

tralized with CaCO_3 and the excess carbonate removed by filtration; (b) pyruvic acid adjusted to pH 6.6 with NaOH.

These data show that pyruvic acid was converted to acetic and butyric acids, carbon dioxide and hydrogen. It is of interest that butyric acid under these conditions of pH above 6.3 is not reduced by *Cl. butylicum* to butyl alcohol. The significance of this critical pH will be discussed in a subsequent communication. However, when the pH is less than 6.3, butyric acid is reduced to the alcohol. Table II shows the results from 2 experiments in which a mixture of pyruvic and butyric acids was adjusted to pH 4.8 with NaOH and K_2HPO_4 . Although butyric acid was reduced to butyl alcohol, it is to be noted that the reduction was not complete. In experiments with mixtures of formic and butyric acids as well as with lactic and butyric acids no enzymatic activity was manifested. Practically the same molarity of these compounds added was recovered unaltered. It must be considered, however, that the above experimental conditions are somewhat removed from those which obtain in a normal fermentation.

Unpublished results obtained in our laboratory indicate that isopropyl alcohol as well as traces of acetylmethylcarbinol is produced from a mixture of pyruvic acid and acetone. Moreover, lactic and formic acids are formed from pyruvic acid when the pH is maintained slightly above 7.0 by addition of sodium bicarbonate.

Conclusions. Pyruvic acid is converted by cell-suspensions of *Cl. butylicum* into acetic and butyric acids, carbon dioxide and hydrogen. Hydrogen donated by pyruvic acid is available for the reduction of butyric acid to butyl alcohol at a suitable pH. Formic acid it not decarboxylated and lactic acid is not dehydrogenated.

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Effects of Androsterone and Testosterone on Oestrous Cycle of Rats.

LUDVIG G. BROWMAN. (Introduced by Carl R. Moore.)

From Hull Zoological Laboratory, The University of Chicago.

Ihrke and D'Amour¹ reported that injections of bull testis-tissue male hormone concentrates prepared by Gallagher and Koch resulted in the suppression of the oestrous cycle of female rats during

¹ Ihrke, I. A., and D'Amour, F. E., *Am. J. Physiol.*, 1931, **96**, 289.

the period of injections. The cycles, as expressed by the vaginal smear, reappeared when injections were no longer given. These observations and others of a similar nature were interpreted in terms of the hypothesis suggested by Moore and Price.² This hypothesis advances possible explanations for the effects produced on the gonads of either sex and their accessories by castration and by the injection of sex hormones and gonadotropic agents.

The hypothesis suggests first, that pituitary hormones stimulate the gonads to produce germ cells and to secrete hormones, and second, that the gonad hormones of either sex have a suppressing action upon the pituitary which results in a diminution of pituitary secretions. One effect of such diminution of pituitary secretions is relative inactivity of the gonads. Moore and Price² had shown that whereas bull testis extracts prevented or repaired castration damage in accessory reproductive organs of the male rat it had injurious effects upon normal young growing testes, presumably by virtue of its suppressing effect upon the pituitary. Ihrke and D'Amour similarly suggested that bull testis-tissue extracts affected the female pituitary so as to make unavailable to the organism sufficient concentration of pituitary secretions to permit the ovaries to continue their normal gametogenic and hormonal activities.

Because the bull testis-tissue male hormone concentrates employed by Ihrke and D'Amour were impure it was deemed advisable to repeat a portion of their work using pure chemical androgenic agents. At the suggestion of Professor Carl R. Moore a brief study was made of the effects of pure crystalline androsterone and testosterone on the oestrous cycle of female rats. These substances, prepared by synthetic processes, were provided through the courtesy of Dr. C. C. Haskell and the CIBA company, whose generosity is herewith gratefully acknowledged.

Experimental. Sixteen normal female rats, about 4½ months old, showing regular oestrous cycles during a preliminary observation period of 16 days as indicated by twice daily vaginal smears, were selected for the experiment. Fourteen of these females received daily subcutaneous injections of sesame oil solutions, some of which contained the pure androgenic substances. The other 2 females were kept in the same cages with the injected animals as untreated controls. Four concentrations of testosterone in sesame oil were injected into paired females as follows: 2 females each received 0.5 mg. daily for 15 days, two 1 mg., two 2 mg., and two 3 mg. Androsterone in sesame oil was injected into 3 animals in different con-

² Moore, C. R., and Price, D., *Am. J. Anat.*, 1932, 50, 13.

centrations: 3, 4, and 5 mg. Three females received injections of sesame oil only in amounts of 0.3, 0.5 and 0.7 cc. These quantities of oil included the range of oil used in carrying the testosterone and androsterone. At the writer's request Professor C. R. Moore presented the oil samples under code number only and their contents were unknown until the termination of the experiment.

The selection of these particular doses was based on the following work. Moore (unpublished) had earlier administered androsterone to normal adult female rats and found that 1 to 2 mg. daily failed to disturb the oestrous cycles, 3 mg. did disturb the cycles, and 4 mg. suppressed them. The present dosage range of androsterone in sesame oil of 3.0, 4.0, and 5.0 mg. was based upon these results in part and also upon the fact that Moore and Price³ determined that 3 mg. androsterone daily were required to replace the effects of the testis on the growth rate maintenance of the seminal vesicle of young mature castrated male rats. It had further been determined for testosterone that 0.3 mg. daily was insufficient to maintain seminal vesicle growth comparable to that found in normal males. Therefore higher dosages were adopted with a range of 0.5 to 3.0 mg. of testosterone daily. It is assumed that the injection doses should be equal to or greater in androgenic activity than the amount of sex hormone normally produced by the testes if the pituitary gland is to be markedly suppressed.

Results. All of the females receiving injections of testosterone or androsterone were in diestrus by the third day following the first injection and remained so throughout the 16-day period of injections. The 3 females receiving injections of sesame oil alone, and the 2 untreated females, continued their regular cycles undisturbed throughout the experiment. One animal of each pair receiving the 4 different levels of testosterone, one animal receiving androsterone, and 3 controls were killed and their tissues weighed and preserved the day following the last injection. The other 2 controls were killed upon reaching mid-diestrus a day or 2 later. Vaginal smears of the remaining animals showed a return to vaginal cornification within 4 to 8 days following the last injection of androsterone or testosterone.

No significant conclusions were reached from a study of the data obtained on recovery of the internal organs, largely because of their variability and because of the small number of animals involved. Females injected with testosterone had somewhat lighter ovaries and heavier uteri than those of females not injected or injected with

³ Moore, C. R., and Price, D., *Endocrinology*, 1937, in press.

sesame oil alone. Animals injected with sesame oil with or without male hormone had lighter pituitaries than normal non-injected controls. Animals injected with sesame oil solutions had an accelerated weight increase during the period of injections over that of their previous weight increase for a similar period just preceding injection, and over that of the normal non-injected controls kept in the same cages.

Conclusions. The observed results demonstrate that daily injections of 0.5 to 3 mg. of testosterone in sesame oil and of 3.0 to 5.0 mg. of androsterone in sesame oil into normal mature female rats leads to a suppression of the oestrous cycles, as expressed by the vaginal smear, during the 16-day period of injection.

These observations agree with the findings of Robson⁴ that testosterone causes total suppression of the oestrous cycle in mice. It seems quite adequate to the writer to assume that the pure crystalline androgenic substances, testosterone and androsterone, have an action upon the pituitary gland of the female rat in a manner consistent with the interpretation offered by Moore and Price. Nothing presented by these experiments, however, would rule out the possibility of a direct effect upon the ovary by crystalline androsterone or a possible neutralization of the action of estrogenic substances upon the female tract by androsterone.

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Effect of Various Degrees of Anoxemia on Secretion of Acid and Chlorides by the Stomach.

CLARK K. SLEETH AND EDWARD J. VAN LIERE.

From the Department of Physiology, West Virginia University, Morgantown, West Virginia.

Alvarez¹ found that when the hemoglobin fell below 12 gm. per 100 cc. of blood the mean gastric acidity fell sharply and the incidence of achlorhydria rose rapidly. Apperly and Cary² showed that when the red cell count of the blood fell below a certain critical level, free acid disappeared from the stomach. The acidity was also found to decrease with an increase in the erythrocyte count above

⁴ Robson, J. M., *Proc. Soc. Exp. Biol. and Med.*, 1936, **35**, 49.

¹ Alvarez, W. C., and Vanzant, F. R., *Proc. of the Staff Meetings of the Mayo Clinic*, 1936, **11**, 385.

² Apperly, F. L., and Cary, M. K., *Am. J. Dig. Dis. and Nutr.*, 1936, **3**, 466.