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† Finkelstein, L. J., Spitzer, J. J., Scott, J. C. Unpublished observation.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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Received June 16, 1960. P.S.E.B.M., 1960, v105.

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

parenteral treatments are required. In recent years, many synthetic compounds with progestational activity have been prepared and some were found to be active by mouth. In hopes that an orally active synthetic progestin would delay estrus in dogs, 17α-acetoxyprogesterone (Prodox*) was evaluated. 17α-acetoxyprogesterone has been shown to be an orally active progestin and to inhibit ovulation in the rabbit(9). The present work was initiated to determine: (1) if 17α-acetoxyprogesterone would delay estrus in the bitch when administered orally, (2) what dose would be required, and (3) possible deleterious effects of drug administration on the bitch and her subsequent fertility.

Methods. Open Beagle and mongrel bitches weighing approximately 10 kg were employed. For some dogs, 17α-acetoxyprogesterone was incorporated in commercial dog feed checkers (200, 50 and 10 mg lb feed) and fed *ad lib.* Since each dog consumed about ½ lb of medicated checkers daily, dose of 17α-acetoxyprogesterone was approximately 10, 2.5 and 0.5 mg kg respectively. For other dogs, 17α-acetoxyprogesterone as a glycerine-sorbitol suspension (25 mg/cc) was administered once daily on the tongue at 4 and 2.5 mg kg levels. These dogs were fed nonmedicated checkers *ad lib.* Duration of treatments varied from 23 to 70 wks. Estrus was determined by a combination of procedures carried out at weekly intervals. Vaginal smears (stained with 0.1% toluidine blue)(10), gross inspection and palpation of the external genitalia and teasing with stud dogs were employed. Exploratory laparotomies to observe ovaries and uteri were made during the 26th wk of experiment on control dogs and dogs in Groups 2, 4, and 6. Histopathological examinations were made of ovaries and uteri of several dogs that died as a result of evisceration accidents following laparotomies or were killed by penmates during the course of experiment. Whelping data were obtained after completion of treatment schedules. Bitches were bred during either first or second estrual period after cessation of treatment. During the experiment, various chemical and physiological tests were completed on some dogs to characterize possible toxic effects due to administration of 17α-acetoxyprogesterone. These tests were completed during the 6th mo and again during the 16th mo.

* Trade-Mark Reg. U. S. Pat. Off., The Upjohn Co.

TABLE I. Activity of Orally-Administered 17α-Acetoxyprogesterone on Delaying Estrus in Dogs.

Group	No. dogs	Treatment mg/kg	Treatment Duration (wk)	No. dogs showing estrus
1	14			14 (100%)
2*	7	10	36	0
3†	3	4	32	0
4*	9	2.5	70	0
5†	3	2.5	32	2 (66.7%)
6*	8	.5	23	7 (87.5%)

* Drug incorporated in feed; fed *ad lib.*
† Drug in suspension; administered once daily.

Results. When 17α-acetoxyprogesterone was incorporated in dog feed and fed at levels of 10 and 2.5 mg/kg, estrus did not occur. Lengths of treatment periods were 36 and 70 continuous wks respectively. At 0.5 mg/kg, estrus was noted in 7 of 8 dogs during a 23-wk treatment period. 17α-acetoxyprogesterone in suspension form administered once daily succesfully inhibited estrus at the 4 mg/kg level in 3 dogs for 32 wks but not at the 2.5 mg/kg level. The 14 control dogs cycled normally at approximately 6-mo intervals (Table I).

Gross *in situ* observations made on ovaries and uteri of control and treated dogs revealed that unregressed corpora lutea were present in ovaries of dogs from Group 1 and 6 but were not present in Group 2 and 4 dogs. Regressed corpora lutea were observed in some dogs in each of these groups. Coiled, corkscrew-like uteri and/or hypertrophied uteri were observed in 80% of the dogs in Group 2, in 44% of Group 4 dogs and in 33% of dogs in Group 6. Uteri of Group 1 dogs were unaltered.

Gross and microscopic examination of ovaries and uteri from treated dogs that died or were killed revealed changes consistent with progestational therapy. Of particular interest were the findings in 3 dogs from Group 2 where average duration of treatment had been 28 wks. Their ovaries were normal grossly. Microscopically, there appeared to be an in-

crease in number of primary follicles. Development of vesicular follicles was inhibited at a rather definite stage (maximum size of 1300 μ) and atresia followed. No evidence that ovulation had occurred was observed. By gross observation, uteri of 2 of 3 dogs were slightly enlarged and displayed several soft, segmented nodules in both horns. Uterus from the third dog was of normal size and shape. Microscopically, the endometrial glandular epithelium in the 2 dogs had undergone hyperplasia with rather extensive papillated mucous-cyst formation. In the third dog, the endometrium was in a relatively quiescent state.

After cessation of treatment, the dogs were maintained to determine time of return to estrus and fertility. Control dogs, Group 1, bred on the third heat after start of experiment, whelped an average of 5 puppies/litter of which 94% were alive at birth. Dogs in Group 2 had first heats an average of 6.5 months after treatment had ceased. These bitches were bred on the first heats, conceived and whelped an average of 5.1 puppies/litter of which 86% were alive at birth. First post-treatment heats in Group 3 dogs occurred at about 1.5 months. The dogs were bred on this first heat and whelped an average of 5.7 puppies/litter with 88% alive at birth. Dogs in Group 4 averaged 2.5 mos to show first heat post-treatment. Four of these bitches were bred on the first heat and 5 on the second heat. In the former group, 2 poor litters, 1 pseudopregnancy, and 1 death occurred whereas in the latter group, 4 puppies/litter were whelped of which 83% were alive. Whelping data on bitches in Groups 5 and 6 are not applicable since delay of estrus was not accomplished in all cases.

Although the primary purpose of the experiment was to observe hormonal effects, safety studies were conducted simultaneously in some of the dogs. No significant deleterious changes in clinical signs, body weight, blood chemistry, urinalysis values, liver morphology or function, kidney function or necropsy findings occurred in bitches on 10 mg/kg for 6 mos and on 2.5 mg/kg for 16 mos.

Discussion. 17α-acetoxyprogesterone incorporated in dog feed and fed *ad lib.* successfully delayed estrus in bitches at the 2.5 mg/kg or higher levels. The 0.5 mg/kg level was ineffective. In contrast, when 17α-acetoxyprogesterone was prepared in suspension form and administered once daily, 4.0 mg/kg was required to delay estrus and 2.5 mg/kg was ineffective. Thus the dose of 17α-acetoxyprogesterone required to inhibit estrus in bitches appeared to vary with frequency of administration. Higher dose level was required when treatment interval was lengthened. Explanation for this finding may be that frequent intake of the drug during a 24-hour period as compared with once-a-day intake minimizes peaks and valleys in blood levels. This point merits further study.

Mechanism of action of 17α-acetoxyprogesterone in delaying estrus was not clearly defined in this limited study. However, the microscopic picture observed in ovaries from dogs on long-term treatment suggested that Graafian follicle maturation was inhibited and ovulation was prevented.

Various 17α - acetoxyprogesterone treatments did not affect future fertility or breeding usefulness of the animals. Gross and microscopic changes observed in ovaries and uteri of treated animals apparently were of a temporary nature. The whelping data were good except for a few dogs in Group 4 that were bred on the first post-treatment heat. For maximum fertility, it would appear reasonable to suggest that bitches be bred no sooner than 6 mos after cessation of treatment.

Previously treated bitches returned to estrus with the usual characteristic signs. No aberrant heats were observed. Close synchronization of heats in dogs within each group did not occur. To forecast with accuracy when previously treated bitches will return to estrus, would appear difficult as great variation occurred. Some dogs returned to estrus within 2 wks after treatment ceased and others took as long as 8 mos to return.

Toxicity studies completed during experiment indicated that the drug was completely safe for dogs.

Summary. When tested for ability to de-

lay estrus in female dogs. 17α-acetoxyprogesterone successfully inhibited estrus during 70-wk treatment period. When incorporated in dog feed and fed *ad lib.*, a minimum dose of 2.5 mg kg was effective. When administered once daily in suspension form, a dose of 4 mg kg was required. Histopathological examination of ovaries of treated dogs revealed inhibition of development of vesicular follicles. Bitches previously treated with 17α-acetoxyprogesterone returned to cycle, were bred and whelped normal, healthy litters of puppies. It was suggested that bitches be bred no sooner than 6 mos after cessation of treatment to insure maximum fertility. Drug was judged completely safe for dogs.

The author is indebted to members of Depts. of Pathology and Endocrinology. The advice of Drs. R. L. Johnston, R. A. Runnells, and R. O. Stafford, The Upjohn Co., and Dr. R. K. Meyer, Univ. of Wisconsin, is gratefully acknowledged.

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Received June 16, 1960. P.S.E.B.M., 1960, v105.

Calcium Requirements of Various Species of *Azotobacter*.* (25997)

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In a reexamination of the trace metal requirements of *Azotobacter vinelandii* O, Esposito and Wilson(1) demonstrated that under defined conditions a quantitative difference existed in level of calcium ion necessary for its growth on molecular and on ammonium nitrogen. The effect of deficient calcium was primarily on length of the lag phase rather than total growth and was definitely associated with the phosphorus metabolism of the organism(2). When it was later established (3) that this requirement could be met by acetate, ethanol or malate, a survey was undertaken to determine how general these findings were among the Azotobacteriaceae. Over a period of 3 years about 25 strains have been examined with the results summarized in this report.

Materials and methods. Esposito and Wilson(1,2,3) have furnished the essential details of technics employed including the special methods for providing a medium with known concentrations of the calcium ion. Further information is given in the thesis of J. A. Bush(4). In most trials urea was used as a source of combined nitrogen to eliminate the complication of change in pH which accompanies utilization of ammonium salts by many of the strains. These are listed in Table I. The inoculum was grown on the modified Burk's medium(1) with no or 20% of Ca^{++} normally added. Size of inoculum was usually 0.1% so maximum calcium carryover was 1.2×10^{-7} M in comparison with the 6×10^{-4} M $CaSO_4 \cdot 2\ H_2O$ (23.4 ppm Ca^{++}) used in the complete Burk's medium.

Results. Calcium requirements of different strains. To present the results obtained with all the strains listed in Table I is neither practical nor necessary; typical findings observed with a single strain of 3 of the species are

* Supported in part by grants from Rockefeller Fn. and Research Comm. of Graduate School from funds by Wis. Alumni Research Fn.