

well as amino acids for normal growth. Woolley¹¹⁻¹³ has demonstrated the role of strepogenin for normal growth in bacteria, rats, mice and chicks.

Summary. 1. The amino acids essential for the growth of *Tritrichomonas foetus* were determined in a medium of known concentration. This flagellate utilizes nitrogen in approximately the same way as do higher organisms. The amino acids essential for *T. foetus* were determined to be arginine, glycine, tryptophane, histidine, isoleucine, leucine, lysine, threonine, methionine, phenylalanine, proline, serine, and valine.

2. Serum contains some factor capable of improving the media tested.

3. Casein, lactalbumin and wheat germ

¹¹ Sprince, H., and Woolley, D. W., *J. Am. Chem. Soc.*, 1945, **67**, 1734.

¹² Woolley, D. W., *J. Biol. Chem.*, 1945, **159**, 753.

¹³ Woolley, D. W., *J. Biol. Chem.*, 1946, **162**, 383.

protein were tested for their growth properties. Results indicate the possible use of *T. foetus* to determine the biological values of proteins.

4. With whole protein, growth is relatively low. In digests, growth improves until the percentage of soluble nitrogen reaches 50%. As digestion continues and more nitrogen goes into solution, the growth rate decreases. This decrease may be due to destruction of strepogenin.

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Effects of Various Sex Hormones on Excretion of Pregnan diol Early in Pregnancy.*

M. EDWARD DAVIS AND NICHOLAS W. FUGO.

From the Department of Obstetrics and Gynecology and the Department of Pharmacology, The University of Chicago and The Chicago Lying-in Hospital.

Smith and Smith¹ reported that the administration of diethylstilbestrol to a patient during pregnancy would increase the excretion of pregnandiol in the urine. They concluded that synthetic estrogens will stimulate steroid metabolism in the placenta increasing the amount of available progesterone. These authors suggested the use of increasing amounts of diethylstilbestrol to prevent the accidents of late pregnancy, beginning with 30 mg per day at the 16th week of the gestation and increasing the daily dose

5 mg each week until the 35th week. Furthermore, it has been recommended by others that large amounts of steroid hormones be administered early in pregnancy to prevent abortion and to treat threatened abortion.

The purpose of the present study is 2-fold: (1) to determine if the sex hormones will increase the production of progesterone by the placenta early in pregnancy and, (2) to study human fetuses from mothers who have received large amounts of these substances in order to establish that no untoward changes have resulted. The second phase of this study is extremely important in view of the changes induced in the reproductive organs of litters of experimental animals by the ad-

* This work has been done under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

¹ Smith, O. W., Smith, G. V. S., and Hurwitz, D., *Am. J. Obst. and Gynec.*, 1946, **51**, 411.

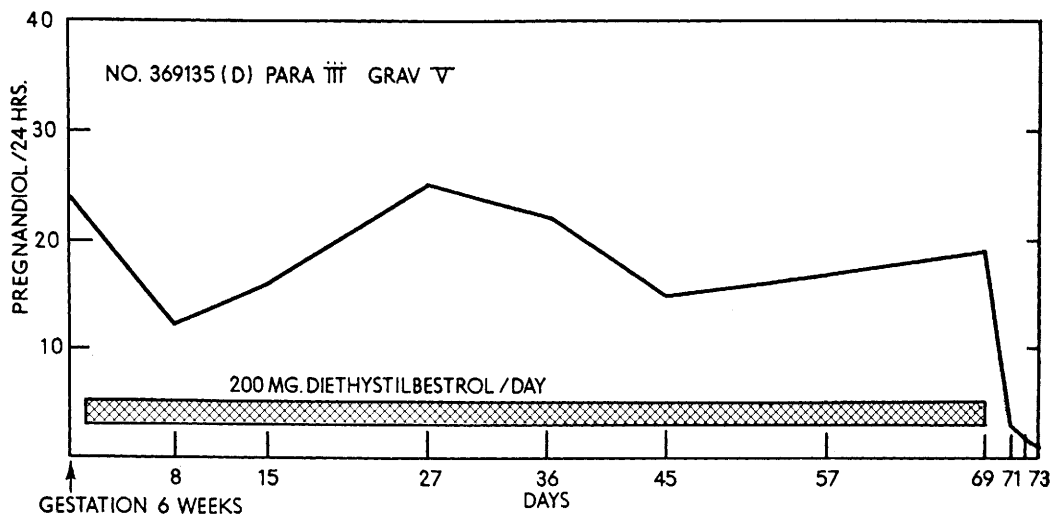


FIG. 1.

Pregnan diol excretion in a patient who had a normal pregnancy. The daily oral administration of 200 mg stilbestrol beginning at 6 weeks and continuing for 69 days did not influence the normal curve. The pregnan diol disappeared promptly at the termination of the pregnancy.

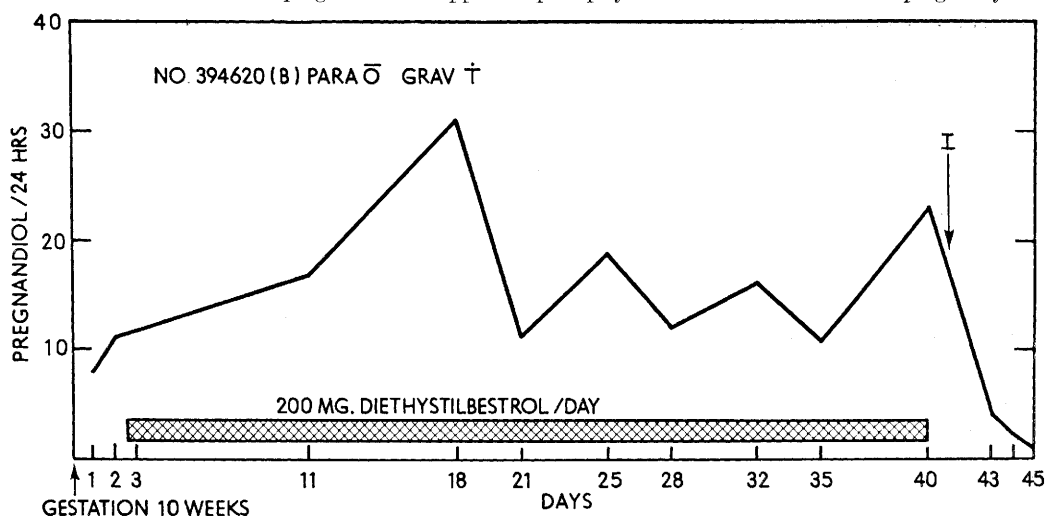


FIG. 2.

The administration of diethylstilbestrol was instituted at 10 weeks gestation and continued for 40 days. No obvious effect on pregnan diol excretion can be seen.

ministration of androgens and estrogens during pregnancy (Greene, Burrill and Ivy²) and will be reported separately at a later date.

Methods. This group of patients comprise 15 women with normal gestations. In 7 the pregnancy was interrupted at 12 to 16 weeks because of some serious maternal complication for which therapeutic abortion was advised. One of the patients had a spontane-

ous abortion at about 14 weeks gestation. The remaining 7 have delivered at term or are continuing their pregnancies.

Pregnan diol determinations were made by a method described in a previous communication.³ Three hormonal preparations were used, diethylstilbestrol, testosterone propionate and progesterone. The diethylstilbestrol was administered orally while the other 2 were given in oily solutions by intra-

² Greene, R. R., Burrill, M. M., and Ivy, A. C., *Physiol. Zool.*, 1942, **15**, 1.

³ Davis, M. E., and Fugo, N. W., in publication.

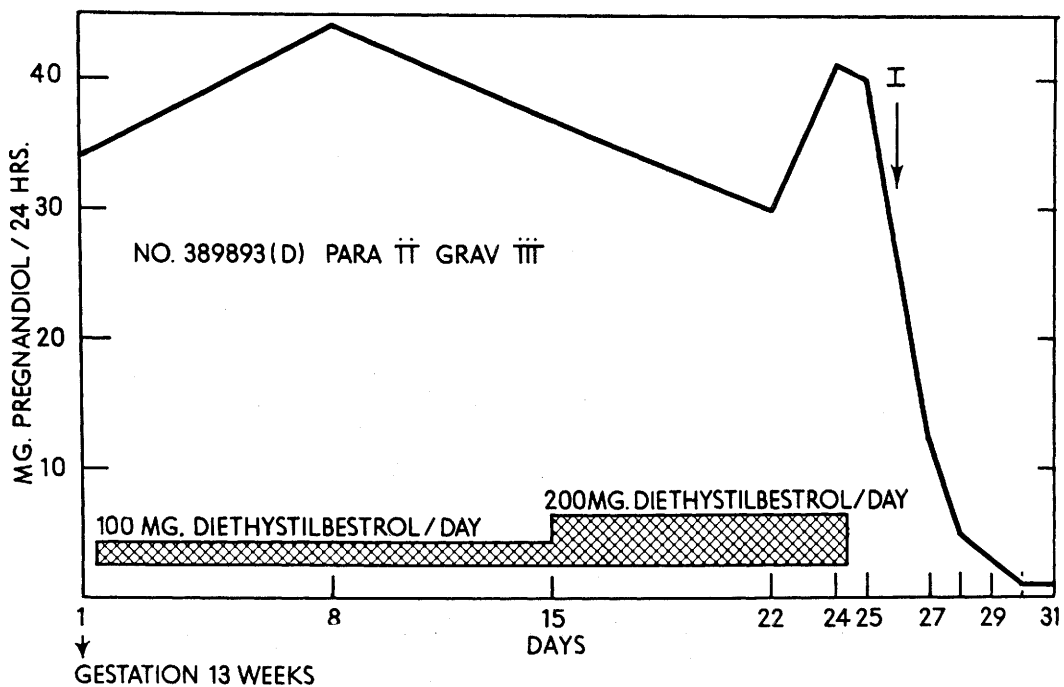


FIG. 3.

Diethylstilbestrol was first administered at 13 weeks of a normal gestation in 100 mg amounts daily for 15 days without effect. The increase in the daily amount to 200 mg did not show any appreciable change.

muscular injections. Several pregnandiol determinations were made in each patient prior to the onset of therapy in order to establish the level of excretion for each individual. The excretion of pregnandiol was followed continuously and at daily or tri-weekly intervals throughout the period during which the hormones were administered and in those patients subjected to therapeutic abortion, until pregnandiol disappeared from the urine. In the remaining cases the determinations were continued throughout pregnancy.

Results. Diethylstilbestrol. Diethylstilbestrol was administered in varying amounts to 10 patients. The administration of this estrogenic substance began at 4 to 10 weeks gestation in most patients and continued for at least 6 or 8 weeks. Most of the patients received 50 mg daily although 2 patients received 100 mg and 2 200 mg. The following figures are representative of the group.

In Fig. 1, 200 mg of diethylstilbestrol was administered daily beginning at 6 weeks gestation (that is about the time of the second missed menstrual period) and continued

for 69 days when the pregnancy was terminated by hysterotomy. The size of the fetus was compatible with a gestation of 16 weeks. No change from the normal is apparent in the pregnandiol excretion. The normal rise in the daily output begins at about 15 or 16 weeks gestation. In Fig. 2 a similar amount was administered over a period of 38 days but the onset of therapy commenced at 10 weeks. Pregnancy was again terminated at about 16 weeks and the size of this fetus was comparable to the previous one. There was no appreciable increase in the daily excretion of pregnandiol and the amounts were within normal limits. In Fig. 3, only 100 mg of diethylstilbestrol was administered for a period of 2 weeks, following which the amount was doubled. However, there was no appreciable rise in the amount of pregnandiol excreted although the daily excretion in this individual was considerably greater than in the other 2.

In the entire group of patients it was apparent that the administration of 50 to 200 mg of diethylstilbestrol per day did not in-

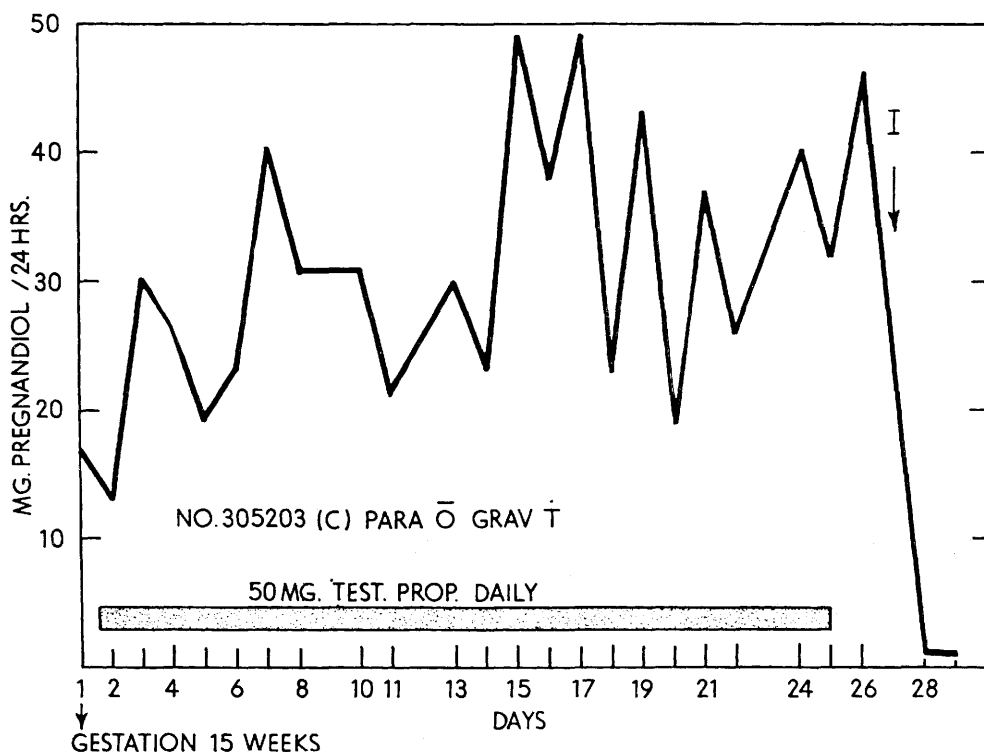


FIG. 4.

The daily intramuscular administration of 50 mg of testosterone propionate was instituted at 15 weeks gestation and continued for 25 days. This androgen did not alter the normal output of pregnandiol and the curve of excretion is typical for this period of pregnancy.

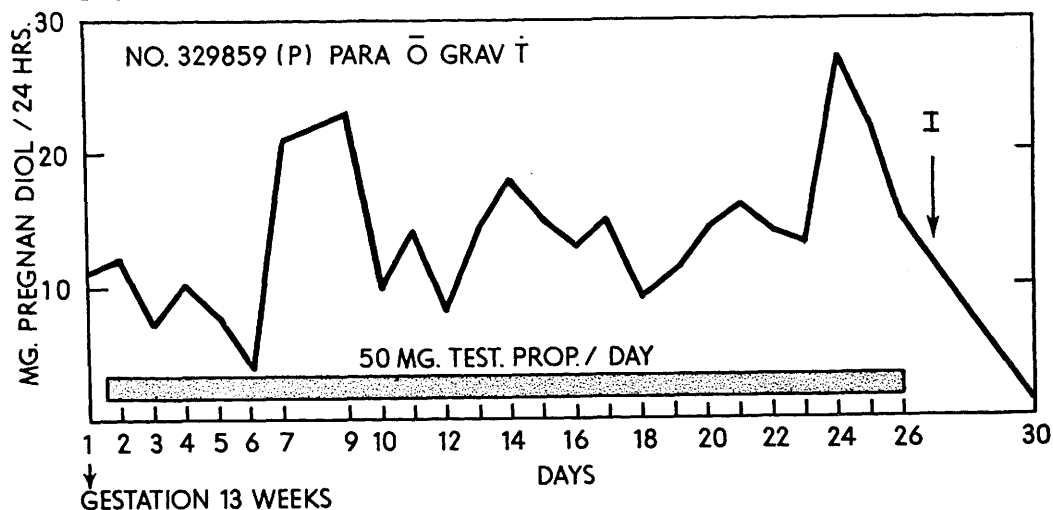


FIG. 5.

The administration of testosterone propionate was started when this patient was 13 weeks pregnant and continued for 26 days for a total of 1300 mg. No effect on pregnandiol excretion can be demonstrated.

fluence the excretion of pregnandiol in the urine. We can assume that during the first 16 weeks of the 38-week gestation this syn-

thetic estrogen does not affect the production of progesterone by the placenta as determined by pregnandiol output. In a previ-

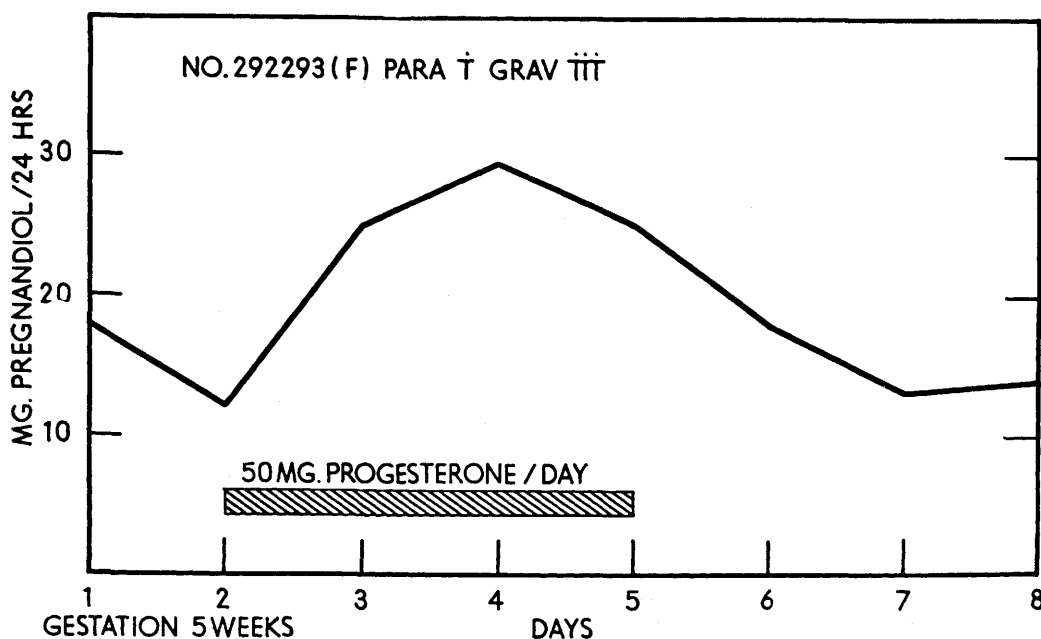


FIG. 6.

Progesterone administered at 5 weeks gestation produces a marked rise in pregnandiol excretion.

ous communication the authors³ have suggested that diethylstilbestrol may affect the pregnancy profoundly by increasing the uteroplacental circulation thereby promoting an improvement in the uterine environment for the fetus. It is recognized that the corpus luteum is the chief source of progesterone early in pregnancy but in that period between 12 and 16 weeks a well formed placenta is present.

Testosterone propionate. Testosterone propionate was administered to 2 patients in order to determine if androgens could influence the metabolism of progesterone and the excretion of pregnandiol. The chemical configuration of the testosterone molecule conforms closely to that of progesterone. The first patient received 50 mg of the hormone in an oily solution by intramuscular injection each day for a period of 25 days or a total of 1250 mg. Therapy was instituted at about 15 weeks and when the fetus was delivered by abdominal hysterotomy its size and development was compatible with 18 or 19 weeks gestation. The second patient received 50 mg of testosterone propionate each day for 26 days for a total of 1300 mg. Androgens were started at about 13 weeks

gestation and the pregnancy terminated at about 17 weeks. Both of these fetuses were normal externally.

Signs of virilism began to manifest themselves when the patients received about 1000 mg of testosterone. Acne appeared on the face. A fine growth of hair could be discerned. There was some prominence of the clitoris. The most striking change was in the voice, the pitch of which dropped rapidly from high soprano to a husky masculine alto. The voice has not recovered the former range although all other findings have disappeared.

There was no obvious change in the pattern of pregnandiol excretion (Fig. 4 and 5). The usual daily variations were present but the tendency of the curve was upwards after the 15th week. At the termination of the pregnancy the pregnandiol output dropped precipitously and disappeared at the end of 72 to 96 hours. Thus, androgens like estrogens do not influence the excretion of pregnandiol in the first half of the gestation.

Progesterone. Progesterone was administered to 3 patients for varying periods at 5, 7 and 10 weeks gestation. Fifty milligrams of progesterone in oil was injected intramuscularly daily in 2 patients and in a single

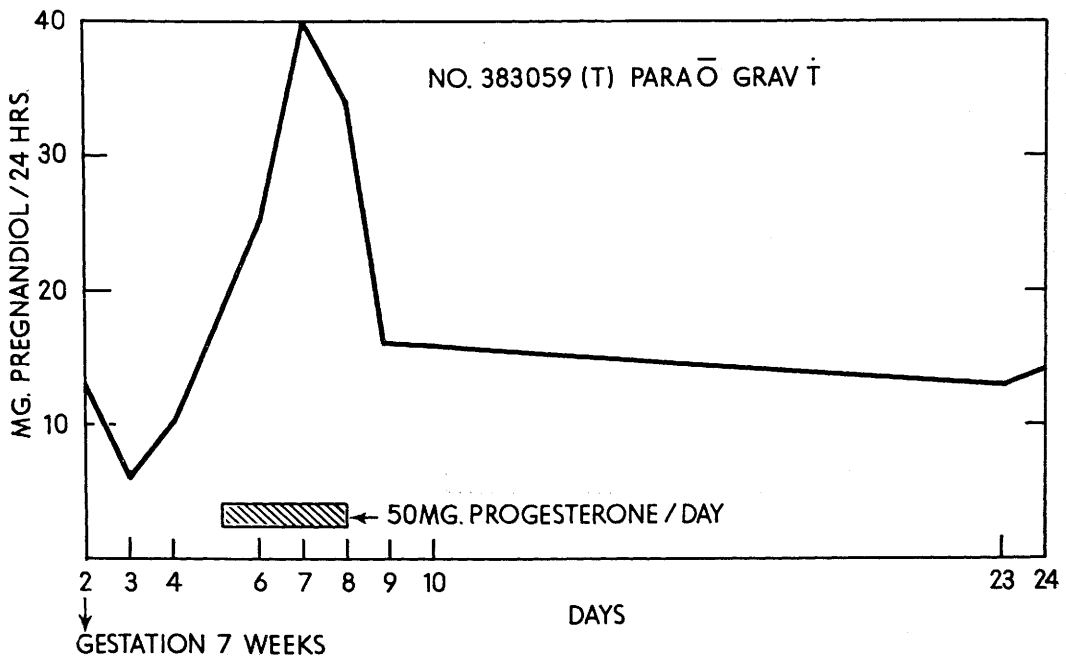


FIG. 7.

The administration of progesterone in 50 mg daily doses at 7 weeks gestation resulted in a 30 to 35% recovery of this steroid as pregnandiol.

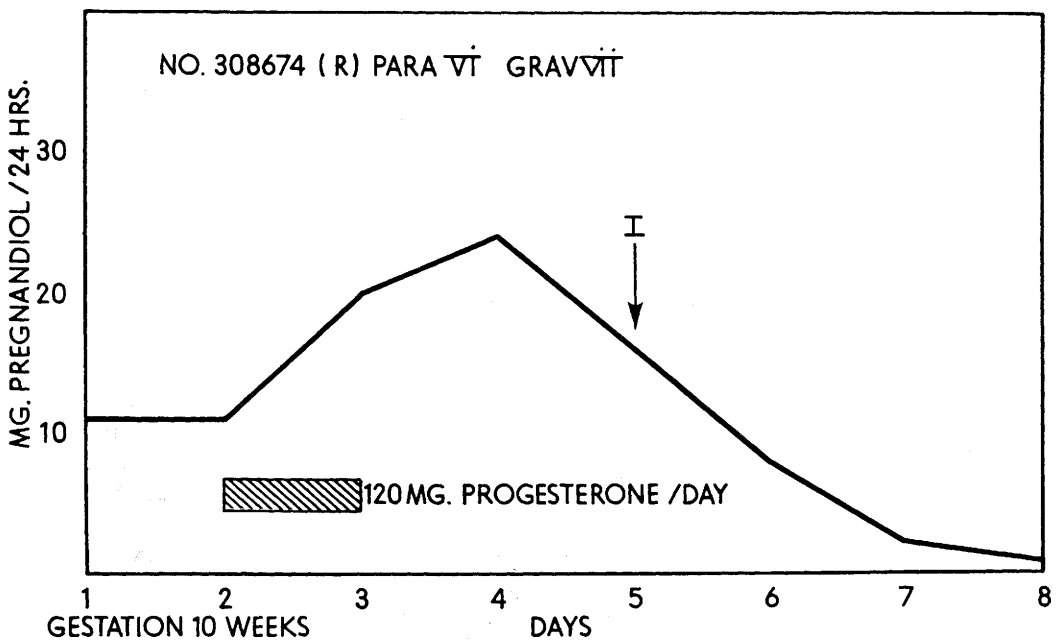


FIG. 8.

The single administration of 120 mg of progesterone to a patient at 10 weeks gestation resulted in a more prolonged increase of pregnandiol excretion but in a decrease in the total amount of the steroid recovered as pregnandiol.

dose of 120 mg in the third. In each instance there was an immediate increase in the excretion of pregnandiol. The effect of this hormone disappeared within 24 hours after the last injection (Fig. 6, 7, 8).

If the increase in pregnandiol above the average level excreted in each patient was calculated, about 30 to 35% of the progesterone injected could be accounted for as pregnandiol. Thus, in Fig. 6, 150 mg of progesterone was injected over a 3-day period and at least 50-60 mg of additional pregnandiol was recovered from the urine during this period. In the nonpregnant individual in the preovulatory phase it is possible to recover less than 10% of the progesterone as pregnandiol. The activity of the corpus luteum in the postovulatory phase and in the first trimester of pregnancy must exercise some effect on the metabolism of progesterone so that a much greater percentage can be accounted for as the inert metabolite, pregnandiol. It was also observed that a higher percentage of progesterone is recovered as pregnandiol when the steroid is administered in small divided doses than as a single large dose (Fig. 8).

Discussion. Diethylstilbestrol and testosterone propionate do not influence the excretion of pregnandiol during the first 16

weeks of normal gestation. On the contrary, progesterone is metabolized efficiently so that at least 30 or 35% is recovered as pregnandiol. If it is desirable to increase the amount of progestational hormone available during early pregnancy, it is more logical to administer progesterone in liberal amounts rather than diethylstilbestrol. However, it is the impression of the authors that there is no lack of progesterone in most of the pregnancies which threaten to end during the early months. Decreased pregnandiol levels may be an index of poor placental function.

Summary. Diethylstilbestrol, testosterone propionate and progesterone were administered to 15 patients with normal pregnancies in order to study the effect of these substances on progesterone metabolism and pregnandiol excretion. Diethylstilbestrol and testosterone when administered daily in large amounts over long periods during the first 16 weeks of gestation did not alter the normal excretion of pregnandiol. The administration of progesterone was followed by the prompt recovery of a considerable portion of the injected steroid as pregnandiol.

We wish to thank E. R. Squibb & Company for their liberal supply of diethylstilbestrol and progesterone.

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A Gelatinous Variant of *Pseudomonas aeruginosa*.

EDWIN W. SCHULTZ.

From the Department of Bacteriology and Experimental Pathology, School of Medicine, Stanford University, Calif.

Pseudomonas aeruginosa is generally recognized as highly variable. Among the variations exhibited are those of colony form. During the course of cultural examinations made on a case of third-degree burn in 1943, a variant was isolated which seems not to have been described before. It appeared in small numbers as an unusual colonial form when the organism was streaked on a modified Sabouraud's medium. The modification

of this medium consisted of the addition of a 0.5% glycerol to the usual Sabouraud's maltose-peptone agar. Subsequent observations showed that the appearance of this variant depended on the presence of the glycerol in the medium.

The initial observations were as follows: among the usual colonial forms a small number of exceptional colonies were recognized on a few of the plates. These were few