stored at this temperature would not be so apt to be injured by gases from apples or other fruits. It appears that a temperature of 34° to 36° is best for carnations if they are kept in a room free from ethylene.

In the light of these findings it seems desirable that results of flower storage investigations which might have been influenced by the gases from ripening fruit should be repeated. It is also suggested that the effect of ethylene, whatever its source, on plants and plant parts other than cut flowers should be fully investigated as a factor in storage problems.

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THE RELATION OF INTERNAL SURFACE TO INTERCELLULAR SPACE IN FOLIAGE LEAVES

THE relation of the exposed cellular area of the mesophyll of the foliage leaf to the volume of intercellular space has been of considerable interest because the relation has an important bearing on transpiration rate and on other types of gas exchange. Although the volume of intercellular space was measured by Unger as early as 1854 and has been measured by several investigators since, the relation of internal surface to volume of intercellular space has been largely a matter of conjecture.

In a sample of twenty leaves from four alfalfa plants, the coefficient of correlation (r) between the internal-external surface ratio and the volume of intercellular space per sample area, as shown in Table 1,

TABLE 1

THE COEFFICIENT OF CORRELATION (r) AND ITS LEVEL OF SIGNIFICANCE (P) BETWEEN INTERNAL-EXTERNAL SURFACE RATIO AND VOLUME OF INTERCELLULAR SPACE AND BETWEEN INTERNAL-EXTERNAL SURFACE RATIO AND PERCENTAGE VOLUME OF INTERCELLULAR SPACE OF FOLIAGE LEAVES

Leaf samples	Intercellular space	r	P
Alfalfa	Volume	+0.874	< 0.01
Alfalfa	Percentage volume	+0.629	< 0.01
16 species	Volume	+0.463	< 0.10
16 species	Percentage volume	+0.071	> 0.10

was +0.874; and between the internal-external surface ratio and the percentage volume of intercellular space, the coefficient of correlation was +0.629. Although the correlation coefficient is higher between the internal-external surface ratio and volume of intercellular space than between the internal-external surface ratio and percentage volume of intercellular space, for both values the probability of chance occurrence (P) is less than 0.01, and the correlation coefficients are highly significant. The relation of the internal-external surface ratio to other mesophyll factors is expressed by the equation

$$R = \frac{t\,v\,(1-v)\,K}{d}$$

where R = the internal-external surface ratio, t = leaf thickness, v = percentage volume of intercelluar space, d = cell diameter, and K = a constant.

Random samples of leaves of sixteen different angiosperm species from various parts of the world showed no significant correlation (+0.071) between the internal-external surface ratio and the percentage volume of intercellular space, but showed a moderate positive correlation (+0.463) between the internal-external surface ratio and the volume of intercellular space (Table 1). For the latter value, P lies between 0.10 and 0.05 (Table 1); thus the correlation coefficient is not significant.

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THE ENZYMATIC DEACETYLATION OF HEROIN AND CLOSELY RELATED MORPHINE DERIVATIVES BY BLOOD SERUM

IN preliminary experiments designed to study the effect of morphine on choline esterase activity it was found that morphine salts were precipitated as the alkaloidal base in bicarbonate Ringer solution. An attempt was made to obviate this difficulty by using the more soluble and physiologically more active diacetylmorphine (heroin).[1] An apparent stimulation of the activity of choline esterase led to the measurement of the effect of serum on heroin. It was found that rabbit and human blood sera deacetylate diacetylmorphine.

The measurements of the rates of deacetylation were made with Barcroft manometers at 37.5° C. under an atmosphere of 95 per cent. oxygen and 5 per cent. carbon dioxide. The serum was tipped from a side arm of the manometric flask into a bicarbonate-containing solution of the acetylated morphine, and the carbon dioxide liberated was measured manometrically at desired intervals.

Observations were made using sera from six male albino rabbits, all fed Purina rabbit chow and lettuce. Sera (0.05–0.5 cc) from three of the animals, when added to diacetylmorphine (5.0 mgm), caused a rapid liberation of carbon dioxide corresponding in quantity to 85 per cent. of the theoretical for the hydrolysis of both acetyl groups. The other three animals hydrolyzed the heroin more slowly, and liberated carbon dioxide corresponding to 85 per cent. of the theoretical

[1] I am indebted to Dr. L. F. Small, of the National Institute of Health, for furnishing the morphine derivatives and for consultation on their chemistry.

for one acetyl group. Repeated observations over a period of two months always gave identical results for each rabbit.

By substituting monoacetylmorphine for the diacetylmorphine it was found that the animals that were able to remove both acetyls from heroin were able to hydrolyze the 6-carbon acetyl in monoacetylmorphine. Sera from the other three rabbits did not liberate acetic acid from the monoacetyl compound. Therefore, all the rabbits were able to remove the 3-carbon acetyl group, but the sera of only three of the animals hydrolyzed both acetyl groups present in diacetylmorphine.

Further work using diacetyldihydromorphine as the substrate showed that all six rabbits were able to remove acetic acid from this compound, equivalent to approximately 100 per cent. of the theoretical for one acetyl group. Again, however, there was a distinct separation of the rabbits into two groups of three animals on the basis of the rate at which hydrolysis took place. The sera from the three rabbits that were unable to deacetylate monoacetylmorphine hydrolyzed the dihydrodiacetylmorphine at a much slower rate.

At this point it was predicted that the diacetyldihydromorphine was hydrolyzed at the 3-carbon, and subsequent determinations with monoacetyldihydromorphine as substrate proved this to be true, since none of the animals were able to deacetylate this compound.

Preliminary experiments have shown that human blood serum is able to deacetylate heroin, but at a distinctly slower rate than any of the sera from the rabbits so far investigated.

Physostigmine inhibits the activity of the enzyme responsible for the deacetylation of the acetylated morphine derivatives. This indicates the possibility that the enzyme might be choline esterase. However, all six rabbit sera have almost identical capacity for hydrolyzing acetylcholine. Also, the human sera so far investigated have much higher concentration of choline esterase than the rabbit sera and at the same time a lesser capacity for the hydrolysis of heroin.

From these results it seems probable that, in the rabbit at least, the difference in potency of heroin and morphine might be fundamentally due to physical factors such as solubility rather than chemical structure, since it appears likely that the animal, in the final analysis, is reacting to morphine, whichever of the two alkaloids is injected. The same applies to monoacetylmorphine.

It would be of considerable interest to determine whether the esterase attacking the acetylated morphines is present in the tissues, especially the central nervous system and to extend the investigation to include other species of animals. It is also possible that certain other morphine derivatives, after entering the body, are converted into morphine. If so, this would be of considerable aid in clarifying some of the similarities and differences in physiological activity that have been found among the chemical compounds related to morphine. The investigation of these possibilities and others not so obvious is now planned.

This is a preliminary report and will be published in detail elsewhere.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A TECHNIQUE FOR THE INTRAVENOUS INOCULATION OF CHICK EMBRYOS

IN the course of experimentation on the growth of various viruses in chick embryos, the need of a simple technique for the inoculation of embryos directly into the blood stream became very evident.

The method of Goodpasture, *et al.*,[1] consisting in removal of that portion of the shell over a vein or the air sac, application of mineral oil to the membrane to render it transparent and then picking up the veins for injection and subsequent searing of these veins, was tried. While some success by this method may be noted, it has several disadvantages, notably difficulty encountered in injection due to the mobility of the vein during inoculation.

[1] Polk, Buddingh and Goodpasture, *Am. Jour. Path.*, 14: 1, January, 1938.

Secondly, there was considerable hemorrhage on withdrawal of the needle even after cauterization of the vein at two points before withdrawing the needle.

A third difficulty is that the removal of the shell cap over the air sac exposes a large area and subsequent maintenance of sterile conditions is difficult even after sealing with Scotch tape.

Accordingly, the following procedure was developed at the laboratories of the Pathological Division of the Bureau of Animal Industry.

Ten- to eleven-day-old embryos were found to be the youngest which could be easily injected routinely. The eggs are candled to locate a vein of the terminal sinus which is fairly straight and which lies embedded in the chorio-allantoic membrane.

This section of vein is then marked on the shell for about 1–1.5 cm, and an arrow indicating direction of blood flow is marked nearby.