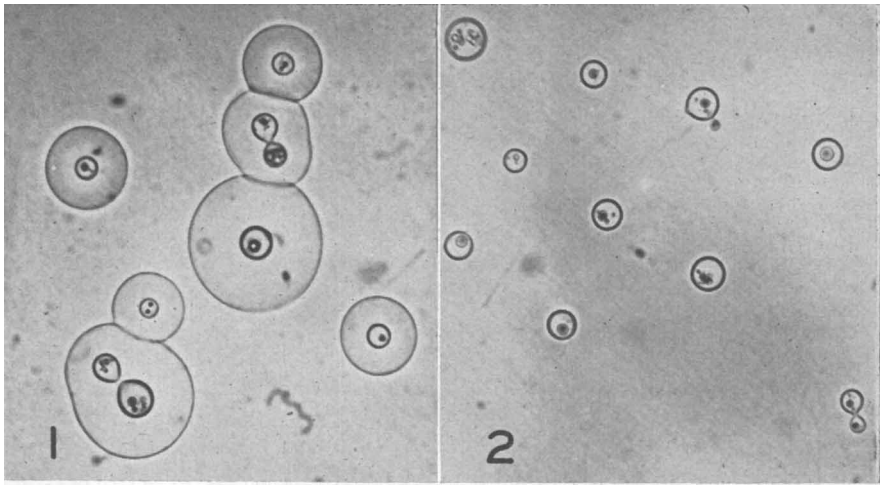

FIG. 1. *Cryptococcus* cells from the sediment of the patient's spinal fluid plus *Cryptococcus* rabbit antiserum. FIG. 2. *Cryptococcus* cells from the same suspension plus normal rabbit serum. Note the prominence and the sharply outlined borders of the capsules§ in Fig. 1 whereas only faint and equivocal halos are evident in the control mount illustrated in Fig. 2. The magnification of the illustrations is × 560. The cells in Fig. 1, including the capsules which comprised from ⅔ to ¾ of the total measurement, range from about 160 to 300 μ in diameter, but larger cells, some of which were as much as 475 μ in diameter were not uncommon in the sediment from the spinal fluid.

amounts of reactive material than would samples from earlier stages or milder forms of cryptococcal infection. However, the relatively large amounts of the reactive material that occurred in this case and the ease and speed of the serological detection suggest that tests upon body fluids of patients may have some practical importance in the study of human cryptococcosis. That question, however, can be determined only by trials with materials from a reasonably large number of patients. We are now attempting to collect material for that purpose, and would appreciate receiving samples from clinical workers who encounter cases of cryptococcal infection.

Summary. Serologically reactive substances ("soluble antigens") which were readily detectable by precipitation and by complement fixation with *Cryptococcus* antiserum were found in the spinal fluid, blood serum, and urine of a patient with cryptococcal meningitis. Absorption of the antiserum with solutions of purified *Cryptococcus* polysaccharide removed its capacity to react with the fluids of the patient. The reactive substances in the patient's fluids were relatively heat stable and it is probable that they represent capsular polysaccharides of the fungus with which the patient was infected.

Serological reactions of the Quellung type were obtained with the capsules of *Cryptococcus* cells from the centrifuged sediment of the spinal fluid.

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Influence of Female Hormones on Motility of Cat's Uterus and Its Responses to Oxytocics. (18925)

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The uteri of cats chosen at random are frequently quite insensitive to α-hypophamine (Pitocin) and synthetic oxytocics. To overcome this limitation an extended study was

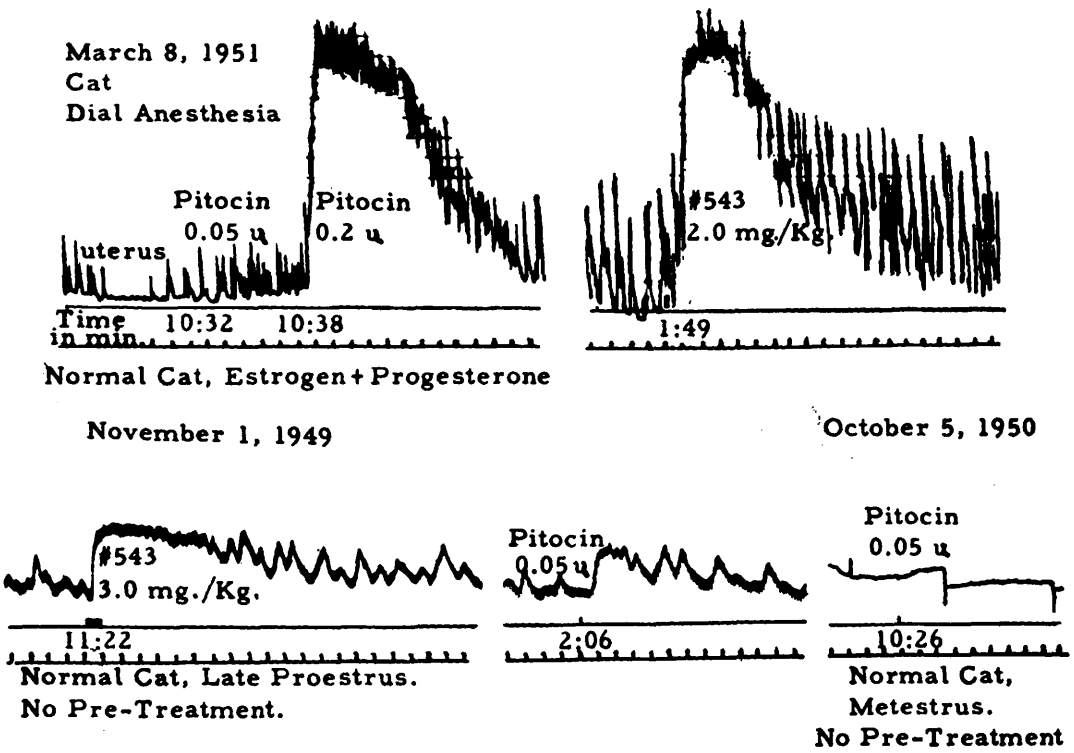

FIG. 1. *Mar. 8.* Response to pitocin and synthetic oxytocics in cat primed with 1.0 mg α-estradiol dipropionate (intramusc) on first and third days and 1.0 mg progesterone (intramusc) on sixth, seventh and eighth days. Experiment on ninth day. Units of pitocin in total dose. Preparation 543 : 2-methyl-6-N-methyl-N-(β-piperidino-ethyl) amino pyridine monohydrochloride. In this, and the subsequent figures, the recordings of blood pressure and respiration have been omitted to conserve space since the changes were not notable.

initiated to determine if suitable hormonal treatment of the cats prior to experimental use could assure uteri responsive to drugs. Wick and Powell(1) earlier reported that priming cats with estrogen increased the responsiveness of the uteri, but cats so treated did not always present active uteri. A method for rendering a uterus responsive to drugs and the experiments upon which it was based, totaling over 200, are described below.

Method. The method of recording the movements of the cat's uterus *in vivo* has been previously described(2). The Trendelenburg modification(3) of filling the belly with Tyrode's solution was employed and a glass tube was snugly fitted into the abdominal opening so the partially withdrawn loop was submerged in the fluid. This yielded longer-lasting preparations than those exposed to air. The following hormonal treatment of cats was found to provide uteri highly responsive to oxytocics over a period of many hours. For 8 consecutive days 0.3 mg of α-estradiol dipropionate was injected intramuscularly; on the last 3 days 1.0 mg of progesterone was also injected. This treatment yielded on the ninth day a uterus extremely reactive to drugs throughout the day. An alternative method that yielded almost as sensitive a uterus and eliminated the need for daily injections was the injection of 1.0 mg of α-estradiol dipropionate on the first and third days and the injection of 2.0 mg of progesterone on the sixth, seventh, and eighth days. The cat was then usable on the ninth day (Fig. 1).

Results of hormonal treatment of juvenile,

1. Wick, H. J., and Powell, C. E., *J. Am. Pharm. Assn.*, 1942, v31, 46.
2. Craver, B. N., Seip, P., and Cameron, A., *Fed. Proc.*, 1946, v5, 172.
3. Trendelenburg, P., *Z. f. Biol.*, 1913, v61, 67.

TABLE I. No. of Cats Used in Various Experiments.

	Normal	Progesterone	Estrogen	Estrogen + Progesterone
Normal	25	5	75	44
Pregnant	12	3	3	3
Juvenile	1	3	4	6
Castrate	1	8	5	14

castrated, and pregnant cats. Experiments were performed with juvenile, castrated, and pregnant cats to define more clearly if possible the influences of gestational and estrogenic hormones, given alone or together, on the motility of the uterus and its responsiveness to drugs. Table I summarizes the number of animals used in each group.

A. *Juvenile cats.** Estrogen alone in doses of 1.0 mg on the first and third days yielded a uterus on the ninth day slightly responsive to drugs for a short interval. The same was true for the uterus of cats used on the fifth day which had received four consecutive daily doses of 5.0 mg of progesterone. Juvenile cats given 1.0 mg of estrogen on the first and third days and 5.0 mg of progesterone on the sixth, seventh and eighth days, presented uteri quite sensitive to oxytocics on the ninth day. The uteri of the cats untreated with hormones were entirely unresponsive to all drugs (Fig. 2).

B. *Castrated cats.* In general castrated cats responded like juvenile cats except that such cats given α-estradiol dipropionate alone or in combination with progesterone offered uteri somewhat more responsive to uterotonics. Even this activity, however, did not match that exhibited by the uterus of a responsive normal cat after the injection of oxytocics (Fig. 3).

C. *Pregnant cats.* The uteri of untreated pregnant cats were unpredictable in their behavior. They varied from complete lack of responsiveness to drugs to extreme sensitivity toward them. With several cats in roughly the latter half of pregnancy, not injected with hormones, a single drug injection initiated a hypermotility that continued unabated throughout the day. A similar finding for the uteri of pregnant sheep, *in vitro,* was reported by Ambache and Hammond(4). Attempts to diminish the motility of these pregnant uteri with high doses of antispasmodics were of no avail, an observation in agreement with the work of Diehl and Hundley(5). These variations, as studied in over a dozen untreated pregnant cats, did not seem correlated with the duration of gestation. In pregnant cats estrogen alone, as well as estrogen plus progesterone, initiated an extraordinary motility and sensitivity to synthetic oxytocics, but the responses subsided in time. Cats primed with progesterone alone exhibited greatly prolonged responses of the uterus to synthetic uterotonics (Fig. 4).

Correlation of vaginal cytological studies with subsequent uterine responsiveness to oxytocics. Only the uteri of cats in estrus or proestrus exhibited a responsiveness to oxytocics. Not infrequently even the uteri of cats in these two stages of the estrus cycle proved unreactive. Sometimes cats treated with an estrogen alone exhibited the cytological changes of estrus, but despite that were unresponsive to drugs.

In a considerable series of experiments efforts were made to restore the responsiveness of a uterus that had lost its activity to oxytocics. The drugs so employed included aqueous estrone sulfate, α-estradiol dibenzoate in oil, progesterone in oil, testosterone in oil, glucose, desoxycorticosterone glucoside, choline and vitamin B complex.† The use of the latter two agents was suggested by the report of Peet and Sampson(6) that rats on a choline deficient diet yielded non-motile uteri. Kraatz and Gruber(7) were unable to confirm this observation although they did

* So adjudged if they did not possess permanent back molars. Histological sections of the uterus were also made.

4. Ambache, N., and Hammond, J., Jr., *J. Physiol.,* 1949, v108, 270.

5. Diehl, W. K., and Hundley, J. M., *Am. J. Obst. and Gynec.,* 1948, v56, 288.

† The authors thank Lederle Labs., Inc., for a generous supply of Folbesyn.

6. Peet, M., and Sampson, M. M., *Science,* 1948, v107, 548.

7. Kraatz, C. P., and Gruber, C. M., *Science,* 1949, v109, 310.

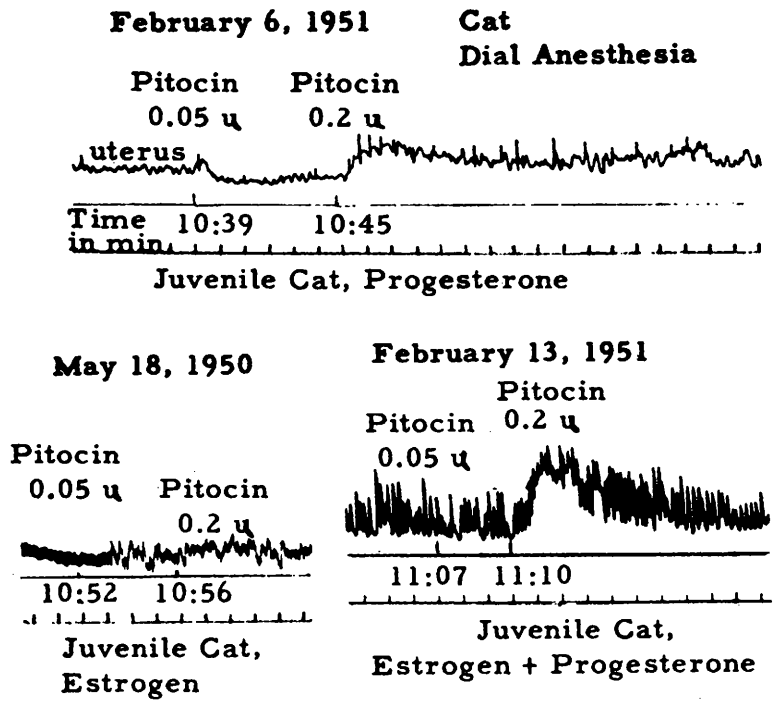

FIG. 2. *Feb. 6.* Units of pitocin in total dose. 5 mg doses of progesterone (intramusc) for 4 consecutive days. Experiment on fifth day. *May 18.* 1 mg α-estradiol dipropionate (intramusc) on first day and third day. Experiment on ninth day. *Feb. 13.* 0.3 mg α-estradiol dipropionate (intramusc) first through eighth day and 5 mg progesterone (intramusc) on days 6, 7 and 8. Experiment on ninth day. Compare with normal cat in late proestrus stage that received no pre-treatment (Fig. 1).

note that their diet contained small amounts of choline. This lack of agreement suggested that other members of the B-complex might have played a role, and for that reason the material was tried. All such experiments met with complete failure.

Discussion. Since the studies of Keye(8) and of Knaus(9) it has been rather generally accepted that an estrogen augments the motility of the uterus and progesterone opposes that action. Much of the work upon which this concept was based was done in rabbits, and it is entirely possible that the cat's uterus does not react in the same fashion. The studies of Knaus on the rabbit, confirmed by Reynolds and others, have made it clear that the uterus of this species responds differently from the cat to drugs and hormones. Bickers and Woods(10) among others, have emphasized the marked species differences in uterine physiology. Since progesterone enhances the responsiveness of the cat's uterus to drugs, this species would appear closer to the human in its physiologic reactions than the rabbit, since Kurzrok, Wiesbader, Mulinos, and Watson(11) reported that progesterone occasionally caused an increase in the tonus and amplitude of the contractions of the human uterus and could, if administered in the postmenstrual phase of the cycle, precipitate cramps. Wilson and Kurzrok(12) later reported that contractions were more marked, if less frequent, in the luteal phase of the cycle. They reported that posterior pituitary

8. Keye, J. D., *Bull Johns Hopkins Hosp.*, 1923, v34, 60.

9. Knaus, H. H., *Arch. Gynak.*, 1930, v141, 374.

10. Bickers, W., and Woods, M., *Am. J. Obst. and Gynec.*, 1949, v58, 1099.

11. Kurzrok, R., Wiesbader, H., Mulinos, M. G., and Watson, B. P., *Endocrinology*, 1937, v21, 335.

12. Wilson, L., and Kurzrok, R., *Endocrinology*, 1938, v23, 79.

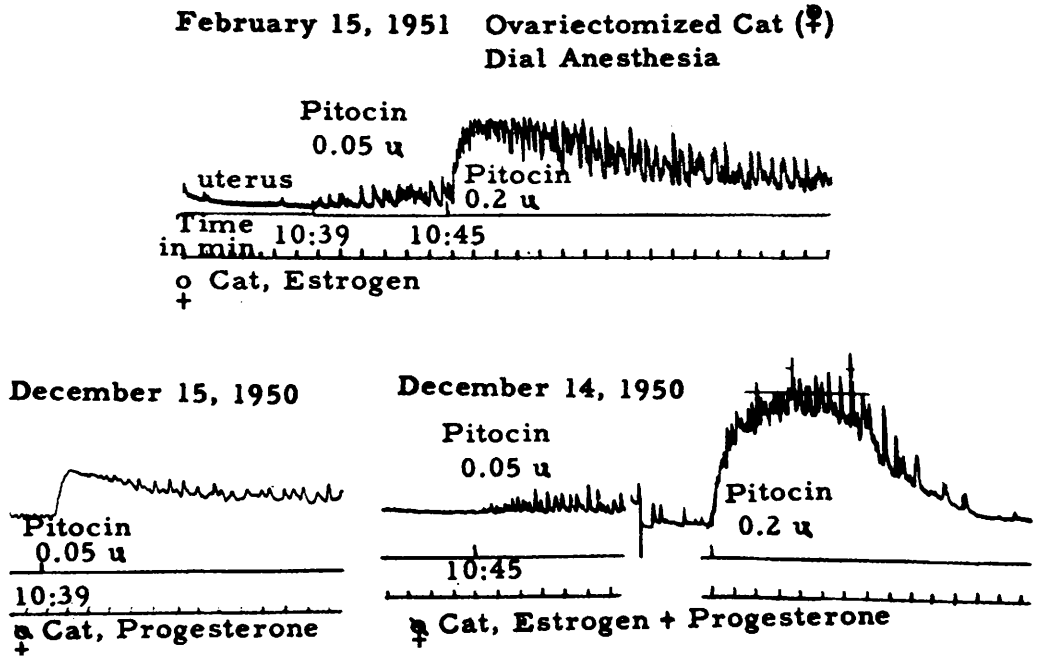

FIG. 3. *Feb. 15.* 1 mg α-estradiol dipropionate (intramusc) on first and third days. Experiment on ninth day. Pitocin units in total dose. *Dec. 15.* 5 mg progesterone (intramusc) on 4 consecutive days. Experiment on fifth day. *Dec. 14.* 0.3 mg α-estradiol dipropionate (intramusc) first through ninth day and 5 mg progesterone (intramusc) on days 8 and 9. Experiment on tenth day.

preparations gave reactions in all phases of the cycle and that the contractions of greatest amplitude were in the luteal phase. Others confirmed and extended this work(13,14) indicating that the effects of estrogens and progesterone on the human uterus must be viewed more as complementary than antagonistic. Recently Henry, Browne, and Venning(15) have entirely discarded the idea of an antagonistic action between estrogens and progesterone on the human uterus and presented evidence that the action must rather be synergistic. The present work would definitely support this concept in respect to the cat. Henry, Browne, and Venning(15) have stated that the maximal muscular efficiency of the uterus "requires adequate amounts of the two hormones in suitable proportions, and acting for a sufficient length of time." The present experiments are in accord with their views. For satisfactory results the hormonal preparation of the animals had to be initiated at least a week before the proposed experiment. The need for adequate proportions is emphasized by two facts, that pregnant cats, demonstrably with a good supply of gestational hormone, sometimes required supplementary estrogen to be responsive, and secondly that some normal, mature animals given only estrogen were not responsive, presumably due to a lack of endogenous progesterone. This suggests that above certain minimal amounts it is perhaps the ratio of the quantities of the two hormones present that determines the reactivity of the uterus to drugs.

Summary. A method has been described of treating cats with an estrogen plus progesterone to assure uteri *in vivo* highly responsive for hours to oxytocics. Modifications of this method applied to juvenile and castrated cats indicated that either type of

13. Szarka, A., *Gynaecologia,* 1946, v122, 338 (Abstracted in *J. Obst. and Gynec., of British Empire,* 1947, v54, 702).

14. Moir, C., *Tr. Edinburgh Obst. Soc.,* 1934, v54, 93.

15. Henry, J. S., Browne, J. S. L., and Venning, E. H., *Am. J. Obst. and Gynec.,* 1950, v60, 471.

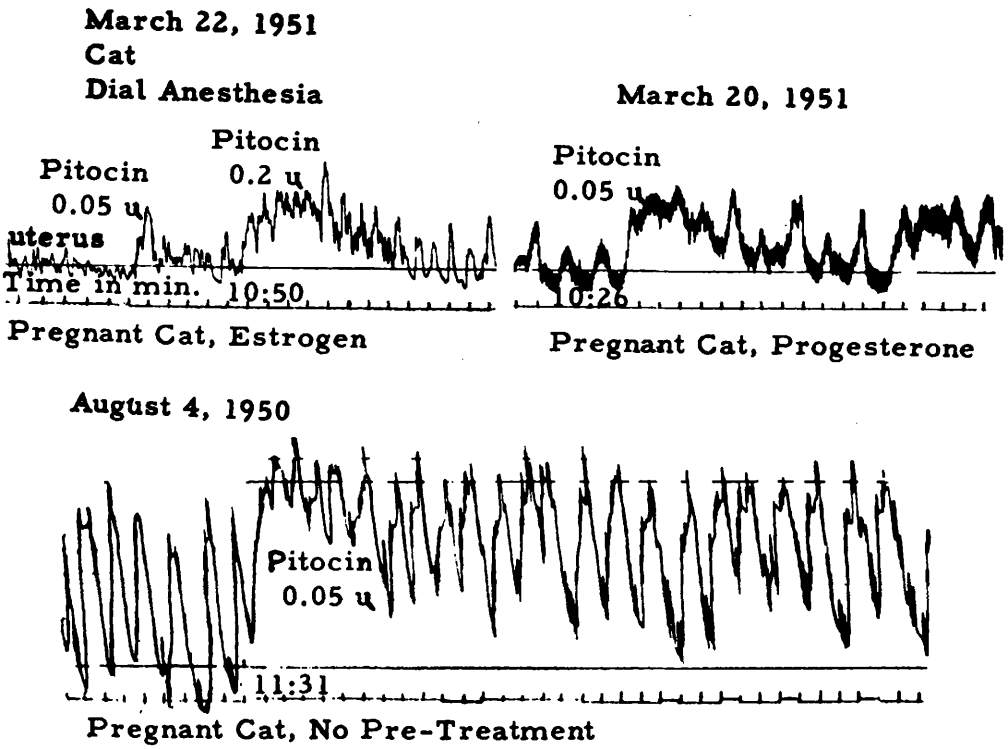

FIG. 4. *Mar. 22.* 1 mg α-estradiol dipropionate (intramusc) on first and third days. Experiment on sixth day. Units of pitocin in total dose. *Mar. 20.* 5 mg of progesterone (intramusc) on 4 consecutive days. Experiment on fifth day. *Aug. 4.* Cat in late stage of pregnancy. Responses of this nature were about as common as those demonstrating a complete lack of reactivity.

hormone would establish some brief uterine responsiveness but that both together were required to give a maximal and prolonged sensitivity to drugs. The two hormones in the cat are synergistic, or at least mutually complementary, and not antagonistic in respect to their effects upon the sensitivity of the cat's uterus *in vivo* to drugs.

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Paper Chromatography of Protein Mixtures and Blood Plasmas. (18926)

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A technic for the 2-dimensional chromatography of blood plasmas of protein mixtures on filter paper has been described by Franklin and Quastel(1). It was found that the addition of surface active agents such as the "Tweens" or "Spans" appeared to facilitate separations of the protein constituents and extend the protein "pattern." The technic adopted at present is to add "Tween 85" or "Tween 81" to the plasma and use hemin as the protein marker. A mixture of the benzidine reagent and hydrogen peroxide is used to detect the hemin after employing *M*/10 sucrose solution as developing agent in the

1. Franklin, A. E., and Quastel, J. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 803.