Production of Gonadotropin Antibodies in Rhesus Monkeys (*Macaca mulatta*)[1] (34485)

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Production of antibodies against gonadotropic hormones has been demonstrated in a variety of mammalian species (1, 2). Some of these animals, especially rabbit and horse, have been used primarily as a source of antigonadotropic serum. The effect of active as well as passive immunization on the reproductive organs and sexual behavior has been more thoroughly studied in the rat (3–6). Published reports on the effects of antigonadotropins in the monkey are limited and mostly relate to immunization with pregnant mare's serum (PMS) (7–9). In the present investigation female rhesus monkeys were injected with a sheep anterior pituitary preparation to determine the effect of active immunization on the reproductive system. The extent of cross-reactivity of the resulting antihormone was also studied, and an attempt was made to define the stage at which the antigonadotropins appear in the blood during the period of immunization.

Materials and Methods. Five female monkeys were used for immunization with a sheep anterior pituitary (SAP) preparation[2] (10). The SAP powder was dissolved in physiological saline (4 mg/0.5 ml) and injected subcutaneously. Three monkeys (F62, 14, and 25) were injected daily with 4 mg of SAP. One monkey (10) was injected with the same daily dose but on alternate days while monkey 21 received 4 mg SAP emulsified with 0.5 ml of complete Freund's adjuvant[3] every fifth day.

Blood was drawn into a heparinized syringe from the femoral vein and was centrifuged immediately and the plasma frozen.

Changes in sex skin color of the treated monkeys were evaluated and graded from 1+ to 4+ by use of a series of color standards. All animals were checked daily for menstruation, and any uterine bleeding recorded on two or more consecutive days was regarded as menstruation. Laparotomies were performed at two different stages during the treatment period. At the time of laparotomy the ovaries and the uterus were measured in three directions; viz., length, width and height; and the ovaries were transilluminated and the approximate number and size of the follicles recorded.

The antigonadotropin (anti-SAP) activity of monkey plasma was determined by a bioassay based upon the inhibition of ovarian and uterine weight increase to injected gonadotropins in 20-day-old Holtzman rats. Plasma was administered in six subcutaneous injections given over a period of 3 days. The last five injections of the plasma were given concurrently with SAP solution so that each assay rat received the total amount of the antigen over 2½ days.

The biological cross-reactivity of anti-SAP in the monkey was measured by testing the plasma from immunized monkeys against FSH,[4] HCG,[5] pregnant mare's serum gonad-

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[2] Obtained from Dr. W. H. McShan, Dept. of Zoology, University of Wisconsin. Yield of SAP powder 5.11 mg/g fresh tissue. Extraction was carried through Sephadex G-100 fractionation of Step 2 of the procedure in the appropriate reference.

[3] Obtained from Difco Laboratories.

[4] Obtained from Endocrinology Study Section, NIH.

[5] Obtained from International Hormones, Inc.

TABLE I. Effects of SAP Injections on Ovarian and Uterine Size.

Monkey no.	Day[a]	Size (mm)[b]		
		Right ovary	Left ovary	Uterus
F62	13	10 × 6 × 4	11 × 6 × 4	12 × 9 × 6
	30	7 × 4 × 4	7 × 3 × 4	10 × 7 × 5
14	12	19 × 15 × 15	18 × 14 × 14	19 × 15 × 15
	29	10 × 6 × 4	9 × 8 × 7	14 × 14 × 9
25[c]	12	10 × 6 × 5	—	14 × 15 × 9
	29	8 × 4 × 4	—	13 × 10 × 6

[a] Day 1 is the day of first injection.
[b] Length × width × height.
[c] Left ovary removed.

otropin (PMS),[6] and an acetone-desiccated powder of monkey pituitaries. All hormones except PMS were injected in a manner similar to that used for the SAP solution. PMS was given in a single injection to assay rats on Day 21 of life. The monkey plasma and the gonadotropins were always injected subcutaneously at different sites. The rats were killed by cervical dislocation during the day after the last injection, and the ovarian and uterine weights were recorded.

Results. Effect of SAP on the reproductive organs of monkeys. Tables I and II and Fig. 1 summarize the changes in the ovaries, uterus, and sex skin of monkeys actively immunized with SAP.

Monkey F62 was an immature animal (30 months old) and consequently had not yet begun to cycle. This animal responded to SAP treatment as indicated by the considerable development of the sex skin, increase in the intensity of its color, and the swelling of the vulva. The sex skin color was maximum on Days 9 and 10. However, with continuation of SAP injections, there was a gradual waning of the sex skin color and by Day 15 of treatment the sex skin color had regressed to 1+. This was accompanied by bleeding beginning on Day 16 and a decrease in size of uterus and regression of ovaries and follicular development.

In monkey 14 SAP injections were begun

TABLE II. Effect of SAP Injections on Ovarian Follicles.

Monkey no.	Day[a]	No. of follicles[b]						Change in follicle size and number
		Right ovary			Left ovary			
		L	M	S	L	M	S	
F62	13	0	2	5	0	3	9	
	30	0	0	5	0	0	6	Decrease
14	12	2[c]	0	≥10	1[c]	5	≥10	
	29	0	0	≥10	0	0	≥10	Decrease
25	12	0	2	≥10	—	—	—	
	29	0	0	≥10	—	—	—	Decrease

[a] Day of first injection is regarded as Day 1.
[b] L = 4 mm or more in diameter; M = >1 mm <4 mm in diameter; S = 1 mm or less in diameter.
[c] Cystic follicle.

[6] Antex Leo, obtained from Henley and Co., Inc.

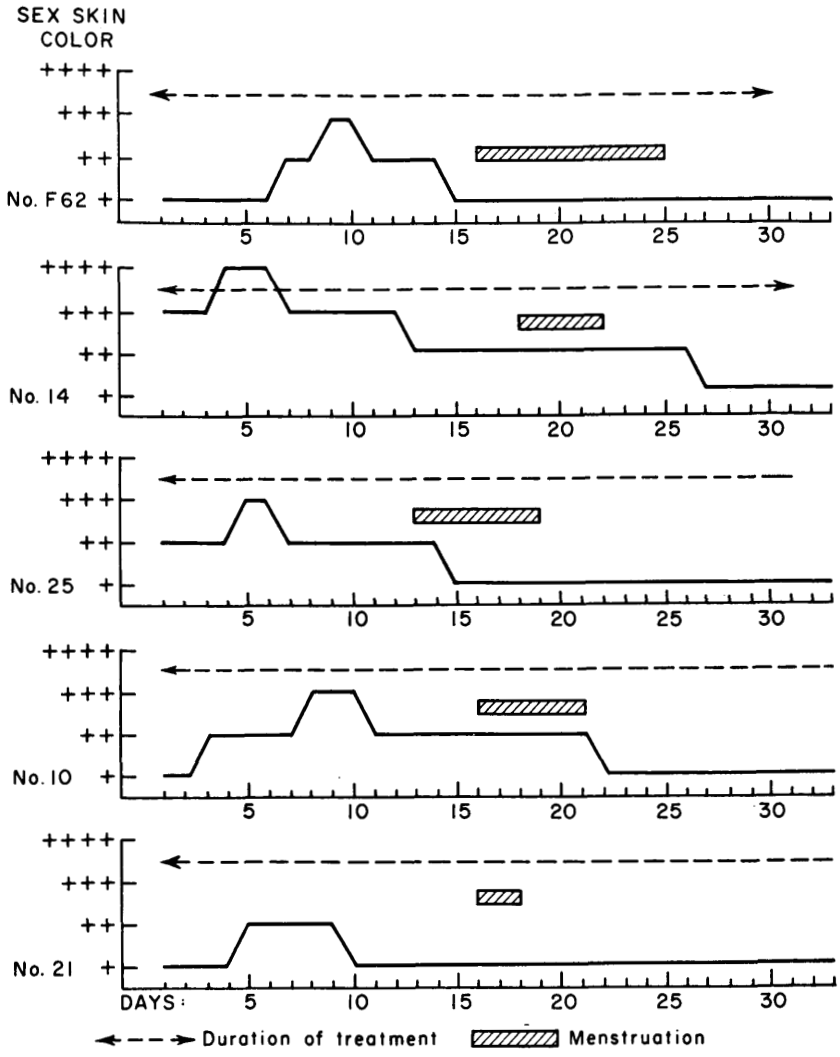

FIG. 1. Changes in sex skin color and incidence of uterine bleeding in SAP-treated monkeys.

on Day 13 of the menstrual cycle. The sex skin color at this time was 4+, and it was after Day 26 of treatment that the sex skin color showed a regression to 1+. Ovaries of this animal examined on Day 12 of treatment possessed cystic follicles ranging from 4 to 10 mm in diameter. However, by Day 29 no evidence of cystic follicles was evident, and the size of the ovaries was greatly reduced.

Treatment in monkey 25 was started on Day 25 of the cycle. An increase in sex skin color was noted on Day 5 of treatment but this lasted for only 2 days and gradually regressed to 1+ by Day 15. Uterine bleeding occurred from Day 13 to Day 18. The ovary and uterus examined on Day 29 were definitely regressed as compared to their condition on Day 12 of treatment.

Monkey 10 received the first injection on Day 8 of the cycle. The pattern of sex skin changes and the onset of menstruation were essentially the same as in monkey 25.

Monkey 21, although more than 3 years old, had never shown any indication of a menstrual cycle. Administration of SAP in Freund's adjuvant caused a slight increase in

TABLE III. Anti-SAP Activity of Plasma from SAP-Immunized Monkeys (14 and 25).

Treatment[a]		Day[b] —	0	7	14	21	28
				Ovarian weight (mg)[c]			
SAP[d]		26.7 ± 0.3					
SAP + plasma[e]	(14)		24.0 ± 2.0	42.0 ± 3.2[f]	44.0 ± 1.5[f]	29.0 ± 1.5	15.0 ± 2.5[f]
SAP + plasma	(25)		23.7 ± 1.2	21.7 ± 1.2	46.7 ± 2.6[f]	20.3 ± 0.8[f]	14.7 ± 2.1[f]
No treatment		15.0 ± 2.0					
Plasma	(14)		15.3 ± 1.2		12.3 ± 0.8		10.3 ± 0.8[g]
Plasma	(25)		12.7 ± 0.3		13.0 ± 1.1		10.3 ± 0.3[g]
				Uterine weight (mg)[c]			
SAP		96.7 ± 2.9					
SAP + plasma	(14)		77.0 ± 2.5[f]	145.0 ± 8.3[f]	145.0 ± 3.2[f]	119.3 ± 7.5[f]	20.7 ± 1.2[f]
SAP + plasma	(25)		91.7 ± 2.0	89.7 ± 9.2	137.3 ± 5.6[f]	141.3 ±12.8[f]	25.0 ± 2.0[f]
No treatment		31.7 ± 2.9					
Plasma	(14)		28.7 ± 1.3		28.0 ± 0.5		23.3 ± 1.8[g]
Plasma	(25)		30.0 ± 1.5		30.3 ± 3.2		24.7 ± 1.6[g]

[a] Three rats in each group.
[b] Day of blood collection.
[c] Expressed as mean ± standard error.
[d] Dose of 0.8 mg.
[e] Dose of 1.8 ml.
[f] Significantly different from SAP group. $p < .05$.
[g] Significantly different from No-treatment group. $p < .05$ [least significant difference (lsd) test was used to determine the significance of differences between the means of organ weights (14)].

sex skin coloration lasting from Day 6 to Day 11. Bleeding was observed on Days 17 and 18.

Biological assay of plasma from immunized monkeys. Antigonadotropic activity was absent in all plasma samples collected during the third week of immunization. Plasma obtained during the fourth week of immunization, however, invariably possessed anti-SAP properties. The anti-SAP activity of the plasma of monkeys 14 and 25 at various stages of immunization is summarized in Table III. A dose of 0.8 mg of the antigen was selected for the assay since it was the minimum amount which caused a significant increase in the weight of the uterus and the ovary. It was evident at the end of the fourth week of immunization that the plasma of all monkeys completely inhibited the uterine and ovarian stimulation induced by the antigen. It is also noteworthy that plasma collected during the early period of immunization caused a significant increase in the uterine and ovarian weights above that of the rats treated with SAP or SAP + plasma obtained prior to immunization (progonadotropic effect). The results of the biological cross-reactivity tests of anti-SAP plasma with gonadotropins from different sources are summarized in Table IV. One and eight-tenths milliliters of immune plasma completely inhibited the ovarian and uterine response produced by different gonadotropins.

Discussion. Antigonadotropic activity of the plasma was not detectable during the third week of immunization, but the plasma collected during the fourth week prevented the gonadotropic activity of the antigen. The time of appearance of antigonadotropin in the circulation appeared fairly constant.

A diminution of the sex skin color, decrease in the size of the uterus and ovaries, and disappearance of larger ovarian follicles in the immunized monkeys at the time of the appearance of antibodies in the blood suggest a cross-reactivity of the anti-SAP with the monkey's endogenous gonadotropins. Similar changes in the reproductive organs of

TABLE IV. Antigonadotropic Activity of Anti-SAP Plasma.[a]

Treatment[b]	Ovarian wt (mg)[c]	Uterine wt (mg)[c]
0.8 mg SAP	31.0 ± 1.1	94.7 ± 3.7
0.8 mg SAP + plasma[d]	9.3 ± 0.8	21.0 ± 1.0
300 μg FSH	34.3 ± 0.8	129.3 ± 3.1
300 μg FSH + plasma	9.7 ± 0.6	22.7 ± 0.3
2.5 IU HCG	19.3 ± 0.8	144.0 ± 2.0
2.5 IU HCG + plasma	12.7 ± 0.8	27.3 ± 4.3
10 IU PMS	30.0 ± 1.0	97.0 ± 2.3
10 IU PMS + plasma	13.0 ± 0.5	26.0 ± 2.3
6 mg monkey pit. prep.	21.3 ± 0.9	118.0 ± 2.3
6 mg monkey pit. prep. + plasma	11.3 ± 0.9	31.3 ± 1.8
No treatment	13.7 ± 1.2	34.7 ± 1.7

[a] Pooled plasma from monkeys 10 and 21.
[b] Three rats in each group.
[c] Expressed as mean ± standard error.
[d] Dose of 1.8 ml.

female rhesus monkeys have been reported by Selkurt (11) who injected antigonadotropic sera produced in the pony against sheep pituitary glands. In the present study the cross-reactivity of anti-SAP with monkey gonadotropin was further confirmed by direct bioassay of the immune plasma against a monkey pituitary preparation. It is of interest that in monkey 21, which received the antigen-adjuvant mixture, the initial sex skin response was very slight and the uterine bleeding lasted for only 2 days. The relatively slight gonadotropic response in this animal was presumably due to small amounts of antigen administered. This, however, did not affect the time of appearance of antigonadotropic activity in the blood or the gonadotropin-neutralizing capacity of the plasma. It is probable that high titers of antihormones can be developed by smaller amounts of antigen than employed for most of the monkeys in this study, if the antigen is injected with adjuvant.

The immunological cross-reaction of antigonadotropins has been reviewed by Geschwind (1). The anti-SAP activity developed in the monkey exhibited a range of cross-reactivity with gonadotropins of diverse origins. Comparable results have been obtained by immunizing horse, mice, and rabbits with sheep pituitary preparations (2, 11, 12). Antihormones produced against sheep gonadotropins tend to be more species aspecific (1) than those produced against crude human pituitary preparations (13) or pregnant mare's serum gonadotropin (8). It may be postulated, therefore, that species specificity of a particular antigonadotropin is mainly dependent on the source of antigen rather than the degree of its purification.

The progonadotropic activity of the plasma obtained during the early part of immunization in monkeys has been observed for other species by various workers (3, 12). Geschwind (1) has suggested the possibility of the formation of an incomplete antibody which, through its association with gonadotropins, retards the breakdown and excretion of the latter thus bringing about an augmentation of the gonadotropic activity of the antigen.

Summary. Five female monkeys were immunized to a sheep anterior pituitary (SAP) preparation. The antigonadotropic activity of the plasma was assayed by neutralization of rat ovarian and uterine response to simultaneously injected antigen. Antigonadotropic activity appeared during the fourth week of immunization and was accompanied by a marked regression in the sex skin color and decrease in the size of uterus and ovaries. The anti-SAP developed in the monkey showed a biological cross-reactivity with ovine FSH, HCG, pregnant mare's serum gonadotropin (PMS), and a monkey anterior pituitary preparation. A progonadotropic effect of plasma was evident during the early part of immunization.

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