TECHNICAL PAPERS

Effect of Certain Substances on the Prevention of Diabetogenic Action of Alloxan

SACHCHIDANANDA BANERJEE[1]

Department of Mitra Research in Diabetes, School of Tropical Medicine, Calcutta, India

Archibald (*1*) described a method of estimation of alloxan by its reaction with o-phenylene diamine. Weinglass, Frame, and Williams (*6*) showed that when 3,4-diaminotoluene, o-phenylene diamine, and sodium bisulfite were injected intravenously in rabbits followed by the injection of alloxan, the hypoglycemic and diabetogenic actions of alloxan were prevented. While developing a sensitive test for the determination of a minute amount of alloxan it was shown (*2*) that alloxan reacted with 1,2-dimethyl-4-amino-5(d-1′-ribityl-amino)-benzene, forming riboflavin. It was of interest to attempt to discover whether the same reaction, whereby the diabetogenic action of alloxan would be prevented, would take place *in vivo* in rabbits and rats. Action of nicotinic acid on the production of alloxan diabetes was also studied accidentally, and it was observed that it did prevent the diabetogenic action of alloxan in rabbits and rats. In order to explain the mechanism of action of nicotinic acid it was of interest to study the action of calcium pantothenate, glycine, benzoic acid, p-aminobenzoic acid, ascorbic acid, pyridoxine-hydrochloride, pyridine-dicarboxylic acid, and 2-phenyl quinoline-4-carboxylic acid in inhibiting the action of alloxan.

1,2-dimethyl-4-amino-5(d 1′-ribitylamino)-benzene. On the first day, a 10 per cent solution of this substance in a 200-mg./kg. dose was injected into the marginal ear vein of each of 6 rabbits which were fasted overnight and kept in separate metabolism cages for the collection of urine under toluene. The injection was immediately followed by one consisting of a 10 per cent solution of alloxan (100 mg./kg.), and the ear, along the course of the vein, was then stained yellow. Since none of the animals excreted sugar, these injections were repeated in the same doses on the next day, and blood was taken at intervals of 1 hour for 5 hours in 3 of the rabbits. Fasting blood was again taken from all 6 rabbits on the third morning. Blood sugar was determined by the method of Hagedorn and Jensen (*4*). Blood sugar changes in 3 of the rabbits are given in Table 1. All 6 showed normal blood sugar on the third day. Six control rabbits, receiving alloxan (100 mg./kg.) for only a day, did develop diabetes and excreted sugar in the urine. A dose of 400 mg./kg. of 1,2-dimethyl-4-amino-5(d-1′-ribityl-amino)-benzene was found to be toxic in rabbits.

Intraperitoneal injections of 1,2-dimethyl-4-amino-5(d-1′-ribitylamino)-benzene (400 mg./kg.) and alloxan (200 mg./kg.) were made, one after another, in 6 rats averaging 100 grams in weight. No hyperglycemia or glycosuria was observed. When injected with alloxan only, all developed diabetes.

Nicotinic acid. Nicotinic acid and alloxan in doses of 100 grams/kg. were injected intravenously, one after another, in 5 rabbits. None of the rabbits excreted sugar in urine on the second day. After repeating the injections as before, samples of blood were taken at intervals of 1 hour, as mentioned previously. Blood sugar values are shown in Table 1. When both nicotinic acid and alloxan were injected in a dose of 200 mg./kg. in 3 rabbits, 2 of the animals developed hypoglycemic convulsions and died, while the third developed neither hypoglycemia nor diabetes.

TABLE 1

Substances used	Rabbit No.	Weight of rabbit (grams)	Fasting blood sugar (mg. %)	Blood sugar after intravenous injections of different substances and alloxan (mg. %)					
				1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	24 hr.
1,2-Dimethyl-4-amino-5-(d-1′-ribitylamino)-benzene	1	1,080	90	152	111	111	112	141	90
	2	1,720	98	195	204	208	146	92	92
	3	1,370	114	—	—	—	—	205	110
Nicotinic acid	1	1,750	100	—	—	—	—	—	93
	2	1,440	96	300	313	275	91	91	90
	3	1,400	121	156	196	128	—	—	100
	4	1,157	94	76	85	94	62	78	85
	5	1,300	107	117	149	—	—	—	100
Calcium pantothenate	1	1,450	105	268	315	313	284	254	385
	2	1,730	75	213	51	Convulsion and death			

In experiments with 6 rats with an average weight of 100 grams, intraperitoneal injections of nicotinic acid and alloxan (100 grams/kg.) for 3 consecutive days produced neither hyperglycemia nor glycosuria. All the rats, however, developed diabetes when injected with alloxan only on the fourth day.

Pantothenic acid. Calcium pantothenate (400 mg./kg.) and alloxan (200 mg./kg.) were injected intravenously in 2 rabbits for 1 day only, and blood samples were taken as before. Results are given in Table 1.

As the injections of calcium pantothenate together with alloxan showed constant hyperglycemia in 1 rabbit, the effects of intravenous injections of 100 mg. of calcium pantothenate alone on the blood sugar of normal and alloxan diabetic rabbits were studied. Results are given in Table 2.

Glycine. Glycine (60 mg./kg.) and alloxan (100 mg./kg.) were injected as before for 1 day only in 4 rabbits. No hypoglycemia was observed, but all the animals excreted sugar in urine and showed hyperglycemia on the succeeding days.

Benzoic acid, p-aminobenzoic acid, and ascorbic acid. Benzoic acid and p-aminobenzoic acid were dissolved in water

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with the addition of normal sodium hydroxide, and a watery solution of ascorbic acid was brought to pH 3.5 with sodium hydroxide. Separate injections of these substances, in doses of 100 mg./kg., followed by injection of alloxan (100 mg./kg.), were made in 3 groups of rabbits, each containing 2 rabbits, as before. Since none excreted sugar on the following day, the injections were repeated. Animals showed persistent hyperglycemia and glycosuria from the third day.

Pyridoxine hydrochloride. Pyridoxine hydrochloride (150 mg./kg.) and alloxan (100 mg./kg.) were injected in 3 rabbits and the injections repeated on the second day. All the animals developed glycosuria and hyperglycemia.

Pyridine-dicarboxylic acid and 2-phenylquinoline-4-carboxylic acid. These substances, in doses of 125 and 200 mg./kg., respectively, were separately injected in 2 groups of rabbits, each containing 3 animals. The injections were immediately followed by the injection of alloxan (100 mg./kg.). Since the urines of all the animals were sugar free on the next day, the injections in the same doses were repeated. None developed either hyperglycemia or glycosuria.

TABLE 2

Rabbits	Rabbit No.	Weight of rabbit (grams)	Fasting blood sugar (mg. %)	Blood sugar after intravenous injection of calcium pantothenate (mg. %)			
				¾ hr.	1½ hr.	2¼ hr.	3 hr.
Normal	1	1,360	101	119	115	121	124
	2	1,380	89	131	—	151	122
	3	1,710	101	126	105	100	86
Alloxan-diabetic	1	1,800	516	562	535	598	593
	2	2,200	410	426	464	464	521
	3	1,175	264	300	400	—	441

The results are summarized in Table 3.

Although 1,2-dimethyl-4-amino-5(d-1′-ribitylamino)-benzene prevented the diabetogenic action of alloxan in rabbits and rats, it could not prevent the initial hyperglycemia generally observed after intravenous injection of alloxan in rabbits. In view of the yellow coloration of the veins through which the substances were injected, it might be suggested that the same condensation as studied *in vitro* (*2*) also took place *in vivo* and alloxan thereby removed from the field of its action. As the condensation of 1,2-dimethyl-4-amino-5(d-1′-ribitylamino)-benzene and alloxan might yield riboflavin *in vivo*, like the condensation studied *in vitro*, the reverse reaction might also be possible in the system. Riboflavin under some circumstances may break up and liberate alloxan in the system, which may play some role in the etiology of diabetes mellitus.

Nicotinic acid in most of the rabbits studied could not prevent the initial hyperglycemia which might be due to the secretion of adrenalin as a consequence of the injections. None of the rabbits, however, developed hypoglycemia or persistent hyperglycemia and glycosuria. Nicotinic acid also inhibited the diabetogenic action of alloxan in rats. When nicotinic acid and alloxan were injected in 200-mg./kg. doses, the hypoglycemic and diabetogenic actions of alloxan were not prevented in 2 of the rabbits studied. The doses of the substances were roughly equimolecular, and it might be possible that in the system, when the dose of alloxan was high, all the alloxan could not combine with nicotinic acid, and hence its diabetogenic action could not be prevented.

Calcium pantothenate could not prevent the diabetogenic action of alloxan, but in 1 rabbit there was persistent hyperglycemia just after the injections of calcium pantothenate and alloxan. The effect of intravenous injection of calcium pantothenate alone on the blood sugar of normal and diabetic rabbits showed that calcium pantothenate raised the blood sugar slightly in normal rabbits and considerably in alloxan-diabetic rabbits. The claim of Vecchio (*5*) that pantothenic acid considerably reduces the blood sugar level in children could not be confirmed in our experiments with rabbits.

Glycine, benzoic acid, p-aminobenzoic acid, and ascorbic acid also could not prevent the development of alloxan diabetes. These observations indicate that the carboxyl group of nicotinic acid is not responsible for the action. Pyridoxine hydrochloride also could not prevent the action of alloxan, which indicates that the pyridine ring is likewise not active. Pyridine-dicarboxylic acid and 2-phenylquinoline-4-carboxylic acid, which, like nicotinic acid, contain both the carboxyl group and the pyridine ring, could inhibit the diabetogenic action of alloxan. It seems, therefore, that the entire nicotinic acid molecule combines with alloxan and prevents its action. It has been suggested by Dunn, *et al.* (*3*)

TABLE 3

Substances used	No. animals used	Dose of substance (mg.)/ kg./day	Dose of alloxan (mg.)/ kg./day	No. of animals which developed persistent hyperglycemia and glycosuria
1,2-Dimethyl-4-amino-5(d-1′-ribitylamino)-benzene	Rabbits 6	200 2 days	100 2 days	0
	Rats 6	400 1 day	200 1 day	0
Nicotinic acid	Rabbits 5	100 2 days	100 2 days	0
	Rats 6	100 3 days	100 3 days	0
Calcium pantothenate	Rabbits 2	400 1 day	200 1 day	1 (another died)
Glycine	Rabbits 4	60 1 day	100 1 day	4
Benzoic acid	Rabbits 2	100 2 days	100 2 days	2
p-Aminobenzoic acid	Rabbits 2	100 2 days	100 2 days	2
Ascorbic acid	Rabbits 2	100 2 days	100 2 days	2
Pyridoxine hydrochloride	Rabbits 3	150 2 days	100 2 days	3
Pyridine-dicarboxylic acid	Rabbits 3	125 2 days	100 2 days	0
2-Phenylquinoline-4-carboxylic acid	Rabbits 3	200 2 days	100 2 days	0

that possible defects in the metabolism of purines or of alloxan in man may play some role in the etiology of diabetes. If the diet contains sufficient nicotinic acid, it might combine with alloxan so formed and thereby prevent its action. Nicotinic acid thus might play some part in the prevention of diabetes.

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Transmission of the Bunchy Top Disease of Papaya (*Carica papaya* L.) by the Leaf Hopper *Empoasca papayae* Oman

FRANCISCO SEÍN, JR., and JOSÉ ADSUAR

Agricultural Experiment Station, University of Puerto Rico, Río Piedras, P. R.

In 1946 Adsuar (*1*) reported the successful transmission of papaya bunchy top by a leaf hopper of the genus *Empoasca*, presumably identical with the one previously used experimentally by Jensen (*2*) in 1938. In their experiments, Adsuar and Jensen used as vectors the leaf hoppers collected on diseased papaya plants in the field but did not identify them specifically. Specimens collected on papaya in Puerto Rico were described by P. W. Oman (*3*) as *Empoasca papayae*, but as more than one species of *Empoasca* may occur on papaya, the specific identity of the vector used by Adsuar and Jensen can only be presumed.

Specific identification of the females of the genus *Empoasca* being practically impossible, the senior author followed a simple method of elimination which permits the use of males only as vectors. Specimens of both sexes collected from diseased papaya trees were exposed to the vapors of ether to render them quiet long enough to permit the separation of the males from the females under the binocular. The desired number of males were released inside cellophane casings inserted on the upper part of the stem and tender leaves of healthy papaya plants.

The identification of the 169 male leaf hoppers recovered dead two or three days later, when the casings were removed, was confirmed by P. W. Oman, of the U. S. Department of Agriculture, as his *Empoasca papayae*.

Out of 30 healthy papaya plants, on each of which from 5 to 10 males were isolated, 9 plants developed the symptoms of bunchy top in about a month and a half. Thirty similar check plants, kept with the others in a greenhouse from which leaf hoppers were excluded, remained healthy. This experiment conclusively demonstrates that *Empoasca papayae* Oman is a vector of the bunchy top disease of papaya.

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Fluorescein as an Agent in the Differentiation of Normal and Malignant Tissues[1]

GEORGE E. MOORE[2]

Department of Surgery, University of Minnesota Medical School, Minneapolis

For many years investigators have noted and described the differential appearance of various tissues under ultraviolet light. In 1934, Danckwortt (*1*) wrote an extensive monograph encompassing the entire subject.

Several authors have reported the use of ultraviolet light as an aid in distinguishing neoplastic tissue. Some have even claimed a specific fluorescence for certain tumors. This, however, has never been consistently substantiated.

Herly (*2*), in 1944, reported that differences in the macroscopic appearance of benign and malignant tumors of the breast were enhanced when viewed under ultraviolet light. Using this technique, in a series of 200 breast tumors suspected of malignancy only one error in diagnosis was acknowledged.

Since October 1946, sodium fluorescein has been injected into patients subjected to laparotomy for gastric carcinoma, with the hope that it might accentuate the differences in appearance of normal and malignant tissues as revealed under ultraviolet light. Initially, 5 cc. of 20 per cent sodium fluorescein was injected intravenously after the viscera were exposed, and then inspected with an ultraviolet lamp emitting rays at about 3,600°. In these first cases no difference in fluorescence of normal and tumor tissues was noted. Next, the dye was injected at various times prior to the operation. It became evident that, when the interval between injection and examination was between 3 and 8 hours, a difference between normal and malignant tissues could be observed. Carcinomatous implants of tumor tissue on the peritoneal surfaces were readily seen. These fluoresced with a vivid yellow color. When, however, the tumor tissue was situated more than a few millimeters below the surface, no fluorescence was observed. This might be related to the fact that ultraviolet light can penetrate only a few millimeters of tissue.

TABLE 1

OBSERVED CORRELATION BETWEEN FLUORESCENCE OF TUMOR OR TUMOR FRAGMENTS AND ULTIMATE HISTOLOGICAL DIAGNOSIS

Site of tumor	Total cases	Correlation*		
		Good	Poor	Failure
Gastrointestinal tract	17	11	3	3
Brain and spinal cord	12	11	1	0
Miscellaneous	17	9	2	6
Total	46	31	6	9

* Good indicates high grade of fluorescence with verified microscopic diagnosis; poor, only slight fluorescence; and failure, that the tumor or tumor fragments did not fluoresce.

To date, 46 neoplasms have been examined with this tech-

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[2] Senior Research Fellow with the National Institute of Health, U. S. Public Health Service.