treatment decreased it. Unlike the other 3 brain areas studied, the hypothalamus appears to be indifferent to orchidectomy, with or without E_2 administration.

Our observations on the effect of orchidectomy followed by E_2 injection on the AChE activity in the cerebral cortex are contrary to the findings of James and Kanungo[7] who claimed that a similar dose of E_2 causes an increase in AChE activity at all ages. This discrepancy may be due to the fact that they used the whole cerebral hemisphere as against the cortex in our study. The response of the amygdala, on the other hand, seems to agree with Kamberi's preliminary report that AChE activity is diminished by gonadectomy in the adult rat and augmented by E_2 replacement therapy[8]. Although gonadectomy decreases the enzyme activity in young[7] and adult rats[7,8], we did not observe any decrease in all the brain areas studied. This may be due to the slight differences in the ages at gonadectomy. We, however, noticed a significant increase in the AChE activity in the adenohypophysis.

From previous demonstrations by autoradiography[9], and binding studies[10] that sex steroids act in the hypophysis, preoptic area, hypothalamus and amygdala we may speculate, from our results, that orchidectomy and E_2 administration could regulate AChE activity by facilitatory and inhibitory mechanisms at different target sites. It is suggested, then, that the hormonal regulation of AChE may play a significant role in neuroendocrinological and behavioural mammalian events.

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The induction of diabetes in rats by intramuscular administration of streptozotocin[1]

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Summary. Streptozotocin administered i.m. was as effective a diabetogenic agent as when administered i.v. This is useful, particularly for the induction of diabetes in small animals.

As part of a study on the hypoglycaemic properties of some local remedies, experimental diabetes was induced in rats with streptozotocin. Following difficulties encountered with i.v. administration to small animals the drug was injected into the thigh muscles of the leg and its effects studied. Streptozotocin given i.m. produced diabetes of dose dependent severity similar to its effects after i.v. administration as reported by Junod et al.[3].

Materials and methods. Male albino Sprague Dawley rats weighing between 100 and 300 g were used. The experimental groups were matched for weight for each set of experiments.

For the induction of diabetes the animals were fasted for 16 h. Individual doses of pure streptozotocin (Upjohn Research Labs, USA) were prepared by weighing and immediately before injection dissolved in 1 ml citrate buffer pH 4.5. Following the injection, the animals were allowed food and water ad libitum for the remainder of the experiment.

Blood samples for glucose determination were taken from a

Table 1. Severity of hyperglycaemia in rats 24 h after streptozotocin administration

Dose mg/kg wt	No. of rats studied	Degree of hyperglycaemia induced** Severe	Moderate	Mild	None	No. with glycosuria	No. with ketonuria	Average survival rate after 10 days
120 mg	7	7	-	-	-	7 (100%)	7 (100%)	0%
60 mg	13	13	-	-	-	13 (100%)	13 (100%)	46%
45 mg	60	60	-	-	-	60 (100%)	12 (20%)	100%
40 mg	60	46	6	4	4	60 (100%)	12 (20%)	100%
30 mg	50	10	25	3	12	35 (70%)	2 (4%)	100%
20 mg	21	-	-	-	21	12* (57%)	0	100%

*Transient glycosuria; **Mild = > 140 mg and < 200 mg%; Moderate = > 200 mg and < 300 mg%; severe = > 300 mg%.

Table 2. Plasma insulin of rats injected with 30 mg/kg streptozotocin

	Random blood glucose (mg%)	Insulin (μU/ml)	No. of animals
Normoglycaemic from start of experiment	100 ± 5.3*	49 ± 5.9	12
Normoglycaemic after 3 to 21 days	101 ± 8.2	28 ± 4.6	9

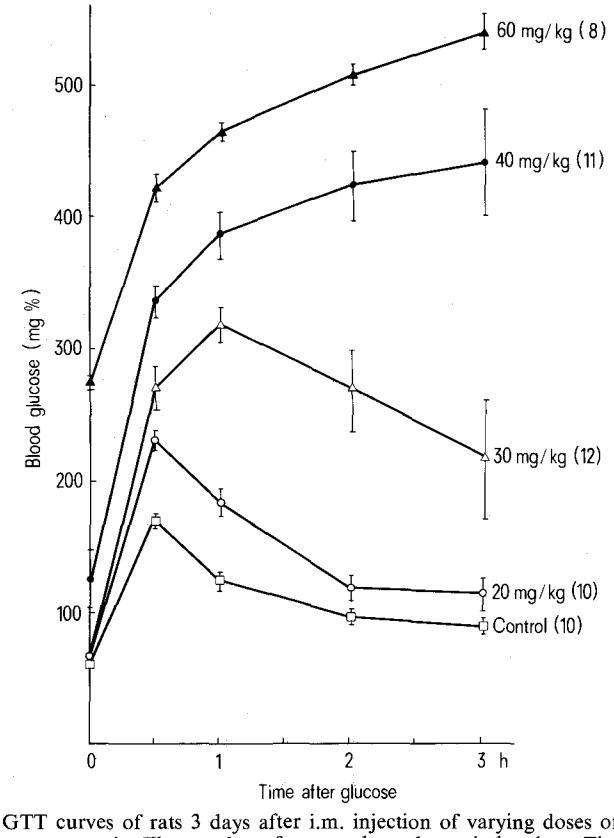
*Mean ± SEM.

nick in a tail vein and collected in small tubes containing a trace of solid heparin. Samples were collected just before injection of the drug, 24 h later and at intervals thereafter. Blood glucose was estimated by the method of Hugget and Nixon[4]. The weights of the animals, 24 h urine volumes, urine glucose and the presence of ketone bodies were recorded throughout the experiments.

Plasma immunoreactive insulin was determined only at the end of an experiment since blood taken from the tail often haemolyzed. 5 ml of blood was withdrawn from the abdominal aorta of the rat, which was anaesthetized with pentobarbitone sodium (23 mg/kg). Insulin was determined by radioimmunoassay using a commercial kit (Radiochemical Center, England)[5].

Glucose tolerance tests were carried out according to the methods of Wexler and Fisher[6] and Cole and Harned[7]. Blood samples were taken from the tails of fasted rats 30 min, 1, 2 and 3 h after the i.p. administration of 10% w/v glucose solution (35 ml/kg)[8].

Results. The mean blood glucose of 42 normal untreated rats was found to be 97±20 mg% (mean±SD). Treated animals were classified as hyperglycaemic if their glucose level exceeded this mean by 2 SD i.e. greater than 140 mg%. Table 1 shows the effect of streptozotocin on the blood glucose of rats 24 h after administration. Streptozotocin 120, 60 or 45 mg/kg induced severe hyperglycaemia in all animals within 24 h. The hyperglycaemia was accompanied by glycosuria and polyuria. Ketonuria occurred in all rats given 120 and 60 mg/kg but in only 20% of those given 45 mg/kg. Following streptozotocin 40 mg/kg, the incidence of glycosuria and ketonuria was similar to that produced by 45 mg/kg but the degree of hyperglycaemia varied.



GTT curves of rats 3 days after i.m. injection of varying doses of streptozotocin. The number of rats used are shown in brackets. The glucose tolerance test was carried out as in 'Materials and methods'. The Mean±SEM are shown at each time period.

With lower doses of streptozotocin the pattern of effects induced was more variable. With 30 mg/kg, 76% of the rats treated exhibited hyperglycaemia which varied from mild to severe. Only in the more severely affected animals did glycosuria persist and only in 20% of these did ketonuria occur. About a quarter of the least affected rats recovered spontaneously and were normoglycaemic after 3–21 days. Their insulin levels at the end of the experiments were however significantly lower ($p < 0.01$) than those which had remained normoglycaemic throughout (table 2). Transient glycosuria was observed in 2 of the 12 rats which remained normoglycaemic. Streptozotocin 20 mg/kg failed to induce hyperglycaemia in any of the rats studied. Mild glycosuria, observed in about 50% of the rats, disappeared completely after 24 h. The highest doses of streptozotocin used showed unacceptable lethality. In rats given 120 mg/kg none survived for longer than 3 days and with 60 mg/kg only half remained after 10 days. In contrast, 45 mg/kg caused no deaths and the animals survived for more than 3 months without treatment and were useful as control subjects for experiments in diabetes. The changes induced appeared to be permanent. Animals given lower doses tended to recover spontaneously; this was most marked in those that weighed least and these animals were also the most resistant to the initial effects of the drug. The response of the treated rats to a glucose load was a more sensitive indication of the toxicity of streptozotocin than was the development of endogenous hyperglycaemia (figure). 3 days after injection, even rats given 20 mg/kg showed significantly raised glucose tolerance curves at 30 and 60 min ($p < 0.001$) after the administration of glucose (3.5 g/kg i.p). Following streptozotocin, 30 mg/kg, the GTT curve was still above the control 3 h after glucose was given although the animals were normoglycaemic initially (30 min, 1 and 2 h $p < 0.001$ and 3 h $p < 0.02$). The pattern of the GTT curves for 60 mg/kg and 40 mg/kg were similar. The values for the 60 mg/kg were however higher at fasting ($p < 0.001$) 30 min ($p < 0.001$) 1 h ($p < 0.01$ and 2 h ($p < 0.02$), with a smaller variation due to the death of the more severely affected animals.

Discussion. These experiments show that streptozotocin can be administered i.m. to successfully induce a diabetic state in rats. We find this a more convenient procedure than injecting i.v. In our animals the dose of 45 mg/kg was the optimum, since it produced permanent changes in glucose metabolism without causing any deaths. Given i.m. streptozotocin appeared to be more potent than originally reported by Junod et al.[3] for its administration i.v. Thus, we observe appreciable lethality with 60 mg/kg i.m. and ketonuria in some rats given 30 mg/kg i.m. In the earlier study, no animals died following 60 mg/kg i.v. and 100 mg/kg i.v. was required to produce ketonuria. We did not, however, investigate whether these differences were due to the route of administration employed or to differences in the animals and their environmental conditions.

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