 ml. 0·1 N hydrochloric acid, and the plate (0·75 mm layer) dried at 90°–95° C for 30 min.

A concentrated benzene or petroleum ether extract of crops or foodstuffs can usually be applied directly to the plate since interfering substances (which give dark brown or black stains on colour development) remain at or near the origin spot. With petroleum ether extracts of stomach contents there is sometimes more interference, but adequate purification can be achieved by partitioning with acetonitrile and spotting the acetonitrile extract.

D. P. Braithwaite

Veterinary Research Laboratory,
P.O. Kabete, Kenya.

[1] Gibbs, H. D., *J. Biol. Chem.*, **72**, 649 (1927).
[2] Menn, J. J., Erwin, W. R., and Gordon, H. T., *J. Agric. Food Chem.*, **5**, 601 (1957).
[3] Fearon, W. R., *Biochem. J.*, **38**, 399 (1944).

PHYSIOLOGY

Oxygen Depletion of a Limited Reservoir by Human Conjunctiva

Although the passage of oxygen across the tear-cornea interface is perhaps the best-known example of a direct pathway[1–3] from atmosphere to tissue, other superficial areas can also be demonstrated to receive oxygen in similar manner.

An ocular tissue particularly interesting in this respect is the conjunctiva. A significant flux across its air-exposed surface might not be unexpected since the maximum oxygen tension of its endogenous[4] vessels can be no more than that of arterial blood, 100 mm mercury, which is considerably less than that of air (155 mm mercury). Also bulbar conjunctiva, especially in close proximity to the cornea, might serve as an oxygen pathway between the atmosphere and the cornea.

To measure oxygen flux across the air-conjunctiva interface, a flat surfaced polarographic oxygen electrode (Clark type[3,5], membrane covered) unit, 6 mm in diameter, was placed in direct contact with unanæsthetized human bulbar conjunctiva. The polyethylene membrane, fitted over the entire end of the housing, served both as an oxygen reservoir and the limiting boundary for a thin layer of *p*H 9 borate buffer solution (0·1 M in potassium chloride) bathing the platinum-reducing electrode. This membrane ensures good electrical stability and is the oxygen reservoir of known volume for quantitative analysis. By using a 12μ-thick membrane as the oxygen reservoir directly below a 25μ-diameter oxygen cathode, the observed drop in current is almost entirely controlled by the decrease in oxygen tension of the membrane and tissue immediately below the cathode.

Current from the electrode was measured with a Beckman model 160 physiological gas analyser and was