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.

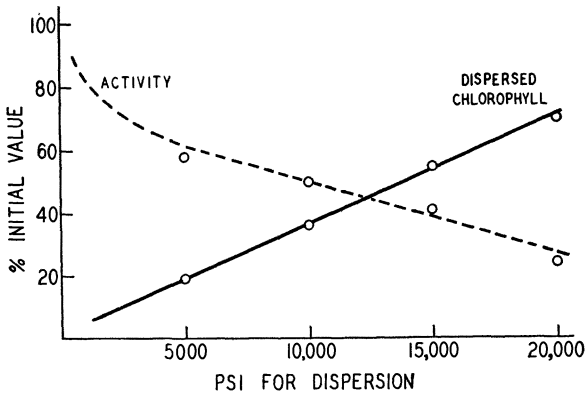

FIG. 2. Completeness of dispersion of chloroplasts and loss of activity at different pressures.

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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[2] With the technical assistance of E. Iannucci, P. Integlia, and F. Ferraiolo.

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

made it clear that no rise in systemic arterial pressure occurred during or following the adrenalin injections. At the same time it was noted that the accelerating action of adrenalin upon the heart was not inhibited by the Dibenamine premedication. As a control, two dogs were given Dibenamine hydrochloride alone, 20 mg/kg, intravenously, each day for 3 days.

Autopsies were performed promptly with the termination of each experiment and histologic study of the tissues was carried out.

Animals of the first two groups—which received massive doses of adrenalin alone—showed striking changes in the coronary and pulmonary arteries, aorta, and myocardium. There were no comparable cardiovascular lesions, except for a few scattered areas of myocardial necrosis in the animals of the third group, which received Dibenamine followed by adrenalin. No cardiac or arterial changes were found in the dogs of the fourth group, given Dibenamine alone.

Segmental necrosis of many of the small coronary arteries and arterioles, as well as extensive hemorrhages and necrosis of the pulmonary artery and aorta, was present in the dogs given adrenalin. Hemorrhage was prominent also in the coronary vessels, often occurring, as in the aorta, at the origin of branches. Medial necrosis of the coronary arteries was frequently accompanied by periarteritic cellular inflammatory exudate or by perivascular fibrosis, depending on the age of the lesion. Necrosis of the aortic adventitial vasa vasorum was prominent. An occasional necrotic arteriole of the gastric submucosa was also encountered. There were no renal or cerebral arterial lesions. Interstitial myocardial edema and focal myocardial necroses were observed. Ten of the 12 dogs receiving adrenalin alone had many lesions of the types described. Two had only minimal changes.

The necrosis of coronary arteries observed in the present experiments following injections of adrenalin reproduces faithfully the acute arterial changes often encountered throughout the body in rapidly developing hypertension in man. Similar arterial necroses are well known in dogs and other animals following certain types of experimentally produced renal insufficiency (*5*).

In the present experiment pretreatment with Dibenamine abolished the rise in systemic pressure of subsequent adrenalin injections and prevented the development of arterial lesions. This suggests that in the untreated groups the severe hypertension was a critical factor in the etiology of these changes. Recent experiments of Byrom and Dodson (*1*) have suggested a direct relationship between increased intra-arterial pressure and necrosis of renal arterioles. Experiments are now in progress to see if adrenolytic drugs will prevent the development of acute arterial damage that follows other experimental procedures.

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The Presence in Animal Organs and Human Blood of a Peptide Detected by Paper Chromatography

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Recently, Borsook *et al.* (*1*) reported the existence of a peptide fraction in various tissues of several animal species. This fraction was obtained from extracts of tissues by means of chromatography on starch columns. In this laboratory we have detected a similar or perhaps identical fraction in several organs of the rat, in human blood and tumors, and in Witte's peptone. The fraction was detected first, and isolated later, exclusively by means of paper chromatography (details for the isolation technique to be reported later). Analytical data revealed that this peptide fraction contains 14% nitrogen and is made up of at least the following amino acids: tyrosine, methionine, proline, arginine, hydroxyproline, leucine and/or phenylalanine, alanine, serine, glycine, threonine, glutamic acid, aspartic acid, and lysine.

At present there are insufficient data to warrant the assumption that this fraction is a single peptide. The chromatographic behavior of the fraction does not vary with the tissue of origin. In every case the R_f values were found to be 0.95 in phenol and 0.05 in 2,4-lutidine. Furthermore, the ratio of the value of amino nitrogen after hydrolysis to the value before hydrolysis averages 14.5, which is in close agreement with the value of 14.2 reported by Borsook for "peptide A."

It is conceivable that we are dealing with the same peptide detected by Borsook in various organs of several animal species.

Reference

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