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Inhibition of Diabetogenic Action of Alloxan.*

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(Introduced by George R. Minot.)

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Administration of alloxan to animals results in marked changes in blood sugar[1-5] and in selective destruction of the islets of Langerhans.[3-5] The literature describing confirmation of these observations has been summarized by Joslin[4] and by Kennedy and Lukens.[5] Certain chemical and biochemical actions of alloxan can be inhibited by various substances. Bernheim[6] observed that alloxan accelerates the oxidation of ethyl alcohol by surviving liver tissue and that 3,4-diaminotoluene, phosphate buffer, pyrophosphate, bisulfite, and cyanide inhibit this accelerating effect. Alanine, methionine, cysteine, hemoglobin, and the chloride and sulfate ions did not affect this action of alloxan. Alloxan solutions are converted to alloxanic acid salts rapidly by the addition of bicarbonate, according to Labes and Freisburger.[7] Jacobs[8] noted inhibition of the "peroxidase-like effect" of an alloxan and aldehyde mixture by hydrazine, hydroxylamine, cyanide, bisulfite, and cysteine, whereas alcohols and amino acids other than cysteine did not inhibit this effect. His experiments indicated also that alloxan reacts with reducing agents like paraphenylenediamine, benzidine, pyrogallol, and iodides.

An investigation was made of the effect of these and similar substances in preventing alloxan from producing changes in the blood sugar and selective destruction of the islets of Langerhans.

Method. After having determined the amount of alloxan necessary to produce diabetes, rabbits and rats were injected with one of the supposedly inhibitory substances a few minutes before the injection of alloxan.

Rabbits, fasted for from 18 to 24 hours, and rats were used as experimental animals. For the rabbit experiments the alloxan was dissolved in physiological saline solution and was injected intravenously as a single dose; except where noted it was injected immediately after the injection of the supposedly inhibitory substance. The rabbits were then fasted for 48 hours, and blood sugar determinations were made at varying times during the first 12 hours, at 24 hours, and at 48 hours after the injection of alloxan. Blood sugar determinations were made by a colorimetric method combining zinc deproteinization and ferricyanide reduction.[9] Rats were injected subcutaneously with single 300 mg/kg doses of alloxan,[10] and the supposedly inhibitory substances were injected intraperitoneally. Macroscopic examinations were made of the organs of all animals, and microscopic examinations were made of pancreas tissue in representative rabbits and in all rats. In many animals microscopic exminations were made also of the liver, kidneys, adrenals, gonads, thyroid, parathyroids, heart and lungs.

The *in vitro* "peroxidase-like effect" of a mixture of alloxan and aldehyde was deter-

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mined according to the method described by Jacobs.[8] A few drops of fresh solutions of alloxan and formaldehyde were mixed with small amounts of various substances, and the mixture diluted to 5 cc with distilled water. Then a few drops of hydrogen peroxide and of an acetic acid solution of benzidine were added, and the solution was observed for the development of blue color.

Results. Single intravenous injections of alloxan in doses of 150 mg/kg caused the usual blood sugar changes and islet damage[4] in 48 of 50 control rabbits; doses of 175 mg/kg were effective in 15 of 16 rabbits. When the alloxan was injected immediately following administration of 3.4-diaminotoluene, orthophenylenediamine or sodium bisulfite the usual hypoglycemic and diabetogenic effects, as shown by blood sugar determinations and histological examinations of the process, did not occur (Table I.) The substances that did not inhibit noticeably these alloxan effects even when they were used in doses approaching lethal quantities were: sodium bicarbonate, sodium phosphates (pH approximately 7.0), sodium pyrophosphate, sodium cyanide, phenylhydrazine, hydroxylamine hydrochloride, cysteine hydrochloride, paraphenylenediamine, sodium pyrogallol, and lysine hydrochloride. The maximum dosages, in mg/kg, of the non-inhibiting substances given intravenously to rabbits were: sodium bicarbonate 500, sodium phosphate 500, sodium pyrophosphate 40, sodium cyanide 10, paraphenylenediamine 108, and lysine hydrochloride 180. The maximum dosages of substances given intraperitoneally were: phenylhydrazine hydrochloride 20, and hydroxylamine hydrochloride 10. The maximum dosage of cysteine hydrochloride was 500 mg/kg given subcutaneously.

When 120 mg/kg or more of diaminotoluene were injected within a period of 5 minutes before or after the injection of 175 mg/kg doses of alloxan, 19 rabbits did not show the usual blood sugar and islet changes following from the administration of alloxan alone. Administration of less than 100 mg/kg doses of diaminotoluene did not change appreciably the usual blood sugar and islet disturbances in 7 rabbits following simultaneous injection of alloxan. When the interval between diaminotoluene and alloxan injections was 15 min or longer, the usual blood sugar and islet cell changes resulting from alloxan administration occurred. In no animal were macroscopic or microscopic lesions of liver, heart, thyroid, parathyroids, adrenals, gonads, kidneys and lungs observed that could be attributed to the effects of diaminotoluene.

After injection of orthophenylenediamine in doses of 108 mg/kg or more, 9 rabbits that received 150 mg/kg doses of alloxan did not show the usual hypoglycemia, hyperglycemia or marked islet cell changes. One rabbit injected with a 54 mg/kg dose of orthophenylenediamine immediately before the 150 mg/kg dose of alloxan died with hypoglycemic convulsions and showed marked necrosis in all the islets. In none of these rabbits were lesions observed that could be attributed to the effects of the orthophenylenediamine on macroscopic and microscopic examinations of the liver, kidneys, heart, and adrenals.

After injection of sodium bisulfite in doses of 80 to 130 mg/kg 8 of 13 rabbits did not develop the usual fatal hypoglycemia and marked islet necrosis usually resulting from the 150 mg/kg dose of alloxan. No histologic changes were observed in liver, kidneys, heart, or adrenals that could be attributed to the effects of sodium bisulfite administration. None of the rabbits that survived the first 48 hours after injection of alloxan and diaminotoluene, orthophenylenediamine, or sodium bisulfite developed hyperglycemia afterwards.

On microscopic examination the sections of pancreas from rabbits receiving adequate doses of 3,4-diaminotoluene, orthophenylenediamine, or sodium bisulfite within 5 min of the alloxan administration showed no lesions (Table I.). In the remainder of the series the pancreas showed either necrosis of all islets, necrosis of most cells in scattered islets with other islets that looked normal, or necrosis of scattered cells in some islets in which many other cells looked normal.

The 300 mg/kg dose of alloxan caused marked islet cell necrosis in all of the control rats and in the ones treated with paraphenylenediamine, sodium pyrogallol, potassium iodide, sodium sulfite and sodium metabi-

TABLE I.
Effects of Inhibiting Agents on Diabetogenic Action of Alloxan.

No. of Rabbits	Dose of alloxan mg/kg	Inhibiting substances*	Dose mg/kg	Animals with changes in blood sugar: No. of rabbits	Hypoglycemia: Fatal	Hypoglycemia: with recovery	No change	Animals with necrosis in islets: All islets	Scattered islets	Scattered cells	No lesions	Not examined
1	250	3,4-diaminotoluene	200	1	0	0	1	0	0	0	1	0
1	200	"	250	1	0	0	1	0	0	0	1	0
2	195	"	250	1	0	0	1	0	0	0	1	0
		"	230	1	0	0	1	0	0	0	1	0
19	175	"	240	1	0	0	1	0	0	0	1	0
		"	230	1	0	0	1	0	0	0	1	0
		"	210	1	0	0	1	0	0	0	1	0
		"	200	7	0	0	1	0	0	5	0	2
		"	200†	1	0	0	1	0	0	1	0	0
		"	120	4	0	0	4	0	0	2	0	2
		"	240‡	1	1	0	0	1	0	0	0	0
		"	210§	1	1	0	0	1	0	0	0	0
		"	240‖	2	2	0	0	2	0	0	0	0
8	150	"	30	2	2	0	0	1	0	0	0	1
		"	61	2	2	0	0	0	0	0	0	2
		"	73	1	1	0	0	1	0	0	0	0
		"	98	2	2	0	0	2	0	0	0	0
		"	244	1	0	0	1	0	0	0	0	1
9	150	Orthophenylenediamine	108	6	0	0	6	0	0	3	0	3
		"	130	2	0	0	2	0	0	0	1	1
		"	54	1	1	0	0	1	0	0	0	0
13	150	sodium bisulfite	80	2	1	0	1	0	0	0	1	1
		" "	90	1	0	0	1	0	0	0	1	0
		" "	104	3	0	1	2	0	1	0	0	2
		" "	115	3	2	0	1	0	0	0	1	2
		" "	125	3	0	0	3	0	0	0	1	2
		" "	130	1	0	0	1	0	0	0	1	0

* injected intravenously
† " 5 min. before alloxan
‡ " 15 " after "
§ " 30 " " "
‖ " 15 " before "

sulfite.

The *in vitro* "peroxidase-like effect" of alloxan and aldehyde[8] was inhibited by thiourea, sodium thiosulfate, and sodium sulfite, but large doses of these substances failed to inhibit the diabetogenic action of alloxan injected simultaneously or immediately afterwards; sodium metabisulfite administration also did not affect noticeably the usual alloxan effects. The maximum dosages, in mg/kg, given intravenously to rabbits were: thiourea 300, sodium thiosulfate 680, sodium sulfite 120, and sodium metabisulfite 100.

Discussion. In flavin synthesis alloxan unites with orthodiaminodimethylbenzene, and it is thought that 3,4-diaminotoluene reacts similarly with alloxan. A yellowish coloration observed along the ear veins of the rabbits after injection of the diaminotoluene and alloxan indicates that this reaction may occur in the blood of these animals. A similar reaction may explain the protective effect of orthophenylenediamine. Apparently most of the alloxan is removed by such reactions before the alloxan can damage the islet cells irreversibly. The work of other investigators[11, 12] suggests that this damage occurs within the first 5 minutes after intravenous injection of alloxan. Failure of the diaminotoluene to protect against the alloxan when the interval between injections of the two substances was greater than 5 min is confirmatory evidence for this suggestion.

Bisulfite also inhibits the diabetogenic action of alloxan, apparently by combining with it.[6] This protective action was not demonstrable consistently unless almost lethal doses of 125 mg/kg or more of sodium bisulfite were injected. An explanation of the failure of other compounds that combine chemically with alloxan to inhibit its diabetogenic action is not available from these experiments. Lack of proper concentrations or pH conditions in the blood stream for some of these reactions to occur may be responsible in some cases.

Summary. It was attempted to inhibit the selective destructive effect of alloxan on islet cells and the resultant blood sugar changes by administration of substances that combine chemically with alloxan or that inhibit some of its chemical actions. Of these substances 3,4-diaminotoluene, orthophenylenediamine, and sodium bisulfite exhibited definite inhibitory effects.

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Serological Reactions of Levans Synthesized from Sucrose and Raffinose by Bacterial Enzymes.*

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Two classes of polysaccharides, dextrans[1-5] and levans,[6-9] have been synthesized from sucrose through the action of cell-free enzymes of bacterial origin. In the case of the leuconostoc dextrans, the enzymatically synthesized polysaccharides were shown to have serological properties similar to those of the polysaccharides formed in living cultures of the bacteria from which the enzymes came. A demonstration of the same feature for the enzymatically synthesized levans at first appeared impracticable because there were no data in the literature to indicate any serological reactivity on the part of the "natural" levans

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