a luteolytic substance produced by the endometrium reaches the ovary. Failure to prolong pseudopregnancy in the second trial of the first experiment apparently was due in part to regeneration of blood vessels, as an extensive adhesion was observed which macroscopically revealed the presence of regenerated blood vessels. An increased level of the uterine luteolytic substance in the general circulation by the time of the second induction of pseudopregnancy may also have contributed to luteal regression. Ligature of the communicating blood vessels between the ovary and the uterus apparently does not completely eliminate luteolytic substances in the general circulation, since the duration of the first pseudopregnancy in this group was shorter than in the hysterectomized rats. A lymphatic circulation connecting the ovaries and uterus has not been established for the rat, but this possibility cannot be ruled out at this time.

Summary. The effects of sham operation, bilateral ligature of blood vessels joining the uterus to the ovary, and hysterectomy were determined on the length of pseudopregnancy in rats. Hysterectomy or bilateral ligature of blood vessels joining the uterus to the ovary significantly increased the length of pseudopregnancy when compared to sham-operated controls. These results indicate that the circulation between the uterus and ovaries is important in local control of luteal lifespan in the rat.

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The Precipitin Band as a Mechanical Barrier Revealed by Gel Diffusion Studies of Gonadotropic Hormones* (32922)

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That all components which do not cross-react in a multiple precipitating antigen/antibody system behave independently as if each were present alone in agar gel double diffusion preparations is a widely accepted point of view (1–4). However, in the course of a study in our laboratory of the antigenic relationship between gonadotropic hormones where a hyperimmune rabbit antiserum to ovine luteinizing hormone (AOLH) was utilized, it was observed that a heavy precipitin line sometimes acted as a nonspecific mechanical barrier, interfering with the free diffusion of heterospecific antigens as well as unrelated antibodies. In this study AOLH completely absorbed with sheep tissues ($AOLH^{st}$) re-

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acted with ovine luteinizing hormone (OLH) in agar double diffusion preparations to form seven precipitin lines, and a hormonal relationship was established for each line (5). Among the seven lines in the OLH/AOLHst reaction, three were antigenically related to lines which developed when AOLHst serum was cross-reacted with pregnant mare's serum gonadotropin (PMSG). Information pertinent to the present report came from observations of the effect of the presence of a heavy early-appearing line in the PMSG/AOLHst reaction on certain nearby unrelated lines and from a study of the effect of selective removal of this line by treatment of PMSG with urea.

Materials and Methods. The development, absorption, and characterization of the hyperimmune AOLH serum used in this study has been reported elsewhere, and the technique of gel double diffusion has also been described (5–8). The hormones most relevant to this study were NIH-LH-S7 and PMSG from Ayerst Laboratories (Equinex, Lot K-34967, 2800 IU/mg) which was involved in experiments of Figures 1c and d. Other hormones have been characterized in other studies (5, 8). Urea treatment of PMSG was carried out at a concentration of 3 mg of PMSG/ml of 8 *M* urea (Merck, reagent grade) in 0.06 *M* phosphate buffer, pH 7.0, at 37°C for 24 hours. Without further treatment PMSG in this solution was added to the experimental wells.

Results and Discussion. Interference with

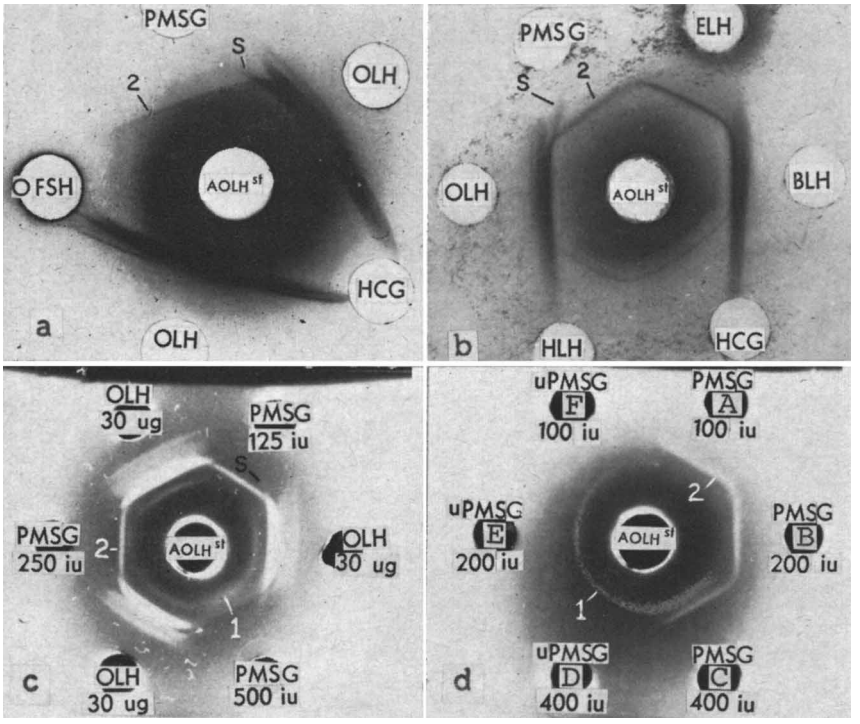

FIG. 1. a–d. Agar double diffusion studies of the reaction between antiserum to ovine luteinizing hormone absorbed with sheep tissues (AOLHST) and various gonadotropic hormones. (a) and (b) are photographs of stained preparations and have been published elsewhere(5, 6). In (a, b, c) the letter "s" refers to the spur of the second PMSG specific line which is of no concern to the present report. The weak line of (d) (line 1) has been retouched. OFSH = ovine follicle-stimulating hormone; PMSG = pregnant mare's serum gonadotropin, OLH = ovine luteinizing hormone; HCG = human chorionic gonadotropin; ELH = equine luteinizing hormone; BLH = bovine luteinizing hormone; HLH = human luteinizing hormone, uPMSG = PMSG treated with 8 *M* urea at 37°C for 24 hours. Line 1 = FSH contaminant in PMSG; line 2 = PMSG-specific component.

free diffusion of antibodies by a heavy unrelated precipitin line was demonstrated by experiments illustrated in Figs. 1a and b. In these studies, precipitin line complexes formed opposite the wells containing either OLH or BLH (bovine luteinizing hormone). Within 7 days, most of these lines had lengthened and reached the neighboring well containing ovine follicle-stimulating hormone (OFSH), human chorionic gonadotropin (HCG), or human luteinizing hormone (HLH). In contrast, extension of the outer line complex into the vicinity of the wells containing PMSG and equine luteinizing hormone (ELH) did not occur.

It will be noted that the precipitin lines representing cross-reaction with AOLH opposite FSH, HCG, and HLH were weak, whereas those opposite PMSG (line 2) and ELH were strong. Presumably the latter interfered with the passage of antibody outward through the barrier into the area opposite the PMSG and ELH wells, but the weak lines produced by FSH, HCG, and HLH offered less interference. Since there is no deviation of the shortened lines (see legend) extending into the PMSG or ELH area, there can be no question of an interaction with PMSG or ELH which might have influenced their development. The heavy line formed by ELH produced the same phenomenon, indicating that the mechanical barrier effect was not unique for PMSG.

Further pertinent evidence came from observations of the effect of urea treatment on the antigens of PMSG. In an earlier study (5) three antibodies in the AOLH serum were found to cross-react with PMSG. One of these was due to an FSH contaminant and the other two were shown to be specific for PMSG. Two of the three precipitin lines characteristically resulting from the PMSG/AOLHst reaction are demonstrated in Fig. 1c. Line 1 represents the FSH contaminant and line 2 represents one of the two PMSG-specific antigens. The second of the PMSG-specific lines (labeled s) appears as a spur in these preparations and is irrelevant to the present study. As shown in Fig. 1c, the optimal dose of hormone for detecting the outer PMSG-specific line (line 2) and insuring the formation of a rapidly appearing strong line was about 100–200 IU. Reliable detection of the inner FSH line (line 1) required a larger testing dose of 400 IU or more, while 100 IU invariably gave negative results. The PMSG lost some of its biological activity after treatment with 4 *M* urea at 37°C for 24 hours, but the precipitating power was not affected.

Figure 1d shows that after treatment with 8 *M* urea the precipitating ability of the PMSG-specific component under consideration was destroyed, but not that of the FSH contaminant. As a result the heavy early-appearing precipitin line (line 2) was removed from the uPMSG/AOLHst system, and a clear FSH line (line 1) appeared in all three instances where urea-treated PMSG was used (wells D, E, F). At no time during the observation period which lasted 14 days was a line of corresponding clarity discernable in the same area opposite wells A and B containing untreated PMSG. Since identical concentrations of both untreated and urea-treated PMSG were tested, the absence of an FSH line facing wells A and B cannot be attributed to insufficient amounts of FSH in these reservoirs. Wells F and A contained equal amounts of FSH, as did also wells E and B, hence the formation of an FSH line must have depended on the presence or absence of the preexisting strong outer line. Presumably the early formation of a dense aggregate by the outer precipitin line (line 2) interfered with the free diffusion of FSH from wells A and B across that line. The resulting failure of a clearly recognizable line to form may have been due either to a low FSH concentration on the other side of the barrier or to a delayed FSH diffusion rate beyond the barrier.

It remains to be seen whether this is a phenomenon unique for the conditions of this study. Oudin (1) promulgates the concept of independency of antigens which do not cross-react, but adds that "this is probably not true outside certain limits; a very dense zone of precipitin could constitute an obstacle to diffusion of antigen." Results of a study by Feinberg (3) are suggestive of a mechanical block. Illustrations presented by this author (Figs. 12 and 13) can be interpreted as demonstration of the unmasking of an extra precipitin line due to fibrinogen, when a heavy line due to ovalbumin was removed in the "in-

tragel specific absorption preparations" which he described. Finally, Wilson and Pringle (2) demonstrated that the presence of a heavy lysozyme precipitin line interfered with the diffusion of human serum albumin. However, they attributed the interference to the formation of complexes with other proteins by the basic lysozyme molecule. A similar explanation is unlikely in the experiments described above, for if this were true a direct proportion between the concentration of the antigen contributing to the barrier line and the degree of interference would be expected. The reverse was true as indicated in Fig. 1c and d where untreated PMSG was used.

Comparisons of untreated hormone and hormone treated with 4 and 8 *M* urea makes it clear that the presence of urea in the gel medium played no part in the result, since PMSG treated with 4 *M* urea produced precipitin patterns identical to those produced by untreated PMSG when identical concentrations of the hormone were compared.

Apparently there are two prerequisites necessary for successful demonstration of the mechanical interference by a precipitin line. First, the interfering line must form early and build up in strength quickly. And, secondly, the precipitin lines being inhibited should be formed under unfavorable conditions such as a disproportionate antigen/antibody ratio, resulting in a labile combination readily upset by outside influences, or antigen and/or antibody scarcity resulting in a relatively long incubation period before detectable precipitin lines form.

If the point of view expressed herein is correct, it follows that due consideration should be given to this phenomenon. When multiple antigen/antibody systems are being studied in agar gel diffusion preparations, the presence of a strong rapidly appearing precipitin line may affect the length, intensity, form or number of lines developed by serologically unrelated precipitin systems.

Summary. Under certain experimental conditions, a specific antigenic component of pregnant mare's serum gonadotropin (PMSG) reacted with a hyperimmune antiserum to ovine luteinizing hormone (LH) to form an early-appearing heavy precipitin line in agar gel double diffusion preparations. It was demonstrated that it interfered with the free diffusion of some unrelated antibodies in a multiple precipitating antigen/antibody system. In addition, removal of this line by treatment of PMSG with urea unmasked a well-defined line identified in another study as a contaminant of follicle-stimulating hormone (FSH). Failure of the latter line to appear under similar conditions when untreated PMSG was used was ascribed to the interference with diffusion of the FSH contaminant through the strong mechanical barrier formed by the specific component of PMSG.

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