

logical properties were studied. The antigens that precipitated antibodies from the sera of rabbits injected with the crude DNA preparation were concentrated in a fraction consisting essentially of DNA and only about 2% protein. This fraction did not elicit the formation of precipitating antibodies, whereas a protein-rich fraction with no reactive antigens did. Treatment of the reactive antigens with DNase altered some of their properties. On the basis of present evidence, it is suggested that the DNase-sensitive antigens are complexes containing DNA.

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### Effect of ACTH on Sex Ratio of the Albino Rat.\* (26464)

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Mammalian spermatogenesis results in an equal number of male and female producing spermatozoa. The actual sex ratio of most species, however, differs characteristically from the expected theoretical value of unity (1).

The factors which enter habitually into the reproductive process of those species and modify the sex ratio (number of males per 100 females) could be paternal, e.g. excess mortality of one type of spermatozoon after spermatogenesis or diminished aptitude of one type of spermatozoon for fertilization. Such and other differential properties of the 2 types of spermatozoa have been demonstrated and have become the subject of considerable research. On the other hand, the maternal milieu interne could be such as habitually to favor survival of, or fertilization by, one type of spermatozoon. These two possibilities may be largely synonymous.

In addition to these normal and species

specific deviations from unity of the sex ratio further changes in sex ratio occur in several well authenticated situations. The old observation that the human sex ratio rises in war time was again verified during the second world war and statistical analysis suggests that this rise was not due to age of the parents or to the birth order(2). In many species including man, it has also been noted that the first born or the first litter tends to have a significantly higher sex ratio than the species as a whole. In the rat with a species specific sex ratio of 105 the ratio for first litters is 122(3). In some animals (cattle, sheep, pigs, wolf hounds) statistics indicate that the relative number of female births tends to rise during the cold months of the year. King has confirmed this for the rat(3).

These observations suggest that stress may be able to alter sex ratio. The possibility that the adrenal cortex may be involved in mediating these effects is strengthened by 2 observations of King and Donaldson made in 1929, i.e. before the relation between adrenal cortex and stress responses was understood. King recorded the sex ratio of the

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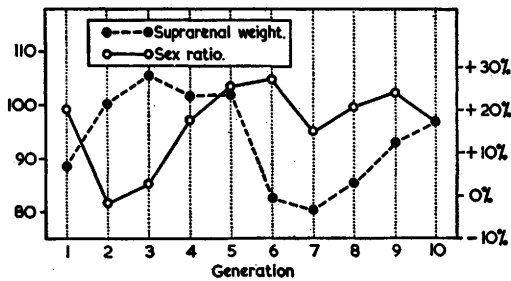


FIG. 1. Relation of adrenal wt to sex ratio in 10 generations of Gray Norway Rats. (Adapted from King and Donaldson, *Am. Anatomical Memoirs* No. 14.) Figures on left give No. of males per 100 females; figures on right, % increase of adrenal wt over standard adrenal wt for albino rats.

gray Norway rat during 10 generations in captivity while Donaldson showed changes in adrenal weight in the same animals over the same period(4). I have superimposed these 2 graphs upon one another to show the striking inverse relation between them (Fig 1). It is seen that rises in adrenal weight correspond to falls in sex ratio.

**Design of Experiment.** The animals were female albino virgin rats. Female litter mates, kept in the same cage and under the same conditions throughout experiment served as controls. At the end of each experiment the experimental animals and their litters were killed and checked for abnormalities whereas control litters were allowed to grow up for further experiment. Insofar as inherited tendencies are involved, the experiment was deliberately designed with a bias against production of differences between control and experimental groups, since all experimental groups except the first were the offspring of control animals.

Control animals were given .05 ml saline, experimental animals .05 ml (1 unit) long acting ACTH subcutaneously every second

day. On the day after first injection the animals were mated with a normal untreated male of the same strain. At the end of one week both injections and mating were interrupted for one week to minimize any possible effect of ACTH on pregnancies. Pregnant animals were isolated, and injections and mating were resumed with the remainder. This weekly alternation was continued until all animals had become pregnant or until 6 weeks had elapsed. Sex ratio of the litters was recorded as soon as possible after birth. The same sire was used for as many succeeding experiments as possible. Only 6 sires were utilized throughout the investigation.

The investigation was carried out in 2 series. In the first series (in Glasgow) Corticogel (Crookes) was the ACTH used. The second series (in Dunedin) was carried out in the same manner except that Cortrophin ZN (Organon) was used.

**Results** are given in Table I. They are essentially the same in both series, which may therefore be considered together. ACTH medication had no adverse effects on fertility. The number of animals which failed to be fertilized within 6 weeks was very small and roughly equal in both control and experimental groups. Average litter size compares well with that recorded for large well looked after colonies and is the same in both control and experimental groups. Sex ratio at birth, however, is significantly depressed in the treated animals. This is seen both in the aggregate figures and in proportion of predominantly female litters. The change is a generalized shift and not a clear cut all-or-none phenomenon.

**Discussion.** It is reasonable to assume that this effect is produced after the male

TABLE I.

|                                     | Series 1 |         | Series 2 |         | Total   |         |
|-------------------------------------|----------|---------|----------|---------|---------|---------|
|                                     | Control  | Treated | Control  | Treated | Control | Treated |
| No. of animals born                 | 114      | 186     | 293      | 183     | 407     | 369     |
| " " litters                         | 15       | 25      | 33       | 19      | 48      | 44      |
| Avg size of litters                 | 7.6      | 7.3     | 9        | 10      | 8.47    | 8.38    |
| No. of predominantly female litters | 3        | 13      | 12       | 10      | 15      | 23      |
| Males : Females                     | 68:46    | 89:97   | 159:134  | 81:102  | 227:180 | 170:199 |

$$\chi^2 = 9.24, \text{ i.d.f., } .001 < P < .01.$$

TABLE II. Distribution of Litter Sizes.

| No. in<br>litter | Control | Treated | No. in<br>litter       | Control | Treated |
|------------------|---------|---------|------------------------|---------|---------|
| 3                | 1       | 2       | 9                      | 11      | 7       |
| 4                | 0       | 1       | 10                     | 10      | 7       |
| 5                | 3       | 2       | 11                     | 3       | 6       |
| 6                | 4       | 3       | 12                     | 2       | 1       |
| 7                | 2       | 5       | 13                     | 0       | 1       |
| 8                | 12      | 9       |                        |         |         |
|                  |         |         | Total No. of litters   |         |         |
|                  |         |         | 48                  44 |         |         |

ejaculate has entered the maternal passages. Is it produced before or after fertilization of the ovum? Does ACTH act by a differential effect on foetal mortality? If ACTH increased male foetal mortality, the experimental litters should be smaller, and if it acts by reducing female foetal mortality they should be larger than control litters. Average litter size is the same in both groups. If ACTH acts simultaneously in both directions the effect should be most noticeable in the smaller litters. Experimental litters with less than 10 animals were found to have a sex ratio of 95 against one of 77 for litters with more than 10 animals. It is therefore unlikely that the ACTH effect was on foetal mortality. Table II showing the very similar distribution of litter size in the 2 groups does not suggest that litter size was in any way connected with the ACTH effect.

Another possibility, namely that the change in overt sex ratio may not correspond to the nuclear sex ratio i. e. that ACTH may have produced females with male nuclear sex cannot be excluded. However, no malformations were observed in any of the experimental animals and there is nothing in the literature to suggest that complete reversal of the genotypic sex can be produced in offspring by moderate stimulation of the mother's adrenal cortex.

The most reasonable hypothesis seems to be that the effect was on primary sex ratio.

i. e. on number of ova fertilized by either type of sperm. The vaginal, uterine or ovarian environment seems to have been modified so as to increase the chances of female producing spermatazoa to fertilize.

Is this effect mediated through the adrenal cortex or is it a non-specific effect of ACTH or of one of its impurities, diluents, or suspending agents? In view of King's observations (Fig. 1) and in view of the use of a different type of ACTH in the 2 series the former seems more likely.

The size of the sex ratio depression obtained more than abolishes the species specific bias towards male conceptions shown by the rat, but the effect of ACTH will not necessarily be in the same direction in other species.

The animals used were young virgin females and to some extent the effect of ACTH resembles therefore that of normal maturation, since the sex ratio of first litters tends to be high in the rat. However, ACTH depresses the sex ratio much more than would be compatible with a mere speeding up of maturation.

*Summary.* Subcutaneous administration every second day of one unit of long acting ACTH to female albino rats before and during mating significantly depresses the sex ratio at birth of their first litters.

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