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Effect of Age and Sex on Sensitivity to D-Tubocurarine in the Rat.* (29480)

SHELDON M. WOLF,[§] RICHARD L. SIMMONS,[†] AND WILLIAM L. NASTUK
(With the assistance of Carl S. Ingber)

*Departments of Physiology, Neurology, and Surgery, Columbia University College of Physicians
and Surgeons, New York City*

Myasthenia gravis occurs most frequently in women of reproductive age(1,2,3). Although the basis for the sex difference in the incidence of this disease is unknown, it is conceivable that the work of Gallagher and Koch(4) has some bearing on this problem. These investigators found that male Wistar rats are more resistant to the effects of curare than are females. One of many possible explanations for this difference in susceptibility to d-tubocurarine might be that in sexually mature females there is a relatively smaller safety margin in neuromuscular transmission. However, Gallagher and Koch do not comment on this point. We felt that it would be interesting to confirm the existence of a sex linked difference in sensitivity

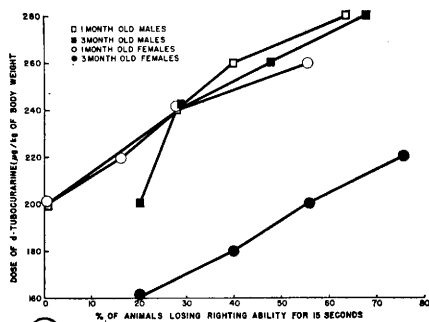
to d-tubocurarine, to study its pattern of emergence with age, and to examine the effect of gonadectomy. Therefore the following study was undertaken.

Methods. Albino Sprague-Dawley rats which had been allowed food and water *ad libitum* were used for these experiments. Male and female littermates, either one or 3 months of age, were randomly selected, weighed, and each was injected intraperitoneally with a single calculated dose of d-tubocurarine (Intocostin, Squibb). The d-tubocurarine (dTC) solution used contained 60 μ g per ml in normal saline. The test for curare sensitivity is based on the assay method described by Collier *et al*(5). In our procedure, as soon as an animal showed any decrease in its normal motor activity, it was placed on its side, on a level surface. The normal animal invariably righted itself immediately and began moving about. In this work, we considered a result to be positive if the animal failed to right itself completely in a 15 second period after

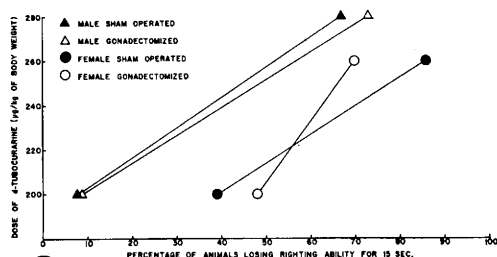
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[§]Present address: Southern California Permanente Medical Group, Los Angeles 27, Calif.

[†] Clinical Fellow, Am. Cancer Society.



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FIG. 1. Effect of various doses of d-tubocurarine on ability of 1- and 3-month-old male and female rats to right themselves within 15 seconds of being placed on their sides.

FIG. 2. Effect of 2 doses of d-tubocurarine on the ability of 3-month-old gonadectomized and sham operated male and female rats to right themselves within 15 seconds of being placed on their sides.

it was placed on its side. After receiving d-tubocurarine, the righting responses of injected animals were tested every 2 to 3 minutes until either a positive result was obtained, or 45 minutes had elapsed. This duration of test period was chosen because it was found that no animal ever lost the ability to right itself more than 35 minutes after injection of dTC. Each animal was injected and used only once.

Complete gonadectomy was performed on animals one week old through a bilateral incision in the groin or lumbar region. Sham operations, performed at the same time, consisted of exposing the gonads and then closing the incision. Each litter contained both gonadectomized and sham operated animals. Testing with curare was performed when these animals were three months old.

Results. Fig. 1 depicts the results of the first experiment. Each point was established by testing 25 animals. Statistical analysis reveals that there is no significant difference

between the curare sensitivity of one-month-old males, one-month-old females, and 3-month-old males. The 3-month-old sexually mature females are significantly more sensitive (at the 1% level) than are the other groups.

Probit analysis was performed on all 4 assays under the restriction of a common slope. The assumptions of linearity and validity were not contradicted by chi square tests which were performed. In each of the 4 assays a 99% confidence interval for the median effective dose of d-tubocurarine was estimated. These values are:

females—one-month-old	243-286 $\mu\text{g/kg}$
males— " "	253-291 "
females—3-months-old	175-211 "
males— " "	237-329 "

By use of Fieller's theorem, 99% confidence intervals for the relative potencies were computed. If the confidence interval includes the potency of one, no statistical difference in sensitivity was established at the 1% level.

Relative potency:

1-month-old males to 1-month-old females (.88 to 1.12)

3-month-old males to 3-month-old females (.69 to .84)

1-month-old females to 3-month-old females (1.22 to 1.55)

3-month-old males to 1 month-old males (.94 to 1.46)

Fig. 2 shows the results of the second experiment. Each point was established by testing from 23 to 30 animals. Statistical analysis reveals that there is no significant difference between the curare sensitivity of sham operated and gonadectomized males, or between sham operated and gonadectomized females. There is a significant difference between the sham operated animals of each sex, and between the gonadectomized animals of each sex.

Relative potency:

Male gonadectomized to male sham operated 1.13 to 0.83

Female gonadectomized to female sham operated 1.20 to 0.88

Male gonadectomized to female gonadectomized 1.37 to 1.01

Male sham operated to female sham operated 1.45 to 1.07

Discussion. It is apparent from these results that between the ages of one and 3 months, the female rat develops a marked increase in sensitivity to the paralytic effects of curare. Our results are also in close agreement with those obtained by Gallagher and Koch even though their end point, the determination of the LD50, was different from our own. From our data it is not possible to determine whether the increased sensitivity in the females is due to an increased rate of peritoneal absorption of dTC, an increased sensitivity to dTC at the neuromuscular junction, or a decreased rate of removal of the drug from the end plate areas. These questions can only be answered by more experiments. For example, it would be useful to determine *in vitro* whether there is any difference in the sensitivity of male and female mammalian neuromuscular preparations to d-tubocurarine.

Since gonadectomy fails to abolish the sex difference in curare sensitivity it appears that this difference is not mainly determined by the sex hormones but by some other sex linked genetic mechanism.

Our results suggest that it is necessary to consider the age and sex of animals used in any comparison of d-tubocurarine with other drugs capable of producing muscle relaxation. Since a search of the literature has not revealed a reference dealing with a difference in sensitivity to d-tubocurarine of

human females *vs* human males, a clinical evaluation of this point is warranted.

Summary. One- and three-month-old male and female rats were injected intraperitoneally with d-tubocurarine in various doses. These animals were then placed on their sides and their ability to regain the upright position determined. No difference in sensitivity to d-tubocurarine was found to exist between one-month-old females, one-month-old males, and 3-month-old males. The 3-month-old females, however, were significantly more sensitive than any of the other groups. Gonadectomy performed at age one week failed to abolish the sex difference in curare sensitivity in animals tested at 3 months of age.

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Inhibition of Adrenal Cholesterolgenesis *in vitro* by AY-9944, 22,25-Diazacholesterol and by Triparanol. (29481)

M. L. GIVNER AND D. DVORNIK (Introduced by C. Chappel)
(With technical assistance of Sheila Gosselin)

Department of Biochemistry, Ayerst Research Laboratories, Montreal, Canada

The over-all course of hepatic cholesterolgenesis is known and, with the exception of some steps involved in conversion of lanosterol to cholesterol(1,2) the normal sequence of reactions constituting the biogenetic pathway is well established(3). It is generally

believed that the extrahepatic biosynthesis of cholesterol follows the same pathway. Similarity of the hepatic and adrenal patterns of cholesterol biosynthesis depends on the presence of similar enzyme systems in both organs. A method of revealing the existence