EFFECT OF ADRENALINE ON SUCCINIC DEHYDROGENASE ACTIVITY AND MORPHOLOGY OF THE MID-PIECE OF MICE SPERMATOOZOA

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Summary. The mid-piece of fully matured mouse spermatozoa showed differential dehydrogenase activity. With the decreased activity, the breadth and area of the mid-piece decreased. Injection of 0.06 mg of adrenaline/male decreased the percentage of spermatozoa with high dehydrogenase activity.

Mukherjee & Ayyagari (1969) reported that administration of adrenaline reduced the area and breadth of the mid-piece of fully matured mice spermatozoa. This reduction was thought to be due to a reduction in the blood supply (and hence oxygen supply) to the spermatozoa. As succinic dehydrogenase is involved in oxidative and anaerobic metabolic processes of spermatozoa, the object of the present experiment was to determine (i) if the succinic dehydrogenase activity was reduced by adrenaline injection and (ii) if the activity was correlated in any way with the area and breadth of the mid-piece of mouse spermatozoa.

Forty, colony-bred, adult, male albino mice were allotted at random to control and experimental groups, each consisting of twenty mice. The origin of the mouse colony and the amount (0.06 mg in 0.06 ml saline) and mode of administration of adrenaline tartrate to each mouse of the experimental group were the same as reported previously (Mukherjee & Ayyagari, 1969). Each mouse of the control group received a total of 0.06 ml saline in six equal doses over 2 days. As before, the mice of the two groups were maintained in a room where the air temperature varied between 25 and 27° C.

Two hours after the last injection, mice of the two groups were killed by dislocation of the neck. From each mouse, the two vasa deferentia were dissected out and cleaned externally. Their contents were squeezed out with the aid of a pair of forceps into a drop of normal saline and thoroughly mixed. The drop was then transferred into an agglutination tube, maintained at 37° C, containing a drop of 0.1 M-sodium succinate and a drop of incubating medium. The composition of the incubating medium was 10 mg of Nitro B.T. (B.D.H.) in 10 ml of 0.1 M-phosphate buffer, pH 7.5. After incubation for 1 hr, four smears were made from each of the control and experimental males. The smears were dried in air, fixed in formol saline and stained with eosin Y according to the method described by Hrudka (1965).

The 160 slides from the forty mice, eighty for each group, were coded and examined unmounted under a microscope with an oil-immersion objective and blue filter. Fifty spermatozoa per slide were selected at random and the dehydrogenase activity of their mid-pieces was graded as follows:

Classification (Plate 1)

- ++++ = Mid-piece exhibiting intensely stained, large-sized and densely packed granules (Fig. 1, 1)
- +++ = Mid-piece exhibiting moderately sized granules with intense staining (Fig. 1, 2)
- ++ = Mid-piece exhibiting intensely stained, loosely-packed granules in one region and packed granules in another region (Fig. 2, 3)
- +
- = Mid-piece exhibiting small and loosely-packed granules (Fig. 1, 4)
- = Mid-piece without stained granules (Figs. 1 and 2, 5)

To study the mensuration characteristics of the mid-piece with different succinic dehydrogenase activity, two out of the four coded slides from each of the ten males of the control and experimental groups were used. A total of forty slides, twenty from the control and twenty from the experimental group were examined. Under a projection microscope, at a linear magnification of $\times 6.169$, spermatozoa, two for each of the five classes, were selected at random from each slide. The mid-pieces of the selected spermatozoa were drawn on separate sheets of paper. There were ten drawings from each slide. The drawings were

TABLE 1

GROUP MEANS, ANGULAR PERCENTAGE AND ANALYSIS OF VARIANCE OF THE MID-PIECE CHARACTERISTICS

Group means	d.f.	Succinic dehydrogenase activity				
		++++	+++	++	+	-
Control mean		29.11	32.16	1.96	30.73	20.10
Experimental mean		22.63	26.75	1.82	30.73	33.05
<i>Analysis</i>						
Groups	1	1680.13	1171.86*	0.75	0.001	6713.41**
Mice within group	38	638.80**	191.36**	25.85**	497.75**	699.56**
Slides within mice	120	54.19	40.93	13.67	105.83	55.94

* $0.025 > P > 0.005$.

** $0.005 > P$.

coded to veil their classification before measurements were made on them for length, breadth and projected area. The projection microscope used and the method of measurement have already been described (Mukherjee & Ayyagari, 1969). The average mid-piece length, breadth and area were determined for each class of spermatozoa present on a slide.

The percentages of spermatozoa with different classes of mid-piece were transformed into angles and statistically analysed. The group means and details of analysis of variance are given in Table 1. The mean square between mice within a group was used to test the mean square between groups. The results of the analysis showed that administration of adrenaline decreased the proportion of spermatozoa with '+++', and increased the proportion of spermatozoa with

PLATE 1

FIGS. 1 and 2. Mid-pieces of mice spermatozoa with different dehydrogenase activity.

(Facing p. 376)

PLATE 2

FIGS. 1 to 4. Mid-pieces of mice spermatozoa showing dehydrogenase activity. Figs. 1 and 2 of the control group and Figs. 3 and 4 of the experimental group.

(Facing p. 377)

'—', succinic dehydrogenase activity. To test this result, four slides from four males of the control and experimental groups were selected at random and photographed. The micrographs confirmed the results obtained (Plate 2).

Group means of the mensuration characteristics of the mid-piece of different classifications are given in Table 2 and the details of an analysis of variance of the characteristics are given in Table 3. The mean squares due to interactions were tested against the error mean squares and were found to be statistically non-significant. They were, therefore, pooled for mid-piece area and breadth, and the pooled error mean square (with 156 d.f.) was used to test the mean squares between classes. To test the mean square between mice within a group, interaction mean squares between classes and mice within groups were used. As

TABLE 2
GROUP MEANS OF THE MENSURATION CHARACTERISTICS OF THE MID-PIECE

Characteristics	Group means	Succinic dehydrogenase activity				
		++++	+++	++	+	—
Mid-piece area (μ^2)	Control	18.04	15.74	15.41	14.30	12.17
	Experimental	17.65	16.10	15.77	14.72	12.31
Mid-piece length (μ)	Control	21.89	21.78	20.98	21.59	21.16
	Experimental	21.96	21.79	21.13	21.13	21.66
Mid-piece breadth (μ)	Control	0.824	0.712	0.721	0.662	0.547
	Experimental	0.804	0.739	0.718	0.677	0.567

TABLE 3
ANALYSIS OF VARIANCE OF THE MENSURATION CHARACTERISTICS OF THE MID-PIECE

Analysis	d.f.	Mid-piece area (μ^2)	Mid-piece length (μ)	Mid-piece breadth (μ)
Classes	4	168.441**	0.166	0.3437**
Groups	1	1.724	0.147	0.0014
Mice within group	18	3.844**	1.313*	0.0048**
Slides within mice	20	0.476	0.316	0.0012
Classes $\times$ Group	4	1.432	0.555	0.0031
Classes $\times$ Mice within groups	72	1.079	0.397	0.0020
Error	80	0.819	0.625	0.0014
Pooled error	156	0.955	—	0.0017

* $0.025 > P > 0.005$.

** $0.005 > P$.

the error mean square for mid-piece length was more than the interaction mean square, it was used to test the mean square between classes and between mice within a group. For all the three characteristics of the mid-piece, mean square between groups was tested against the mean square between mice within a group.

As the means of the mid-piece area and breadth varied between classes, the differences between means were tested according to the method described by Snedecor (1957). It was found that for both mid-piece area and breadth, the differences were significant, except between classes showing '+++' and '++' activity.

From the results of the present experiment, it was concluded that administra-

tion of adrenaline to mice reduced the percentage of spermatozoa with high dehydrogenase activity. The results also indicated that, with the decrease in the dehydrogenase activity, the area and breadth of the mid-piece also decreased. As the mid-piece area and breadth of spermatozoa with the same dehydrogenase activity did not vary between the control and the experimental groups, it was also concluded that reduction in the area and breadth of the mid-piece of mice spermatozoa as reported previously by Mukherjee & Ayyagari (1969) was due to a reduction in the number of spermatozoa with high dehydrogenase activity in the semen samples obtained after adrenaline injection. Beatty & Sharma (1960) and Beatty (1962) reported strain differences in the length and breadth of the mid-piece of mouse spermatozoa. It appears from the present result that the succinic dehydrogenase activity of the mid-piece may also vary between different strains of mice.

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