During this period of 100 days opposite selection was continued in the main (A) lines until we have a difference of 126±3.27 generations between the fast and the slow lines. Throughout this same period the lines subjected to balanced selection (A_1) maintained a difference in division rate of 119±2.16 generations, while the difference in the case of the reversed lines (A_2) had dropped to 89±3.84. Thus (taking into consideration the probable error) we find that this difference, which was cumulative, has persisted through the period of balanced selection, but tends to disappear under the influence of reversed selection. Selection has apparently been effective in establishing two diverse sets, differing in regard to the physiological character, division rate.

A more detailed analysis of these experiments will be presented in another paper. The effect of selection is now being studied in *Paramecium calkinsi,* a form which differs from *Paramecium aurelia,* in that endomixis has never been demonstrated, and apparently does not occur. The experiments are also being repeated on the hypotrich, *Stylonychia pustulata,* for the purpose of comparison with the two holotrichous forms mentioned above.

[1] Jennings, H. S., *Proc. Am. Phil. Soc.*, 1908, xlvii, 393.
[2] Jennings, H. S., *Genetics*, 1916, i, 407.
[3] Middleton, A. R., *J. Exp. Zool.*, 1915, xix, 451.

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Diabetes Mellitus: An Experimental Study on the Etiology of the Disease.

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A survey of the literature gives no convincing evidence that the disease is caused by bacteria. It seemed possible that it may be caused by a filterable virus.

Experiments were made on rabbits to determine whether it is possible to obtain evidence of the presence and action of a filterable virus. It seemed probable that the causative organism is thrown off from the patient's body in the urine. The urine of diabetic patients was therefore passed through Berkefeld filters, and the filtered urine was injected into the circulation of rabbits in doses of

2 cc. Two rabbits were injected with the urine of each patient, each animal receiving only one injection.

After the rabbits had been injected they were confined in metabolism cages which permitted the collection of the urine. Daily analysis of urine was carried out, using Benedict's quantitative reagent.

Diabetes mellitus is generally a slowly progressing disease and it was believed that if it is transmissible to rabbits evidences of infection would probably be delayed for some time.

Experiment 1. The first pair of rabbits was injected with the filtered urine of patient Q on January 12, 1926. Almost daily urine examination failed to show the presence of glucose until 22 days later, when a trace of sugar was found in the urine of rabbit No. 1 (female). Rabbit No. 2 (male) first showed glucose in the urine on the 42nd day after inoculation.

A peculiar phenomenon in the experimental glycosuria in rabbits has been the irregular periodicity of the occurrence of glucose in the urine. After glucose was demonstrated for several successive days it disappeared, but reappeared more or less irregularly. After glucose had been demonstrated for several months in the urine of these 2 rabbits they were taken out of the metabolism cages. Rabbit No. 2 was killed 152 days after inoculation, and Rabbit No. 1 was kept over the summer, and again placed in a metabolism cage 250 days after inoculation. Glucose was not found again in the urine of this rabbit until 279 days after inoculation, when it was present to the amount of 1.426 per cent, and continued to be voided in varying amounts for several days when it was again absent. The glucose in this specimen permitted active fermentation with gas formation by Fleischman's yeast at 37°C.

Experiment 2. The filtered (Berkefeld) urine of the patient (Q), when inoculated into serum-broth medium and grown in the incubator under aerobic conditions, appears to permit development of the causative agent, though no organisms could be demonstrated, and there was no visible change in the medium. After cultivation for 56 days this culture was inoculated into the circulation of normal rabbits in 0.1 cc. amounts. Glucose appeared in the urine in a shorter time (in 7 to 8 days, rabbits Nos. 5 and 6) than in those inoculated with the filtered urine of the patient, indicating that the culture contained many more organisms than the diabetic urine of the patient.

Experiment 3. Two rabbits were inoculated with a later culture,

No. 8 with heated culture and No. 9 with unheated culture, but these failed to develop glycosuria in 28 days, when they were killed.

Experiment 4. The urine of rabbit No. 5 (inoculated with a serum broth culture of the filtered urine of the first patient (Q), and incubated for 56 days) was filtered on the 18th day after inoculation, and this inoculated into the circulation of rabbit No. 7. This rabbit showed glycosuria in 7 days after inoculation.

Experiment 5. Some of the filtered urine of patient Q, received on January 10, was placed in sterile ampoules, and kept at room temperature. On October 28 rabbits No. 10 and No. 11 were given intravenous injections of 2 cc. of the urine. After 7 days rabbit No. 11 had a trace of sugar in its urine, while rabbit No. 10 first showed a trace of sugar after 11 days. The sugar disappeared from the urine after several days, and reappeared later. So far these rabbits have not shown large amounts of sugar, but in all probability will show greater quantities as the disease progresses.

Experiment 6. Some of the urine of patient Q, received on January 10, was inoculated into serum broth and cultivated for 56 days. A portion of the culture was used for experiment 2, and a portion was reinoculated into serum broth. Some of the subculture was used for experiment 3, and the remainder was reinoculated into serum broth. This subculture was inoculated into fresh serum broth 20 days later, and grown at room temperature under vaseline seal for 6 months, when 0.5 cc. of the culture was injected intravenously into rabbit No. 12. This rabbit had a trace of sugar in its urine 16 days after inoculation.

Experiment 7. Diabetic urine from a second patient (W) was filtered and inoculated into the circulation of 2 normal rabbits in 2 cc. dose. These rabbits (Nos. 3 and 4) showed no glucose in the urine until 28 days after inoculation, and then in only small quantities. Again on the 35th day small amounts of glucose were found. On the 38th day rabbits Nos. 3 and 4 were in a comatose condition and had to be killed. Rabbit No. 4 had a small quantity of urine in the bladder at autopsy, and this showed a slight reaction for glucose with Benedict's solution.

Several of the rabbits, when killed, showed slight congestion of the pancreas, but on sectioning and staining no definite pathologic changes could be demonstrated. There was, apparently, a suggestion of degeneration of the islands of Langerhans, but the alterations were not marked. In rabbits that have been under experiment for longer periods of time microscopic sections of the pan-

creas and other internal organs may possibly show more convincing evidence of pathologic changes.

The results of these preliminay experiments suggest that *Diabetes mellitus* is caused by a filterable virus; that the disease can be transmitted to rabbits by intravenous administration of a single dose of 2 cc. of the urine of diabetic patients, passed through Berkefeld filters to remove bacteria; that the filtered urine of diabetic patients contains an ultravisible organism which multiplies in serum-broth medium, since cultures 56 days old produced glycosuria in rabbits more promptly and to a more marked degree than freshly filtered urine.

This transmissibility of *Diabetes mellitus* to rabbits, the passage of the disease to other rabbits through the intravenous injection of filtered urine of inoculated rabbits, and the transmission of the disease through the inoculation of serum-broth cultures of the filtered urine of diabetics, indicates the infectious nature of the disease. A number of normal rabbits have been kept under observation, and so far none have been encountered with sugar in their urine. A number of rabbits have been injected with human non-diabetic urine, and, so far, none of these rabbits has developed glycosuria.

The fact that the virus causing *Diabetes mellitus* can be cultivated opens the way for the development of specific prophylactic and therapeutic measures against this disease. Time has not been available to develop this line of investigation, but it is hoped that by bringing the results of this preliminary study to the attention of other investigators work in these several fields will be stimulated.

There is no doubt that systematic study of the blood of normal and inoculated rabbits will aid in the solution of the problem of the etiology of diabetes, and at the same time elucidate the irregularity of the appearance of glucose in the urine of inoculated rabbits.

More detailed study of the pathology of infection in rabbits is also needed to determine whether the changes in the pancreas and other internal organs are similar to those in man. For this study it will be necessary to keep inoculated rabbits under observation for a year or more so as to permit the development of alterations in the pancreas and other internal organs.

The relation of the form of diabetes in children, which is often rapidly fatal, to the slowly progressing disease in adults, may also be elucidated through experiments on animals.