Purified flavin prepared from liver by a process described elsewhere, has been administered to 5 patients. The material was given by intramuscular injection, in amounts (5 cc.) representing 500 gm. of fresh liver. The criteria of effect were the hemoglobin, erythrocyte, and reticulocyte response. The 5 patients studied in this manner gave uniformly negative results. Since no effect was noted it is not necessary to record the protocols. The following statement is representative. Five cc. of flavin solution were given on alternate days until a total of 25 cc. had been injected. No significant change in the blood picture was noted during this period. The patient was then given commercial liver concentrate, which was followed by the typical reticulocyte response, generally on the fourth day.

One patient was treated by oral administration of Lilly's liver extract "343" which had been irradiated for 24 hours with a 300 watt light bulb. The irradiation was done at 15° in 10% NaOH solution, a treatment proved to change the flavin into photoflavin. After irradiation the material was neutralized, evaporated, and extracted with 80% alcohol. The alcohol extract was evaporated and the residue fed to the patient. A positive response was obtained, showing that the photolysis of the flavin had not impaired the hematopoietic activity of the material.

From these observations it may be concluded that liver flavin, given intramuscularly to patients with pernicious anemia does not exert an hematopoietic response; and that liver extract, the flavin of which has been converted to photoflavin, is still active.

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## Influence of Estrogenic Substance upon Experimental Syphilis of the Adult Male Rabbit.

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It is well known that the human female is more resistant to syphilitic infection than the male, especially during the child bearing period of life. This difference has been attributed to the influence of pregnancy.[1, 2] Corroborating the observations on human individ-

[1] Moore, J. E., *Arch. Int. Med.*, 1922, **30**, 548.

[2] Moore, J. E., *Bull. Johns Hopkins Hospt.*, 1923, **34**, 89.

uals, there is experimental evidence that the pregnant rabbit inoculated at the time of conception reacts to infection less severely than either the normal female or male rabbit.[3] A similar sex variation has been reported in normal rabbits.[4]

The essential nature of the forces responsible for this immunologic dissimilarity of the sexes is not known. In seeking an explanation, it is reasonable to assume that some endocrine function peculiar to the female and closely related to reproduction may play a dominant rôle in the protective mechanism of this sex. In this connection it has been shown that the reaction of the rabbit to syphilis depends in part upon the integrity and balance of the glands of internal secretion.[5]

One of the most striking phenomena of pregnancy related to the activity of the glands of internal secretion is the elaboration of an estrogenic substance, or substances, which as pregnancy progresses can be demonstrated in large amounts in the circulating blood[6] and in the urine.[7] As it is known that certain secondary sex characters of the female depend upon the presence of this substance, it has occurred to us that the resistance of the female to syphilitic disease may be an analogous character of sex dependent upon the same or similar factors.

The following experiment was undertaken for the purpose of observing the effect of estrogenic substance upon the course of syphilitic infection in the adult male rabbit. The feminizing effects of the prolonged administration of this substance on the animals employed in this experiment have already been described.[8]

Estrogenic substance was prepared by extracting acidulated human pregnancy urine with butyl alcohol by the method described previously.[8] The extract was heated to a temperature of 100°C. for at least 12 hours during the process of extraction. After removal of the solvents the residue was taken up in olive oil and assayed for its estrogenic content. The quantity required to produce estrus in 50% of 20 ovariectomized, sexually mature, albino rats was the rat unit employed in the experiment.

Mature, male albino rabbits, 16-17 months of age, from a pure

3 Brown, W. H., and Pearce, L., *Am. J. Syph.*, 1920, **4**, 593.

4 Chesney, A. M., *J. Exp. Med.*, 1923, **38**, 627.

5 Pearce, L., and Van Allen, C. M., *J. Exp. Med.*, 1926, **43**, 297.

6 Frank, R. T., Frank, M-L., Gustavson, R. G., and Weyerts, W. W., *J. A. M. A.*, 1925, **85**, 510.

7 Aschheim, S., *Arch. f. Gynäk.*, 1927, **132**, 179.

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

albino stock were used. The animals had been caged and fed from birth under uniform conditions, and had never been mated.

The strain of *Treponema pallidum* employed was that originally isolated by Nichols. The immediate source of virus was the testicular tissue of a rabbit with a progressing syphilitic orchitis. On May 1, 1934, when the inoculations were made, the strain had undergone 31 serial animal passages since being brought to this laboratory in 1927.

From 20-60 rat units of estrogenic substance in olive oil were injected subcutaneously once a day, 6 days a week, into 8 rabbits. A second series of 8 rabbits were injected in like manner with a corresponding amount of plain olive oil. Injections were continued in each case for 250 days, or as long as the animal survived.

Fifteen days after the first injection, each animal of both series was inoculated with 0.3 cc. of a saline emulsion of testicular tissue containing from 2-3 actively motile organisms to the microscopic field. The 2 groups were inoculated alternately so that no 2 animals from the same group received material from the same syringe. All inoculations were made into the right testis.

A primary orchitis developed in each animal. The mean incubation period for the estrin group was 16.4 days, and for the olive oil group, 20.3 days. This difference is probably not significant. The primary testicular reaction of the 2 groups differed particularly with respect to its chronicity. The mean duration of orchitis in the former group was 58.4 days as compared with 141 days in the control group. Healing of testicular lesions in those animals treated with estrogenic substance coincided with enlargement of the nipples, the first manifestation of the physiologic activity of the estrogenic substance, and in all animals except one the period of primary orchitis was terminated with the establishment of lactation. Milk could be expressed from the mammary glands of these animals 80 days after the first injection of the estrogenic preparation, which was 65 days after inoculation with *T. pallidum.*

Scrotal chancres developed in 7 of the control rabbits and in 4 of the test rabbits. The scrotal lesions of the latter group originated as circumscribed foci in the scrotal skin and were not direct extensions of testicular lesions.

There was a significant difference in the incidence of metastatic orchitis between the 2 groups. Six control animals developed lesions of the uninoculated testis at a mean time of 69.5 days after inoculation, but not a single rabbit treated with estrogenic substance developed metastatic foci of the uninoculated testis, and in

only 1 animal was there a transient localized infiltration of the head of the epididymis. The 2 rabbits in the control group without metastatic lesions of the testis, developed ulcerative reactions of the scrotum, and 2 test animals showed similar reactions.

An important criterion of the effectiveness of the resistance of the rabbit to syphilis is the character and extent of the generalized metastatic lesions. With respect to the location and number of these, the control animals had in all 16 detectable lesions as compared with 5 in the treated animals. The distribution of lesions was as follows: Control group: periosteum and bone 11, skin 3, and eyes 2. Test group: periosteum and bone 2, skin 0, and eyes 3. There was, moreover, a distinct delay in the appearance of generalized lesions among the animals treated with estrogenic substance. This was particularly true with respect to the periosteal and bone lesions. Among those animals developing reactions in the skeletal tissues, the difference between the 2 groups in the mean time of appearance of the lesions was 57 days, while the mean duration of such lesions in the control group was 28 days and in the test group, 14 days.

A further means of comparison is in the behavior of the 2 groups with respect to the number of cases in which resolution and healing of lesions was complete at the expiration of the experiment. Seven of the 8 animals injected with plain olive oil survived for 227 days. All of these had active lesions at that time, while only 2 of the 6 surviving animals treated with estrogenic substance presented signs of active infection.

A final comparison of the difference in susceptibility may be made on the basis of the actual number of days during which the animals were free from detectable manifestations of disease. For this summary only those rabbits surviving the full period of 227 days are included. In the control group (7 rabbits) there was a total of 105 days of latency and in the test group (6 rabbits), a total of 651 days. As the phenomenon of latency in syphilis may be taken as a measure of the effectiveness of the forces opposing the infection, this difference is especially significant and indicates that the feminized male rabbits possessed a more adequate degree of protection against infection than the normal male rabbits.

*Conclusions.* Under the conditions of the experiment, the estrogenic substance employed exerted an inhibitory influence upon a syphilitic infection when administration of the preparation was begun shortly before inoculation. The first evidence of this effect, in the form of resolution of the primary orchitis, coincided with the

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.

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## 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.