

Basement membrane thickness was measured in two groups of spiny mice (A. cahirinus): one with spontaneous diabetes, the other with streptozotocin-induced diabetes. A statistical analysis of the morphometric results of the two groups showed a significant basement membrane thickening in the group with induced diabetes when compared to the spontaneously diabetic group.

Capillary basement membrane thickening is a frequent observation in diabetic microangiopathy. However, the question whether basement membrane thickening has to be considered as a direct manifestation of the hereditary diabetic trait, or if it results from long-standing hyperglycemia and subsequent metabolic disorders has yet to be resolved.

The study of microvasculature in human diabetic retinopathy is rendered difficult by the fact that material for ultrastructural studies can only be obtained after enucleation (mostly for intraocular tumors) and not from biopsy. The material obtained at autopsy does not give, in general, satisfactory ultrastructural preservation due to the long delay between death and fixation of the tissue. Therefore, it appears necessary to study possible microvascular changes in diabetic animals. Retinal lesions comparable to some extent to the ones observed in human diabetes have been reported in streptozotocin-diabetic rats.

In this study, retinal capillary basement membrane thickness of a group of spontaneously diabetic Acomys was compared with a group of streptozotocin-induced diabetic animals. The results seem to indicate that experimentally induced hyperglycemia may induce or enhance capillary basement membrane thickening in the retina.

Material and methods. Twelve spiny mice (A. cahirinus) were used in this study and raised under normal laboratory conditions. A group of normoglycemic animals had intraperitoneal injections of 100 mg. per 100 grams of body weight of streptozotocin in order to induce, experimentally, a diabetic state (= induced group). Two months after the injection they were weighed and then killed by injections of nembutal. Of these animals, two were 20 months old, three were 24 months old, and two were nine months old, the average age being 19 months. Another group consisted of five spontaneously diabetic animals (= spontaneous group). At the time of death, three animals were 19 months old and two were 21 months old, the average age of this group was 20 months. No hypoglycemic therapy was administered to either group.

The metabolic state of the animals was checked periodically with tests for urine glucose. In the spontaneous group, urine sugar was over 6 Gm. per liter in three animals and between 1 and 4 Gm. per liter in two animals, none had ketonuria; glycemia at the time of death was about 320 mg. per cent (range 130 to 529 mg. per cent). Immunoreactive insulin (IRI) was 22 mU. per milligram (range 15 to 28 mU. per milligram) in the pancreas and 134 mU. per milliliter (range 48 to 296 mU. per milliliter) in serum. In the induced group, urine sugar was over 6 Gm. per liter in six animals and between 1 and 4 Gm. in one animal, five animals had ketonuria; glycemia was about 440 mg. per cent (range 292 to 627 mg. per cent). IRI was 5.7 mU. per milligram (range 2.8 to 10.5 mU. per milligram) in the pancreas, and 76 mU. per milliliter (range 14 to 237 mU. per milliliter) in serum.

The eyes were enucleated, opened at the equator, fixed in 4 per cent phosphate-buffered glutaraldehyde, and postfix in 1 per cent osmium tetroxide. They were then dehydrated in a graded series of alcohol and embedded in Epon 812. Semithin and thin sections were cut on a Porter Blum MT-2 ultramicrotome. Thin sections were stained with lead citrate and uranyl acetate and observed in a Philips 300 electron microscope.
Fig. 1. Distribution curve of measurements of basement membrane width of retinal capillaries based on measurements in (A) seven Acomys with experimentally induced diabetes (1,680 measurements) and (B) in five spontaneously diabetic Acomys (1,200 measurements).

Table I

<table>
<thead>
<tr>
<th>Variable</th>
<th>Spontaneous diabetes</th>
<th>Induced diabetes</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basement membrane thickness</td>
<td>1242 ± 17 Å</td>
<td>1508 ± 14 Å</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Circumference</td>
<td>20.51 ± 0.49 μM</td>
<td>20.29 ± 0.41 μM</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Weight</td>
<td>68 ± 5.0 Gm.</td>
<td>54 ± 4.2 Gm.</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Micrographs were taken at the single tap-setting M5 (= 6.300 x). Basement membrane thickness (BMT) was measured using the method of Siperstein, Unger, and Madison, except that 16 instead of 20 measurements per capillary were used. Fifteen capillaries per animal were measured. The circumference of each capillary was measured using a curvimeter. An extended variance analysis was used in order to compare weight, circumference, and BMT of the 2 groups.

**Results.** The results can be summarized in Table I.

In the spontaneous diabetic group the average individual BMT ranged from 1,029 Å (minimum) to 1,381 Å (maximum), whereas in the experimentally diabetic group it ranged from 815 Å to 2,616 Å.

In the induced diabetic group, there is a great incidence of higher values not seen in the spontaneously diabetic group; there is no such difference for the lower values (600 to 1,500 Å) between the two groups (Fig. 1).

No significant differences between the two groups were observed in the capillary circumference nor in the animals' weight.

**Discussion.** In this study it was shown that BMT in the group with experimentally induced diabetes was greater than that observed in the spontaneously diabetic group. No such differences were found between the two groups' weight or retinal capillary circumference.

In the statistical analysis, the age of the animal was not taken into account since the period of observation and the average age of the animals in the two groups was not notably different. As such, the BMT in the streptozotocin group can hardly be explained as being the result of an age-dependent phenomenon as it has been recorded in different tissues of normoglycemic Acomys and rats.\(^4\), \(^6\), \(^7\)

The dispersity in the BMT of the induced diabetic group may not necessarily be the result of the experimental conditions since, even in normoglycemic Acomys, BMT was found to exhibit an important intra- and inter-individual variation especially in old animals. In 12-months-old normoglycemic animals the width varied from 1,005 Å to 1,211 Å (individual average), and in animals of 36 months it ranged from 1,405 Å to 2,034 Å (individual average).\(^4\)

The metabolic state of the animals in the two groups compared was not identical and might account for the observed differences in BMT. In fact, the induced group had more ketonuria,
higher blood-glucose, and very low pancreatic and serum IRI-levels. The metabolic state of this group corresponds almost to the ketogenic, catabolic diabetic group with a strongly reduced life expectancy as described by Junod and co-workers.1

In conclusion, therefore, the results of our study suggest that BMT in induced diabetes may be a consequence of long-standing hyperglycemia and subsequent metabolic disorders due to the β-cytotoxic effect of streptozotocin.

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Key words: diabetic microangiopathy, streptozotocin, retina, Acomys cahirinus, basement membrane thickening, morphometry, spontaneous diabetes, electron microscopy.

REFERENCES


The relationship between pre-exposure fundus temperature and the temperature rise which produces a threshold burn was examined in rabbit eyes exposed to an Argon c. w. laser (4,880 A) for 10 seconds. The posterior pole of the eye was surgically exposed and a 20-micron tip diameter probe was inserted into the ocular fundus to measure temperature rises. The temperature rise for threshold burns linearly increased as pre-exposure fundus temperature decreased, implying a constant threshold temperature. Threshold temperature was indirectly predicted to be 52.4° C., using a system with an estimated error of ± 1° C.

The need for understanding the nature of retinal injury from intense light increases each year. Consequently, development of a reliable model of retinal damage has been actively pursued.1 However, such a model requires accurate temperature measurement associated with retinal lesion formation. Such temperature measurements have been made in rabbit and monkey eyes with thin-film copper-nickel microthermocouples specifically designed for measuring temperature transients in tissue at The University of Texas.3 These temperature measurements have shown good agreement with temperatures predicted with a finite difference solution to the heat conduction equation in the retina.4

The threshold lesion temperatures (temperatures associated with formation of a minimum ophthalmoscopically visible lesion) measured with these microthermocouples have also shown agreement with computed temperatures based upon corneal power necessary to produce a threshold burn. Ward and Bruce1 estimated threshold lesion temperatures by correlating body temperature with threshold retinal irradiance. They assumed a linear relation between retinal irradiance and body temperature. Furthermore, they predicted that a threshold lesion due to a 100 ms. exposure would require a fundus temperature at the site of the burn of 44° C. This would represent a temperature rise of 7° C. with respect to normal body temperature.