Effect of vibration on sulfomucopolysaccharide metabolism in the eye. JOLANTA DABROWSKA.

The metabolism of $^{35}$S sodium sulfate was studied in the eyes of guinea pigs and rabbits subjected to vibration. The investigations included determination of radioactivity of whole eyeballs of guinea pigs and single eyeball tunics of rabbits, i.e., retina-choroid-sclera investigated jointly as a tissue complex, and cornea and lens studied separately, besides that, autoradiographic investigations were performed. The animals were subjected to a vertical, sinusoidal vibration of 42 Hz frequency and 1 mm. amplitude. The duration of one exposure was three hours and they were repeated daily for three to six days. Incorporation of the isotope into the eyeballs of animals subjected to vibration was decreased and this decrease was related to the length of exposure suggesting that vibration changes the metabolism of sulfomucopolysaccharides in the eye.

With the development of modern techniques, medicine has been faced with the problem of diagnosis and treatment of diseases caused by vibration. Vibration leads to changes in many organs which are jointly responsible for the so-called vibration syndrome. Changes develop in this syndrome in the organs with high connective tissue content. The eyeball, particularly the cornea and sclera, contains great amounts of acid mucopolysaccharides (AMPS) which are one of its components. The activity of acid mucopolysaccharide metabolism is very high in these tissues.

It was assumed at the onset of these investigations that an insight into the metabolism of sulfomucopolysaccharides may be gained from the amount of incorporated $^{35}$S sodium sulfate which is incorporated nearly completely into sulfomucopolysaccharides and in only a negligible percent into sulfur-containing amino acids.

Materials and methods. Isotope investigations were carried out on 37 guinea pigs (74 eyes) weighing 350 to 500 grams and on 32 male rabbits (64 eyes) weighing 2,000 to 2,500 grams. Clinical investigations were performed on 25 rabbits (20 of them belonged to the group subjected previously to isotope studies). Isotope investigations included measurements of radioactivity of eyeballs of control and experimental animals, and autoradiographic examinations. For isotope investigations, the experimental animals were divided into three groups: a control group, a group of animals subjected to vibration for three days, and a group subjected to it for 6 days. The animals were subjected to vertical, sinusoidal vibration of 42 Hz. frequency and 1 mm. amplitude during three hours daily. Three days before death, the animals received $^{35}$S sodium sulfate intraperitoneally in doses of 0.5 μCi per gram of body weight (guinea pigs) or 0.7 μCi per gram (rabbits). For autoradiographic investigations the dose was 2 μCi per gram. The animals were killed and the eyeballs were obtained immediately dissecting them from all surrounding tissues, fixing in absolute alcohol, homogenized eyeballs: cornea, lens and the tissue complex comprising the sclera, choroid, and retina. Radioactivity was decreased more in the group of animals exposed to vibration for six days than for three days. Autoradiographic investigations were carried out by the method of Pelt on the eyeballs of guinea pigs from all experimented groups. The blood vessels of the ocular conjunctiva were observed and ophthalmoscopy of the fundus was performed in rabbits before vibration and then 1, 3, and 6 days after the end of exposures.

Results. Isotope investigations. Measurements of radioactivity of eyeballs of guinea pigs. In the group of guinea pigs exposed to vibration during 3 days (Table 1) radioactivity was lower than in the control group. The difference was statistically significant. Similarly, in the group of animals exposed to vibration for six days, the radioactivity of eyeballs was decreased and the significance of the difference was confirmed statistically. Radioactivity was decreased more in the group of animals exposed to vibration for six days than for three days.
Table I. Radioactivity of eyeballs of guinea pigs (impulses per minute per 1 Cm. of dry tissue)

<table>
<thead>
<tr>
<th>Group</th>
<th>Arithmetic mean</th>
<th>Standard error</th>
<th>Standard deviation</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>204.8</td>
<td>15.1</td>
<td>62.2</td>
<td>—</td>
</tr>
<tr>
<td>Vibration 3 days</td>
<td>138.0</td>
<td>7.9</td>
<td>30.6</td>
<td>p &gt; 0.001</td>
</tr>
<tr>
<td>Vibration 6 days</td>
<td>121.7</td>
<td>9.4</td>
<td>42.0</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

Table II. Radioactivity of rabbit cornea and radioactivity of tissue complex (sclera, choroid, retina) from rabbit eyeballs (impulses per minute per 100 mg. of dry tissue)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cornea</th>
<th>Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arithmetic mean ± standard error</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Control</td>
<td>452 ± 31.4</td>
<td>151.0</td>
</tr>
<tr>
<td>Vibration 3 days</td>
<td>377 ± 18.8</td>
<td>77.8</td>
</tr>
<tr>
<td>Vibration 6 days</td>
<td>308 ± 27.0</td>
<td>111.8</td>
</tr>
</tbody>
</table>

Measurements of radioactivity of eyeballs of rabbits. In the experiments, the cornea, lens, and tissue complex (sclera, choroid, and retina) were studied separately. The works of Dohlman and Bostrom and Larsen demonstrated that choroid and retina contain only a small proportion of mucopolysaccharides. It is supposed that the results of radioactivity determinations in these two tissues, together with the cornea, reflect mainly the mucopolysaccharide content of the sclera (Table I).

In the group of rabbits exposed to vibration for three days, the radioactivity of the cornea was lower than in the control group. The difference was statistically significant (p = 0.05). Similar changes were found in the group of animals exposed to vibration for six days and the difference was again confirmed statistically (p = 0.05). Radioactivity was decreased more in the group exposed to vibration for six days than for three days (Table II).

In the tissue complex (sclera, choroid, and retina) of rabbits exposed to vibration for three days, radioactivity was lower than in the control group. The difference was, however, not significant statistically (p > 0.05). On the other hand, in the group of animals exposed for six days, radioactivity was statistically significantly lower than in the control group (p < 0.05).

No difference was observed in the radioactivity of rabbit lens between the experimental and control groups.

Comparing the autoradiograms of cornea in the control group with those in the experimental group, one gains the impression that the silver granules are more numerous in control animals. The autoradiograms of sclera seem quite similar.

Clinical investigations. During macroscopic observation of the ocular conjunctiva of rabbits exposed to vibration no changes were observed in the form of hyperemia or blanching during, as well as after, exposure to vibration. Ophthalmoscopy of the fundus of the same rabbits on the first, third, and sixth days during exposure, as well as afterward, failed to demonstrate any difference in the appearance of the fundus in relation to that before exposure.

Discussion. The results obtained indicate that vibration may reduce the metabolism of sulfomucopolysaccharides in the eyeball as evidenced by decreased incorporation of 35S sodium sulfate. This is suggested by measurements of the radioactivity of whole guinea pig eyeballs as well as measurements of the radioactivity of various tunics of the eyeball in rabbits such as the cornea and the tissue complex. These changes were more pronounced in the cornea than in the tissue complex because the cornea contains 5 times more AMPS than the sclera. Autoradiography confirmed the results obtained in isotope investigations demonstrating reduced incorporation of 35S sodium sulfate in the cornea of the animals exposed to vibration.

A correlation was also found between the intensity of these changes and the duration of vibration; after six days vibration the metabolism of sulfomucopolysaccharides was more reduced than after three days. No significant changes were observed in the radioactivity of the lens which may be explained as a result of low content of sulfomucopolysaccharides, lack of blood vessels, and low metabolic rate.

Lack of changes in clinical observation in the eye blood flow suggests that these changes cannot be explained by disturbances of eye hemodynamics.

The pathways and mechanism of these changes remain unexplained as yet. It is believed usually that short-lasting exposure of the organism to vibration is a stress factor stimulating the sys-
Increased secretion of glycocorticosteroids may lead to suppression of the metabolism of sulfonmucopolysaccharides of the eyeball. Many authors separate as a special entity, disturbances due to the so-called vibration syndrome. Separation of effects of stress from direct effects of vibration is very difficult and it is impossible in the present study. It is worth adding that the interpretation of $^{35}$S sulfate incorporation into AMPS was based on previous publications.

From the Laboratory of Vision Protection, Railway Health Service Research Center, Warsaw, Poland. Submitted for publication Aug. 13, 1974. Reprint requests: Dr. J. Dąbrowska, Mokotowska 19, 00-560 Warsaw, Poland.

Key words: mucopolysaccharides, metabolism, vibration, cornea, sclera, $^{35}$S sodium sulfate.

REFERENCES

Anti-inflammatory effectiveness of topically administered corticosteroids in the cornea without epithelium. ALLAN KUPFERMAN AND HOWARD M. LEIBOWITZ.

The present studies demonstrate that modification of the derivative of a given steroid base alters its anti-inflammatory potential as measured by suppression of leukocyte invasion of the cornea. A comparison of each drug's corneal bioavailability with its anti-inflammatory effectiveness shows the acetate derivative of prednisolone to be a more potent anti-inflammatory agent than the phosphate derivative. Similarly, the free alcohol derivative of dexamethasone proved to be more potent than the phosphate derivative. Increasing the concentration of prednisolone acetate from 0.125 per cent to 1.0 per cent results in a significant increase in its anti-inflammatory effectiveness in the cornea following topical administration. The same increase in prednisolone phosphate concentration does not produce a significant increase in its ability to suppress polymorphonuclear leukocyte infiltration of the cornea. When the epithelium of the injured cornea is intact, prednisolone acetate, 1.0 per cent ophthalmic suspension, is the most effective of the corticosteroid preparations studied. In the absence of an intact epithelium, prednisolone acetate, 1.0 per cent ophthalmic suspension, again produces the greatest mean reduction in polymorphonuclear leukocyte infiltration of the cornea although here one cannot demonstrate a statistically significant difference from the anti-inflammatory effect produced by prednisolone phosphate, 1.0 per cent ophalmic solution, or dexamethasone alcohol, 0.1 per cent ophthalmic suspension. Overall, therefore, prednisolone acetate 1.0 per cent is the most effective of the topical agents studied for suppression of corneal inflammation.

Commercially available ophthalmic corticosteroid formulations differ in their ability to suppress corneal inflammation. Among the preparations studied to date, prednisolone acetate, 1.0 per cent ophthalmic suspension, most effectively suppressed a corneal inflammatory response when the epithelium of the involved cornea was intact. However, variations in the differential solubility of these corticosteroids and the lipophilic nature of the corneal epithelial barrier raise the possibility that the relative anti-inflammatory effectiveness of these formulations might be different when the corneal epithelium is absent. The results of our investigation of this possibility are reported here.

Methods. With the exception of epithelial debridement prior to treatment, the methodology used in these experiments was identical to that reported previously. Six commercial ophthalmic corticosteroid preparations were studied. They were (1) prednisolone acetate, 0.125 per cent ophthalmic suspension; (2) prednisolone acetate, 1.0 per cent ophthalmic suspension; (3) prednisolone phosphate, 0.125 per cent ophthalmic solution; (4) prednisolone phosphate, 1.0 per cent ophthalmic solution; (5) dexamethasone alcohol, 0.1 per cent ophthalmic suspension; and (6) dexamethasone phosphate, 0.1 per cent ophthalmic solution. The ophthalmic suspensions were placed in a shaker for 15 minutes immediately prior to ocular administration to ensure an even distribu-