has been reported by Hartman, Firor and Geiling[10] and by Smith, Tyndale, Dotti and Engle[11] who also found that partial hypophysectomy did not produce the marked increase in sensitivity which followed complete removal of the gland. The present experiments indicate that insulin responses may be normal when only half of the anterior lobe is left intact.

Infundibular connections between the hypothalamus and pars distalis, however, are not essential for normal insulin sensitivity in the monkey, and are therefore probably not essential for normal carbohydrate metabolism.

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Assay of Progesterone by Intrauterine Application in the Rabbit.

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The crop gland test for the lactogenic hormone as performed by Lyons and Page,[1] the local feather test for estrone by Greenwood and Blyth,[2] and the intravaginal test for estrogenic substance by Yerby,[3] serve as examples of the efficacy of the local application of a hormone for analytical purposes. The application of progesterone to the uterus has been shown by McGinty, Anderson and McCullough[4] to be a more sensitive test for its presence than introducing the hormone into the blood stream.

Previous biological assay or test procedures for the presence of progesterone have involved the subcutaneous or intramuscular injection of the hormone over a period of 5 days, removal of the uterus on the sixth day and subsequent histological examination of the uterus. The criterion of the activity of the hormone of the corpus luteum was the degree of progestational proliferation in evidence in

10 Hartman, C. G., Firor, W. M., and Geiling, E. M. K., *Am. J. Physiol.*, 1930, **95**, 662.

11 Smith, P. E., Dotti, L. B., Tyndale, H. H., and Engle, E. T., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 247.

1 Lyons, W. R., and Page, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1049.

2 Greenwood, A. W., and Blyth, J. S., *Quart. J. Exp. Physiol.*, 1935, **25**, 267.

3 Yerby, D., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 496.

4 McGinty, D. A., Anderson, L. P., and McCullough, H. B., *Endocrinol.*, 1939, **24**, 829.

the uterus. The test for progesterone as described by Corner and Allen[5] utilized as test animals bilaterally oöphorectomized mature rabbits. The degree of proliferation in the uterus was expressed by them in terms of + to ++++ reactions, + reactions being those of least progestational proliferation.

Clauberg[6] and later McPhail[7] modified this procedure to the extent that immature rabbits were used as test animals. These animals were primed with subcutaneous injections of estrone for a period of days preceding the intramuscular injection of progesterone. Immature rabbits as treated by Clauberg and McPhail were found to be more sensitive to the presence of progesterone, since divided daily injections totalling 0.5 mg brought about a uterine proliferation equal to injections totalling approximately 1 mg in the mature rabbit.

McGinty and his collaborators found that single intrauterine doses of progesterone in amounts of 5 to 0.5 gamma caused a local proliferation of ++ to +++ which is equal to that proliferation caused by the injection of 0.5 mg of progesterone in the McPhail test. Applications of amounts of progesterone from 0.5 to 0.125 gamma in all cases produced a definite proliferation. However the decreasing intensity of the reaction indicates that these small doses are approaching the minimum required for a recognizable progestational action to occur within the uterus.

The following experiments were carried out to test the results as reported by McGinty, Anderson and McCullough.

Immature female rabbits, average weight 1239 g, were treated with 150 international units of amniotin over a period of 6 days. On the seventh day they were anesthetized with ether, the abdomen was opened and the uterus exposed. A segment of each horn of the uterus was isolated by two ligatures approximately 3 cm apart; care was taken to leave an adequate blood supply to the segment. A third ligature was applied loosely between the others and close to the lower ligature. The progesterone-containing solution to be applied was forced into the lumen of the isolated segment by means of a syringe inserted beneath the loose ligature, which was gradually tightened as the solution was injected and the needle withdrawn.

Crystalline progesterone* was applied in a 0.1 cc solution of peanut oil or lanolin in dilutions as indicated in Tables I and II. The

5 Corner, G. W., and Allen, W. M., *Am. J. Physiol.*, 1929, **88**, 326.

6 Clauberg, C., *Zbl. f. Gynak.*, 1930, **54**, 2757.

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

* The crystalline progesterone used in this study was generously supplied by the Schering Corporation through Dr. Erwin Schwenk.

isolated segment in the opposite horn was injected with peanut oil, lanolin or blood serum from the non-pregnant guinea pig as required for a control.

Mature rabbits when used were bilaterally oöphorectomized at the time of the application of progesterone in addition to the above procedure. The mature rabbits were not primed with estrone. All animals were killed 72 hours following the application of progesterone, the injected segments were removed and examined histologically. The degree of reaction is expressed according to the McPhail scale.

Results. The control segments in all but 2 cases exhibited no trace of progestational proliferation. These 2 cases were found in the control horn after injection of 1 mg and 0.3 mg of progesterone into the opposite horn. This fact would seem to indicate that the action of locally applied progesterone remained local up to a maximum dose, at which point there was seepage into the blood stream.

The progesterone-injected segments of the immature rabbit uteri showed a positive reaction in all but one animal, this being one of those injected with 0.25 gamma, as is shown in Table I. Lanolin when used as an alternative solvent for the progesterone, produced results similar to that of the peanut oil solution as indicated in Table II. However in all cases the variability of the reaction as compared

TABLE I.

Rabbit wt, g	Progesterone in 0.1 cc peanut oil, gamma	Reaction	Control peanut oil, cc	Reaction
1245	1000	++++	.1	+ to ++
1245	300	++++	.1	+
1232	100	0 to +	.1	0
1264	25	0 to +	.1	0
1069	5	++	.1	0
1416	1	+	.1	0
1470	1	+	.1	0
1700	1	+	.1	0
1530	0.5	++	.1	0
1480	0.25	+	.1	0
1818	0.25	0	.1	0
736	0.25	+ to ++	.1	0
854	0.25	+ to ++	.1	0
1013	0.25	++	.1	0
3200*	100	0	.1	0
2900*	100	0	.1	0
2841*	1	0	.1	0
2554*	0.5	0	.1	0
2841*	0.5	0	.1	0
2727*	0.25	0	.1	0
3009*	0.25	0	.1	0

* Bilateral oöphorectomy at time of application of progesterone, mature rabbits.

TABLE II.

Rabbit wt, g	Progesterone in 0.1 cc Lanolin, gamma	Reaction	Control Lanolin, cc	Reaction
963	10	+ to ++	.1	0
1135	1	+	.1	0
1226	1	+ to ++	.1	0

TABLE III.

Rabbit wt, g	Pregnancy serum, cc	Reaction	Control non-pregnancy serum, cc	Reaction
1192	.4*	++ to +++	—	—
1084	.2	+ to ++	.2	0
1205	.2	+ to ++	.2	0
1432	.1	0	.1	0

* Ether-soluble contents of 0.4 cc of pregnancy serum, dissolved in 0.2 cc of peanut oil.

to the amount of progesterone administered indicates that this test as it stands would be impractical as a quantitative test.

Mature rabbits were used with the thought that they might exhibit a more predictable or constant reaction. However amounts of progesterone from .25 to 100 gamma failed to cause a recognizable proliferation of the mature uterine endometrium.

In addition to a corroboration of the results of McGinty, Anderson and McCullough, it was found that the immature rabbits to which blood serum from the pregnant guinea pig was applied by intrauterine injection (Table III) exhibited a + to ++ reaction with application of 0.2 cc of serum. A stronger reaction occurred in the uterus to which the ether-soluble portion of 0.4 cc of pregnancy serum, dissolved in 0.2 cc of peanut oil, was applied. The uterus of the rabbit to which 0.1 cc of pregnancy serum was applied showed no reaction. The pregnancy serum was obtained from 2 guinea pigs, 2 weeks after mating.

Blood serum from the non-pregnant guinea pig was injected for control purposes. The control segments in all cases exhibited no recognizable progestational activity.

A number of factors may be involved in the variability of reaction encountered with the intrauterine application of progesterone. If the total proliferation produced by a given amount of progesterone were always equal, a slight variability in the size of the segment isolated would alter the apparent progestational proliferation as encountered in a single histological section of the whole segment. The size or

volume of the isolated segment may vary because of differences in the calibre of the segment and the bore of its lumen. The length of the segment isolated is of course subject to experimental error. The reaction to progesterone may also vary according to differences in the blood supply of the isolated segment. Finally the reaction to progesterone in the individual may be subject to individual variation caused by the interaction of other endocrine secretions.

It is apparent, however, from McGinty's work that the local application of progesterone is superior as a qualitative test to the previous methods of intramuscular injection because of its relative sensitivity.

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Indoluria and Its Relation to Sulfur Deficiency.

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Indole is a product of the action of bacteria on protein. Whether this action takes place in the gastrointestinal tract or in the body proper the organism is faced with the problem of the disposal of the resulting indole. Normally, as far as can be determined by analysis of excretions, the indole is oxidized to indoxyl, this in turn conjugated with potassium hydrogen sulfate and the indican thus formed is excreted in the urine. It is conceivable that if oxidation is deficient, if the liver fails in its function of conjugation, or if the supply of sulfate is deficient, free indole may appear in the urine.

Indoluria has been reported by various investigators, Cantelli,[1] Schour and Rosengarten,[2] Forbes and Neale[3] but Vaughan[4] failed to observe it. It has also been shown by Vaughan[5] and Carnes and Lewis[6] that the urine contains some compound which is readily acted upon by *E. coli* to produce indole.

If the indoluria is due to a lack of sulfate to effect the conjugation, the sulfur deficiency should be manifested elsewhere in the body. It is known that practically all the urinary sulfate enters the organism

1 Cantelli, O., *Riforma Medica*, 1922, **38**, 481, abstract in *J. Am. Med. Assn.*, 1922, **79**, 508.

2 Schour, M., and Rosengarten, C., *Klin. Wochschr.*, 1930, **9**, 1751, 1968.

3 Forbes, J. C., and Neale, R. C., *J. Lab. Clin. Med.*, 1934-35, **20**, 1017.

4 Vaughan, S. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 623.

5 Vaughan, S. L., *J. Lab. Clin. Med.*, 1937, **22**, 399.

6 Carnes, H. E., and Lewis, G. T., *J. Lab. Clin. Med.*, 1938, **23**, 459.