Our experiment shows that certain immunological characteristics which with a few exceptions have been attributed only to protein antigens may also be demonstrated in some bacterial polysaccharides, at least this is true with the soluble substance derived from *B. rhinoscleromatis*. Through the use of properly prepared polysaccharides it was possible in our hands to induce the formation of antibodies in rabbits and to induce active sensitization in guinea pigs.

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Relationship Between Female Sex Hormone and Dewlap in the Rabbit.

CH'UAN-K'UEI HU AND CHESTER N. FRAZIER.

From the Division of Dermatology and Syphilology, Department of Medicine, Peiping Union Medical College, China.

Frazier and Mu[1] noted that prolonged administration of an estrogenic substance, extracted with butyl alcohol from the urine of pregnant women, feminized male rabbits. Among the physiological and anatomical changes observed was the development of a ruff, a prominent fold of loose skin encircling the anterior part of the neck. In well-developed cases the skin was so redundant that it could easily be pulled over the nose of the animal. A similar condition in normal rabbits is known to breeders as dewlap.[2] In order to ascertain the significance of this formation of skin, we have examined the rabbits in our breeding colony for incidence of dewlap, and have also made some observations on adult male rabbits and on immature male and female rabbits treated with estrogenic substance. Another series of female rabbits was subjected to ovariectomy. This we did on the assumption that dewlap is related physiologically to the female sex hormone of the animal.

The rabbits of the breeding colony were examined on 3 occasions, 6 months apart, in the spring and fall. Both male and female animals and their young were included in this study. They were of different breeds with a variation in age from birth to 3 years. In the case of the adult females, the physiological status (resting, pregnant, or lactating) and the number of pregnancies were taken into account.

1 Frazier, C. N., and Mu, J. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 997.

2 *Guide Book and Standard*, Chicago, Ill., The Am. Rabbit and Cavy Breeders Assn., 1932-1933.

The dewlap is best observed in profile. The positive observations were classified in 3 grades depending upon the prominence of the fold of loose skin in front of the neck. A grade I fold may not be shown in a profile photograph, in which case it can be demonstrated only by grasping the skin between the fingers. Folds of grades II and III are easily seen in profile.

The observations on normal animals may be summarized under the following headings.

Sex. Table I shows that 53.6% of the 250 adult female rabbits had a dewlap, but that only 7% of the 200 adult males had the condition. Furthermore, in the male rabbits the fold of skin was usually very small, that is, no more than grade I. In the majority of instances, the females presented a dewlap of either grade II or III.

TABLE I.

Adult male rabbits*			Adult female rabbits*		
No. of animals	No. with dewlap	Dewlap %	No. of animals	No. with dewlap	Dewlap %
200	14	7.0	250	134	53.6

*Six or more months of age.

Age. There was not a single case of dewlap among 200 male and female animals under 3 months of age. Among 50 male and female animals between 3 and 6 months of age no case of dewlap occurred. In 189 adult females whose exact ages were known, a dewlap was present in 51% of 86 animals between 6 and 12 months of age, and in 61% of 103 animals more than 12 months of age.

Physiological Status. Our observations indicated no correlation between the incidence of dewlap and the number of pregnancies in the females, or the number of matings in the males. Neither pregnancy nor lactation was found to have any definite influence on the incidence of dewlap, although a few lactating does seemed to have a less prominent fold than when they were in the resting stage.

Breed. The main breeding lines showed no significant differences in the incidence of dewlap. However, these were not pure stocks. Most of the animals had the coat colors of the Dutch and Chinese albino breeds. A few were English, and others were of a gray colored breed.

Dewlap in Estrinized and Ovariectomized Rabbits. The experimental observations were made on groups of male animals receiving injections of estrogenic substance and on groups of ovariectomized adult females and their normal litter-mates. The effect of estrogenic substance on immature male and female animals was tested

also. All injections of estrogenic substance were given daily, 6 times a week, by the subcutaneous route. The estrogenic substance was extracted from the urine of pregnant women by the same method described previously.[1, 3]

Dewlap developed in all of the 24 adult male rabbits in the course of from one to 3 months following the daily injection of 20-60 rat units[4] of estrogenic substance.

Two immature male rabbits, aged one month, were treated with the estrogenic substance starting with a dosage of 3 R.U. daily. This was doubled once, and again, in 3 and 8 weeks respectively, and finally increased to 18-30 R.U. a day. Both animals began to show the dewlap at a little over 7 months of age. It was quite prominent in one of the 2 rabbits while the animal was receiving 30 R.U. a day, but disappeared entirely after the dose of estrogenic substance was reduced to 15 R.U. a day. The dewlap did not return during a period of 3 months through which the treatment at this dosage was continued. This observation suggests that the response is not initiated by small amounts of the estrogenic substance in the circulating blood and can be sustained only when relatively large amounts of the substance are present.

A third male, aged 2 months, was treated with an initial dose of 15 R.U. a day. This dose was doubled once, and then again, in 3 and 9 weeks respectively. In spite of this large dose the animal, like the above 2, did not develop the dewlap until it had passed the seventh month of age.

One immature female was subjected to the same treatment as the first 2 immature males which were its littermates. This female, like the 3 males, did not begin to show the dewlap until it, too, had attained the age of 7 months.

Effect of Ovariectomy. Four sets of female rabbits, totaling 31, were ovariectomized at the age of 4-6 months. Both ovaries and fallopian tubes were removed together with the surrounding fatty tissue. Not a single case of dewlap was observed in a period of 4-6 months after the operation. Of 23 non-operated female litter-mates, 11, or 48%, developed a dewlap.

Ovariectomy not only prevented the development of a dewlap but also had a marked effect upon it after its full development. Three adult females, aged 10 months or over, with a grade III dewlap were ovariectomized. In all of the 3 animals the dewlap was re-

[3] Frazier, C. N., Mu, J. W., and Hu, C. K., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 65.

[4] Coward, K. H., and Burn, J. H., *J. Physiol.*, 1927, **63**, 270.

duced from grade III to grade I. The reduction was not appreciable in the first month following ovariectomy, but gradually and progressively took place in the next 2-2½ months, and then halted. One animal was observed as long as 8 months after the operation, while the other 2 were observed 4 months.

Summary and Conclusion. Judging from the evidence at hand we believe that the dewlap is normally a secondary sex character of the adult female rabbit, and that its presence is dependent upon the action of a substance (or substances) related primarily to the ovary. It may develop to a limited degree in the male rabbit in which case it is assumed that the female sex hormone is present in amounts sufficient to initiate and maintain the existence of the dewlap. The following observations support these conclusions: (1) A prominent dewlap was present in about 50% of the normal adult females and in only 7% of the adult males examined. When present in the male animal the fold of skin was usually so small that it could not be seen in profile. (2) The administration of a butyl alcohol extract of the urine of pregnant women induced a pronounced dewlap in all treated male animals. The developmental process was reversible, the fold of skin disappearing when the administration of estrogenic substance was reduced. (3) The dewlap failed to develop in animals ovariectomized before the age of sexual maturity, but did develop in normal female litter-mates with a frequency equal to that observed in the breeding colony. (4) When ovariectomy was performed after the appearance of the dewlap, it gradually underwent almost complete involution.

It is apparent that the female sex hormone did not exert its influence directly upon the skin during the development of the dewlap. In the breeding colony, the animals normally showed no evidence of dewlap before they were 6 months old, the age at which the rabbit reaches adult status. In spite of the daily administration of estrogenic substance, even in large amounts, normal immature male and female rabbits did not respond with a dewlap until they had reached the age of 6 months. It would seem, therefore, that the action of the female sex hormone depends upon an intermediary to stimulate the growth of the dewlap, and that this intermediary factor is present only in the adult animal.