earliest manifest physiological action of the substance on the mammary glands. Further evidence of a similar nature was in the absence of metastatic orchitis, and in the delayed appearance and shorter duration of generalized lesions. The effectiveness of the defensive reaction was particularly well demonstrated by the predominance of latency in the treated animals. The degree of protection afforded the testis, which as a rule is highly vulnerable to syphilitic infection, was the most noteworthy feature of disease resistance in the feminized males.

8252 C

Cocaine Recovery from Rabbit and Cat Aqueous Humor.

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In 1930 the statement was made "At present there is no method sufficiently delicate to determine quantitatively the amount of cocaine present in the anterior chamber as it arrives from the conjunctival sac after clinical instillation."[1]

Recently, a method[2] was developed whereby cocaine can be quantitatively recovered from body fluids. The essential features of this method follow:

To the deproteinized fluid supposedly containing cocaine, is added 1 cc. of a 1% solution of phosphomolybdic acid in normal sulphuric acid. After mixing and centrifuging, the precipitate is washed with water which, after another centrifuging, is decanted and discarded. One cc. of N/5 sulphuric acid, 1 cc. of 2% aqueous solution of hydroquinone, and after shaking, 1 cc. of carbonate-sulphite solution are successively added to the precipitate. This develops a color which is read in the Duboscq microcolorimeter against a standard prepared by adding the above reagents to 1 cc. of a solution of approximately equal alkaloid content.

With solutions of cocaine in aqueous humor the probable analytical error is 20% or less with the concentrations employed, which

[1] Yonkman, F. F., *J. Pharm. and Exp. Therap.*, 1930, **40**, 195.

[2] Walker, B. S., and Walker, E. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 148.

concentrations are approximately at the limit of colorimetric reading.

By this method we have succeeded in recovering cocaine from rabbits' aqueous humor in so small a concentration as 1-18,000, and 1-25,000 in aqueous humor of the cat.

Normal rabbits and cats of both sexes and various breeds received, after pupillary measurements, several instillations of 4% cocaine until the cornea became desensitized and a pupillary dilatation of 2 mm. or more occurred under uniform lighting. This usually required from ½ to 1½ hours. The animals were then lightly anesthetized with ether; the eyes were washed with normal saline in most cases, and the aqueous humor was extracted with the hypodermic needle and tuberculin syringe by means of the sclero-corneal approach used by Koppanyi.[3] In some instances the same rabbits were used for similar experiments after 48 hours, since the aqueous humor extraction works no particular hardship on the eye if carefully done and followed by protargin wash.

Our data were obtained from 50 experiments on 25 animals. Of these, 24 yielded recoverable cocaine, 5 served as controls and 16 failed to show cocaine in the aqueous humor although respective pupils were noticeably dilated. Of the failures recorded, 5 appeared in 6 cat experiments, the remaining 11 appearing in the rabbit eyes. However, in 2 instances attempted, 0.12 mg. of cocaine was regained from 0.15 mg. added to the clear, control aqueous humor, suggesting extremely minute amounts of cocaine responsible for pupillary dilatation in some eyes.

Conclusions. The amount of cocaine recovered from anterior chambers of rabbit and cat eyes, whose pupils were dilated at least 2 mm. after conjunctival administration varied from 1-5,000 to 1-25,000. Beyond this dilution no recovery was encountered even though the pupils in these failures of recovery were dilated 2 mm. or more. We feel justified in stating that failure to recover cocaine in these instances can be accounted for on the basis of amounts of cocaine physiologically active but too small to be recoverable by this procedure. This is supported by experiments[2] in which known amounts of cocaine were added to control aqueous humor and recovered almost *in toto,* but not beyond the limits of 1-25,000. Further, our stock solution (4%) checked accurately against fresh cocaine standard, even after 3 and 4 weeks' usage.

These findings are of interest in view of the fact that in an earlier paper[1] on the physiological action of cocaine on isolated steer irides, the following conjecture was offered: " . . . it would seem that

[3] Koppanyi, T., *J. Pharm. and Exp. Therap.,* 1930, **38,** 113.

1:10,000 or perhaps more safely, 1:15,000 or 1:20,000 concentration of cocaine more closely approximates the concentration found at the iris sufficient to produce dilatation."

At present, we are studying cocaine recovery following clinical application in man.

8253 P

Influence of Hormones on Agglutinin Response to B. Pertussis in Immature Rats.

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The inability of immature animals to respond to active immunization with the degree of demonstrable antibody production found in mature animals has been noted by numerous investigators.[1] Our experiments carried out in infantile, adolescent, and mature rabbits and rats indicated that increase in ability to form agglutinins to *B. pertussis* and *B. typhosus* was approximately coincidental with sexual maturation.

In an attempt to determine more accurately the effect of increasing age on agglutinin production and to correlate this, if possible, with some easily determined index of sexual development, the following experiment was performed. Ten groups of 5 male rats each were selected. The age of the groups increased by increments of 7 ± 1 days, the youngest group being 4 days old. All the rats were injected intraperitoneally with total doses of 9,000 million *B. pertussis* in 6 injections: 1,000 million per day for 3 days, 4 days' rest, and 2,000 million per day for 3 days. On the 17th day of the experiment, the animals were weighed and bled. Seminal vesicles and testes were weighed as an approximate measurement of the degree of sexual maturity. As the rats advanced in age, the results showed a general correlation between the rise in agglutinin titer on one hand, and the increase in testes, seminal vesicle, and body weights on the other. For example, the average body weight, seminal vesicle weight, testes weight, and agglutinin titer of 4-day-old rats were 34 gm., 8 mg., 182 mg., and 120, respectively; whereas those of 74-day-old rats were 211 gm., 511 mg., 2660 mg., and 2480.

[1] See review of literature by Baumgartner, L., *Yale J. Biol. and Med.*, 1934, **6**, 403.