min) at this step, although not essential, is helpful in removing very finely precipitated particles. The clear supernatant liquid is precipitated in 4 volumes 95% ethyl alcohol, the fibrous precipitate is washed in more alcohol, then in absolute alcohol, and finally in ether, and dried.

Further treatments are in large measure determined by the properties of the different preparations and of the individual making the preparation. In general, the material should be redissolved in 2.0 *M* NaCl, and treated again with the detergent, centrifuged, and precipitated in alcohol, then dissolved in distilled water, and precipitated with alcohol. This final alcohol precipitation should be repeated at least three times.

The product obtained is white, fibrous, hygroscopic, and readily soluble in water, to give a perfectly clear but viscous solution.

The principal merits of the preparation lie in the exploitation of the capacity of the chloroform and of the efficiency of the detergent for the removal of protein from the DNA–protein extract. The efficiency of the chloroform treatment will determine the necessary extent of the detergent treatment; the alcohol precipitations are a final purification, primarily for the removal of the detergent.

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The Existence of Antifibrinolysin Activity in Platelets[1]

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Recent studies of fibrinolysin (plasmin) and antifibrinolysin (antiplasmin) have advanced our knowledge of this enzyme system (*1–6*) considerably, owing largely to chemical purification work and methods for quantitative measurement of activity. Plasma of most animals examined has been found to contain profibrinolysin and antifibrinolysin (*7*). Quantitative studies show that antifibrinolysin concentration of plasma may fluctuate in various diseased states, and especially interesting is the rise in its concentration with pteroylglutamic acid deficiency in chicks (*1, 2*).

Apparently, in all the interesting studies of plasma, no one has attempted to find, or by chance found, antifibrinolysin in platelets or other formed elements. Its existence in platelets came to our attention when we were studying the platelet clottable factor which is evident in our platelet extracts. It was of interest to determine whether the clottable factor of platelets is fibrinogen itself or a substance with properties nearly like fibrinogen. Among other things, in pursuit of this problem, an attempt was made to lyse the platelet clottable material with purified fibrinolysin, prepared by Loomis (*8*). It was found that the clottable factor would not lyse even though strong solutions of purified fibrinolysin were mixed with it. This finding could be interpreted to mean that the clottable factor was itself resistant to fibrinolysin. Another possible interpretation, however, was that the platelet clottable factor preparation contained antifibrinolysin, and subsequently many experiments have confirmed this.

In a number of experiments an attempt was then made to determine the quantity of antifibrinolysin present in platelets and to compare this quantity with the concentration of normal plasma. For this purpose, bovine materials were used throughout. Platelets were obtained by commonly used differential centrifugation procedures, and a suspension in physiological saline solution was made. This suspension contained about 30 times more platelets, by actual count with the phase contrast microscope, than are found in cow blood. This platelet suspension was frozen and subsequently thawed. This freezing and thawing, in our experience, is an effective way of liberating materials of platelets. The thawed preparation was lightly centrifuged to remove any particulate material. A few units of purified thrombin (*9*) were then added to clot out the so-called clottable factor first found to be in platelet extracts by Ware, Fahey, and Seegers (*10*), all added reagents having been found to contain no apparent antifibrinolysin activity. The assay (*1*) for antifibrinolysin content of this platelet preparation showed that it contained 2310 u antifibrinolysin/ml. Quantitative data on the various volumes involved were carefully kept. Simultaneously, oxalated bovine plasma was found to contain 222 u antifibrinolysin/ml. This is approximately the same as found by Guest, Ware, and Seegers (*1*). By assuming a hematocrit of 45% and disregarding a correction for anticoagulant volume, it was possible to estimate the distribution of antifibrinolysin activity in plasma and platelets. The platelet compartment contained approximately 70% as much antifibrinolysin as was obtained from the plasma of the same sample of blood. Even though the experiment was done several times, this cannot be regarded as an exact quantitative statement, but, rather, as an order of magnitude concept, because there were some quantitative considerations that could not be taken into exact account.

From our experiments there is no way of knowing whether the antifibrinolysin of platelets is the same substance as that of the plasma. However, the discovery of antifibrinolysin activity in platelets brings up that question for future consideration, for it is

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possible that the antifibrinolysin activity of plasma is derived from the platelets.

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Protection Against Alloxan Diabetes by Mannose and Fructose

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In a previous communication (*1*) from this laboratory, it was reported that glucose, given prior to the injection of diabetogenic doses of alloxan, prevented diabetes in rats.[2] The present communication reports the results of similar experiments done with the other physiologically important hexoses—namely, mannose, fructose, and galactose.

Rats fasted overnight were given diabetogenic doses (40 mg/kg) of alloxan intravenously at different intervals of time after intravenous administration of varying doses of the above sugars. Blood sugar values of the rats were then determined daily for 7 days. The urine was also tested daily for the presence of sugar during the experimental period. A blood sugar value over 180 mg% for 2 consecutive days and the presence of sugar in the urine for a similar period of time were considered to indicate a diabetic condition in the animals. The results are given in Table 1.

Thus mannose and fructose, given prior to the injection of diabetogenic doses of alloxan, prevented diabetes in rats. Galactose had no such preventive action. The present results, considered together with those given in the previous communication (*1*), show that the preventive abilities of glucose and mannose are practically of the same order. Mannose is possibly slightly less effective than glucose. Fructose, however, is obviously much less effective.

Inhibition of essential sulfhydryl enzymes of the β-cells has been suggested as the cause of the diabetogenic action of alloxan (*2*). Reversal of the action of alloxan by sulfhydryl compounds (*3*) has added strength to this hypothesis. Mammalian hexokinase is a sulfhydryl enzyme, being inactivated by alloxan and reactivated by cysteine (*4*). There is considerable evidence (*5, 6*) that this enzyme is fundamentally related to the condition of diabetes in animals—the activity of the enzyme being inhibited in diabetes. Hexokinase is a nonspecific enzyme acting on glucose, mannose, or fructose. The affinity of fructose for the enzyme is, however, low compared to that of glucose or mannose for it (*7*). In addition to the above nonspecific hexokinase, mammalian tissues may contain a specific fructokinase, which does not act on glucose or mannose (*7*). Galactose is attacked by another specific enzyme, galactokinase (*8*). The ordinary hexokinase is presumably present in the β-cells; for, like all other cells, the β-cells must also ordinarily obtain energy from the oxidation of glucose, the first step in the process being catalyzed by the above enzyme.

The results obtained show that the relative abilities of the hexoses to prevent the diabetogenic action of alloxan are directly comparable to their affinities for hexokinase.[3] They also show a close similarity with the results obtained on the protection of yeast hexokinase against inactivation by proteolytic enzymes by the above sugars (*7*). One therefore wonders whether the protection against the diabetogenic action of alloxan by glucose, mannose, and fructose is due to a protection of the hexokinase in the β-cells against alloxan poisoning and whether inhibition of β-cell hexokinase is the primary cause of the diabetogenic action of alloxan. It is also interesting to speculate whether the results reported here offer any clue to the understanding of the selectivity of alloxan for the

TABLE 1

EFFECT OF PREVIOUS ADMINISTRATION OF MANNOSE, FRUCTOSE, AND GALACTOSE ON THE PREVENTION OF DIABETES CAUSED BY INTRAVENOUS ALLOXAN (40 MG/KG) IN RATS

Sugar injected	Dose of sugar injected (g/kg)	Interval between injection of sugar and alloxan (min)	No. rats used	No. diabetic rats
Mannose	1	5	6	0
	1	10	6	5
	2	5	5	0
	2	10	6	0
	2	15	6	6
Fructose	2	5	5	5
	5	5	6	0
Galactose	2	5	5	5
	5	5	5	5

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[2] Similar results (unpublished) have been obtained with rabbits.

[3] It may also be noted that the relative abilities of the hexoses to protect against alloxan diabetes are directly comparable to their effectiveness to alleviate hypoglycemia in animals.