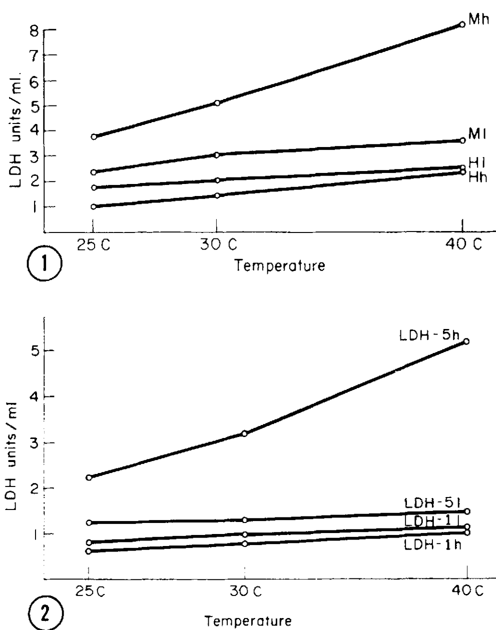

FIG. 1. Effect of temperature on LDH activity. M = breast muscle, H = heart muscle. l = 3.3×10^{-4} M pyruvate, h = 6.6×10^{-3} M pyruvate. One unit of LDH equals the amount of enzyme utilizing 1 μmole of NADH per minute.

FIG. 2. Effect of temperature on LDH 1 and LDH 5 at high and low pyruvate concentration. The individual isozymes were obtained by elution from starch gel after electrophoresis of heart and muscle supernatants for 16 hr at 4°C. h = 6.6×10^{-3} M pyruvate, l = 3.3×10^{-4} M pyruvate.

muscle homogenate, and isolated breast muscle LDH 5 both have greater activity at high pyruvate concentration than at low, especially at the physiological body temperature of the chicken (40°C). The LDH in the supernatant of a chicken heart muscle homogenate, and isolated LDH 1 react best at low pyruvate concentration, the difference, however, is less marked at 40°C. These data are in accord with the observation of Cahn *et al* (3) on the differential sensitivity of LDH 1 and LDH 5 to pyruvate concentration.

Summary. Spectrophotometric analysis of chick lactate dehydrogenase activity showed that the LDH in breast muscle supernatant and isolated LDH 5 had greater activity at high pyruvate concentration than at low. The difference was more marked at 40°C than at lower temperatures. Heart muscle LDH, and isolated LDH 1 reacted best at low pyruvate concentration. The difference was less marked at 40°C than at lower temperatures.

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Production of Gonadotrophin Antibodies in Mouse Peritoneal Fluid.* (31622)

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The production of antibodies against protein hormones has provided the basis for sensitive and specific assays for these hormones. A variety of animal species has been used to produce antibody, ranging from the common rabbit to guinea pigs, sheep, goats and horses. Use of the mouse as a source of antibody has been limited primarily because of the difficulty in obtaining large amounts of serum. Munoz(1), however, reported that antigens (egg albumin or beef serum albumin) mixed with Freund's adjuvant and injected intraperitoneally into mice caused the development of large amounts of peritoneal fluid (ascites fluid) containing specific antibody in high concentration. Subsequent in-

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vestigators also have demonstrated significant levels of antibody in mouse ascites fluid after immunization with egg albumin(2-4), virus (5,6), *Staphylococci*(7-9), *Salmonella*(8), human gamma globulin(4), human myosin(4) and sheep red cells(10). The fluid was formed in large volumes and could be easily removed from the same animal on repeated occasions.

The production of hormone antibodies in the mouse has not been reported previously. In the present study groups of mice were immunized with either a crude sheep anterior pituitary preparation or human chorionic gonadotrophin emulsified with adjuvant. The resultant ascites fluid was assayed biologically and shown to have high antigonadotrophin activity.

Materials and methods. I. *Immunization and induction of ascites fluid.* Male and female mice of the Swiss-Webster strain, 2 months of age and weighing 25 to 30 g, were received from the Rolfsmeyer Co., Madison, Wis. The mice were immunized with either a crude sheep anterior pituitary (SAP) preparation‖(11), or human chorionic gonadotrophin (HCG).¶ The antigens were dissolved in physiological saline and emulsified with an equal part of complete Freund's adjuvant,** to a *water in oil* emulsion by mechanical shaking for 30 minutes. The emulsion was injected immediately after preparation.

Each mouse received 3 intraperitoneal injections, the second and third 7 and 19 days after the first. Each injection consisted of either 7.5 mg of SAP powder or 500 international units (IU) of HCG in 0.5 ml of the saline-adjuvant emulsion. Twenty-seven male and thirty female mice were immunized with the SAP preparation in 3 experimental groups; one group of 9 female mice was immunized with HCG. Control mice received injections of the same volume of a saline-adjuvant emulsion. Ascites fluid produced by these mice will subsequently be referred to as "control ascites fluid."

II. *Collection of ascites fluid.* Mice were observed daily, but fluid was collected only from those mice in which abdominal distension was marked. The first collection was 3.5 to 4 weeks after the first immunizing injection. At the peak of ascites production collections were made every 4 or 5 days.

Ascites fluid was collected from anesthetized mice by puncturing the lower abdomen with a sterile No. 18 gauge syringe needle. The fluid drained freely into iced centrifuge tubes. The average volume of ascites fluid obtained from one mouse at a single collection was 3 to 4 ml; the maximum amount was 15 ml. The onset of production and the amount of fluid formed were extremely variable among individual animals; however, female mice were consistently better producers of ascites fluid than male mice.

Ascites fluid collected on a given day was pooled according to the treatment and sex of the mice. The fluid was centrifuged at 3000 rpm for 30 minutes at 2-4°C, after which the supernatant was frozen in small lots for subsequent assay.

III. *Biological assay of ascites fluid.* Biological assay of the antigonadotrophin activity of the ascites fluid was based upon inhibition of the ovarian and uterine response to injected gonadotrophins in immature female rats. Assay rats were received from the Holtzman Co., Madison, Wis., at 21 days of age.

Ascites fluid from SAP-immunized mice (SAP-ascites) was assayed against a homogenate of pooled adult male rat pituitaries instead of the antigen since the supply of antigen was limited. Each assay rat received a total of 1 ml ascites diluted to 2.25 ml with physiological saline and the equivalent of ⅓ of a pituitary homogenized in 2.25 ml saline. The ascites solution and pituitary homogenate were administered subcutaneously at different sites beginning on the afternoon of the rats' 21st day of life and continuing twice daily (mid-morning and late afternoon) for the next 4 days. Control groups were injected in the same manner. The rats were sacrificed

‖ SAP: Obtained from Dr. W. H. McShan, Dept. of Zoology, Univ. of Wisconsin. Nitrogen content—2.26 mg/g fresh issue; Yield of SAP powder—2.5037 mg/g fresh tissue. Extracton was carried through the Sephadex G-100 fractionation of Step 2 of the indicated reference.

¶ HCG: International Hormones, Hicksville, L. I., N. Y. Control No. C-161.

** Complete Freund's adjuvant: Difco, Bacto Adjuvant, Complete Freund, Difco Laboratories, Detroit, Mich. Control No.: 464123 and 472160.

TABLE I. Antigonadotrophic Activity of Ascites Fluid from Female SAP-Immunized Mice.

Treatment of assay rats*	Rat ovarian weight (mg) (mean ± standard error)					
Group I	(Day 27)†	(Day 41)				
Pit. homog. + saline	52.7 ± 8.4	43.6 ± 5.7				
Pit. homog. + SAP ascites	25.8 ± 4.6‡	20.0 ± 1.3‡				
Pit. homog. + control ascites	74.3 ± 4.1	46.5 ± 5.2				
SAP ascites + saline	15.7 ± .8	14.8 ± 1.6				
Control ascites + saline	16.1 ± .6	14.1 ± .5				
Saline + saline	18.7 ± 1.1	16.8 ± 1.2				
Group II	(Day 23)	(Day 27)	(Day 31)	(Day 36)	(Day 41)	(Day 45)
Pit. homog. + saline	45.9 ± 6.6	77.5 ± 7.9	77.5 ± 7.9	77.5 ± 7.9	45.9 ± 6.6	45.9 ± 6.6
Pit. homog. + SAP ascites	77.9 ± 5.7	43 9 ± 7.0‡	40.9 ± 9.1‡	56.9 ± 4.1	21.9 ± 1.7‡	20.9 ± 1.9‡
Pit. homog. + control ascites			62.2 ± 10.5		44.8 ± 5.1	
SAP ascites + saline	14.1 ± .6	15.7 ± .6			15.9 ± 1.2	16.5 ± 1.5
Control ascites + saline					15.1 ± .7	
Saline + saline	16.7 ± 1.1	15.8 ± 1.0	15.8 ± 1.0	15.8 ± 1.0	16.7 ± 1.1	16.7 ± 1.1
Group III	(Day 25)	(Day 29)	(Day 40)	(Day 46)	(Day 57)	(Day 67)
Pit. homog. + saline	56.1 ± 7.1	56.1 ± 7.1	60.4 ± 3.9	60.4 ± 3.9	60.4 ± 3.9	60.4 ± 3.9
Pit. homog. + SAP ascites	59.0 ± 10.1	76.5 ± 5.5	15.2 ± 1.2‡	17.9 ± 1.0‡	20.9 ± 2.3‡	19.1 ± .9‡
Pit. homog. + control ascites	45.3 ± 5 6	67.0 ± 7.5				50.5 ± 4.9
SAP ascites + saline	16.4 ± .9	17.1 ± 1.0		16.4 ± .7		
Control ascites + saline	17.2 ± 1.0	17.3 ± .8		15.7 ± 1.4		16.0 ± .8
Saline + saline	17.5 ± .8	17.5 ± .8	12.6 ± .7	12.6 ± .7	12.6 ± .7	12.6 ± .7

* 5 rats in each assay group.
† Day of ascites fluid collection.
‡ Significantly less than pituitary homog. + saline group, $p \leq .01$.

the morning after the last injections and the weights of ovaries and uteri recorded.

Ascites fluid from the HCG-immunized mice (HCG-ascites) was assayed against 3 doses of HCG from the same lot as the immunizing HCG. Each assay rat received a total of 0.5 or 1 ml ascites fluid diluted to 1.25 ml with saline and either 2.5, 5 or 10 IU of HCG in 1.25 ml saline. The ascites and HCG solutions were administered subcutaneously at different sites beginning on the afternoon of the rats' 21st day of life and continuing twice daily (mid-morning and late afternoon) for the next 2 days. Appropriate control groups were injected in the same manner. Rats were sacrificed the morning after the last injections, and the ovarian and uterine weights recorded.

The biological cross-reactivity between anti-HCG and either sheep or rat pituitary gonadotrophins was tested. In 5 subcutaneous injections each assay rat received a total of 1 ml of day 54 anti-HCG ascites fluid diluted to 1.25 ml with saline and either 7.5 mg SAP powder or ⅓ of a rat pituitary homogenized in 1.25 ml saline.

Duncan's new multiple range test(12) was used to determine the significance of differences between the means of ovarian weights.

Results. Three groups of both male and female mice were immunized with the SAP preparation. Tables I and II summarize the results of biological assays of the SAP-ascites fluid. Only the ovarian weights of the assay rats are included in the tables. In general, the uterine weight paralleled the ovarian weight response.

The ovarian weights of rats injected with pituitary homogenate and saline differ from experiment to experiment because of a variation in the gonadotrophic potency of the donors' pituitaries. Therefore, antigonadotrophic activity can be measured only by comparison with controls in the same assay.

Day 1 is defined as the day of the initial antigen injection. Ascites fluid from Group I SAP-immunized female mice was first collected on day 27 and showed high antigonadotrophin activity. The mean ovarian weight of rats which received concurrent injections of SAP-ascites and pituitary homogenate was not different from the saline controls and was significantly reduced ($p \leq 0.01$) from the mean ovarian weight of rats which received pituitary homogenate and saline. SAP-ascites collected from the same females on day 41

TABLE II. Antigonadotrophic Activity of Ascites Fluid from Male SAP-Immunized Mice.

Treatment of assay rats*	Rat ovarian weight (mg) (mean ± standard error)			
Group I	(Day 45)†	(Day 55)	(Day 63)	
Pit. homog. + saline	42.9 ± 4.8	52.7 ± 8.4	42.9 ± 4.8	
Pit. homog. + SAP ascites	49.3 ± 5.3	53.3 ± 4.8	19.3 ± 1.1‡	
SAP ascites + saline	17.3 ± .7	17.5 ± .7	20.0 ± 2.2	
Saline + saline	18.0 ± 1.2	18.7 ± 1.1	18.0 ± 1.2	
Group II	(Day 23)	(Day 27)	(Day 45)	
Pit. homog. + saline	45.9 ± 6.6	30.8 ± 4.7	45.9 ± 6.6	
Pit. homog. + SAP ascites	76.7 ± 4.6	22.2 ± 1.9	26.7 ± 3.2‡	
Pit. homog. + control ascites		31.9 ± 3.0	62.5 ± 5.9	
SAP ascites + saline	17.1 ± .8	16.8 ± 1.5		
Control ascites + saline			16.7 ± .6	
Saline + saline	16.7 ± 1.1	16.1 ± .8	16.7 ± 1.1	
Group III	(Day 25)	(Day 29)	(Day 57)	(Day 67)
Pit. homog. + saline	56.1 ± 7.1	56.1 ± 7.1	60.4 ± 3.9	60.4 ± 3.9
Pit. homog. + SAP ascites	59.6 ± 5.5	59.0 ± 5.7	18.9 ± 1.0‡	18.2 ± 1.0‡
SAP ascites + saline	16.3 ± .8	17.3 ± 1.1		
Saline + saline	17.5 ± .8	17.5 ± .8	12.6 ± .7	12.6 ± .7

* 5 rats in each assay group.
† Day of ascites fluid collection.
‡ Significantly less than pituitary homog. + saline group, $p \leq .01$.

was also able to inhibit gonadotrophin activity of the rat pituitary homogenate. Insufficient ascites fluid was obtained from Group I male mice on day 27 for assay; it was day 45 before appreciable fluid developed. Ascites fluid collected from males on days 45 and 55 had no detectable antigonadotrophin activity. However, ascites collected on day 63 had very high antigonadotrophin activity.

The first ascites fluid from Group II SAP-immunized mice was collected on day 23. Neither male nor female fluid had antigonadotrophin activity. Fluid from females on days 27 and 31 partially inhibited rat ovarian weight stimulation, indicating some antigonadotrophin activity. However, complete inhibition was not attained until day 41. Similarly, Group II SAP-ascites from male mice was inactive on day 27 but active on day 45. This group of males was particularly poor in producing ascites fluid; no fluid was obtained after day 45 although the females continued to produce fluid in abundance.

The Group III SAP-immunized mice first yielded ascites fluid on day 25. These samples as well as those collected on day 29 had no antigonadotrophin activity whether obtained from female or male mice. Complete inhibition of ovarian weight stimulation was obtained on day 40 from female ascites fluid and day 57 from male ascites fluid.

Neither the control nor the SAP ascites fluid injected together with saline had any effect on rat ovarian or uterine weight. It will be noted, however, that in 5 early fluid collections there was a significant *increase* in the mean ovarian weight of ascites + pituitary homogenate-treated groups above that of the respective pituitary homogenate + saline-treated group. This increase was observed with both control and SAP ascites fluid from both male and female mice (*e.g.*, Group II female, day 23, pituitary homogenate + SAP-ascites).

Table III summarizes the results of assays of HCG-ascites produced in female mice. The initial fluid collection on day 25 completely inhibited the stimulatory activity of up to 10 IU of concurrently injected HCG. Assay of 3 later collections showed continued high anti-HCG activity. One-half ml of ascites fluid per assay rat from day 39 also completely inhibited the stimulatory activity of 10 IU of HCG when injected concurrently. The threshold volume of ascites fluid which will inactivate 10 IU of HCG was not determined. There was *no* inhibition by anti-HCG ascites fluid of either SAP powder or rat pituitary homogenate.

TABLE III. Anti-HCG Activity of Ascites Fluid from Female HCG-Immunized Mice.

	Day of ascites fluid collection			
	25	34	39	54
Treatment of assay rats*	Rat ovarian weight (mg) (mean ± standard error)			
2.5 IU HCG + anti-HCG‡	15.4 ± .7†	17.7 ± .7†	16.8 ± 1.4†	
5 " " + "	15.2 ± .6†	17.3 ± .5†	14.9 ± .6†	
10 " " + "	14.6 ± .9†	16.1 ± 1.0†	14.4 ± .4†	16.2 ± .9†
2.5 IU HCG + 0.5 ml anti-HCG			14.8 ± 1.1†	
5 " " + " "			16.7 ± 1.2†	
10 " " + " "			16.3 ± 1.2†	
2.5 IU HCG + saline	22.3 ± 2.4	20.7 ± 1.5	22.7 ± 1.2	
5 " " + "	27.5 ± 2.0	26.4 ± 1.3	25.2 ± 1.1	
10 " " + "	31.2 ± .9	35.5 ± 1.5	29.4 ± .8	34.5 ± 1.2
2.5 IU HCG + control ascites‡	19.1 ± 1.1	20.8 ± 2.6	22.7 ± 2.4	
5 " " + " "	29.5 ± 2.4	24.5 ± 3.1	27.8 ± 1.2	
10 " " + " "	31.5 ± 1.9	36.6 ± 2.4	27.8 ± 1.2	
Anti-HCG + saline		16.1 ± 1.3	16.0 ± .8	17.5 ± .4
Control ascites + saline	16.1 ± .8	19.3 ± .9	16.2 ± 1.1	
Saline + saline	16.6 ± 1.0	17.9 ± 1.1	17.0 ± 1.3	16.0 ± .8
Anti-HCG + rat pit. homog.				40.6 ± 3.1
Anti-HCG + SAP prep.				77.2 ± 5.6
Rat pit. homog. + saline				38.2 ± 2.1
SAP prep. + saline				68.5 ± 1.9

* 5 rats in each assay group.
† Significantly less than HCG-treated group, $p \leq .01$.
‡ 1 ml unless specified.

Discussion. The data presented demonstrate that mouse ascites fluid is a satisfactory source of gonadotrophin antibodies. The anti-SAP ascites fluid contained antibody capable of completely blocking the biological activity of rat pituitary gonadotrophin. Likewise, the anti-HCG ascites fluid completely inhibited the biological activity of as much as 10 IU of HCG. Anti-HCG ascites fluid did not inhibit the biological activity of either SAP powder or rat pituitary gonadotrophin.

The substance present in both control and SAP ascites fluid which augments the gonadotrophic response of rat pituitary homogenate was observed only in early collections. Other investigators studying the development of gonadotrophic anti-hormones in animal sera have reported similar progonadotrophic responses in the early part of the immunization period(13-17). No satisfactory explanation has been given for this progonadotrophic phenomenon. In our experiments augmentation occurred despite the fact that the ascites and pituitary homogenate were injected at different sites. Since both control and SAP ascites fluid augmented the pituitary homogenate, the progonadotrophic response was not dependent on the presence of hormone antigen. It would seem that the assay animal's own pituitary is not involved in the augmentation since injection of control or SAP ascites fluid without pituitary homogenate had no effect on ovarian weight. Augmentation was not observed with anti-HCG ascites fluid; antibody activity was already present in the first collections. According to Snook and Cole(17) antiserum to HCG rarely gives a progonadotrophic response with its antigen.

The major difficulty of this method for obtaining antibody is that not all mice produce abundant ascites fluid. The onset of production and the amount of ascites fluid formed is variable among mice, and production is not always continuous. In our experiments about 60% of male and 90% of female mice eventually produced some ascites fluid, with about half of this number being consistent producers. Munoz(1) reported that about 50% of his mice developed ascites fluid, while other workers(5-8) have reported that nearly all inoculated mice developed ascites fluid, although the volume was extremely variable.

Similarly, the onset of antibody synthesis is variable among mice. Antibody in some mice appeared as early as day 25, although

in the majority it was not present until after day 40. Once antibody appeared in the ascites fluid it continued to be present in subsequent collections up to day 67, when the experiments were terminated.

The advantages of this method of obtaining antibody are obvious. The method is simple, uses an inexpensive animal from which fluid can be obtained repeatedly, and allows the production of a satisfactory amount of antibody within a relatively short period of time. Qualitatively, mouse peritoneal fluid is very similar to mouse serum and is as concentrated a source of precipitating antibody as is mouse serum(3).

Hiramoto(4) has reported that only 2-3 mg of total antigen is required per mouse for immunization, which is an obvious advantage compared to the amount required for the rabbit. The amounts of antigen given in our experiments, therefore, may have been higher than necessary for antibody production.

Summary. Antibodies to a sheep anterior pituitary (SAP) preparation and human chorionic gonadotrophin (HCG) have been developed in mouse ascites fluid. The ascites fluid was biologically assayed by the rat ovarian weight response to concurrently injected gonadotrophin. The anti-SAP ascites fluid contained antibody capable of completely blocking the biological activity of rat pituitary gonadotrophin. Likewise, the anti-HCG ascites fluid completely inhibited the activity of as much as 10 IU of HCG. Anti-HCG ascites fluid did not inhibit the biological activity of either SAP powder or rat pituitary gonadotrophin.

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Induction of Hypothermia by Dimethyl Sulfoxide in Rats Exposed to Cold: Tissue and Enzyme Changes. (31623)

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Recently we reported that injection of rats with dimethyl sulfoxide (DMSO) before exercise in a rotating cage markedly increased changes in serum enzyme levels attributable to exercise and increased fatty deposits in the heart, liver and striated muscle(1). Although DMSO apparently affected membrane permeability, the precise mechanism of such an effect was not known. We thought that further studies on the effects of DMSO on rats subjected to other stresses might clarify the nature of its effect on membranes. Exposure to cold was chosen for such a study.

After intraperitoneal injection of DMSO