of *in vitro* tuberculin hypersensitivity has been further elucidated.

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17268. Alloxan Subdiabetes in Rabbits Detected by Modification of Glucose Tolerance by Adrenal Cortex Extract.

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The subdiabetic state may be defined as a phase of pancreatic diabetes characterized by subtle metabolic disorders; these disorders, in contrast to overt diabetes, are unaccompanied by hyperglycemia or glycosuria, and, in contrast to latent diabetes, are not accompanied by impaired glucose tolerance as determined by conventional methods. The existence of such a state, which might or might not be a prediabetic state, has been suspected by many students of human diabetes mellitus.

Although latent diabetes has been demonstrated in various animals,[1-3] subdiabetes and prediabetes have only been demonstrated by retrospective analysis of the records of mothers of overweight babies[4] and of the records of partially pancreatectomized animals.[5,6] The present studies were undertaken in an attempt to produce experimental subdiabetes, and, by exploiting the adrenal cortex-insulin antagonism,[7] to devise a method for its detection.

Methods and Materials. Mongrel and white rabbits weighing 2.5-3.6 kg were used. Their diet consisted of oats, hay, carrots, cabbage, lettuce, and water *ad lib.* The animals were starved for 18-20 hours prior to each experimental procedure, including the alloxan injections. Sugar was determined on ear vein blood by the method of Hagedorn and Jensen;[8] no anticoagulant was used, and the precipitated samples were all permitted to stand, without fluoride, until the end of the third hour, when the determinations were made. Urinary sugar was not followed.

Glucose tolerance was tested by the intravenous injection, during about one minute, of 3 g of glucose in 50% solution, regardless of weight; this was followed by 2-3 cc of physiologic saline. Fasting, 1, 2, and 3 hour blood samples were drawn. The effect of adrenal cortical hormones on the glucose tolerance was studied by injecting aqueous adrenal cortex extract (Upjohn) intramuscularly, ½ cc/100 g body weight, one half hour before the glucose injection; the entire procedure will be called the *A. C. Tolerance Test.* It should be noted that the fasting blood sugar specimens in all A. C. tolerance tests were drawn one half hour after the injection of adrenal cortex extract (A.C.).

One day or more after the completion of preliminary tolerance tests, alloxan monohydrate (Eastman) was rapidly injected into the ear veins of fasted rabbits in 2.5% solution; further tolerance tests were performed, and subsequent doses of alloxan given, if indi-

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TABLE I.

Animal and wt	Date	Alloxan, mg/kg	Date	Glucose tolerance. Blood glucose level in mg/100 cc Hour 0	1	2	3	Date	Adrenal cortex-glucose tolerance. Blood glucose level in mg/100 cc Hour 0	1	2	3
No. 1			9/9	99	177	101	93	9/23	139	138	115	106
2.9 kg		None										
No. 2			8/26*	111	276	188	—					
2.9 kg	8/30	23										
	10/22	29.3	10/28	101	110	97	95	11/4	77	159	215	206
			11/11	97	111	106	97					
No. 3			9/23	108	104	125	97					
3.0 kg	9/24	22	9/28	113	—	102	117	9/30	92	166	141	127
No. 4			10/7	86	199	139	146					
2.6 kg	10/8	24.2										
	10/15	15	10/26	122	120	110	99	10/28	104	177	139	124
No. 5			10/7	104	154	124	115	10/14	72	173	120	104
2.5 kg	10/15	15										
	10/28	38	11/11	84	208	117	79	11/15	75	213	159	104†
			11/18	113	179	120	88†					
No. 6			11/18	106	154	99	136	11/25	90	238	102	106
3.6 kg	12/7	41	12/16	102	127	101	93	12/19	119	177	145	117
			12/23	102	111	95	90					
No. 7			11/25	95	146	101	65	12/2	—	184	148	131
3.0 kg	12/7	25										
	12/17	20	12/30	101	150	102	99	1/4	113	132	154	127
			1/18	95	150	102	119					
No. 8			9/28	97	170	186	132	9/30	127	241	226	204
2.9 kg		None										

* 7.4 g of 10% glucose injected intraperitoneally.
† Blood specimens drawn 15 minutes after the indicated hour.

cated. The initial and subsequent alloxan doses listed in Table I are approximate since in some instances there were small or moderate amounts of leakage at the hub of the needle, or of subcutaneous infiltration.

Autopsy was performed on rabbit No. 5 within 15 minutes of its death, and on rabbits Nos. 4 and 7 after approximately 8 hours. Pancreas, aorta and kidneys were studied, fixed in formalin, and stained by conventional histologic methods.

Results. Eight rabbits were studied, of which 6 received alloxan on one or two occasions. Blood sugar observations on these animals, before and after alloxan treatment, are presented in Table I. The data in Table I were treated statistically. Five means were computed for each hour of the glucose tolerance tests, using the following series: (1) The initial glucose tolerance tests; (2) the initial A.C. tolerance tests; (3) the glucose tolerance tests following the first effective dose of alloxan; (4) the A.C. tolerance tests immediately following the preceding group; and (5) the ordinary glucose tolerance tests following shortly after the fourth group. Calculations of the standard errors of the differences between each of these 5 blood sugar means were made for each hour (0,1,2, and 3) with the usual equation for small series, and p was determined, in each instance, from Fisher's t table. Where two successive tolerance tests of the same type were performed the later figures were used in computing the means, and only these figures appear in Table I; for the initial glucose tol-

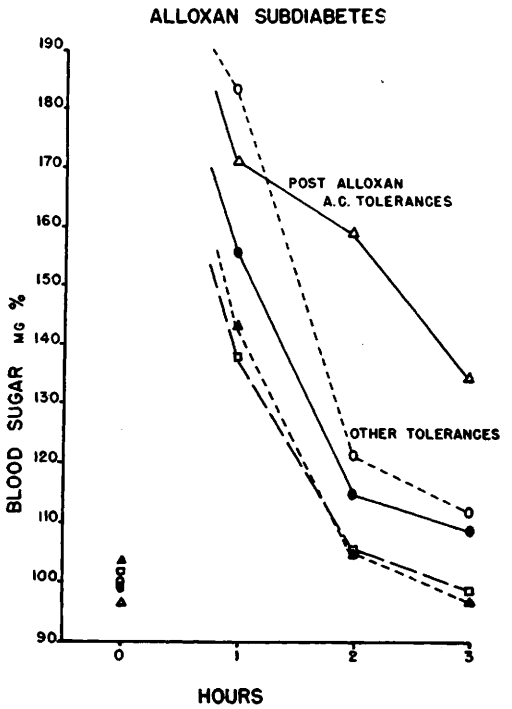

FIG. 1.
Glucose tolerance curves before and after alloxan.
●——● Initial glucose tolerances
○ - - - ○ Pre-alloxan A.C. tolerances
▲ - - - ▲ Post-alloxan glucose tolerances
△——△ Post-alloxan A.C. tolerances
□ – – □ Final glucose tolerances
Each point represents a mean blood sugar value as described in text.

erance tests the earliest figures were used. The control values for Rabbit 2 were not used because of the difference in method (see Table I), and none of the values for Rabbit 8 were used because of its spontaneous diabetes.

Two hours after the injection of glucose the mean blood sugar of the post-alloxan A.C. tolerance tests is 158.8 mg%, this is significantly higher than any of the other blood sugar means at that hour ($p < 0.01$ for all the differences except that between the pre- and post-alloxan A. C. tolerance means, where $p = 0.05$). At three hours the post-alloxan A.C. tolerance mean is 37.2 mg % higher than that of the post-alloxan glucose tolerances; this difference is of moderate significance ($p < 0.05$). No other p values of 0.05 or less were found. Glucose tolerance curves for the five experimental situations have been plotted in Fig. 1, using the same blood sugar means as in the statistical treatment.

Of the 6 rabbits which received alloxan, 4 were retested after a month or more, while one animal (No. 3) died 48 hours after a third dose of alloxan of 25 mg/k, and another (No. 7) died on 5/5 following a month of polydipsia and marked polyuria. Data on the retested animals is presented in Table II. Animal No. 4 subsequently died (4/26) following about 8 weeks of polydipsia and polyuria; it had not been retested since 1/13. Rabbit 5, which developed a diabetic ordinary glucose tolerance, was found to have hind limb paralysis shortly after its last test. Diarrhea supervened and the animal died immediately following the intraperitoneal injection of 60 cc of physiologic saline on the fifth day after this last tolerance test. At autopsy the bladder was found to be distended by about 100 cc of slightly turbid, albumin-free urine containing 179 mg% of sugar. The abdominal aorta exhibited two 0.5 cm long fusiform swellings; the intima in these areas was rough, friable, and pearly with loss of elasticity of the wall. The other organs appeared grossly normal. Histologic sections of the aorta showed medial sclerosis and calcification without significant intimal involvement. The aortas of Rabbits 4 and 7 appeared grossly normal.

Discussion. Intravenous Glucose Tolerance. In order to stabilize test conditions the rabbits were fasted[9] after a period of adequate carbohydrate feeding.[10] A uniform intravenous dose of 3 g of glucose (0.9-1.2 g/kg) was selected for the experiments in order to circumvent variability both in the time and in the degree of absorption of the sugar. Others have found intravenous tolerance methods satisfactory in normal rabbits,[2,11-13] and modification of moderate glucose dosage in

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TABLE II.
Course of Subdiabetic Rabbits.

Animal	Date of last alloxan	Test date	Glucose tolerance test type	Blood sugar level in mg/100 cc Hr 0	1	2	3
No. 2	10/22	1/6	Ordinary	84	119	104	99
		11	A.C.	113	122	120	111
No. 4	10/15	11	Ordinary	88	136	115	102
		13	A.C.	125	221	186	184
No. 5	10/28	6	Ordinary	—	276	247	211
No. 6	12/7	13	Ordinary	88	99	92	99
		18	A.C.	90	164	129	104

proportion to body weight seems unnecessary.[14]

As expected[12,13] the one hour blood sugar levels, while high, showed wide variation. By 2 hours, however, the means of the ordinary glucose tolerance tests had returned to the levels of the fasting means. Sole exception was the mean of the initial tolerance tests, where the 2 hour mean was slightly (15.2 mg%), but not significantly, elevated. This variation is due principally to Rabbits 4 and 8 which later exhibited normal glucose tolerance curves; excitement may account for the form of their initial curves.[15] The return to normal by 2 hours agrees with the previous observations.[2,11-13]

At 3 hours the means of all of the ordinary glucose tolerance tests approximated the fasting means. In occasional rabbits there was a marked rebound from the 2 hour level, amounting to as much as 40 mg%.

Modification of Glucose Tolerance by A.C. The effects of adrenal corticoids[16] and of pituitary adrenocorticotrophic hormone (A.C. T.H.)[17,18] on carbohydrate metabolism are well known. Adrenal cortex extract was chosen for the present experiments, rather than A.C.T.H. merely because it was immediately available. In selecting the dosage the aim was to use a quantity of hormone large enough to modify carbohydrate metabolism, yet small enough so that the glucose tolerance of a normal rabbit would be unaltered. The dose used, ½ cc/100 g body weight, is about 15% of the amount of A. C. which produces hyperglycemia in rats.[19] The preparation used was assayed by the manufacturer as containing "not less than 2.5 rat units/cc", and presumably it contained not much more. From Kuizenga's data[20] it may be calculated that the dosage of 11-dehydro-17-hydroxycorticosterone was approximately 0.04 mg/100 g body weight; this is about 2½% of the dose of corticoid which produces frank hyperglycemia in normal rats.[21]

The choice of dosage proved fortunate, since it did not modify the mean blood sugar levels of 4 rabbits subjected to A.C. tolerance tests prior to the administration of alloxan, while there was significant elevation of the mean 2 hour blood sugar level of the 6 alloxan treated rabbits subjected to A.C. tolerance tests. Since these alloxan treated rabbits had normal ordinary glucose tolerances, the A.C. tolerance test was effective in disclosing im-

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paired carbohydrate metabolism not demonstrated by usual methods. As would be expected, a rabbit with diabetic glucose tolerance (No. 8) showed further impairment of its tolerance when pretreated with A.C.

Alloxan Subdiabetes. In these experiments one or two small doses of alloxan have produced a subdiabetic state in rabbits: namely disturbances in sugar metabolism which are so slight that they are not associated with either hyperglycemia or abnormal glucose tolerance. These slight physiologic disturbances have been demonstrated, in every instance, by the use of the A. C. tolerance test. This state is not discussed in the experimental literature, but it was probably produced by Shipley and Rannefeld.[1] These observers noted progressive and persistent impairment of glucose tolerance in rats injected with repeated 25 mg/kg doses of alloxan. In four of their rats, which had normal fasting blood sugars at the end of their alloxan courses, anterior pituitary extract produced a transient fasting hyperglycemia.

Three reports concerned with pancreatic islets suggest the presence of subdiabetes. These changes were reported in nondiabetic alloxan treated rats,[22] in 95% pancreatectomized rats prior to the onset of either latent or manifest diabetes,[5] and in one nondiabetic rabbit which received two 50 mg/kg doses of alloxan.[23] In each report the histologic findings were similar, namely hypertrophy and hyperplasia of the β cells in the larger islets, succeeded by β cell degranulation and degeneration, as few as 1/5 of the islets could be involved. It seems not unlikely that comparable changes occurred in the present experiments.

The course of subdiabetes in these rabbits remains under observation. Progression has been observed in one animal, and maintenance of the subdiabetes for as long as 15 weeks has been the rule in the others. It would not be surprising if remission ultimately occurred in some animals, since spontaneous remission of manifest alloxan diabetes has been reported.[24,25] None of the morbid anatomical changes seen in human diabetes mellitus were found in the three animals thus far autopsied.

Summary and conclusions. 1. Small doses of adrenal cortex extract (A.C.) do not modify the glucose tolerance of fasted normal rabbits.

2. One or two small doses of alloxan (less than 40 mg/kg) do not produce more than transient changes in the blood sugar level or glucose tolerance of normal rabbits.

3. Six rabbits, so treated, showed a significant impairment of glucose tolerance when pretreated with A.C. (A.C. tolerance test); the hormone effect was transient.

4. By definition the treated rabbits had alloxan subdiabetes: they had impaired carbohydrate metabolism unaccompanied by fasting hyperglycemia, glycosuria, or impaired ordinary glucose tolerance.

5. Alloxan subdiabetes persisted in the rabbits for the duration of the experiment (up to 15 weeks).

6. A hormone sensitized glucose tolerance test, such as the A.C. tolerance test, should prove useful in studying problems in human subdiabetes and prediabetes.

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