Effect of Strain Differences on Alloxan Diabetes in Albino Rats

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Studies in this laboratory on the details of the alloxan reaction in albino rats of Wistar parentage have revealed a number of features hitherto not reported in this or other species of experimental animal.[1, 2, 3] These features include:

1. Greater susceptibility of females than of males to alloxan diabetes, a sex difference not eliminated by prepubertal gonadectomy.
2. Marked and delayed hypoglycemic phase of the alloxan reaction occurring at about the 24th to 37th hours after injection.
3. Inability of severely diabetic animals to maintain high fasting blood sugars and the frequent occurrence of severe fasting hypoglycemia, a demonstration that alloxan diabetes in Wistar rats is of a complex and unorthodox type.

It seemed strange that these characteristics of alloxan diabetes, so marked in the Wistar derivative, were not described by other workers who have observed alloxan treated rats. It appeared likely that the explanation might lie in strain differences. The present work, which explores this possibility, demonstrates that such a strain difference does in fact exist and implies diversity between strains in endocrine endowment relating to carbohydrate metabolism.

EXPERIMENTAL

Observations of the alloxan reaction in 72 male and 72 female albino rats of Wistar origin which were reported previously[1] are compared herein with those from a similar experiment using 68 male and 66 female albinos of the Osborne-Mendel strain.* These were bred in our own colony from descendants of a small stock secured from The Connecticut Agricultural Experiment Station in New Haven.† In designing the work the Osborne-Mendel rat seemed of special significance because investigation of this hereditary line in other laboratories has demonstrated it to be physiologically unique. Not the least interesting characteristic of the strain from our viewpoint is its normally lowered tolerance for glucose which was discovered by Cole and Harned[4] and referred to by them as the "diabetic trait."

Animals in the age range of 200 to 280 days were used. These were fasted 96 hours prior to injection of alloxan monohydrate (Eastman) which was given in 3 per cent solution subcutaneously in a dose of 20 mg. of the monohydrate per 100 gm. body weight. Fifteen minutes after injection the animals were given their usual ration of Purina Laboratory Chow *ad libitum*. Blood samples for glucose determination were taken from the tail before alloxan was given and at various intervals thereafter up to 61 hours. Not more than eight or nine samples of 0.1 ml. volume were

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*The Osborne-Mendel strain of albino rat is a pure line maintained by The Connecticut Agricultural Experiment Station having been bred there since 1910 without accessions from elsewhere. It originated from a few animals purchased in a local pet shop by Thomas B. Osborne for some early nutrition work at the Experiment Station. This breed of rat was used in the now classic experiments in nutrition by Thomas B. Osborne in collaboration with Lafayette B. Mendel of Yale University. Thus, although the parent breeding colony has always remained at The Connecticut Agricultural Experiment Station, it sometimes has been referred to as the "Yale strain." The development of this stock as regards its accelerating growth rate over the years of inbreeding and special nurture is described by Osborne and Mendel[5] and later by Mendel and Hubbell.[6]

†The authors are grateful to Dr. Hubert B. Vickery and Dr. Rebecca B. Hubbell for breeding stock from The Connecticut Agricultural Experiment Station colony.

taken from any one animal to avoid excessive handling and blood loss.

A variety of measures was used to assess the resulting diabetic state. Postprandial blood sugar was determined one and three weeks after alloxan and the fasting glucose tolerance two weeks after alloxan. At one and three weeks also, urinary glucose and acetone (qualitative) were determined on samples collected during a standard 18-hour period on a diet of fluid milk. During this period a record of body weight was maintained. The details of these tests and methods are described in an earlier paper.[1]

RESULTS

The over-all incidence of diabetes in alloxan treated rats among the two strains is presented in table 1. Here it is seen that the Osborne-Mendel animals evidence a far greater sensitivity to the compound than those of Wistar origin. Of 144 Wistar rats so treated, only 31 terminated fatally whereas there were 62 fatalities among 134 Osborne-Mendel rats. Twenty-six per cent of the Wistar males but only nine per cent of the Osborne-Mendel males were resistant to alloxan. It is clear that while the two strains differ from each other in sensitivity to alloxan both strains show the same sort of sex divergence in susceptibility, namely, that the females are more severely affected than the males, a difference originally demonstrated in the Wistar strain.[1] Sixty-three per cent of the Osborne-Mendel females but only 30 per cent of the corresponding males developed diabetes which terminated fatally. Although alloxan treated animals classed in the subgroup "Fatal" under "Severe Diabetes" died with high blood sugars and had every evidence of severe diabetes, it is possible that other toxic damage may have contributed to the fatal issue, for it is well known that alloxan injury is not limited exclusively to the pancreas.

Comparison of established severe alloxan diabetes in the two strains of animals in the postprandial state is furnished by table 2. As regards blood sugar level, males and females of both strains are about comparable showing averages in the range of 400 to 450 mg. per 100 cc. Likewise, urinary glucose levels are not very different though all groups show a wide variability as indicated by large standard deviations. Strain and sex differences are apparent however in the matter of urinary ketone body excretion. That the Osborne-Mendel strain is more susceptible to ketonuria is demonstrated by the fact that three weeks after alloxan injection 4 per cent of the Wistar male diabetics and 72 per cent of the Osborne-Mendel male diabetics show ketonuria. The sex difference originally observed in diabetic ketonuria in the Wistar strain[1] is not as readily apparent in the Osborne-Mendel strain on the basis of the present data. A lower proportion of Osborne-Mendel males than females show ketonuria one week after alloxan but by three weeks the qualitative incidence appears the same. However, if quantitative estimates of urinary ketone bodies had been made the sex difference might also have been apparent in Osborne-Mendel rats at the third week, for many of the males at that time evidenced faint traces of acetone while the females gener-

TABLE 1
Diabetes in alloxanized rats

Animal Strain	Type Reaction*	Male No.	Male Per cent	Female No.	Female Per cent
WISTAR	Severe diabetes	40	56	64	89
	Fatal	12	17	19	26
	Mild or transient diabetes	13	18	2	3
	Resistant	19	26	6	8
	Total	72		72	
OSBORNE-MENDEL	Severe diabetes	57	84	62	93
	Fatal	20	30	42	63
	Mild or transient diabetes	5	7	1	2
	Resistant	6	9	3	5
	Total	68		66	

*Type reaction.

Severe diabetes—Marked glycosuria in both strains ranging from 4.0 to 12.0 per cent. If not terminated fatally the postprandial blood sugar at three weeks was above 270 mg. per 100 cc.

Weight loss was generally observed in these animals during the three week period and was more severe in the Osborne-Mendel animals amounting to 10 to 35 per cent of the body weight.

Fatal (subgroup)—Developed severe diabetes but failed to survive one week after injection of alloxan.

Mild or transient diabetes—Showed some symptoms of diabetes but by three weeks had recovered or showed postprandial blood sugars below 270 mg. per 100 cc. Little or no glycosuria at three weeks and no weight loss.

Resistant—All others.

TABLE 2
Blood sugar, glycosuria and ketonuria in alloxan diabetic rats

Animal Strain	Number and Sex	Time After Alloxan	Postprand. Bloodsugar	Average Urine Vol.	Urine Glucose*	Cases Showing Ketonuria†
		Weeks	mg./100 cc.‡	ml.	gm./100 cc.	Per cent of total
WISTAR	28 Males	1	402±79	41	4.7±2.0	12
		3	424±74	55	5.7±1.1	4
	45 Females	1	426±74	50	6.3±1.8	50
		3	418±72	54	7.0±1.7	61
OSBORNE-MENDEL	37 Males	1	439±68	33	7.2±2.7	38
		3	442±89	49	7.5±2.7	72
	20 Females	1	436±57	28	7.2±2.6	70
		3	457±52	42	7.2±1.8	75

*During this test animals are placed in metabolism cages for an 18 hour period, given water *ad libitum* and whole fluid milk up to a total volume of 60 ml.
†The ketone body test was applied to the same urine sample on which glucose was determined.
‡± indicates standard deviation.

ally showed more substantial amounts.

The dramatic succession of blood sugar changes which occurs during the first 48 hours following alloxan injection, referred to usually as the "triphasic response," has interested nearly all workers who have dealt with this type of diabetes. Figures 1 and 2 present such a study on males and females respectively of the Wistar strain and may be compared with figures 3 and 4 relating to the Osborne-Mendel strain. The data on the sexes have been separated to reveal the sex difference as well as the marked contrast between the two strains. In Wistar stock hyperglycemia increases progressively during the first 20 hours after injection and this is followed by the rapidly-developing, transient relative hypoglycemia. This characteristic low blood sugar phase is observed in every Wistar animal. In a large percentage of the reacting Wistar rats, particularly the more susceptible females, the hypoglycemia is "real" with blood sugars falling well below normal fasting levels. By way of contrast, males of the Osborne-Mendel stock are completely resistant to the development of the hypoglycemic phase, while the Osborne-Mendel females show but a slight tendency to falling blood sugar in the 24 to 30 hour interval.

Most important in the assessment of alloxan diabetes is the oral glucose tolerance test after a 24 hour fast. Data on this test applied to alloxan diabetic and normal subjects of both animal strains are presented in table 3. To emphasize fully the outstanding features of variability in alloxan diabetes within as well as between strains, the high, low and median values and the first and third quartile limits in addition to average values are presented. Animals of the Wistar line in severe diabetes are characterized by relatively low fasting blood sugars. A significant fact is that some of them develop a real hypoglycemia and may even become convulsive, behavior not explainable on the basis of relative supply of insulin alone. In contrast, the Osborne-Mendel line shows generally an intense fasting hyperglycemia with only one male in the present group developing a normal fasting blood sugar and no instance of fasting hypoglycemia. Attention is also called to the fact that the average glucose tolerance curve in the Osborne-Mendel diabetics falls less steeply from the 0.5 to the two-hour period and is thus more typically diabetic than that of the Wistar animals. These differences are especially noteworthy when it is recalled that the diabetic animals of both strains appear to have equal glycosuria and hyperglycemia when fed.

Cole and Harned[4] and later Orten and Devlin[7] demonstrated that untreated Osborne-Mendel rats have an impairment in glucose tolerance compared with Wistar rats. While this difference is hardly apparent in the normal data in table 3, the present results can be explained in that they were obtained by the use of a much less taxing glucose tolerance load.

DISCUSSION

After Cole and Harned's[4] finding that the Osborne-Mendel strain rat may develop progressive impairment of glucose tolerance with age,* Cole, Harned and Keeler[8] studied the genetic aspects of this "diabetic trait" and concluded that the sire exerts a greater influence than the dam upon the glucose tolerance of

TABLE 3
Oral glucose tolerance test* after 24 hour fast

	Wistar					
	Male (26)†			Female (35)		
Time after glucose hour	0	0.5	2	0	0.5	2
Normal Average	86±6‡	125±7	90±6	89±7	128±8	91±6
Alloxan diabetic Average	115±49	252±49	141±53	150±73	276±78	178±80
Highest value	239	378	265	309	435	329
1st quartile	138	290	180	215	356	252
Median	94	253	132	123	262	170
3d quartile	83	224	109	94	235	119
Lowest value	62	165	62	47	129	44
	Osborne-Mendel					
	Male (37)			Female (20)		
Time after glucose hour	0	0.5	2	0	0.5	2
Normal Average	97±14	132±23	105±31	83±7	136±10	97±7
Alloxan diabetic Average	251±91	382±106	329±97	289±89	431±70	366±86
Highest value	454	672	484	568	548	572
1st quartile	326	444	384	327	476	409
Median	272	388	332	285	436	362
3d quartile	165	328	284	237	394	327
Lowest value	94	138	111	138	264	188

*Tests were made on alloxan treated animals two weeks after alloxan injection; 100 mg. glucose per 100 gm. body weight in 20 per cent solution were given by mouth.
†Figures in parenthesis show number of cases represented in average values.
‡± indicates standard deviation.

the offspring. Harned and Cole have proposed that this difference between the Osborne-Mendel and the Wistar strains is due to a difference in anterior pituitary function. As evidence of hyperactivity of the anterior pituitary in the Osborne-Mendel strain they point out its greater hyperglycemic response to epinephrine, a mild degree of insulin resistance, increased urine volumes, accelerated growth rate and higher incidence of sterility. During the long history of the breeding of the Osborne-Mendel strain a remarkable acceleration of growth rate has occurred,[5, 6] and while this enhanced capacity to grow has been largely attributed to improved dietary regimen[10] it also presupposes pituitary growth hormone production at a rate permissive of such growth. The Osborne-Mendel strain reaches a body size one quarter to one third greater than that of the Wistar strain but frank gigantism is not observed. Orten and Sayers[11] have demonstrated that the Osborne-Mendel rat has a diminished ability to store liver glycogen compared with the Wistar rat and they believe it is this condition rather than lack of insulin which causes the "diabetic trait."

All of the comparative work on the two strains of rats, including our present observations, indicate a marked divergence between them and differences in endocrine endowment almost certainly are responsible in part at least. It is, of course, not certain that all of the unique characteristics of the Osborne-Mendel strain compared with the Wistar can be explained on the basis of a single hereditary difference.

If the hypoglycemic phase in the response to alloxan action is due to an outpouring of insulin from the damaged beta cells of the pancreas, as is commonly thought, the absence of any marked hypoglycemia in the Osborne-Mendel rat under these circumstances sug-

*After two years of observing the Osborne-Mendel strain in our own breeding colony, we have found among about 200 males one that at maturity spontaneously developed frank diabetes with continuous glycosuria and hyperglycemia. In all other respects the animal was normal and seemed vigorous.

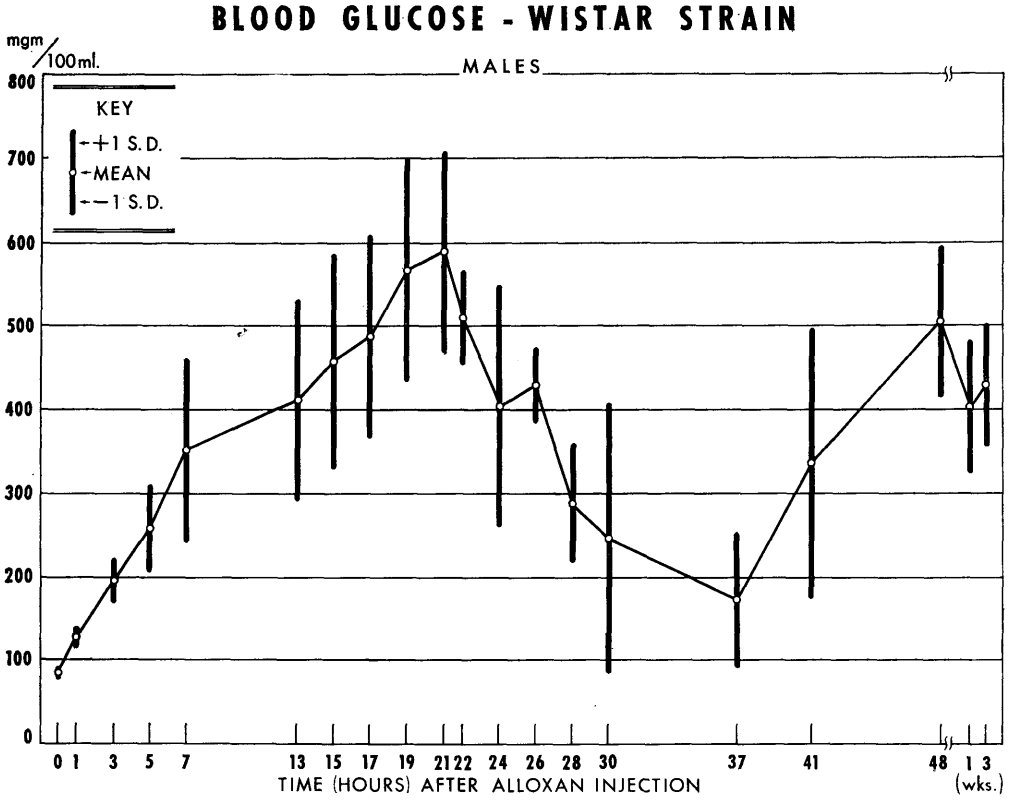
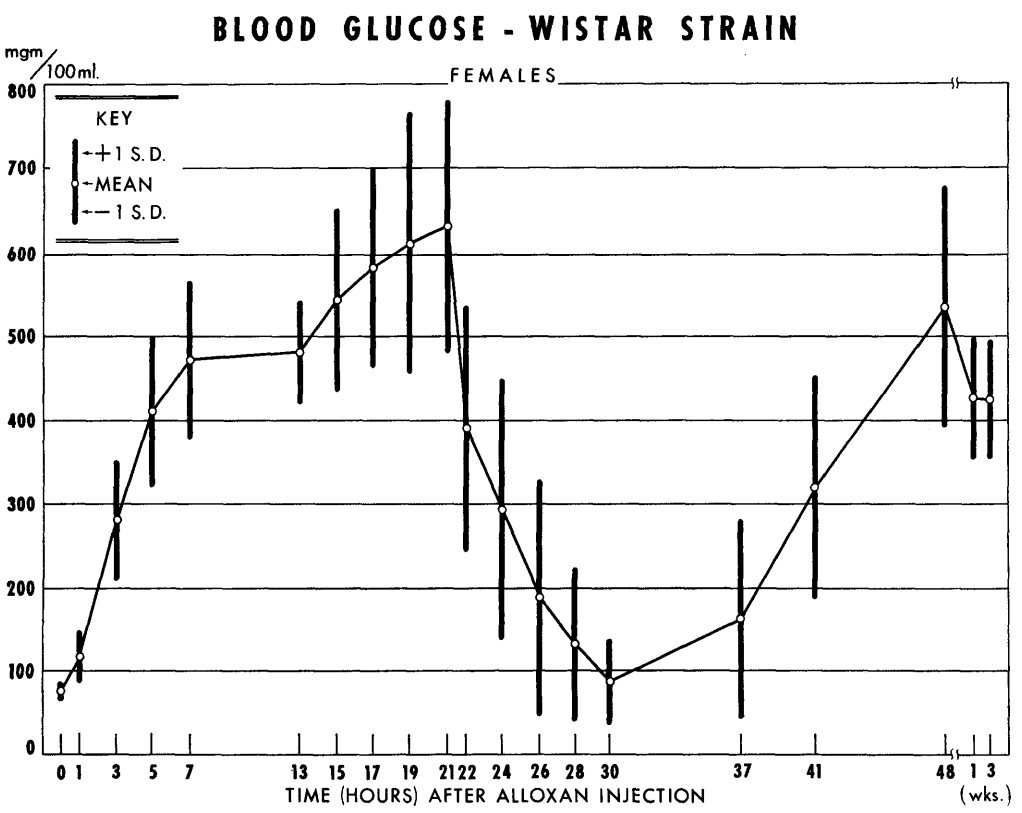
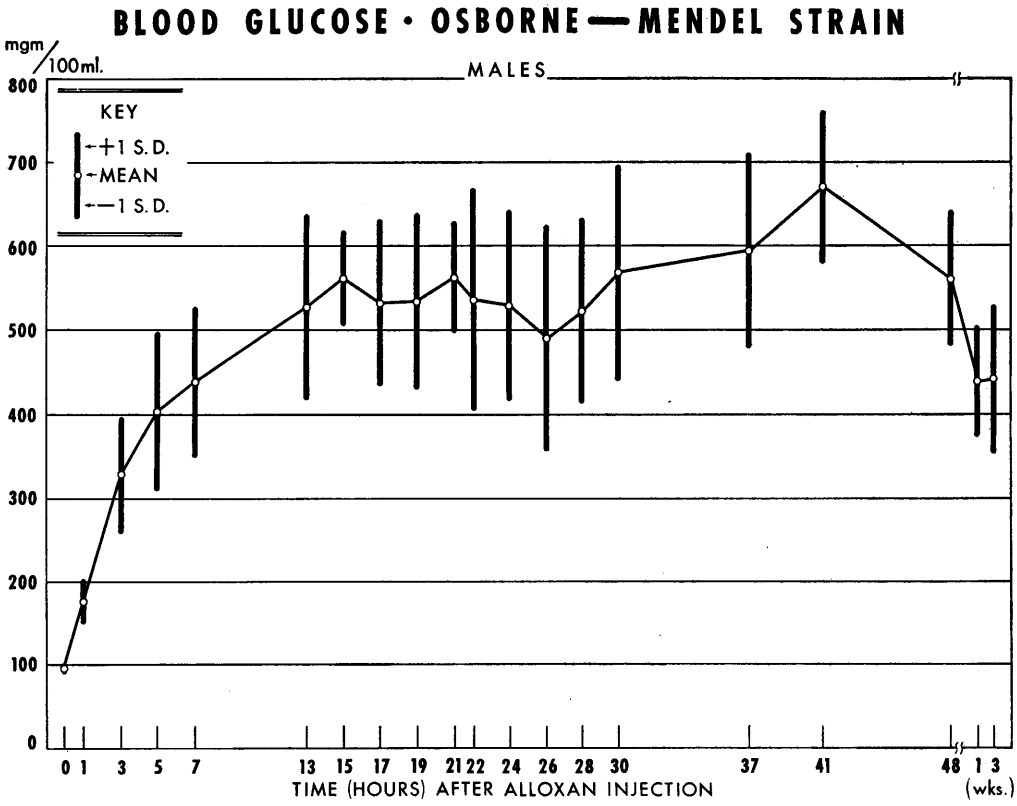


FIGURE 1



Blood sugar changes after alloxan. Data on only those animals developing severe diabetes and surviving at least one week after alloxan injection. Prior to alloxan injection animals were fasted four days. Fifteen minutes after alloxan, food was given ad libitum for the duration of the experiment.

FIGURE 2



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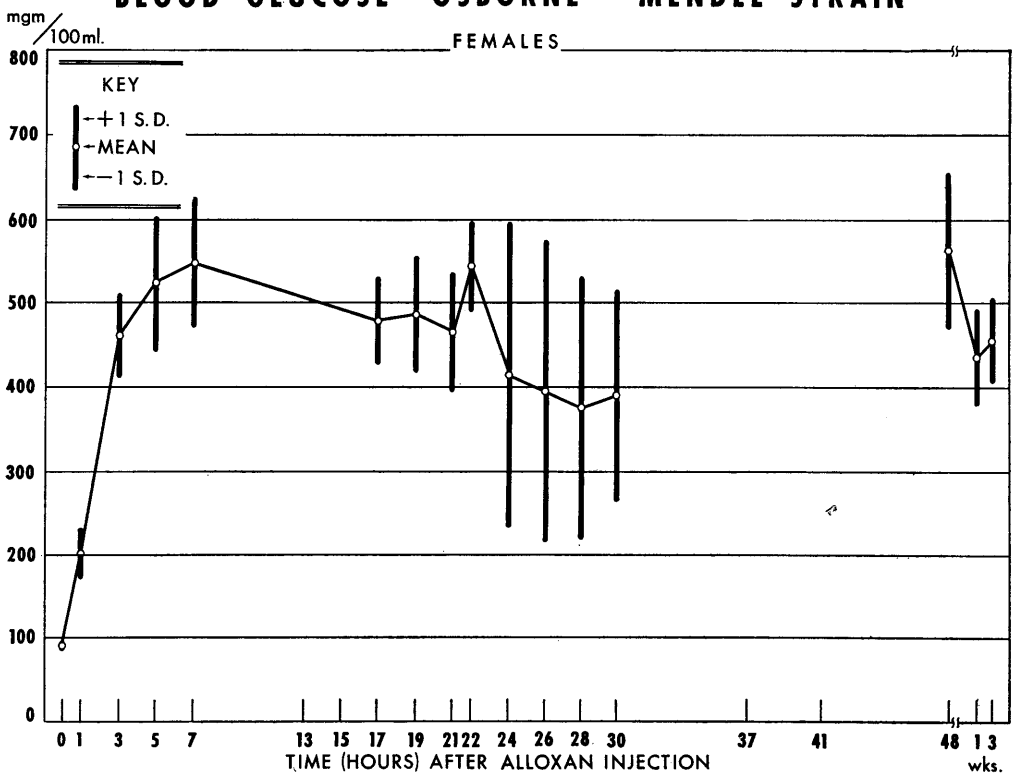

FIGURE 4

gests either a virtual absence of insulin reserves in the pancreas or an insulin resistance not shared by the Wistar strain. In connection with this absence of a pronounced triphasic response in the Osborne-Mendel strain, it is perhaps of special significance to consider the possible role of liver glycogen storage as the mechanism for producing the hypoglycemic phase. Cullimore and others[3] demonstrated that in the Wistar animals the hypoglycemic period was simultaneous with a period of elevated liver glycogen. The observation of Orten and Sayers[11] that the Osborne-Mendel rat has an impairment in the mechanism of liver glycogen storage suggests this as a possible cause of the failure of the strain to give a typical response to alloxan.

The additional difference between the two strains of rats in respect to fasting blood sugar and form of glucose tolerance curves after induction of alloxan diabetes presents difficulties of interpretation. The assumption that insular tissue in the Osborne-Mendel rat is more susceptible to alloxan and consequently that more severe diabetes is produced is attractive and would be sufficient to explain all except the group of Wistar diabetics which develop real hypoglycemia in fasting. It is in this group of animals, which resemble hypophysectomized or adrenalectomized animals, that the diabetes is believed to be modified by some factor other than insulin supply. Experiments are now in progress to examine further points of difference in carbohydrate economy of these two contrasting albino rat strains that might shed some further light on the present problem. Previous work from this laboratory[2] has demonstrated that the gonads have a considerable effect upon the diabetic state produced by alloxan. Castrates of both sexes, in which alloxan diabetes was subsequently induced, show more elevated fasting blood sugars and prolonged tolerance curves than intact control diabetics. This emphasizes one way in which alloxan diabetes depends upon the balance of extrapancreatic endocrine factors, a point of view not inconsistent with current research and clinical opinion regarding spontaneous diabetes.

SUMMARY

A comparative study of alloxan diabetes in the Osborne-Mendel and the Wistar strains of albino rat is reported. The Osborne-Mendel strain differs from the Wistar strain in: (1) greater susceptibility to alloxan; (2) failure to develop any severe hypoglycemic response to alloxan during the 48 hours following its administration; (3) greater susceptibility of the alloxan diabetic males to ketonuria; (4) more elevated fasting blood sugars in severe alloxan diabetes; and (5) more elevated and prolonged diabetic glucose tolerance curves. The findings are discussed with reference to other work that has demonstrated abnormalities in the Osborne-Mendel strain.

SUMMARIO IN INTERLINGUA

Diabete Alloxanogene in Rattos Albin, Influentiate per Differentias de Racia

Es reportate un studio comparative de diabete alloxanogene in le racias Osborne-Mendel e Wistar de rattos albin. Le racia Osborne-Mendel differe ab le racia Wistar in (1) su plus grande susceptibilitate a alloxano, (2) absentia de sever responsas hypoglycemic a alloxano durante 48 horas post su administration, (3) un plus grande susceptibilitate del masculos a cetonuria, (4) plus elevate nivellos de sucro sanguinee in stato jejun in sever casos de diabete alloxanogene, e (5) plus elevate e prolongate curvas de tolerantia a glucosa. Nostre constatationes es discutite con referentia a altere studios que ha demonstrate anormalitates in le racia Osborne-Mendel.

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