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# Relation of Adrenal Gland and Hypophysis to Blood Sugar Levels Following Administration of Alloxan.*

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Following the administration of alloxan and preceding the establishment of diabetes, the blood sugar of alloxan-treated animals showed an immediate rise which was followed by a severe hypoglycemia, the latter resulting from release of insulin by damaged islets of Langerhans.[1-3] Following the hypoglycemic phase a final hyperglycemia ensued. If the adrenal glands were extirpated preceding the administration of alloxan, an initial hyperglycemia did not appear, although the test animals became diabetic.[3] Destruction of the adrenal medulla of rabbits by injection of formalin produced the same result as adrenalectomy.[3] It was concluded that the adrenal medulla is concerned in the initial hyperglycemia.[3]

The present investigation was undertaken to determine the role of the adrenal cortex (and medulla) and hypophysis in governing the reaction to alloxan. Alloxan was injected either intravenously (100 mg/kg) or subcutaneously (200 mg/kg) into young adult rats which had been fasted for 12 hours. Blood sugar determinations were made at intervals after injection, as indicated below, under ether anesthesia.

Fifteen normal rats were injected with alloxan (10 IV and 5 SC). Tables I and II present blood sugar levels of these animals following alloxan administration.

TABLE I.

Blood Sugar Levels in mg % Following Injection of 100 mg/kg of Alloxan Intravenously into Normal Rats.

| | Hours | | | | | | |
|---|---|---|---|---|---|---|---|
| Fasting | 1 | 2 | 3 | 4 | 5 | 6 | 24 |
| 105 | | 124 | | 192 | | 100 | 844 |
| 133 | | 208 | | | 109 | | 500 |
| 134 | 154 | 250 | | | 250 | 244 | 609 |
| 129 | | 250 | 161 | | | 149 | |
| 125 | | | | 263 | | | |
| 138 | | | | 261 | | | |
| 136 | 208 | 148 | | 112 | 132 | | |
| 102 | | | 76 | | 66 | 72 | 323 |
| 128 | | 105 | | | 71 | | 167 |
| 140 | | | | 130 | | | |

TABLE II.

Blood Sugar Levels in mg % Following Injection of 200 mg/kg of Alloxan Subcutaneously Into Normal Rats.

| | Hours | | |
|---|---|---|---|
| Fasting | 4 | 23 | 46 |
| 125 | 98 | 72 | 384 |
| 102 | 122 | 70 | 500 |
| 109 | 113 | 46 | 400 |
| 104 | 89 | 43 | 286 |
| 113 | 118 | 100 | 234 |

A definite initial hyperglycemia was demonstrated within 4 hours after intravenous injection of alloxan in 7 of 10 intact rats. In those injected subcutaneously the hypoglycemic phase appeared at approximately 23 hr, and the initial hyperglycemic phase, if it occurred, sometime between 4 and 23 hr after injection.

Fourteen rats were adrenalectomized 24 hr or more preceding the administration of alloxan (9 IV and 5 SC); 3 animals were operated upon immediately preceding IV injection. Three rats received alloxan IV 4 weeks following adrenal enucleation. By this procedure all of the adrenal glands except their connective tissue capsules are removed.[4,5] From

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3 Goldner, M. G., and Gomori, G., *Endocrinology*, 1944, **35**, 241.

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TABLE III.
Blood Sugar Levels in mg% of Adrenalectomized Rats Following Injection of 100 mg/kg of Alloxan Intravenously.

| Maintenance | Fasting | 2 hr | 4 hr | 6 hr |
|---|---|---|---|---|
| Adrenalectomy performed 24 hr preceding alloxan injection | | | | |
| Salt | 92 | 59 | 45 hge | |
| " | 64 | 40 hge* | | |
| " | 66 | 28 " | | |
| " | 67 | 41 " | | |
| " | 72 | 35 " | | |
| Cortical extr | 105 | | 48 " | |
| " | 108 | 44 " | | |
| Adrenalectomy performed immediately preceding administration of alloxan | | | | |
| | 125 | 89 | 73 | 44 hge |
| Animals possessing only the adrenal cortex (following adrenal enucleation) | | | | |
| | 118 | 161 | 161 | 208 |
| | 120 | 118 | 128 | 133 |
| Animals from which regenerated adrenal cortical tissue was removed immediately preceding administration of alloxan | | | | |
| | 161 | | 98 | 37 hge |
| | 133 | | 85 | 35 " |

* hge = hypoglycemic convulsions.

the latter, functional cortices regenerate—the glands possess no functional medulla. Two animals received alloxan IV immediately following extirpation of regenerated adrenal cortices (previously enucleated glands). Regenerated adrenal cortices were serially sectioned. Of 12 such glands a single island of medullary tissue was found in one. Table III records the blood sugar levels in this series of animals.

Hypoglycemic convulsions appeared within 5 hours in fasting adrenalectomized animals injected with 200 mg/kg of alloxan subcutaneously. Compare with blood sugar levels in intact animals alloxanized with this dose (Table II).

Eight rats were alloxanized 72 hr after hypophysectomy by intravenous injection; 3 additional rats were injected IV following a sham hypophysectomy, and 2 rats were hypophysectomized immediately preceding the IV administration of alloxan. Table IV records the blood sugar levels in this series of animals.

The results indicate that the adrenal cortex rather than the medulla was concerned in the initial hyper- and hypoglycemic reactions to alloxan. In these experiments all of 15 intact animals survived the injection of alloxan without the development of hypoglycemic convulsions. All of 14 adrenalectomized and 9 of 10 hypophysectomized rats similarly treated developed hypoglycemic convulsions within 6 hr after injection. Three of these animals were adrenalectomized and one hypophysectomized immediately preceding the injection of alloxan, indicating that the role of these glands is active, and that the rapid development of hypoglycemic convulsions in alloxanized-adrenalectomized or hypophysectomized animals is not merely secondary to an initially lower blood sugar at the moment of injection

TABLE IV.
Blood Sugar Levels in mg % of Hypophysectomized Rats Following Injection of 100 mg/kg of Alloxan Intravenously.

| Pre-op | Post-op | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| Hypophysectomy—72 hr preceding injection | | | Hours | | | | |
| | 122 | | | | 78 | | hge |
| | 80 | | | | 66 | | " |
| 140 | 125 | 100 | 83 | 42 | hge | | |
| 128 | 172 | 184 | 181 | | | | 59 hge |
| 133 | 113 | | 35 hge* | | | | |
| 128 | 114 | | 76 " | | | | |
| 139 | 95 | | 32 " | | | | |
| 133 | 125 | | 100 | 42 hge | | | |
| Hypophysectomy—immediately preceding injection | | | | | | | |
| Convulsions in 8 hr. | | | | | | | |
| One animal survived injection. | | | | | | | |
| Sham hypophysectomy—72 hr preceding injection | | | | | | | |
| | 125 | | | | 267 | | |
| | 138 | | | | 264 | | |
| | 140 | | | | 130 | | |

* hge = hypoglycemic convulsions.

TABLE V.
Summary of Results Following Intravenous Administration of Alloxan (100 mg/kg)

| No. of animals | Previous treatment | No. with initial hyperglycemia | No. with hypoglycemic convulsions |
|---|---|---|---|
| 10 | none | 7 | 0 |
| 9 | adrenalectomy | 0 | 9 |
| 3 | adrenal enucleation cortical regeneration | 1* | 0 |
| 2 | extirpation of regenerated adrenal cortex | 0 | 2 |
| 10 | hypophysectomy | 0 | 9 |
| 3 | sham hypophysectomy | 2 | 0 |
| Results following subcutaneous administration of alloxan 200 mg/kg | | | |
| 5 | none | ? | 0 |
| 5 | adrenalectomy | ? | 5 |

* 2 animals tested.

[5] Ingle, D. J., and Griffith, J. Q., *The Rat in Laboratory Investigation*, p. 389, 1942. J. B. Lippincott Co., Philadelphia, Montreal, London.

of alloxan. In 3 animals possessing only the adrenal cortex hypoglycemic convulsions did not occur following the injection of alloxan; one of the 2 animals tested showed an initial hyperglycemia. That extra-adrenal chromaffine tissue was not responsible for this protection against the hypoglycemia of convulsive level is demonstrated by the fact that hypoglycemic convulsions occurred in 2 alloxanized animals from which adrenal cortices had been removed. It would seem that the hypophyseal-corticoadrenal mechanism antagonizing the action of insulin is concerned in the initial reaction to the injection of alloxan.

*Summary.* An initial hyperglycemia did not appear in either adrenalectomized or hypophysectomized rats injected with alloxan. With the doses of alloxan used, hypoglycemic convulsions did not appear in any of 15 intact animals whereas convulsions occurred within 6 hr in all of 14 adrenalectomized and 9 of 10 hypophysectomized animals. In the absence of the adrenal medulla the reaction to alloxan was the same as in intact animals; removal of the adrenal cortex (regenerated cortex following adrenal enucleation) resulted in the same reaction as total adrenalectomy.

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## Effect of Mapharsen on Bromsulphalein Retention in Normal Dogs.*

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Various arsenicals have been used as experimental agents to produce and study hepatic damage. Thus, liver injury produced by arsphenamine has been studied in relation to the protein, carbohydrate and fat content of the diet.[1,2] More recently methionine has been reported to counteract the increased icterus index produced by Mapharsen in *protein depleted dogs*.[3] Recently we studied the effect of oxophenarsine hydrochloride (Mapharsen) on the liver function of *normal dogs* and noted (1) that the bromsulphalein retention produced by a single injection of Mapharsen varied considerably and (2) that the dogs rapidly acquired a tolerance to Mapharsen, as judged by the rapid return of the liver functions to normal on subsequent injections.

*Methods.* The dogs used in the experiments were healthy adult animals receiving a stock diet of Purina chow. After control bromsulphalein determinations were made on the animals each dog was injected intravenously with 0.06 g of Mapharsen in 10 cc of distilled water. Bromsulphalein determinations were then made 6 hr and 24 hr after the Mapharsen injection. In some of the dogs the Mapharsen injections were repeated at weekly intervals. The 5 mg/kg dose of bromsulphalein was used to estimate the degree of hepatic dysfunction, and the reported readings were read directly against the 2 mg standards. Therefore, by using the 2 mg standard the retention values may range above 100%. The control dye retentions were between 5 and 12%, so that any retention above 15% was considered as evidence of decreased liver function.[4]

*Results.* Of 17 dogs injected with 60 mg of Mapharsen, 59% showed a retention of bromsulphalein 6 hr later. A bromsulphalein test 24 hr after the mapharsen injection showed

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