as that described by Wright (*2*), this card file has the advantages of lower cost and greater simplicity of matching spectra. It is not readily searched for subject, author, functional groups, and other detailed information possible to a punch card system, but it is recommended for chemical studies paralleled by infrared identification on a limited class of chemicals, most of which may be identified simply by the three strongest bands in the 9–15-μ region. Such a card file has proved valuable in the rapid identification of over 75 purines, pyrimidines, nucleosides, nucleotides, and nucleic acids, using the mineral oil mull method of sample preparation. Infrared identification of nucleic acid hydrolyzates by this method is now being attempted.

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Colloidal Dispersion of Chloroplast Material[1]

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Chloroplasts, isolated from leaves and placed in a suitable medium, are able when illuminated to liberate oxygen from water (*1*). This photochemical activity is little diminished when chloroplasts are broken into fragments barely visible under a microscope. In order to observe the properties of chloroplast material having submicroscopic particle size, it is necessary to prepare colloidal dispersions of the material. These dispersions are consistently and easily prepared by use of the apparatus described here. It is anticipated that other small particles, such as blood cells, unicellular organisms, and homogenates of animal tissue, for example, can be disintegrated in the same apparatus.

It was found impractical by blending, grinding with sand in a mortar, or using a colloid mill to disperse an appreciable fraction of chloroplast material. Exposure of a suspension of chloroplasts to ultrasonic energy resulted in the disintegration of part of the material to colloidal dimensions. Dispersion was much more conveniently accomplished by using high pressure to force a suspension of chloroplasts through a small orifice. Fig. 1 illustrates the device which was used.

It is made as follows: A 1-in. hole is bored 4 in. deep in the center of a 3-in. steel bar 5 in. long. A smaller hole is bored the rest of the way through the bar and is threaded to take a Hoke steel needle valve. A steel piston slightly less than 1 in. in diam has attached to it a leather washer which fits snugly in the bore of the cylinder. The top ¼ in. of a No. 5 rubber stopper makes a watertight seal between the piston and the cylinder. The leather washer is necessary to prevent the rubber from becoming wedged between the piston and cylinder wall. The top of the piston is drilled and tapped to take a ¼-in. bolt. This provides means for attaching a crosspiece to the piston, in order to withdraw it from the cylinder.

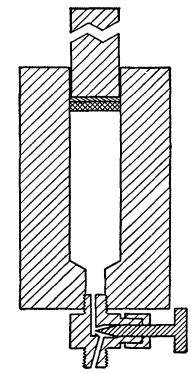

FIG. 1. Dispersion unit.

In operation, 40 ml of a filtered suspension containing 20–30 mg chloroplasts/ml is placed in the dispersion unit. The rubber washer is inserted, broader side down, followed by the piston. The chamber is inverted, the valve opened, and the air forced out. With the valve closed again, the chamber is placed on its base (not shown in the figure) and is put under a 60-ton hydraulic press. After the desired pressure is built up in the chamber, the needle valve is barely opened, so that the liquid runs through at the rate of a few ml/min. During passage of the material through the valve, the pressure is maintained by use of the pump on the hydraulic press. A unit with a smaller bore and with a built-in needle valve for use with laboratory presses has been designed but not tested.

Following passage through the needle valve, the liquid is diluted to contain about 0.5 mg chlorophyll/ml, then it is centrifuged 1 hr at 12,000 × gravity. This empirically determined dilution gives the best yield of material in dispersion at nearly the highest concentration. We consider the chloroplast material which is not sedimented in 1 hr at 12,000 × gravity to be in colloidal dispersion. After separation from the sediment, the dispersion of chloroplast material is dark green, appearing very clear with the light behind it but almost opaque when illuminated obliquely.

Using a pressure of 20,000 psi to force a suspension through the valve, ⅔–¾ of the chloroplast material is colloidally dispersed. In order to do this in one passage through the valve, it was found advisable to freeze the leaves just before isolation of the chloroplasts. Otherwise two passages through the valve were required to attain the same degree of dispersion.

The photochemical activity (*2*) per unit of chlorophyll is about a fourth as great in such dispersions as the activity of intact chloroplasts. Several tests were made to see whether the large loss of activity might be due to the effect of high pressure alone, or to passage of the material through the valve at high pressure. Chloroplast suspensions held 30 min in the chamber at pressures of 5,000, 10,000, 15,000, and 20,000 psi, without passing

[1] The progress of this work was greatly facilitated by a grant from the Research Corporation.

through the valve, lost respectively 7%, 20%, 37%, and 78% of their initial activity. Under these conditions no fragmentation of the chloroplasts was observed. The loss of activity was found to be roughly proportional to the time under pressure. Keeping a chloroplast suspension under a pressure of 20,000 psi for 1 min, 15 min, and 30 min caused the loss of 8%, 44%, and 78% of the original activity. The loss of activity, due to pressure alone, should be small if the time occupied in the pressing operation is made short.

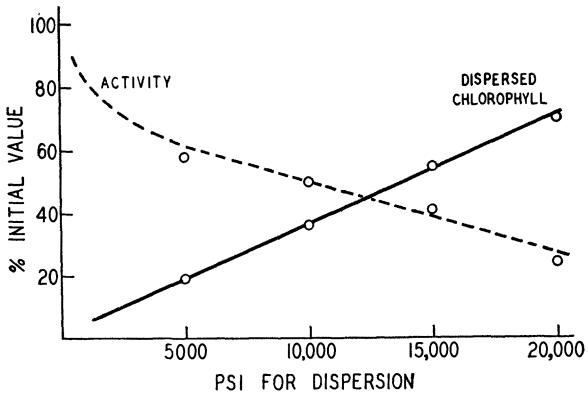

FIG. 2. Completeness of dispersion of chloroplasts and loss of activity at different pressures.

At a pressure of 20,000 psi in each case, chloroplast suspension was forced through the needle valve, set to permit flow at 2, 4, 8, and 16 ml/min. Surprisingly, almost identical yields of dispersed material and losses in activity were observed within this range, that is, the dispersions contained about three-fourths of the total material, with an activity about one-fourth the initial value. The low activity of dispersed chloroplast material seems to be attributable more to its small particle size than to an effect of the pressure used in preparing the dispersion.

Fig. 2 shows the results of forcing chloroplasts through the needle valve at different pressures. In each case the valve was adjusted for a rate of flow between 8 and 10 ml/min. The solid curve shows the percentage of the chloroplast material put into colloidal dispersion, determined by chlorophyll analysis. The broken curve shows the photochemical activity per unit of chlorophyll for the dispersions, compared to the activity of unbroken chloroplasts as 100%.

A dilute suspension of yeast showed about 20% broken cells after passing once through the valve. In collaboration with C. E. Clifton and W. E. Clapper, an experiment was performed in which a dense suspension of *E. coli* was passed through the valve. It showed a several fold increase in glutamic acid decarboxylase activity, presumably due to release of the enzyme from broken cells. The general applicability of the described procedure to a wide diversity of material has not been tested, but we wish here to call to the attention of workers in various fields a simple and possibly useful method for preparing colloidal dispersions of other biological materials.

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Lesions of the Coronary Arteries and Great Vessels of the Dog Following Injection of Adrenalin. Their Prevention by Dibenamine[1]

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The vascular-damaging properties of adrenalin and other vasopressor amines have been extensively investigated. Hueper's review (*3*) indicates that medial necroses and calcifications of the rabbit's and rat's aorta have been the most frequently observed effects. Duff and his associates (*2*) noted also necrosis and hyalinization of the coronary arterioles of rabbits following repeated injections of tyramine. In dogs, consistent arterial changes have not been described following the administration of adrenalin.

The present preliminary note reports the occurrence of segmental necrosis of the coronary arteries, and necrosis and hemorrhage of the media of the pulmonary artery and aorta of the dog following massive intravenous injections of adrenalin. It further reports preliminary experiments indicating that the development of these lesions is prevented by pretreatment with the adrenolytic substance, Dibenamine (*N*, *N*-dibenzyl-β-chloroethylamine).

Four groups of dogs were studied. The first three dogs were given adrenalin intravenously under pentothal anesthesia in sufficient quantity to keep their mean femoral arterial pressures between 220 mm and 280 mm Hg for 30 min. From 8 to 9 ml of standard 1: 1000 adrenalin solution (Parke Davis) was required. These dogs recovered from the anesthesia but died within 24 hr.

In the second series, nine unanesthetized dogs of 10–12 kg were given, on each of three successive days, 1 ml of adrenalin intravenously every 15 min over a period of 1 hr. Their condition remained good throughout, and they were sacrificed between the 4th and 8th day.

The third group of seven dogs were given adrenalin as in the first and second series, but also received Dibenamine hydrochloride,[3] 20 mg/kg intravenously at least 30 min before the adrenalin injection (*4*). Blood pressure determinations carried out on several of these animals

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[3] Received through the courtesy of Smith, Kline, and French Laboratories.