

terol. Ordinary cholesterol, according to Bills,⁸ contains less than 5 parts of ergosterol in 1000 parts or less than 0.5%. We dissolved one gram of cholesterol in 5 cc. of washed chloroform and added 2 cc. of the selenite-hydrochloric acid reagent. A reaction was obtained, which when compared to a series of standards, indicated the presence of 0.25 mg. of ergosterol in the one gram of cholesterol (0.025%). We made up a mixture of 100 mg. of ergosterol-free cholesterol and 0.025 mg. of ergosterol (0.025%) in 5 cc. of chloroform, applied the test and obtained a positive reaction.

Conclusion. A mixture made up in the proportion of three volumes of 2% aqueous solution of sodium selenite and one volume of concentrated hydrochloric acid is useful for the detection of irradiated or non-irradiated ergosterol and calciferol. The reagent is useful in distinguishing ergosterol from cholesterol, since the latter does not react. It is also very serviceable in the detection of small amounts of ergosterol in samples of cholesterol.

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Further Studies of Sex Skin Reactions in *Macaca Mulatta*.

C. BACHMAN, J. B. COLLIP AND H. SELYE.

From the Department of Biochemistry, McGill University, Montreal.

It has previously been reported¹ that the sex skin reactions of rhesus macaques, which can be called forth by injection of oestriol, eventually spread over a wide extra-genital area if the injections of this hormone are continued for longer than a month. Minor degrees of this extra-genital oedema can be observed in many untreated cyclic females at certain periods of the year, but the full manifestations possible after prolonged oestriol treatment have not been observed to date in a considerable experience with untreated macaques. In the further study of these phenomena, it was of interest to see whether the results obtained with oestriol could be duplicated with oestrone. Also, in view of the symmetrical character of the sex skin reactions, it was felt desirable to investigate a few relationships of the segmental nerve supply of the affected areas to the reactions occurring therein.

⁸ Bills, C. E., *Physiol. Rev.*, 1935, **15**, 1.

¹ Bachman, C., Collip, J. B., and Selye, H., *Proc. Roy. Soc. Lond.*, 1935, **117**, 16.

Females only were used. Two adult castrates, one adolescent castrate, and 2 adolescent but acyclic intact animals were injected subcutaneously once daily for 48, 48, 74, 74 and 76 days respectively with 500 γ of crystalline-folliculine (Girard) dissolved in corn oil.

A typical experiment will show that the responses previously elicited by oestriol were duplicated by the use of oestrone. Monkey No. 885, a 4275 gm. female castrate, had been used during the original experiments with oestriol, but had received no treatment for 7 months. The genital reaction appeared on the second day after starting oestrone injections, and attained maximum brilliancy on the 16th day. This local reaction, as in most animals which have been repeatedly treated with oestrogenic hormone, was featured more by coloration than by oedema. An analogous situation exists in nature, where the initial genital oedema which characterizes the sex skin phenomena of pubertal females gradually gives way to an erythematous type of reaction during the menstrual cycles of maturity. After the 25th day, the genital color of Monkey No. 885 varied from day to day, but tended gradually to fade in spite of continued treatment. By the end of the injections on the 48th day, it had almost lost its red shade. Meanwhile, and from the 20th day onward, the pachydermatous oedema of the extra-genital reaction gradually spread over hips, thighs, legs, back and brow in this order, and was still slowly spreading at the end of the experiment. Eight days after the injections were stopped, vaginal bleeding occurred and lasted for 3 days.

Except for individual variations, the responses in the remaining animals were analogous. The second adult castrate was an animal which had also been used during the former experiments with oestriol; its reactions in both experiments were not as maximal as in Monkey 885. Since a sub-maximal result was further observed in one of the 3 adolescents, none of which had previously received oestrogenic hormone treatment, these deviations can probably be ascribed to individual variations of response rather than to qualitative differences in the activity of oestrone and oestriol, or to effects of castration upon the actions of these hormones.

For the investigation of nervous factors in the sex skin reactions, both of the adult castrates used above, and a young male, were utilized. In one of the females, a segment of skin from a reactive area on the thigh was transplanted to the abdomen by means of a 2-stage pedicle graft operation. In spite of the severance of all previous segmental nerve supply, the graft reacted as usual to oestrogenic hormone in its new location. In the second female, the

reverse procedure was applied, that is, transplantation of a piece of skin from a non-reactive area on the abdomen to a denuded site within the genital area; treatment in this animal left the transplant a pale island of skin in brilliantly colored surroundings.

In the male, the left abdominal sympathetic trunk was severed below the fifth lumbar ganglion. Treatment with oestrogenic hormone elicited a temporary but definitely greater degree of swelling in the left scrotal area than in the corresponding area on the opposite side. This was soon obscured, however, by the increasing oedema of the genital reaction as a whole. A complementary type of experiment has already been recorded by Zuckerman,² who was able to inhibit the genital response by section of the sacral nerves in the cauda equina.

Summary. In the sex skin reactions of *M. mulatta* there are no qualitative differences of response to the keto- and trihydroxy-forms of oestrogenic hormone. Although the skin reactions are to a degree under the influence of the autonomic nervous system, the characteristic phenomena are probably determined by inherent structural peculiarities of the genital skin itself.

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Oestrogenic Hormone and the Mechanism of Corpus Luteum Formation in the Rabbit.

CARL BACHMAN. (Introduced by J. B. Collip.)

From the Department of Biochemistry, McGill University, Montreal.

Hohlweg's experiment,¹ in which corpus luteum formation was induced in immature "40 to 50 gm. rats" by the administration of single large doses of dihydroxyoestrin benzoate (Progynon-B), constitutes the crucial evidence in support of the currently favored view that oestrogenic hormone stimulates the output of luteinizing hormone by the anterior pituitary. Attempts to duplicate Hohlweg's attractive experiment in this laboratory have unfortunately been unsuccessful. Single subcutaneous doses of as much as 2000 γ of crystalline oestrone (Folliculine-Girard) in oil have been given to each of 10 female rats, weighing from 40 to 50 gm.—this weight corresponding to an age of 24 days in our colony. At autopsy one

² Zuckerman, S., *Proc. Roy. Soc. Lond.*, 1935, **118**, 22.

¹ Hohlweg, W., *Klin. Wchnschr.*, 1934, **13**, 92.