and the cornification test in rats required slightly more than 0.0005 μg of estradiol benzoate, in both cases dissolved in oil. It is possible that the sensitivity of these tests would approach that of intrauterine injection more closely if smaller areas of target tissue could be used or more delicate cytological criteria employed.

The effectiveness of small amounts of hormone when applied directly to the target organ emphasizes their physiological potential. The effect of estrogen is apparent not only in the epithelium but as far peripherally as the longitudinal muscle. Its concentration there must be exceedingly small since our minimum effective dose, if distributed evenly throughout the tissue of the segment into which it was injected, would provide only 4 molecules of steroid for each cubic micron of tissue.

The small amount of test material required has enabled us to carry out successful preliminary experiments with small pieces of tissue excised from organs of estrogenic interest. Although the rat is the more convenient animal for this purpose, it is possible to make simultaneous observations on progestational activity with the criterion of Hooker and Forbes(4) by using the mouse.

Summary. Very small amounts of test substances can be assayed for estrogenic activity by injecting them into the lumen of the castrate uterus of rat or mouse and observing hypertrophy of the Golgi apparatus and depletion of basal phospholipid of the epithelium. Additional criteria are appearance of glycogen in the uterine muscle of the rat and mobilization of alkaline phosphatase in the mouse.

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Uptake of Free Fatty Acids by Skeletal Muscle During Stimulation.* (25995)

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Recent work from several laboratories has suggested that free fatty acids (FFA) of the plasma might be utilized by skeletal muscles (1,2,3,4). However, direct proof of uptake has been hard to obtain, chiefly because of technical difficulties in obtaining blood from the muscles and in measuring blood flow through the muscle. In the present experiments uptake of FFA by the stimulated skeletal muscle was demonstrated in the dog by simultaneous determinations of blood flow and of arterio-venous FFA differences.

Methods. Adult mongrel dogs of both sexes were studied in postabsorptive state under sodium pentobarbital anesthesia (30 mg/kg i.v.). Using the method of Issekutz *et al.* (5), blood flow was determined in the profunda femoris veins which drain predominantly muscular areas. The femoral vein was prepared on the right side and tied 2-3 cm below the junction of profunda femoris veins. A "T" cannula was inserted into the femoral

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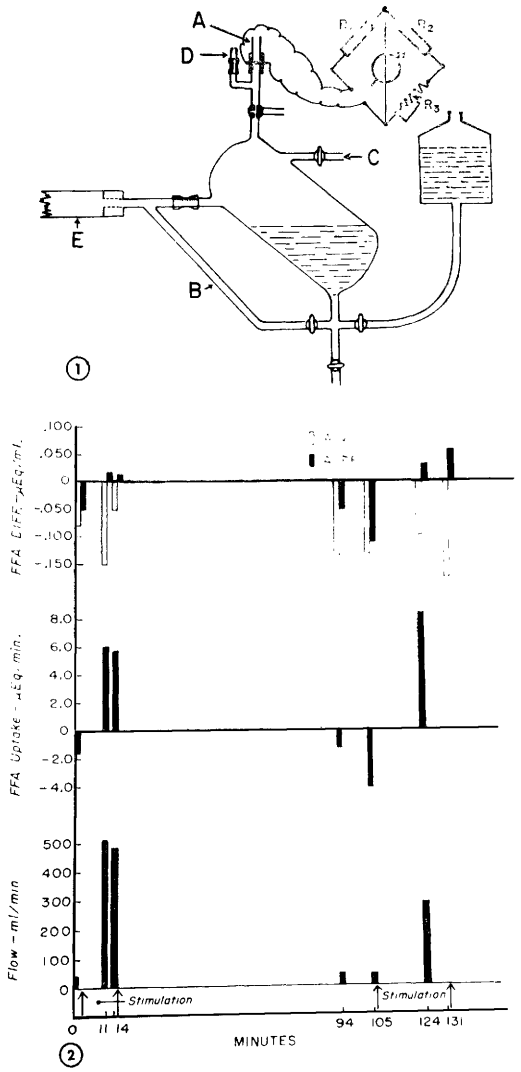

FIG. 1. Schematic representation of apparatus used for blood flow measurements.

FIG. 2. Changes of flow, uptake and A-V difference during repeated stimulation.

vein between the ligature and the deep femoral veins. The long stem of the cannula was connected with a glass tube, 50 cm long and 1 cm wide (Fig. 1, "E"). Cannula and glass tube were filled with physiological saline. The system was kept closed between measurements so that no blood could enter the cannula. Proximally from the profunda femoris veins at about the level of the inguinal ligament, a spring-clamp was applied to the femoral vein, which allowed quick closing or opening of the vessel. All branches of the femoral vein that came from genitalia or abdominal muscles were tied. Closing the clamp forced the blood of profunda femoris veins through cannula into glass tube where it displaced the physiological saline, the level of which was adjusted to provide the necessary pressure gradient. The saline followed by blood entered a glass cylinder at a rate equal to that of the blood flow. The air left the glass cylinder through a hot wire anemometer (Fig. 1, "A") as described by Anrep and Downing(6). A bypass of the air ("D") with empirically selected glass capillaries of various diameters provided a wide range for flow measurements. After reading the deflection of the galvanometer of the Wheatstone-bridge, blood samples were taken from the rubber tubing which connected the cannula with the glass tube. The connection between glass tube and cylinder was then closed and the blood was forced back (*via* "B") by means of compressed air ("C") into the reopened femoral vein, whereby the system was filled again with physiological saline and kept closed until the next measurement. No anticoagulants were used in these studies.

All blood samples were drawn into chilled oxalated tubes, centrifuged, separated and precipitated in the cold. FFA was determined in duplicate analyses using the method of Dole(7).

For direct stimulation of thigh muscles a voltage of 1.3, duration 0.5 millisec. and frequency 10/sec. was used.

Results. Concentration of FFA in the femoral vein was higher than in the femoral artery in agreement with previous findings(8). In 9 out of 11 dogs studied blood from the profunda femoris vein also showed higher FFA concentration than the femoral artery.

Direct electrical stimulation of thigh muscles produced an up to 16-fold increase in blood flow through profunda femoris veins. At the same time, in all but one of the 15 experiments performed on 11 dogs, FFA concentration in profunda femoris veins became lower than in the femoral artery. The veno-arterial FFA difference in the intact, non-stimulated side showed no consistent change during stimulation. No systemic hemody-

namic changes could be detected during stimulation, as judged by blood pressure and heart rate. In the typical experiment shown in Fig. 2, it can be observed that control sample before stimulation shows a negative A-V difference both in intact and cannulated side. During the first stimulation a 16-fold increase in blood flow and a small positive A-V difference developed showing a marked uptake of FFA. Control samples before the second stimulation again showed negative A-V differences on both sides. During the second stimulation blood flow increased about 7-fold with a more marked positive A-V difference on the stimulated side than during first period of stimulation. During both phases of stimulation the negative A-V difference was maintained on the non-stimulated side.

Discussion. In the present studies only an occasional dog showed arterio-venous FFA difference across the skeletal muscle during rest. The lack of more consistency in this respect could be due to the possibility that canine skeletal muscle, unlike human(3), does not remove FFA during rest, or it could be ascribed to the effect of anesthesia. Sodium pentobarbital depresses FFA levels(9) and it also may have a direct effect on metabolism of fatty acids. During direct stimulation of the muscles an uptake of FFA occurred without consistent changes in arterial levels, presumably because the exercise was so well localized, that the other, resting regions of the body compensated for the decrease of FFA in the exercised region.

† Finkelstein, L. J., Spitzer, J. J., Scott, J. C. Unpublished observation.

It is of interest to compare average uptake of 0.1 meq/hr/100 g of skeletal muscle during stimulation with uptake of 0.3 meq/hr/100 g of liver (average of 45 dogs)(10), and that of 0.8 meq/hr/100 g of myocardium (average of 54 dogs).† It should be noted, however, that these values were obtained in different dogs, under slightly different conditions.

Summary. Uptake of free fatty acids by skeletal muscle was determined by simultaneously measuring blood flow through profunda femoris veins, and arteriovenous fatty acid difference. Thigh muscles of the anesthetized dog showed only an occasional uptake of free fatty acids during rest. During direct electrical stimulation of muscles a consistent uptake of fatty acids was found on the stimulated side.

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Utility of 17α-Acetoxyprogesterone in Delaying Estrus in the Bitch. (25996)

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Progesterone has been found capable of inhibiting ovulation in rabbits(1,2), rats(3), guinea pigs(4), mice(5), sheep(6), and cows (7). In the bitch(8), progesterone given by injection will delay estrus for as long as treatment is continued. Progesterone is employed by some veterinary practitioners to delay estrus in dogs but its use is limited as frequent