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# Influence of adrenaline on the diabetogenic effect of alloxan in the rat

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The effect of alloxan and adrenaline on blood glucose was investigated in 84 rats. The diabetogenic action of alloxan, 200 mg/kg, was prevented by mixing it for 10 min with adrenaline, 50 μg/kg, before injection. No histological changes in the islets were observed after the injection of this mixture. Similar doses of adrenaline and alloxan, injected intramuscularly at the same time but separately produced a diabetogenic action greater than that produced by alloxan alone.

SINCE the discovery of alloxan-induced diabetes by Dunn, Sheehan & McLetchie (1943), drugs which can protect the animal against the diabetogenic effects of alloxan have been sought.

Kass & Waisbren (1945) found that the intraperitoneal administration of adrenaline along with the subcutaneous injection of alloxan, diminished the diabetogenic effect of the latter compound. The diabetogenic effect of alloxan has been reported to be antagonised by glutathione acting by virtue of its –SH group (Patterson, Lazarow & Levey, 1949), by nicotinamide, benzamide, propylene glycol propanol and isopropanol (Janes & Schueler, 1955).

Drugs which antagonise the diabetogenic effect of alloxan are of particular interest since alloxan has been used in the treatment of certain malignant neoplasms (Gilman, Hathorn & Lamont, 1957).

The present study, which deals with the effect of adrenaline on the diabetogenic action of alloxan, involved the intramuscular injection in rats of both drugs either separately or as a pre-incubated mixture at one site.

## Material and methods

Eighty-four male adult albino Wistar rats of from 150–200 g were used. All were kept under the same conditions.

The rats were divided into 5 groups as follows:

Group I (alloxan group), comprising 23 rats, were injected intramuscularly with alloxan 200 mg/kg weight.

Group II (alloxan + adrenaline group); 18 rats were injected intramuscularly with an incubated mixture of alloxan, 200 mg, and adrenaline hydrochloride, 50 μg, for each kg weight. Incubation of this mixture was at room temperature (25°) for 10 min.

Group III (separate alloxan and adrenaline group); 13 rats were injected intramuscularly at the same time with alloxan, 200 mg/kg, in one thigh and adrenaline hydrochloride, 50 μg/kg, in the other thigh.

Group IV (adrenaline group); 14 rats were injected intramuscularly with adrenaline hydrochloride 50 μg/kg weight.

Group V (control group); this comprised 16 rats each injected intramuscularly with 0·8 ml of physiological saline.

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*Drugs.* Alloxan monohydrate (Merck) 5% aqueous solution and adrenaline hydrochloride (May & Baker Ltd.) 0·00125% aqueous solutions were used. Solutions were prepared and kept at room temperature, 25°, for 10 min before injection.

*Collection of blood samples.* All rats were fasted overnight before the collection of blood for estimation of blood glucose. Samples of 0·15 ml blood were taken before treatment, and 36 hr, 72 hr, 1, 2 and 3 weeks after the drug by cardiac puncture using needle No. 20. Lithium oxalate, 1 mg/ml blood, was used as an anticoagulant.

*Blood glucose estimation.* Blood samples, 0·1 ml, were measured accurately using dry micropipettes; the glucose concentration in these samples was determined using Somogyi's technique (1945), utilising Nelson's (1944) arsenomolybdate colour reagent. The optical densities were measured using a Zeiss Spectrophotometer at a wavelength of 490 mμ.

*Histological studies.* Three rats from each group were killed at different intervals and their blood glucose levels estimated. The results are not presented in the tables to avoid interference with the mortality rates. Histological sections of the pancreas from these animals were prepared and stained with haematoxylin and eosin to see if there was evidence of histological changes in the islet cells after drug administration.

*Statistical analysis of the results.* The results of the blood glucose estimations were subjected to statistical analysis. The significance of differences between means of the various groups was tested by their $t$ values and the level of P (Burn, Finney & Goodwin, 1952).

## Results

### BLOOD GLUCOSE LEVEL

The blood glucose concentrations in the different groups of animals at different intervals after drug administration are shown in Table 1. The statistical significance of the difference between means of blood glucose in the different groups are presented in Table 2.

From these results, there is no significant difference in the mean blood glucose levels of the animals in the control group and those injected with adrenaline alone or those injected with the incubated mixture of adrenaline and alloxan. The animals of the remaining two groups developed hyperglycaemia. In the rats that received alloxan only, the hyperglycaemia was noticed after 36 hr and was maintained during the first two weeks. On the third week, some animals showed marked hypoglycaemia.

The group of animals receiving alloxan and adrenaline separately at the same time, developed more hyperglycaemia than the group on alloxan. The difference is highly significant (Table 2).

### MORTALITY RATE

The cumulative percentage mortality in each group at different intervals after drug administration is in Table 3. The highest mortality rate was in group III whereas the rate in group II was much lower than either that of group I or III.

TABLE 1. ACTION OF ADRENALINE ON THE DIABETOGENIC EFFECT OF ALLOXAN IN RATS

| Drugs (i.m.) | | Fasting blood glucose mg/100 ml blood | | | | | |
|---|---|---|---|---|---|---|---|
| | | Initial | 36 hr | 72 hr | 1 week | 2 weeks | 3 weeks |
| Group I, 20 animals Alloxan .. .. | Mean | 60·85 | 151·50 | 144·05 | 110·12 | 89·30 | 82·60 |
| | s.d. | ±17·93 | ±75·17 | ±90·13 | ±50·95 | ±32·37 | ±34·34 |
| | s.e. | ±4·01 | ±16·80 | ±20·16 | ±12·36 | ±10·24 | ±10·86 |
| | | (20) | (20) | (20) | (17) | (10) | (10) |
| Group II, 15 animals Adrenaline and alloxan incubated mixture | Mean | 66·00 | 80·14 | 82·46 | 84·67 | 73·10 | 66·42 |
| | s.d. | ±8·31 | ±15·34 | ±18·82 | ±14·49 | ±15·95 | ±21·21 |
| | s.e. | ±2·15 | ±4·10 | ±5·22 | ±3·74 | ±4·60 | ±6·12 |
| | | (15) | (14) | (13) | (15) | (12) | (12) |
| Group III, 10 animals Adrenaline and alloxan in separate sites | Mean | 64·00 | 285·57 | 515·50 | 205·33 | 104·00 | 82·00 |
| | s.d. | ±6·68 | ±90·68 | ±185·60 | ±94·56 | | |
| | s.e. | ±2·11 | ±34·27 | ±75·77 | ±54·66 | | |
| | | (10) | (7) | (6) | (3) | (2) | (1) |
| Group IV, 11 animals Adrenaline .. .. | Mean | 58·45 | 84·27 | 64·50 | 83·27 | 83·10 | 60·09 |
| | s.d. | ±12·65 | ±16·19 | ±14·52 | ±14·36 | ±16·98 | ±8·18 |
| | s.e. | ±3·81 | ±4·88 | ±4·59 | ±4·33 | ±5·37 | ±2·47 |
| | | (11) | (11) | (10) | (11) | (10) | (11) |
| Group V, 13 animals .. Control .. .. | Mean | 62·90 | 76·38 | 74·77 | 87·27 | 81·23 | 65·15 |
| | s.d. | ±7·77 | ±13·70 | ±11·10 | ±8·31 | ±10·92 | ±11·12 |
| | s.e. | ±2·46 | ±3·80 | ±3·08 | ±2·50 | ±3·03 | ±3·08 |
| | | (10) | (13) | (13) | (11) | (13) | (13) |

s.d. = Standard deviation.
s.e. = Standard error of the mean.
( ) = Number of samples.
Variations were due to mortalities or to loss of samples.

TABLE 2. STATISTICAL SIGNIFICANCE OF DIFFERENCE BETWEEN MEANS OF BLOOD GLUCOSE IN VARIOUS GROUPS AS TESTED BY THEIR *t* VALUE AND THE LEVEL OF *P*

| Groups compared | | Initial | Time after drug administration | | | | |
|---|---|---|---|---|---|---|---|
| | | | 36 hr | 72 hr | 1 week | 2 weeks | 3 weeks |
| Alloxan group .. .. | *t* | 1·13 | 4·125 | 2·478 | 1·972 | 1·443 | 1·298 |
| Alloxan + adrenaline group | P | <0·15 | <0·0005 | =0·01 | <0·05 | <0·15 | <0·15 |
| Alloxan + adrenaline group | *t* | 0·664 | 5·938 | 5·702 | 2·205 | | |
| Separate alloxan and adrenaline group .. | P | =0·25 | <0·0005 | <0·0005 | <0·025 | | |
| Alloxan group .. .. | *t* | 0·436 | 4·359 | 3·398 | 1·812 | 0·756 | 1·546 |
| Control group .. .. | P | <0·35 | <0·0005 | =0·0005 | =0·05 | <0·25 | <0·1 |
| Alloxan + adrenaline group | *t* | 0·95 | 0·673 | 1·269 | 0·577 | 1·475 | 0·185 |
| Control group .. .. | P | <0·20 | =0·25 | <0·1 | =0·3 | <0·1 | <0·4 |
| Adrenaline group .. | *t* | 0·98 | 1·275 | 1·854 | 0·80 | 0·303 | 1·281 |
| Control group .. .. | P | <0·2 | <0·1 | <0·05 | =0·2 | <0·35 | <0·1 |

TABLE 3. THE CUMULATIVE PERCENTAGE MORTALITY IN THE VARIOUS GROUPS OF RATS

| Group | Time after drug administration | | | | |
|---|---|---|---|---|---|
| | 36 hr | 72 hr | 1 week | 2 weeks | 3 weeks |
| Alloxan group .. .. .. .. | 0 | 0 | 10% | 40% | 50% |
| Alloxan + adrenaline mix group .. .. | 0 | 0 | 0 | 20% | 20% |
| Separate alloxan and adrenaline group .. | 30% | 40% | 70% | 80% | 90% |
| Adrenaline group .. .. .. .. | 0 | 0 | 0 | 0 | 0 |
| Control group .. .. .. .. | 0 | 0 | 0 | 0 | 0 |

MICROSCOPICAL CHANGES

Twelve hr after administration of alloxan in group I, the nuclei of the beta cells in the islets of Langerhans showed marked pycnosis (Fig. 1).

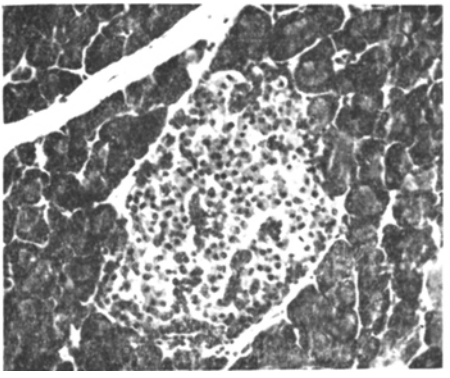

FIG. 1. Islets of Langerhans in a rat 12 hr after i.m. injection of 200 mg alloxan/kg. The nuclei of the beta cells show marked pycnosis. The fasting blood glucose level was 112 mg/100 ml.

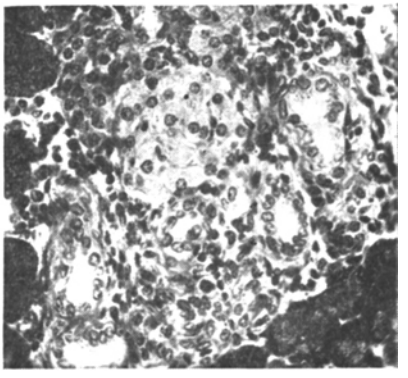

FIG. 2. Islets of Langerhans in a rat 10 days after i.m. injection of 200 mg alloxan/kg. Regeneration in the pancreas is apparent; complex small tubules arranged focally in small islands have begun to appear. The fasting blood glucose level was 57 mg/100 ml.

In the animals that received alloxan only and in those injected with alloxan and adrenaline at the same time by separate injections, there were marked histological changes in the islet cells 36 hr after the drug. Some beta cells were vacuolated and appeared to retain shadows of nuclei which showed various stages of pycnosis, karyorrhexis or karyolysis. The blood sinusoids were dilated and occasionally the islets were infiltrated with leucocytes. In these animals marked hyperglycaemia was observed.

The pancreatic tissue of rats which were injected with alloxan only and were killed 10 days after the drug, was characterised by the appearance of small tubules indistinguishable in all respects from small pancreatic ductules present in normal pancreas (Fig. 2). These were arranged focally in little islands. The tubules were lined by cubical epithelium and the lumen was usually empty. In the animals which recovered from the diabetic condition particularly those which exhibited hypoglycaemia, the pancreatic islets looked almost normal, although many small islets were seen.

Microscopical examination of the pancreatic tissues of the animals of the control group, those injected with adrenaline only and those injected with the mixture of adrenaline and alloxan, showed that there were no relevant microscopical changes in the islet cells.

## Discussion and conclusions

The results show that the groups of animals injected intramuscularly with a mixture of alloxan and adrenaline neither developed hyperglycaemia nor any microscopical changes in the pancreas. However, those animals injected intramuscularly at the same time with alloxan and adrenaline

at different sites, showed hyperglycaemia even more severe than that occurring in the alloxan group and degenerative changes in the beta cells of the islets were observed.

It appears that the process of mixing adrenaline with alloxan abolishes the diabetogenic property of the latter. But, adrenaline injected intramuscularly at the same time as alloxan but at a different site did not protect against the diabetogenic action of alloxan, on the contrary it enhanced the action.

The reduction of the diabetogenic effect of alloxan, 200 mg/kg, after mixing it with adrenaline, 50 $\mu$g/kg, for 10 min at 25° before administration might result from a chemical interaction. Lazarow (1954) pointed out that loss of the diabetogenic action of alloxan results either from an attack on the ketone group or the =NH group. Since the amount of adrenaline is so small compared to the amount of alloxan, it may be that oxidation products of adrenaline resulting from interaction with alloxan might also be responsible for the antagonism of the diabetogenic action. A recent report of the activity of adrenochrome on oxidative phosphorylation showed that at micromolar levels, it decreased both oxygen uptake and phosphorylation (Krall, Siegel & Gozansky, 1962).

The action of alloxan when it was simultaneously administered along with adrenaline at separate sites could be the result of the very rapid destruction of alloxan (Goodman & Gillman, 1957) *in vivo* and also the very rapid disappearance of adrenaline from the blood stream (Lund, 1951) which would not allow much time for any chemical interaction. However, the possibility that the vasodilator action of adrenaline on the blood vessels of the skeletal muscles might enhance the rate of absorption of alloxan from its site of injection in the thigh and hence accentuate the diabetogenic effect must not be overlooked.

In the report of Kass & others (1945), the protection against the diabetogenic action of alloxan administered subcutaneously, by intraperitoneal injection of adrenaline, could be due to delayed absorption of alloxan from the subcutaneous tissue with consequent low blood levels.

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