normal female. It is suggested that the retardation in the involution process is due to the great subcutaneous hyperemia produced by the turpentine applications.

12020

Qualitative Progesterone Assay of Pregnant Cattle AP and Extracts Having Mammary Growth Activity.*†

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It was shown in this laboratory[1, 2] that the duct system of the mammary gland could be experimentally stimulated in castrate male or female animals by the injection of estrogen while a simultaneous injection of estrogen and progestin was necessary to secure complete mammary gland growth. Since this growth could not be secured in hypophysectomized animals, it was suggested that the ovarian hormones might produce their action by stimulating the secretion of mammogenic hormones in the anterior pituitary.[3] It has since been shown that lipid extracts[4] of the AP will stimulate the growth of the mammary duct system and further, that fresh pregnant cattle pituitaries will stimulate the growth of the lobule-alveolar system.[5]

Since Gardner and Hill[6] have shown that progesterone alone will stimulate the growth of the duct system, it seemed desirable to determine whether progesterone was present in the lipid AP extracts of the pituitary in which the mammogen duct factor is present as well as in the fresh pregnant cattle AP which stimulates the growth of the lobule-alveolar system.

In previous studies of this question Corner[7] and Callow and

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1 Turner, C. W., and Frank, A. H., *Mo. Agr. Exp. Sta. Res. Bul.* 145, 1930.

2 Turner, C. W., and Frank, A. H., *Science*, 1931, **73**, 295.

3 Gomez, E. T., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.* 259, 1937.

4 Lewis, A. A., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.* 310, 1939.

5 Mixner, J. P., Lewis, A. A., and Turner, C. W., *Endocrinology*, 1940, **27**, 888.

6 Gardner, W. U., and Hill, R. T., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 718.

7 Corner, G. W., *Am. J. Physiol.*, 1930, **95**, 43.

Parkes[8] reported either negative or questionable responses with pituitary extracts. The development of the more sensitive progesterone assay method of McGinty, Anderson and McCullough[9] is a further reason for a reëxamination of the possible presence of progesterone-like activity of the pituitary.

Procedure. Four immature female rabbits were sensitized over a period of 6 days with 150 i.u. of estrone. On the 7th day their abdominal cavities were opened under ether anesthesia, and the materials to be tested were injected into 3 cm isolated segments of a uterine horn. After 72 hours the isolated test segment as well as a control segment from the opposite uterine horn was removed. These were prepared for histological examination and the degree of progestational proliferation was rated according to the McPhail scale.[10]

Rabbit A received in one uterine segment 400 mg of fresh pregnant cattle AP in a water suspension. Ten out of 10 ovariectomized virgin female mice which were injected with this same material over a period of 10 days at the rate of 368 mg per mouse, responded with lobule-alveolar growth. Similarly 4 of 6 mice injected at the rate of 200 mg per mouse over a 6-day period gave positive lobule-alveolar growth.

Rabbit B received in one segment of the uterus .02 mg crystalline progesterone in .1 cc raisin seed oil. The check segment in the other horn received .1 cc oil only.

Rabbit C received in one segment .0002 mg crystalline progesterone in .1 cc oil. The control segment in the other horn received .1 cc oil only.

Rabbit D received in one uterine segment 10 mg of lipid extract No. 60 which was dissolved in .1 cc raisin seed oil. One-tenth cc of oil was placed in the control segment in the other uterine horn. This extract contained approximately 13 mouse units of the mammogenic duct growth hormone per mg.

Two virgin sexually mature rabbits (E and F) were treated as in the Clauberg test.[11] Eight preliminary daily injections, 100 i.u. each, of estrone were made subcutaneously. In the same manner lipid extracts of AP were administered daily for 5 days. Portions of uterine horns were removed and prepared for histological examination.

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8 Callow, R. K., and Parkes, A. S., *J. Physiol.*, 1936, **87**, 28p.

9 McGinty, D. A., Anderson, L. P., and McCullough, N. B., *Endocrinology*, 1939, **24**, 829.

10 McPhail, M. K., *J. Physiol.*, 1934, **83**, 145.

11 Clauberg, C., *Zentralb. f. Gynäk.*, 1930, **54**, 2757.

TABLE I.
Results.

Rabbit No.	Assay method	Treatment	McPhail rating	
			Test segment	Control segment
A	McGinty	400 mg AP	0	0 to +
B	"	.02 " Progesterone	++	0
C	"	.0002 " "	+	0
D	"	10 " Extract 60 AP	0	0
E	Clauberg	5 " " 46 "	0	
F	"	5 " " 61 "	0	

Rabbit E received 5 mg total of extract No. 46 of cattle AP prepared by vacuum distillation of 86% alcohol used in the Bergman-Turner[12] method of preparing initial extract. The residue was then fractionated in ether. Extract No. 46 constituted the ether-soluble fraction. One milligram of this extract contained approximately 10 mammogenic duct growth mouse units.

Rabbit F received a total of 5 mg of extract No. 61 containing 2 mammogenic duct growth mouse units per mg.

Extract No. 60 (pregnant cattle) and extract No. 61 (cattle pituitary) were prepared by A. J. Bergman by vacuum distillation at a low temperature (30°-35°C) of the acetone-ether used in drying the AP. Further purification was obtained by fractionation with ether. These extracts constituted the ether-soluble fractions.

Discussion. These results are believed to indicate that the mammary gland growth secured either with the fresh material or with extracts of the AP could not be due to the presence of progesterone. The amounts of progesterone administered by Gardner and Hill[6] to obtain duct growth in the male mouse varied from .35 to 1.6 Corner-Allen units (1 C-A. unit = 1 mg) over a period of 14-16 days whereas 10 mg of our extract 60 failed to give a positive progesterone response (Rabbit D).

Further, Mixner (unpublished) observed that 500 gamma of progesterone injected simultaneously with 133 i.u. of estrone over a 10-day period into castrate virgin female mice stimulated lobule-alveolar development in 1 of 4 mice, whereas 1000 gamma with the same estrone dosage caused development in 3 of 4 mice. Two-tenths gamma of progesterone gave a positive uterine response in Rabbit C. Yet a negative progesterone response was secured with 400 mg of fresh cattle AP (Rabbit A).

Summary. Fresh pregnant cattle pituitary in amounts which will stimulate growth of the lobule-alveolar system in castrate female

[12] Bergman, A. J., and Turner, C. W., *J. Biol. Chem.*, 1938, **123**, 471.

mice was found to contain insufficient progesterone to give a positive response by the sensitive McGinty technic. Lipid extracts of the AP which stimulate duct growth in the male mouse were also found to be negative for progesterone. These observations are taken to indicate that neither the mammogenic duct nor lobule-alveolar effects of the AP are due to the presence of progesterone.

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Effect of Selective Poisons on Utilization of Glucose by Yeast.

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Well washed suspensions of *Saccharomyces cerivisiae* (American Type Culture Collection No. 575) may utilize glucose by oxidizing it to carbon dioxide and water, by fermenting it to carbon dioxide and alcohol, and by synthesizing it to cell material. With well aerated suspensions the oxygen consumption, as determined at 37°C by the Warburg technic, is approximately one-third of the theoretical amount required for complete combustion, less than 5% of the sugar is fermented and the remainder appears to be synthesized to cellular material.

The possibility of so altering the course of glucose metabolism by yeast that the substrate will be entirely oxidized has been suggested by studies with a number of microörganisms. Clifton and Logan,[1] using resting suspensions of *Escherichia coli*, showed that glucose is oxidized to completion in the presence of sodium azide or 2:4-dinitrophenol (DNP). Similarly, Winzler[2] showed that M/1250 azide or M/2000 DNP blocks synthesis from acetate by yeast suspensions, the oxidation going to completion in the presence of these poisons.

The following results indicate that sodium azide and DNP block synthesis from glucose by yeast. However, in the presence of these poisons the oxidation does not go to completion but instead fermentation accounts for the bulk of the substrate disappearing from the medium. Typical results are reported in Table I.

In the presence of M/1000 DNP the assimilatory process is in-

[1] Clifton, C. E., and Logan, W. A., *J. Bact.*, 1939, **37**, 523.

[2] Winzler, R. J., *J. Cell. and Comp. Physiol.*, 1940, **15**, 343.