Augmentation of Human Chorionic Gonadotrophin by Plasma and Other Non-Hormonal Substances.* (20643)

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While studying the effects of chorionic gonadotrophin on the human testis, it was observed that the administered hormone could be readily detected in the patients' plasma(1). Furthermore, 24 hours after a single intramuscular injection, the hormonal activity accounted for in the patients' plasma appeared to exceed the total administered dose. Since the method used for assaying chorionic gonadotrophin involved injecting unaltered plasma in rats, it seemed likely that plasma, by some mechanism, enhanced the activity of the hormone as measured in the assay animals. Previous studies have demonstrated that *in vitro* addition of non-hormonal substances, such as eggwhite(2), lemon juice, heme(3), metallic salts(4) and insoluble hydroxides(5), can augment the activity of *pituitary* gonadotrophin preparations, and that these substances probably act by prolonging absorption from the site of injection(4). The experiments described in this report were performed to determine if the phenomenon of augmentation by non-specific materials might also apply to chorionic gonadotrophin and thus account for the surprisingly high levels observed in the plasma of men receiving the hormone.

Methods. Intact female Sprague-Dawley rats, 25 days old at the time of the first injection, were used for assaying chorionic gonadotrophin. All materials to be assayed were administered twice daily for 3 days. Twenty-four hours after the last injection the rats were killed and the uterus and ovaries were weighed. Two preparations of chorionic gonadotrophin were used: a commercial product, "A. P. L.",† and the International Standard.† The following materials were mixed with chorionic gonadotrophin to determine if they were capable of augmenting hormonal activity: unaltered human plasma, boiled plasma, rat serum, egg-white and polyvinylpyrollidone. The effect of plasma obtained from 3 normal men was compared with that obtained from 2 hypogonadal men, one having primary testicular failure with high urinary gonadotrophin levels, the other having hypopituitarism with undetectable gonadotrophins. Materials were prepared for assay by diluting with water so that the total amount administered to each rat was contained in 6 ml, and 1 ml was administered subcutaneously at each injection, except in experiments involving polyvinylpyrollidone, where the volumes were 1.2 and 0.2 ml, respectively. All preparations were kept at 4°C, except those mixtures that were first incubated at 37°C, as indicated in Table II.

Results. Assay of aqueous solutions of chorionic gonadotrophin: The effects of the commercial product "A. P. L." were entirely similar to results obtained with the International Standard (Table I). Compared with the uninjected controls, 0.5 international units elicited no significant change in uterine weight, 1.0 unit produced an approximate doubling of uterine weight, and 2.0 units gave a maximal response. With these doses, ovarian weight did not increase, but with larger doses of 5.0 to 50.0 units a gradual increase in ovarian weight was observed. The consistency of these dose-response relationships is evidenced by the close agreement of our results with those of others(6-8) who have used the uterine weight response of rats for assaying the International Standard. In view of these findings, uterine weight, rather than ovarian weight, was the criterion employed for measuring the biologic

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† The "A. P. L." used in these studies was supplied by Ayerst, McKenna and Harrison, Ltd. The "International Standard for Chorionic Gonadotrophin" was obtained from the U. S. P. Reference Standards, 4738 Kingsessing Ave., Philadelphia.

TABLE I. Assay of Aqueous Solutions of Chorionic Gonadotrophin.

Chorionic gonadotrophin				
Type	Internat. units	Uterine wt, mg	Ovarian wt, mg	No. of rats
Internat. standard for chorionic gonadotrophin	.5	27	9.3	12
	1.0	52	11.1	10
	2.0	121	12.4	20
Uninj. controls*	—	25	10.5	34
"A. P. L."	.5	36	11.4	12
	1.0	56	10.1	15
	2.0	107	12.1	16
	5.0	137	19.1	7
	10.0	110	21.5	4
	50.0	114	37.1	4
Uninj. controls*	—	30	10.4	27

* Values for uninj. controls also apply to data of Tables II and III.

activity of mixtures of chorionic gonadotrophin and augmenting substances. Accordingly, in the experiments recorded in Table II and III, amounts of materials were selected which produced uterine, but not ovarian, weight increases, and therefore only the values for uterine weight are tabulated.

Human plasma added to chorionic gonadotrophin produced a 5- to 10-fold increase in hormonal activity (Table II). The effects of the hypogonadal patients' plasma were no different than those of plasma samples obtained from normal individuals. When mixed with plasma, as little as 0.2 units of chorionic gonadotrophin produced maximal increases in uterine weight. When an adequate amount of hormone was used, quite small amounts of plasma sufficed to enhance hormonal activity, but when the amount of hormone was reduced below a certain level, increasing the amount of plasma failed to produce an effect. For example, the addition of 0.01 ml of subject L.'s plasma to 0.4 unit of hormone produced clearcut augmentation. With 0.2 unit of hormone, a definite effect was noted from 0.4 ml plasma, but not from 0.04 ml plasma. When the amount of hormone was reduced to 0.1 unit, no effect was observed from increasing the amount of plasma to as much as 3.0 ml.

Incubation of the hormone-plasma mixture for 4 to 20 hours at 37°C resulted in a further increase in potency in one experiment with subject E.'s plasma, but in 2 subsequent trials no effect was noted from incubation using plasma obtained from subjects L. and P. (Table II).

Boiled plasma, rat serum, eggwhite and polyvinylpyrollidone produced definite increases in activity of chorionic gonadotrophin, similar to the effects observed with untreated human plasma (Table III).

Augmentation was not observed when plasma and hormone were kept separate and administered at different subcutaneous sites. No increase in uterine weight occurred from the administration of a plasma-hormone mixture to rats castrated three days prior to the first injection.

Discussion. Although the ability of nonspecific materials to increase the activity of *pituitary* gonadotrophin preparations has been repeatedly demonstrated, most previous studies have failed to demonstrate augmentation of chorionic gonadotrophin. Thus, augmentation was not observed when substances such as plasma (2), heme (3), and zinc and copper salts (4) were added to chorionic gonadotrophin, although under the same experimental conditions these materials produced marked increases in activity of pituitary gonadotrophin preparations. Certain differences in experimental procedure may explain why augmentation of chorionic gonadotrophin, which was regularly observed in our studies, was not seen in earlier investigations. First, in most studies of the augmentation phenomenon, ovarian weight has been used for assaying both pituitary and chorionic gonadotrophins. Although a sensitive and useful

TABLE II. Augmentation of Chorionic Gonadotrophin by Human Plasma.

Patient	Plasma, ml	Chorionic gonadotrophin — Type	Chorionic gonadotrophin — Internat. units	Procedure °C	Procedure hr	Uterine wt, mg	No. of rats
L., normal ♂	.1	Internat. standard	.1	4		38	4
	3.0	for chorionic	.1	4		25	4
	.04	gonadotrophin	.2	4		30	4
	.4		.2	4		88	4
	.01		.4	4		67	4
	.04		.4	4		89	4
	.2		.1	37	4	25	4
	3.0		0	4		28	4
P., normal ♂	.1	A. P. L.	.1	4		40	4
	.2		.2	4		80	3
	.4		.4	4		155	4
	.1		.1	37	4–20	46	4
	.2		.2	37	4–20	54	4
	.4		.4	37	4–20	130	3
E., normal ♂	.1	"	.1	4		31	4
	.2		.2	4		29	6
	.5		.5	4		105	6
	.1		.1	37	4	34	4
	.2		.2	37	4	82	4
	.5		.5	37	4	148	4
H., hypopituitarism	.05	"	.05	4		34	4
	.1		.1	4		64	6
	.2		.2	4		132	4
	.5		.5	4		145	4
R., hypergonadotrophic hypogonadism	.3	"	.1	4		71	4
	.75		.25	4		116	4
	1.5		.5	4		140	4

TABLE III. Augmentation of Chorionic Gonadotrophin by Various Substances and Procedures.

Procedure	"Augmentor"	ml	Internat. units chorionic gonadotrophin ("A. P. L.")	Assay rats — Uterine wt, mg	Assay rats — No. of rats
Mix and store at 4°C	Polyvinylpyrollidone 25% in water	.2	.1	30	4
		.2	.2	65	4
		.2	.5	150	4
" " " " "	Raw egg white	.4	.2	57	4
		1.0	.5	102	4
" " " " "	Rat serum	.5	.5	72	6
		2.0	.5	138	4
Human plasma boiled 5 min., coagulum broken, mixed with hormone, stored at 4°C	Boiled human plasma (patient H.)	.5	.5	115	4
Plasma and hormone inj. separate subcut. sites	Human plasma (subject P.)	.5	.5	26	4
Assay rats castrated 3 days prior to inj.	" "	1.0	5.0	33	4

indicator of pituitary gonadotrophin activity, ovarian weight is an insensitive indicator for measuring chorionic gonadotrophin and even with large doses, increases in ovarian weight are not striking. In some studies, such large amounts of chorionic gonadotrophin were used

that almost maximal ovarian weight increases were produced, even without the addition of augmenting materials. Using a vaginal smear technic of assay, Pedersen-Bjergaard and Tønneson(9) have recently observed that the addition of colloidal materials to chorionic gonadotrophin would enhance its activity to the extent that a single injection would produce the effect of multiple injections of an aqueous solution of hormone. Thus, the choice of endpoint may account for failures to observe augmentation. Another important factor in interpreting studies of the augmentation phenomenon is the purity of the hormone preparations employed. With impure extracts, sufficient non-hormonal materials may be present to produce maximal augmentation and further enhancement of activity would not result from adding more "augmentor."

Since augmentation did not occur when plasma and chorionic gonadotrophin were kept separate and administered at different sites, the mechanism of augmentation is probably the same as that advanced for pituitary gonadotrophins, *i.e.* prolongation of absorption from the site of injection. Two other mechanisms might conceivably account for the enhancement of chorionic gonadotrophin activity by plasma: a synergistic action by minute amounts of endogenous gonadotrophin in plasma, and protection against inactivation (10). The first possibility seems unlikely since similar augmentation was observed with plasma from individuals having high, low, and normal endogenous gonadotrophin levels. Protection against inactivation would not seem to be an important factor, since aqueous solutions of the hormone are relatively stable, even when kept at 37°C.

Taking into account the phenomenon of augmentation, very small amounts of chorionic gonadotrophin can be detected in plasma. Considering that 0.2 unit of hormone mixed in plasma can produce a definite increase in uterine weight and that as much as 6.0 ml of plasma can be administered to the assay rats over a three-day period, it is possible to detect 0.03 units of chorionic gonadotrophin per ml of plasma. This is sufficiently sensitive to measure plasma levels of chorionic gonadotrophin in patients receiving therapeutic doses of the hormone(1).

Summary and conclusions. A 5- to 10-fold increase in the activity of chorionic gonadotrophin, as measured in rats, was produced by the *in vitro* addition of unaltered human plasma. Similar augmentation was observed when the hormone was mixed with boiled human plasma, rat serum, egg white and polyvinylpyrollidone. The augmenting abilities of plasma obtained from 2 hypogonadal individuals, the one having high, the other having low endogenous gonadotrophin levels, was no different than that of plasma obtained from normal individuals. Enhancement of hormonal activity did not occur when "augmentor" and hormone were kept separate and injected at different subcutaneous sites. It is concluded that the most likely mechanism of augmentation is prolongation of absorption from the site of injection.

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