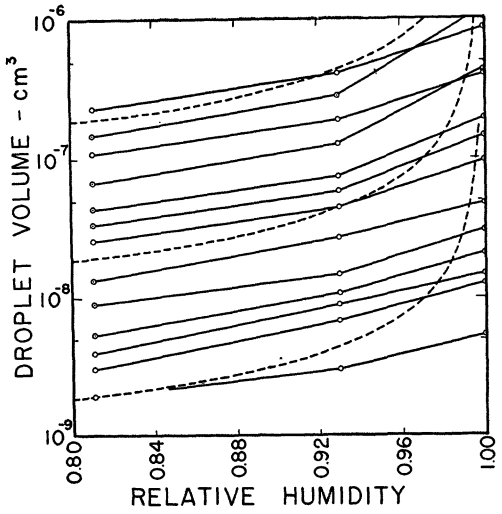

Fig. 1. Relationship between droplet volume and relative humidity for hemispherical droplets of NaCl solution on a Dri Film surface. Solid lines, experimental; dashed lines, calculated.

of Teflon or of glass coated with Dri Film SC-87 (*3*). Both of these surfaces give contact angles very close to 90°, so that the droplets were practically hemispherical. Diameters of the droplets were measured by a microscope, looking through a window in the top of the test chamber. A number of droplets were measured at three values of relative humidity, 1.00, 0.930, and 0.811, maintained by a small tray containing, successively, water, a saturated $(NH_4)H_2PO_4$ solution, and a saturated $(NH_4)_2SO_4$ solution. These solutions were chosen because they maintain relative humidity practically independent of temperature (*4*) in the region of the temperature used (25°C), thus eliminating the need for precise temperature control. After closure of the small chamber, equilibrium was reached essentially in 15 to 20 min for the smaller droplets and in about 1 hr for the largest droplets used. No measurable change in droplet size occurred thereafter in times ranging up to several days.

Representative experimental data are shown in Fig. 1, where the hemispherical droplet volumes are plotted as a function of the relative humidity. The experimental points are shown as circles; the solid lines join points applying to the same droplet. The data shown were taken with the droplets on a Dri Film support, but closely similar results were found with the Teflon support.

For comparison, calculations were made of droplet volume versus relative humidity for three NaCl masses, 5×10^{-10}, 5×10^{-9}, and 5×10^{-8} g, using the *International Critical Tables* data (*5*) for equilibrium concentration versus relative humidity and (*6*) for solution densities. The results are shown as the three dashed curves in Fig. 1. The theoretical curves are asymptotic to the line RH = 1.00 if the Thomson-Gibbs effect is neglected; inclusion of the Thomson-Gibbs effect makes no appreciable difference within the range of the graph but causes the curves to intersect RH = 1.00 several decades above the top of the graph.

Rough measurements of the sizes of the solid NaCl particles left after complete evaporation of the droplets indicate that the NaCl contents of droplets measured at 0.811 relative humidity are probably within a few percent of those calculated by the method used for the theoretical curves. The discrepancy between the forms of the experimental and theoretical curves indicates, however, that at 0.93 relative humidity the mass of NaCl will be underestimated by about 20 percent; at 0.96 relative humidity it probably will be underestimated by about 35 percent.

Although the present measurements refer to NaCl only, it is expected that similar results will be found with other salts. Errors of the afore-mentioned magnitudes may not be very important, of course, in some applications of the isopiestic method. But if it is necessary that errors be kept down to a few percent, humidities apparently should be used that will result in near-saturation of the droplets.

No satisfactory explanation has been found for the apparent difference in vapor pressure behavior between the solution in small hemispherical droplets and in larger, more or less plane, areas. It might be suspected that the discrepancy is due to a decrease in the surface free energy in the neighborhood of the 90-deg contact with the supporting surface; if this is true, it would be expected that continual convection would exist within the droplets, accompanied by gradients of concentrations and temperature. Microscopic observation of suspended particles in the droplets indicates, however, that such convection is not present.

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On the Protection against Alloxan Diabetes by Hexoses

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In earlier communications (*1, 2*) it was reported that preadministration of glucose, mannose, and fructose but not of galactose protected animals from diabetes caused by alloxan. Administration of the sugars after alloxan, however, had no protective action. Evidence for any direct reaction between glucose and alloxan could not be obtained (*1*). From these results, it was suggested that inhibition of beta cell hexokinase by alloxan was possibly the primary step in the dia-

betogenic action of alloxan (*2*). This paper reports the results of an extension of the earlier studies.

Overnight-fasted rats (weight 100 to 150 g) were the animals used. Presence or absence of diabetes was judged as before (*2*).

None of the following sugars and sugar-derivatives given intravenously before a 40-mg/kg intravenous dose of alloxan offered any protection from diabetes: sucrose (5 g/kg), lactose (5 g/kg), D-xylose (5 g/kg), D-arabinose (5 g/kg), L-arabinose (5 g/kg), D-sorbitol (5 g/kg), D-mannitol (5 g/kg), and Na-gluconate (3 g/kg). Maltose (5 g/kg), however, offered protection in all the animals.

The evidence of protective abilities of the sugars, as obtained by the method of preadministration so far followed, was to some extent vitiated owing to the possibility of interference by intermediate breakdown products of the sugars actually administered. In the present series of experiments, therefore, alloxan and the sugar tested were given simultaneously, the alloxan being dissolved in solutions of varying strengths of the sugar tested. Only the sugars that offered protection when given before alloxan—that is, glucose, mannose, fructose, and maltose—were used in these experiments. The results are given in Table 1.

Thus, the ability to protect animals against diabetes caused by alloxan is probably limited to glucose, mannose, and fructose. Their relative protective abilities as judged from 100 percent protection (Table 1) are approximately: glucose : mannose : fructose = 100 : 50 : 15. The protection given by preadministered maltose possibly resulted from glucose produced *in situ* from the maltose.

Table 2 gives the results of the effects of alloxan on succinic oxidase in both the presence and the absence of excess glucose. The succinic enzyme was prepared from pigeon breast muscle by the method of Barron and Kalnitsky (*3*). The results show that the previous presence of excess glucose did not affect the action of alloxan on the succinic enzyme. This confirms the view, earlier expressed from chemical evidence (*1*), that protection against alloxan diabetes by the hexoses is not the result of any direct reaction between the hexoses and alloxan leading to the inactivation of the latter.

The relative protective abilities of the hexoses against alloxan diabetes as described here closely simulate their relative affinities to the ordinary mammalian hexokinase. In view of the hexokinase inhibition theory of alloxan action (*2*), this suggested that the antagonism between the hexoses and alloxan was possibly competitive in nature. Table 3 gives the results of experiments undertaken with a view to obtain *in vivo* information on the point.

The results show (Table 3) that the protection against a given dose of alloxan by a certain dose of glucose could be overcome by increasing the dose of the alloxan used and that the diabetogenic effect of the increased dose of alloxan could again be counteracted by further increment in the dose of the glucose. In view of the fact that alloxan and glucose do not react with each other, the results indicate that the antagonism between glucose and alloxan with respect to the development of diabetes in animals is possibly competitive in nature.

Thus, we believe that the results reported here add further strength to the hexokinase inhibition theory of alloxan action. The competitive nature of the antagonism between glucose and alloxan also possibly throws more light on the selectivity of alloxan for the beta cells.

Table 1. Effect of simultaneous administration of sugars and alloxan on the development of diabetes in rats. Alloxan (40 mg/kg) was given intravenously dissolved in solutions of different strengths of the sugars.

Sugar tested	Strength sugar soln. (%)	Strength alloxan soln. (%)	No. diab. rats/ No. rats used
D-glucose	7	1.0	6/6
	11	1.0	2/8
	12	1.0	0/10
	6	0.5	2/8
	7	0.5	0/10
D-mannose	22	1.0	2/6
	25	1.0	0/10
D-fructose	35	0.5	2/6
	40	0.5	0/10
Maltose	50	0.5	6/6

Table 2. Effect of excess glucose on the inhibition of succinic oxidase by alloxan.*

Contents of vessels during test	Oxygen uptake (μL O_2/30 min)
Enzyme alone (no succinate)	0
Enzyme + glucose (no succinate)	0
Enzyme + succinate	211
Enzyme + glucose + succinate	215
Enzyme + alloxan + succinate	71
Enzyme + glucose + alloxan + succinate	72

* Enzyme activity was measured manometrically by the Warburg apparatus. Each vessel contained 0.4 ml of enzyme, 0.1*M* phosphate buffer, *p*H 7.4, 0.2 ml of 10 percent glucose (or 0.2 ml of distilled water) and 0.2 ml of 0.5 percent alloxan monohydrate (or 0.2 ml of distilled water); 0.1 ml of 1*M* succinate was added from the side bulb immediately after the first reading (15 min after the addition of alloxan). Total volume, 2 ml; gas-air, temp., 38°C; duration of experiment, 30 min.

Table 3. Effect of increased doses of alloxan on glucose-protection against alloxan diabetes in rats. Alloxan was given intravenously dissolved in solutions of glucose of varying strengths.

Dose of alloxan (mg/kg)	Strength alloxan soln. (%)	Strength glucose soln. (%)	No. diab. rats/ No. rats used
40	1.0	12	0/10*
50	1.25	12	6/6
50	1.25	18	0/10
60	1.5	18	6/6
60	1.5	25	0/10

* Reproduced from Table 1.

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* Senior research fellow, National Institute of Sciences of India.
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Detection of a New Inhibitor of the Tricarboxylic Acid Cycle

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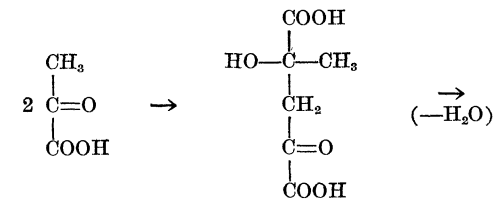
Comparison of different samples of sodium pyruvate (commercial and prepared in this laboratory) showed the presence of variable amounts of a substance that was correlated with an altered behavior of rat heart mitochondria in the oxidation of pyruvate (*1*). The mitochondrial suspension oxidized pure pyruvate completely when small amounts of malate were added, with an oxygen-to-pyruvate ratio of the theoretical value of 5, the tricarboxylic acid cycle being self-perpetuating and operating at an initial Q_{O_2} of between 800 and 1000. Each member of the cycle initiated the oxidation of pyruvate by acting as an oxalacetigen (*2*). However, using a pyruvate containing the impurity, a different pattern of metabolism was exhibited: (i) The cycle was no longer self-perpetuating and, for the utilization of all the pyruvate, an equimolar amount of malate was required; (ii) the oxygen-to-pyruvate ratio dropped to a value arund 3; (iii) the tricarboxylic acids (citrate, cis-aconitate, and isocitrate) no longer acted as oxalacetigens; and (iv) a ketonic substance accumulated as the pyruvate was utilized, the conversion being quantitative. The incorporation of pyruvate into the cycle was not affected by this impurity, as was indicated by the fact that no change was observed in the initial Q_{O_2}. The spectral absorption curve of the 2,4-dinitro-phenylhydrazone of the accumulated substance corresponded quite closely to that of the similar derivative of α-ketoglutarate, and the accumulated substance, therefore, was temporarily assumed to be α-ketoglutarate.

This impurity was found to be present in commercial pyruvic acid and in certain samples of sodium pyruvate prepared from triply redistilled pyruvic acid according to the standard procedure (*3*). Pyruvic acid, originally 99-percent pure, contained no more than 25 percent pyruvic acid after 1 to 2 years. When such pyruvic acid was redistilled under reduced pressure (10 mm-Hg), a fraction distilled over at a higher temperature (105° to 108°C) than the pyruvic acid fraction (55° to 58°C). The higher boiling fraction was a viscous fluid at room temperature from which a waxy crystalline mass slowly formed in the cold, and both liquid and solid material produced the same type of block in the cycle as was previously demonstrated with the impure samples of sodium pyruvate. Sodium pyruvate prepared from triply redistilled pyruvic acid sometimes contained the sodium salt of this impurity in sufficient amounts to produce marked cycle block. Depending on the purity of the original pyruvic acid and the conditions of crystallization of the sodium pyruvate, as much as 25 percent impurity was found in the final product.

A sample of sodium pyruvate containing the impurity was examined by paper chromatography, and it was found that the impurity migrated at approximately the same rate as the pyruvate, indicating that it was a monocarboxylic acid under these conditions. Pyruvate treated for 3 days with 1*N* hydrochloric acid inhibited the oxalacetigenic action of citrate in the mitochondrial preparation 65 percent. It was concluded that the impurity was formed from pyruvic acid. It is known that pyruvic acid is capable of slowly forming a dimer (γ-methyl-γ-hydroxy-α-ketoglutaric acid, I) and that this dimer may lose water to form the cyclic α-keto-γ-valerolactone-γ-carboxylic acid (II). The lactone, furthermore, is believed to exist in a keto-enol equilibrium (II, III). It was considered that



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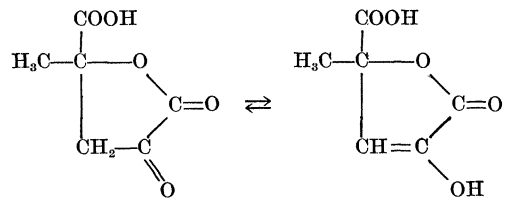

II III

any one of these substances might be involved in the mitochondrial block observed. We therefore prepared the lactone by a method (*4*) in which hydrochloric acid gas was passed for 9 days through a long column of pure, triply redistilled pyruvic acid; the final viscous liquid was allowed to stand over concentrated sulfuric acid in vacuum for 3 days, and a mass of waxy, highly hygroscopic, crystalline material was obtained. The prepared lactone was found to be a potent blocker of the tricarboxylic acid cycle in rat heart mitochondria and, when added with pure pyruvate, produced all the phenomena described here, namely, the failure of the cycle to be self-perpetuating, the inability of the tricarboxylic acids to act as oxalacetigens, and the accumulation of a substance temporarily identified as α-ketoglutarate. The inhibition of the ability of citrate and isocitrate to function as oxal-