Experimental dental caries in the albino rat. The effect of single subcutaneous injections of alloxan on the incidence of dental caries

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Several workers during the last half-century have claimed that human sufferers from diabetes mellitus are especially prone to dental caries. This early work has been reviewed by Nichols & Shaw (1957). Everett, Lal & Phatak (1949) reported that alloxan diabetes resulted in an increased caries score when hamsters were maintained on either cariogenic or non-cariogenic diets. Nichols & Shaw (1957) produced diabetes in some of their caries-susceptible rats by intravenous injection of alloxan monohydrate. They could not demonstrate any significant difference in caries incidence between diabetic and control animals. In our investigation weanling rats of one group were injected subcutaneously with alloxan monohydrate and, though it is doubtful if any of the animals were severely diabetic, the incidence of dental caries in the injected group was significantly higher than it was in the controls.

EXPERIMENTAL

Animals. Sixty-eight rats from our own colony were weaned at 4 weeks and divided into two groups. Group 1 contained thirty-seven animals (twenty males and seventeen females), which were injected subcutaneously in the lower abdominal region with a 2% (w/v) solution of alloxan monohydrate in 0·9% (w/v) NaCl solution. Each animal received 200 mg/kg body-weight, e.g. a 50 g rat was injected with 0·5 ml. of the solution. Group 2 contained seventeen males and fourteen females as controls. The animals were housed and maintained as described earlier (Hartles, Lawton & Slack, 1956).

Diets. All animals received diet HS 1, which is the normal diet of the colony and contains 67% sucrose (Hartles & Lawton, 1957).

Duration of experiment. When 26 weeks old the rats were killed with ether: the teeth were examined and the degree of caries was assessed as previously described (Hartles *et al.* 1956).

RESULTS

Effect of alloxan injection. Within 1 h all the rats had developed a temporary paralysis of their hind legs. Recovery was rapid, however, and within 24 h all animals appeared normal. Of the females injected one died within a week, one after 2 weeks, a third 5 weeks after injection and a fourth after 18 weeks. Three male animals died, one 1 week, a second 3 weeks and the third 4 weeks after injection.

Diabetic signs. All the injected rats had a marked glycosuria 2 weeks after injection.

Positive glucose reactions were again obtained from the urine 4 weeks before the end of the experiment. The animals were kept without food for 18 h before the tests. Glycosuria was rarely observed in fasting controls, but sugar was frequently present in the urine of non-fasting animals. Blood-sugar determinations were made on twenty-four fasting animals, twelve being selected at random from each group 10 weeks after injection. Blood (0·1 ml.) was taken from the caudal vein. The results are given in Table 1.

Table 1. *Blood glucose (mg/100 ml.) of twelve fasting rats from each group, 10 weeks after injection with alloxan monohydrate*

	Group 1 (injected)		Group 2 (controls)	
	Males	Females	Males	Females
	230	168	150	196
	146	138	216	150
	150	185	138	132
	146	212	125	166
	312	170	210	120
	238	184	198	176
Mean with its standard error	204 ± 28	176 ± 10	173 ± 16	157 ± 12

The differences between the males and females of group 1 and of group 2 are not significant ($P < 0{\cdot}4$ and $< 0{\cdot}5$, respectively).

In only one injected male was a blood sugar of over 300 mg/100 ml. recorded. Two of the male control animals tested had blood-sugar values a little above 200 mg/100 ml. The difference between the means for the control and injected animals is of doubtful significance ($P < 0{\cdot}3$).

Growth of animals. For 2 weeks after injection the alloxan-injected rats grew much less rapidly than the controls. The injected male animals continued to gain weight at a lower rate than the controls. After the first 2 weeks the growth curves of the females were almost parallel (Fig. 1).

The mean gains in weight per week (in g) with their standard errors were as follows:

	Group 1 (injected)		Group 2 (controls)
Males	10·7 ± 0·87	Males	13·3 ± 10·37
Females	6·0 ± 0·21	Females	6·6 ± 0·24

The difference in weight increase between the males in groups 1 and 2 is significant ($P < 0{\cdot}01$). The corresponding difference for the females is of doubtful significance ($P < 0{\cdot}1$).

Food consumption. Food supply was unrestricted and was not measured accurately. Food pots were replaced each morning and filled up each evening. It was estimated that the animals of group 1 ate about 10% more than did those of group 2.

Water consumption and polyuria. The animals of group 1 (alloxan-injected) drank more water than those of group 2 (controls). The amount the rats drank was not

measured, but a cage of five control rats normally had to have their 8 oz. bottles of drinking water filled once daily: similar cages containing five injected animals required to have their bottles filled twice daily. The quantity of urine voided by the injected animals was also greater.

Cataract. Two of the male animals of group 1 developed bilateral cataract.

Condition of the molar teeth. In group 1, of the thirty surviving animals twenty-eight had caries. The mean number of carious teeth per rat was 4·7 (5·5 in males, 3·5 in females). The mean caries score was 8·2 (9·9 for males, 5·9 for females). In group 2 (controls) twenty-six of the thirty-one animals had caries, the number of carious teeth

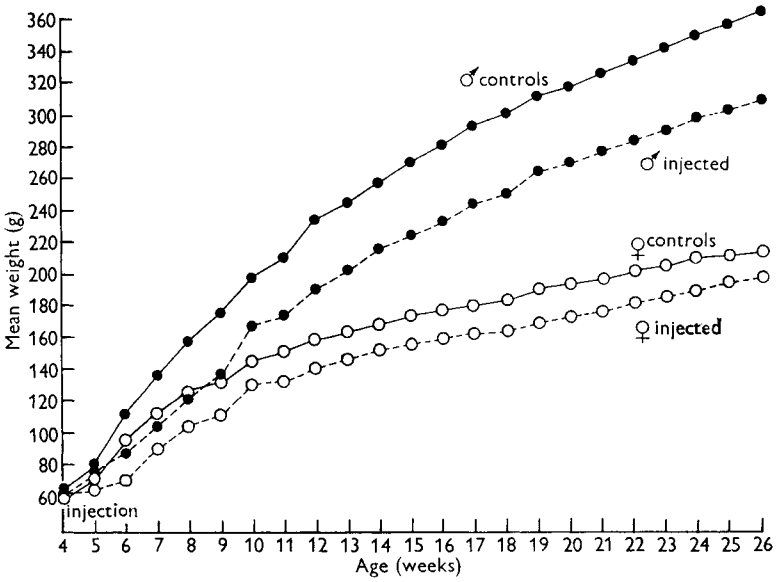

Fig. 1. Mean growth of thirty rats injected with alloxan monohydrate and on a diet containing 67% sucrose (group 1) and thirty-one uninjected rats on the same diet (group 2). ●- - -●, group 1 males; ○- - -○, group 1 females; ●—●, group 2 males; ○—○, group 2 females.

per animal being 2·3 (2·8 in males, 1·8 in females). The mean caries score for these animals was 4·0 (4·9 for males, 2·9 for females). The detailed results are given in Table 2.

In the injected group 1 there were 141 carious teeth, 39% of all molars, of which eighty-five (24%) showed gross carious lesions, which were scored 2 or 3. In the control group 2 there were seventy-two carious molars (19%), of which thirty-nine (10%) were scored 2 or 3.

Table 3 shows the distribution and severity of the carious lesions in the mandibular and maxillary molars of the animals in groups 1 and 2. It is well known that the maxillary molars of the rat are less likely to become carious than the mandibular teeth. One of the notable features of the animals in group 1 compared with their fellows in group 2 was the increase in maxillary caries (21 and 4% respectively).

It can be seen from Table 3 that in the injected group 1 there were about twice as many carious teeth and twice as many teeth scored 2 and 3 as in the control group 2.

Table 2. *Effect of subcutaneous injection of alloxan monohydrate on development of dental caries in rats maintained on diets containing 67% sucrose*

	No. of rats	Age at death (weeks)	No. of rats with caries	No. of carious teeth	No. of carious teeth/rat			Caries score/rat		
					Mandible	Maxilla	Total*	Mandible	Maxilla	Total*
Group 1 (injected):										
Males	17	26	17	94	3·9	1·6	5·5 ± 0·92	7·1	2·8	9·9 ± 1·63
Females	13	26	11	47	2·7	0·8	3·5 ± 0·90	4·6	1·3	5·9 ± 1·87
Total	30	26	28	141	3·5	1·2	4·7 ± 0·63	6·0	2·2	8·2 ± 1·26
Group 2 (controls):										
Males	17	26	15	47	2·4	0·4	2·8 ± 0·45	4·4	0·5	4·9 ± 1·09
Females	14	26	11	25	1·7	0·1	1·8 ± 0·43	2·7	0·2	2·9 ± 0·76
Total	31	26	26	72	2·0	0·3	2·3 ± 0·32	3·6	0·4	4·0 ± 0·70

In the males the differences between groups 1 and 2 in the number of carious teeth ($P < 0·01$) and in the caries score ($P < 0·05$) are significant. For the females the differences are of doubtful significance ($P < 0·1$ and $< 0·2$, respectively). When the results for the males and females of each group are taken together the differences are highly significant ($P < 0·005$).

* Value with its standard error.

Table 3. *Distribution and severity of caries in the mandibular and maxillary molars of rats injected with alloxan monohydrate and of control rats*

	Percentage of molars with caries		Percentage of molars with scores of 2 and 3 (see p. 288)	
	Group 1 (injected)	Group 2 (controls)	Group 1 (injected)	Group 2 (controls)
Males:				
Mandible	66	40	40	22
Maxilla	26	6	16	3
Total	46	23	28	12
Females:				
Mandible	47	27	26	15
Maxilla	13	2	10	1
Total	30	14	18	8
Males and females:				
Mandible	58	34	34	19
Maxilla	21	4	13	2
Total	39	19	24	10

DISCUSSION

We do not claim that our animals exhibited all the signs of severe diabetes. Though they were all glycosuric, with the exception of one male animal there was no dramatic loss of body-weight, but the growth rates of the injected male animals were significantly less than those of the controls. Blood-sugar tests on twelve injected animals showed only one above 300 mg/100 ml. and three between 200 and 300 mg/100 ml. In the control group two animals had blood-sugar values just over 200 mg/100 ml. Duff & Starr (1944) state that the average blood-glucose value in the normal rat is about 100 mg/100 ml. Lazarow & Palay (1946) consider that normal values may lie between 90 and 160 mg/100 ml.; they showed that intravenous injection of alloxan at a level of 40 mg/kg body-weight caused values ranging from 200 to 500 mg/100 ml. Similar values were produced by the intraperitoneal injection of doses of 200 mg/kg body-weight.

From a study of the hyperglycaemic effect of alloxan Bennett & Behrens (1946) report an average blood-sugar value of 129 mg/100 ml. in uninjected control rats, with a range of 113 to 146 mg/100 ml. After injection once with alloxan intraperitoneally at a level of 200 mg/kg body-weight, the mean blood-sugar value rose to 151 mg/100 ml., with a range of 108–333 mg/100 ml. Rats receiving the same dose on two successive days developed a more severe hyperglycaemia, mean values of 736 mg/100 ml. being recorded. All these rats were maintained on stock laboratory diets.

The control rats in our investigation ate a diet containing 67% sucrose and had mean blood-sugar values of 173 mg/100 ml. (males) and 157 mg/100 ml. (females). The corresponding values for the injected animals were 204 mg/100 ml. (males) and 176 mg/100 ml. (females). The high-sugar diet itself therefore appears to have caused an elevation of the blood-sugar values in the control animals, compared with the reported normal values for rats maintained on conventional stock diets.

In both injected and control rats the mean blood-sugar values for the males were greater than for the females. These differences were not significant ($P < 0{\cdot}4$ and $< 0{\cdot}5$, respectively).

Our experiment is not strictly comparable with that of Nichols & Shaw (1957). Their animals, which were frankly diabetic, had received intravenous injections of alloxan at a level of 48 mg/kg body-weight and had blood-sugar values above 315 mg/100 ml. The blood-sugar values of their control animals did not exceed 170 mg/100 ml. Our route of injection was subcutaneous, which is well known to be less reliable in producing diabetes, and our animals were less severely affected. In the Harvard experiment the average number of carious teeth per animal in the controls was the high one of 10·5, the maximum possible value being 12; as the authors point out, they are compelled to seek differences in susceptibility to caries by estimating the extent of the caries lesions rather than by counting their number. Their animals were admirable material for assessing factors that reduce caries but less satisfactory for estimating any increase. Our animals, on the other hand, had a comparatively low incidence of caries, thus providing ample scope for demonstrating any increased incidence or severity.

We are satisfied that our injected rats had more caries than the controls, but we cannot say that this increase was dependent upon the development of severe diabetes.

Evidence is accumulating that there is a relationship between the endocrine system and the salivary glands. Hypophysectomy in the rat leads to histological changes in the submaxillary glands and to an increase in dental caries (Shafer & Muhler, 1955). Bixler, Muhler & Shafer (1955) noted changes in the adrenals after removal of the salivary glands from the rat. Muhler & Shafer (1955) reported that the injection of oestrogens into intact rats caused a greater increase in caries than did the injection of androgens.

The possibility cannot be excluded that the slightly increased food intake may have affected the caries incidence in group 1 animals, but it is unlikely to have been of major importance. We think that the most probable way in which the injection of alloxan can influence the incidence of dental caries is by causing an alteration in the salivary secretions, either directly or indirectly.

SUMMARY

1. Sixty-eight 4-week-old rats from our colony, which is maintained on a diet containing 67% sucrose, were divided into two groups. Thirty-seven (group 1) received single subcutaneous injections of alloxan monohydrate (200 mg/kg body-weight). Thirty of the injected rats survived the experimental period of 5 months. Thirty-one uninjected animals were used as controls (group 2).

2. The injected animals exhibited some of the signs of diabetes, such as glycosuria, polyuria, increased intake of water. The concentration of glucose in the blood of twelve such animals chosen at random was not outstandingly higher than that of a similar number of controls (group 2).

3. The mean number of carious teeth per rat and the mean caries score were significantly higher for the injected animals (4·7 and 8·2) than for the controls (2·3 and 4·0).

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Effect of long-term feeding of albino rats on rice and on rice–tapioca diets on the blood and on the liver

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In a previous paper, Subrahmanyan, Murthy & Swaminathan (1954) reported that replacement of 25% of rice in a poor rice diet by tapioca flour enhanced significantly the growth of young rats. This effect was shown to be due to the supplementary value of the extra calcium present in the tapioca flour. Since partial replacement of rice by tapioca flour caused a slight lowering in the protein content of the diet, the possibility remained that the ill effects, if any, due to it, may not be observed in a period of 8 weeks. It was therefore thought desirable to conduct long-term feeding experiments and to study the effect of prolonged feeding of such diets on the concentration of haemoglobin in the blood, on the red-blood cell count, and on the composition and histological appearance of the liver. A third group of rats on the control rice diet, supplemented with the same amount of calcium (as calcium lactate) as that supplied by tapioca in the rice–tapioca diet, was also included with a view to ascertaining the effect of the calcium supplement on growth and on the liver and blood of rats.

EXPERIMENTAL

Three groups of freshly weaned albino rats weighing between 40 and 48 g (ten in each group and distributed equally according to sex, body-weight and litter) received the diets *ad lib.*; the composition of the diets is given in Table 1. The methods of prepara-