18. *Spontaneous Diabetes Produced by Selective Breeding of Normal Wistar Rats*

By Yoshio GOTO, Masaei KAKIZAKI, and Naoyoshi MASAKI

The Third Department of Internal Medicine, Hirosaki University
School of Medicine, Hirosaki, Japan

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Diabetes mellitus is thought of as a disease of inheritant nature. The mode of transmission, however, is still inconclusive. An autosomal recessive trait theory was postulated by Pincus and White¹⁾ and supported by Steinberg.²⁾ On the other hand, Cammidge³⁾ and Stimmler and Elliott⁴⁾ thought that juvenile onset type was recessive and adult onset type was dominant, and recently a multifactorial inheritance theory has been proposed by Simpson.⁵⁾

In 1968, Craighead and McLane⁶⁾ observed that an inoculation of a variant of encephalomyocarditis virus produced a diabetic state in mice and found that the islets of their pancreas were selectively damaged. This experiment suggested a possibility that diabetes might be a disease of viral infection. If a family has less resistance to the virus, a higher incidence of diabetes would occur in this family and the phenomenon of a higher prevalence of the disease in certain family will resemble an inheritance, just as tuberculosis was thought to be a disease of inheritant nature in the early days. Therefore, a question will arise: is it certainly true that diabetes is a disease of inheritance?

From this background of our present knowledge, we cristalized the following working hypothesis. If animals, selected from normal population, with a glucose tolerance test (GTT) slightly deviated from normal range, are mated, their offsprings may have a more impaired GTT than the parents. If it is so, the repeat of this procedure over several generations would finally produce diabetic animals. If it is not so, diabetic state would be produced only by definite gene(s) unless the occurrence of mutation, or by other factors, such as viral infection, etc. The present study was carried out according to this hypothesis.

Methods. One hundred and thirty male rats and 81 female rats of Wistar strain, supplied from the Nogawa Farm of Japan Clea Co., Kawasaki, were used as foundation stock (P). The oral GTT was performed in the rats, weighing 150–200 g and fed Oriental Chow (MF) ad libitum, after 12 hours fasting and the rats with slight

glucose intolerance were selected as breeding stock. GTT was made as following: 20% glucose solution (2 g/kg) was administered orally by metal catheter attached with 5 ml size syringe and 0.05 ml of blood specimens were collected from tail vessels by cutting its end, prior to, and 30, 60, 90 and 120 minutes after the glucose administration. Urine sugar was tested with Tes-Tape when urinated. Blood glucose was determined by glucose-oxidase method using Glucostat.

Of the 211 foundation stock rats, 9 male and 9 female rats were selected as breeding stock. For breeding, male and female rats were housed together in a cage for 5–10 days. Pregnant rats were housed in an individual delivery-cage in which rice straw were put in. When infant rats were grown up to approximately 80 g of body weight, they were taken out of the delivery-cage and housed in cages separately by sex. By the breeding, 162 offsprings (F_1) were obtained, i.e., 89 male and 73 female rats. GTT was made in these offsprings when their body weight was 150–180 g. According to the result of the test, 12 male and 13 female rats were finally selected as breeding stock and the crossing was made as the same way described above. Two hundred and four offsprings (F_2), i.e., 116 male and 88 female rats were obtained. The same procedure was repeated in this generation.

For the selection of the breeding stock rats, the second GTT was repeated within two weeks in the rats screened by the (first) test and the rats with two impaired tests were chosen. In this experiment, inbreeding, especially sib-breeding was avoided. The grade of glucose intolerance was expressed as the sum of blood glucose values

Table I. Mean glucose tolerance tests of the foundation stock rats, the first and the second generation

Generation	Sex No.	Blood glucose (mg/dl)					
		Fast	30	60	90	120	Sum
P	M 130	79.0 ±12.0	139.4 ±19.0	131.5 ±18.4	107.6 ±16.5	95.4 ±12.5	552.8 ±57.3
	F 81	81.2 ±11.7	142.2 ±17.8	129.8 ±18.5	112.3 ±15.5	103.5 ±13.5	568.4 ±61.1
F_1	M 89	96.5 ±12.9	167.2 ±25.6	149.1 ±21.2	126.7 ±14.6	112.2 ±11.4	649.6 ±61.1
	F 73	93.5 ±9.5	157.6 ±23.0	136.6 ±19.0	121.2 ±12.9	115.8 ±13.9	624.8 ±63.3
F_2	M 116	103.3 ±15.9	192.6 ±32.9	162.8 ±27.3	137.9 ±21.3	123.7 ±17.8	719.8 ±93.1
	F 88	102.3 ±17.0	180.7 ±29.4	152.6 ±33.6	136.8 ±29.2	126.6 ±27.2	698.9 ±113.0

Mean ±S.D.

Table II. Combination of mating in the foundation stock rats and sum of blood glucose of the offsprings (F₁)

No.	Breeding stock						Offspring (F ₁)	
	Sex	Glucose tolerance test					Sex	Sum of blood glucose
		Fast.	30	60	90	120 sum		
1	M	87	177	180	163	131 738	M	789,780,752,778
	F	87	148	159	136	114 644	F	793,794,712,742,730
2	M	92	148	162	144	109 655	M	599,587,572,665,506,645*
	F	93	162	127	114	101 597	F	548
3	M	107	196	169	137	119 728	M	594,627,632,600,615,615,590
	F	103	178	140	126	111 658	F	602,604,656,629,639,715
4	M	87	194	169	124	119 693	M	627,756,657,648,595
	F	87	184	152	129	115 667	F	557,652,615
5	M	96	182	137	113	103 631	M	745,694,713
	F	97	128	148	137	127 637	F	689,595,662,690
6	M	100	148	157	153	125 683	M	729,704,757,623
	F	90	140	150	131	122 633	F	617,709,619,552,629,608*,569*,573*
7	M	72	157	154	127	111 621	M	599,688,679,685,591,618,701,627,662,
	F	90	147	169	129	116 651	F	none 574,662*
8	M	72	176	180	154	127 709	M	636,680,675,743*,654*,555*,632*
	F	94	153	126	113	102 588	F	658,588,585,568,543*
9	M	74	162	240	123	101 700	M	565,559,570,614,578,562
	F	84	150	159	146	143 682	F	567,577,556,573
10	M	92	148	162	144	109 655	M	620,636,614,599
	F	87	184	152	129	115 667	F	605,583,555,592
11	M	107	196	169	137	119 728	M	637,653,557,665,742
	F	97	128	148	137	127 637	F	586,628,574,637,603,643*
12	M	92	148	162	144	109 655	M	628,632,583,712,675,682,606
	F	94	153	126	113	102 588	F	673,682,643
13	M	87	177	180	163	131 738	M	656,689,694,635*
	F	90	140	150	131	122 633	F	692,638,760,624,745,642
14	M	107	196	169	137	119 728	M	699,683,736,674,708,659
	F	87	142	137	128	116 610	F	584,586,587,689,676
15	M	100	148	157	153	125 683	M	556,552,606,603,669
	F	103	178	140	126	111 658	F	594,543,590,562,576,552*,579,583,506,559*
16	M	72	157	154	127	111 621	M	691,753,698,635,672
	F	84	150	159	146	143 682	F	711,679,633

Bold type indicates cases with a positive test for urine sugar.

Asterisk represents cases of retarded growth.

at five time periods, i.e., fasting, 30, 60, 90 and 120 minutes.

Results. The mean GTT of the foundation stock (P), of the first generation (F₁) and of the second generation (F₂) are shown in Table I. The mean blood glucose value becomes high with generation at each time period and the values of F₁ and F₂ are significantly higher in comparison with those of P. The combination of crossing and the sum of blood glucose of the offsprings are shown in Tables II and III. Cases with a positive test for urine sugar were shown in bold type. There were, however, many cases in which urine sugar was not tested

Table III. Combination of mating in the first generation rats and sum of blood glucose of the offsprings (F₂)

No.	Breeding stock (F ₁)							Offspring (F ₂)	
	Sex	Glucose tolerance test						Sex	Sum of blood glucose
		Fast.	30	60	90	120	sum		
1	M	111	234	163	142	139	789	M	674,918,804,726,728,782
	F	104	163	136	131	128	662	F	760,713,689
2	M	104	225	161	146	121	757	M	603,751,697,686,838,650
	F	115	211	181	146	141	794	F	861,628,676,656
3	M	92	180	176	134	119	701	M	608,741,679,735,677
	F	99	217	157	137	132	742	F	609,664, 1005,1090,806,738,823,890
4	M	122	201	149	138	135	745	M	877,900,754,665,650,882,847,950,1040
	F	86	207	160	139	117	709	F	974,694,683,903
5	M	95	163	148	138	118	662	M	591,560,646,602,609,644,685,590
	F	103	173	132	129	121	658	F	684,607,675
6	M	80	164	166	133	119	662	M	847,799,824,734,817,865,861,786,775
	F	102	231	180	144	136	793	F	702,713,622,710
7	M	98	138	134	141	125	636	M	758
	F	103	169	167	142	130	711	F	682
8	M	109	194	148	128	115	694	M	733,649,651,661,705*
	F	99	154	139	140	147	679	F	690,696,749,691,612*
9	M	94	165	187	129	123	698	M	665,625,712,608,588
	F	106	170	154	135	124	689	F	593,644,653,630,577*, 977*
10	M	92	180	176	134	119	701	M	782,885,734, 699,648,586,663,753
	F	115	211	181	146	141	794	F	630,645, 641,714,632
11	M	122	201	149	138	135	745	M	626,897*, 796,932,748,768
	F	99	217	157	137	132	742	F	907*,760*,864,743,603,611*,866
12	M	104	225	161	146	121	757	M	670,779,856,747, 706,913* ,693,644
	F	86	207	160	139	117	709	F	574,569,637,724,623, 1084*
13	M	95	163	148	138	118	662	M	693,617,675,692,704,779,703,678,650
	F	103	173	132	129	121	658	F	664, 656,584
14	M	80	164	166	133	119	662	M	725,848,705,706,694
	F	102	231	180	144	136	793	F	687,694,713,713,715, 629*,657*,690*
15	M	117	145	141	130	123	656	M	651, 663,568,669
	F	87	129	136	123	115	590	F	634,601,585,637*,653,601,608
16	M	98	138	134	141	125	636	M	683,697,602, 690,723,668,646,726
	F	91	186	151	127	137	692	F	686,801,715,609*
17	M	100	152	162	124	115	653	M	624,638,719,673,667,647*
	F	110	195	160	145	150	760	F	619,557,613
18	M	84	163	155	146	121	669	M	660,859,697, 760,661,840
	F	95	188	163	151	148	745	F	713,644,622,576,688,643

Bold type indicates cases with a positive test for urine sugar.

Asterisk represents cases of retarded growth.

because of no urination during the test. In each group of litters, there were few rats of retarded growth and these rats are shown as asterisk in the tables. There was no special pattern of GTT in these rats.

Distributions of the sum of blood glucose in each generation are shown in Fig. 1. It is easy to understand in this figure that the dis-

Fig. 1. Distribution of sum of blood glucose in each generation.

tribution curves shift to the right with the advance of the generation. This indicates that the selective breeding of the rats with a GTT slightly deviated from normal range yields rats with a test more deviated from normal. Some of F₂ had a typical diabetic GTT with a two hour value exceeding 200 mg/dl and a positive test for urine sugar. There was no rats in F₁ which sum of glucose exceeded 800 mg/dl. In F₂, however, 24 of the 116 male rats and 14 of the 88 female rats had the sum of blood glucose exceeding 800 mg/dl and few of them exceeded 1000 mg/dl. The distribution curves of the sum of blood glucose in F₂ are non-symmetrical but steep in left and gradual in right, i.e., a curve tailed to right. This seems to show the effect of the selective breeding.

Comment. This experiment demonstrated that offsprings of the rats with a slight impairment of glucose tolerance had a more impaired tolerance for glucose and some of the second generation had a diabetic GTT although they had no classical symptoms of diabetes

such as polyuria and polydipsia. For the explanation of the present result, the multifactorial inheritance theory seems more reasonable than the theory assuming special diabetes gene(s). The further succession of this study will give many informations on natural history of diabetes including prediabetic stage, prevention of the development of diabetes and of the vascular complications, and so on.

Summary. The present study was undertaken to see the possibility of production of spontaneous diabetes rat by repeating the selective breeding of normal rats with a slight impairment of glucose tolerance. One hundred and thirty male and 81 female normal rats of Wistar strain were obtained from an experimental animal farm as foundation stock and 9 male and 9 female rats were chosen as breeding stock by oral glucose tolerance test. By the crossing, 89 male and 73 female rats were obtained (F_1). The mean glucose tolerance curve of these offsprings was significantly higher than that of the foundation stock. Twelve male and 13 female rats were chosen among the F_1 rats as breeding stock by glucose tolerance test. The crossing of them yielded 116 male and 88 female rats (F_2). The mean glucose tolerance curve of F_2 was significantly higher than that of F_1 and some of them had a diabetic test and a positive test for urine sugar during the test. The result demonstrated the possibility of production of spontaneous diabetes from normal rats by the selective breeding.

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