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Anti-Gonadotropic Substances in Man Following Treatment with Pregnant Mare Serum.*

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The appearance of hormone antagonists in the blood serum as a result of treatment with hormone preparations was discovered by Collip and Anderson.[1] Although preparations of gonadotropic substances from the anterior pituitary gland, pregnant mare serum and human pregnancy urine have been used extensively in the clinics, little consideration has been given to the demonstration of inhibitory substances appearing in the human. The presence of inhibitory substances in the serum following injections of thyrotropic preparations has been reported[2] and similar findings were obtained in 9 patients, with undescended testes, following treatment with massive doses of pregnant mare serum.[3]

The purpose of this study was to determine whether anti-gonadotropic substances appeared in the blood of female patients undergoing treatment with pregnant mare serum gonadotropin (PMS). We wish to express our appreciation to Dr. C. L. Buxton for making this work possible by placing patients from the clinic of the Sloane Hospital for Women at our disposal.

Three women, age 22 to 34, suffering from sterility which was accompanied by dysmenorrhea in 1 case, were treated with a total of 4,800 to 8,900 r.u. (Cole-Saunders) of PMS (Gonadin†) during a period varying from 4 months to over a year. In this group the injections were given only during the middle of the menstrual cycle in an attempt to induce ovulation. One patient with amenorrhea of 10 years' duration served as an uninjected control.

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1 Collip, J. B., and Anderson, E., *Lancet*, 1934, **1**, 76.

2 Collip, J. B., and Anderson, E., *J. Am. Med. Assn.*, 1935, **104**, 965.

3 Rowlands, I. W., and Spence, A. W., *Brit. Med. J.*, 1939, **2**, 947.

† Mare serum hormone "Gonadin" was generously supplied by Dr. Donald Wonder, Cutter Laboratories, Berkeley, California. An international unit of mare's serum gonadotropin is the equivalent of 0.25 mg of the international standard. The Cole-Saunders rat unit is said to be the equivalent of 0.5 mg or twice that of the international standard.

Two male patients who were treated for hypogonadism received a single injection of 200 r.u. of PMS each week for 7 and 9 weeks.‡

As test animals, 21-22-day-old female mice were used. All test mice received a total of 5 r.u. of PMS (Gonadin) and, at a different site, 0.9 or 1.0 cc of the serum to be tested. Injections were made subcutaneously once daily for 3 days and the animals sacrificed 72 hours after the first injection. The weights of the ovaries and uteri were obtained, the latter after removal of intra-uterine fluid. In all, the blood from 7 patients was analyzed for anti-gonadotropic activity. The blood was kept in the refrigerator and the serum test started 24-48 hours after the blood sample was taken.

The results are summarized in Table I, where it is shown that 0.9 or 1.0 cc of serum from patients Nos. 1, 2 and 3 completely inhibited the gonadotropic action of 5 r.u. of PMS. These patients had received a total of 4,800 to 8,900 r.u. during a period of as

TABLE I.

Anti-gonadotropic Activity of Serum from Individuals Following Mare Serum Hormone.

Patient (age)	Condition	Treatment	No. mice	Injections PMS	Injections Serum	Avg ovar. wt mg	Avg uter. wt mg	Neutralization
1 ♀ (25)	Steril.	2/10/39-4/5/40 8,900 r.u.	4	5	1.0	3.2	8.6	Complete
			3	5	0.9	2.8	18.9	"
2 ♀ (22)	Steril. dysmenorrhea	6/21/39-3/1/40 7,200 r.u.	7	5	0.9	2.4	6.3	"
3 ♀ (34)	Steril.	Oct.-Dec. 2,400 r.u. Jan.-Feb. 2,400 r.u.	5	5	0.9	2.6	7.5	"
4 ♀ (21)	Steril. dysmenorrhea	4/17-5/5 1,000 r.u.	2	5	0.9	5.4	35.0	Partial
7 ♀ (26)	Amenorrhea	None	4	5	0.9	9.0	32.1	None
5 ♂ (40)	Hypogonadism	7 wk injections 200 r.u. each	6	5	0.9	11.5	33.2	"
6 ♂ (31)	Hypogonadism	9 wk injections 200 r.u. each	6	5	0.9	6.5	49.6	Partial
		Control Experiments.						
			26	5	None	9.2	34.1	
			10	None	"	3.1	7.9	

‡ We wish to thank Dr. G. W. Fish for placing the male patients at our disposal.

long as a year. These inhibitory substances remain in the blood long after cessation of treatment; blood was collected 4 months after the last injection (Patient No. 3), and yet 0.9 cc of serum completely inhibited 5 r.u. of PMS.

Patient No. 4 (sterility accompanied by dysmenorrhea) received a total of only 5 injections (1,000 r.u.) of PMS, given over a 3-week period. Inhibitory substances were definitely present in the blood 10 weeks after the first treatment.

Patient No. 7 received no treatment and antigonadotropic substances were not detected.

Treatment with Synapoidin,§ following PMS administration, did not interfere with the production of anti-serum to PMS in the one case (No. 1) tested. Furthermore, anti-gonadotropic activity to the Synapoidin was not evident 6 weeks after the first of 14 daily injections.

Summary and Conclusions. The injection of gonadotropic substances of equine origin elicits the production of anti-gonadotropic activity in man. It has been reported previously[3] that daily or biweekly administration of PMS caused anti-gonadotropic serum formation. The data reported here demonstrates that PMS treatment extending over a period of months will build up anti-gonadotropic activity despite the fact that hormone therapy involved only a few injections (4-5) at each mid-period of the menstrual cycle. Furthermore, this activity persists for as long as 4 months after the cessation of treatment.

Definite anti-gonadotropic activity was evident in the serum of patients receiving shorter treatment. The time factor is of importance since anti-serum of the same potency resulted after 9 injections (200 r.u. each) given once a week and after 5 injections (200 r.u. each) administered in 3 weeks. The serum was tested 10 weeks after the first injection in each case.

It is interesting to note that inhibitory substances to human pregnancy urine extracts have never been detected in man,[4, 5, 6] whereas they have been shown to occur after treatment with heterologous extracts. This fact supports the contention that the inhibitory substances are antibody in nature, elicited by an extract of a different species, rather than anti-hormone to the injected active material.

§ Synapoidin consists of pregnancy urine plus Evans' pituitary synergist and was kindly supplied by Parke, Davis & Co.

4 Sulman, F., *J. Exp. Med.*, 1937, **65**, 1.

5 Spence, A. W., Scowen, E. F., and Rowlands, I. W., *Brit. Med. J.*, 1938, **1**, 66.

6 Dorff, G. B., *Endocrinology*, 1938, **22**, 669.