solutions measured fluorometrically contained 1×10^{-2}–100 μg of glafenine/ml of solvent. All glassware should be free of fluorescent contaminants and, therefore, frequently rinsed with distilled water; quartz sample cells are preferably cleaned with nitric acid followed by distilled water.

Determination of Glafenine in Tablets—Ten tablets were weighed accurately, and the average weight was calculated. They were brought to a homogeneous fine powder in a mortar, and a quantity equivalent to 25 mg of glafenine was transferred into a 500-ml conical flask. About 450 ml of ether was added, and the mixture was stirred with a magnetic stirrer for 2 hr. After filtration on a paper filter, the filtrate was diluted to 500 ml with ether. Then 10 ml of this solution was diluted to 100 ml with ether.

An analogous standard solution was prepared by dissolving 25 mg of glafenine in 500 ml of ether; 10 ml of this solution was diluted to 100 ml with ether.

It is advisable to extract the tablets simultaneously with the preparation of the standard solution or during at least the same period to avoid incomplete extraction.

Pure ether was used as a blank solution. Fluorometric measurement was performed at 327-nm excitation and 400-nm emission.

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Gonadotropin-Inhibitory Contaminants in Partially Purified Pharmaceutical Preparations of Human Chorionic Gonadotropin

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Abstract □ Various commercial preparations of partially purified human chorionic gonadotropin, inactivated by heating, inhibited the uterine growth induced in immature mice with the same active gonadotropins as well as spontaneous uterine growth. The more purified preparations of chorionic gonadotropin failed to produce these effects after inactivation by boiling, suggesting that the inhibitory activity is not generated from gonadotropin by the procedure but may be related to some contaminant similar to the gonadotropin-inhibitory substance previously found in human urine.

Keyphrases □ Gonadotropin, human chorionic—various commercial preparations, presence of inhibitory contaminants □ Contaminants, inhibitory—presence in various commercial preparations of human chorionic gonadotropin □ Inhibitory contaminants—presence in various commercial preparations of human chorionic gonadotropin

Human chorionic gonadotropin (I) for clinical purposes is a concentrate of the urine of pregnant women containing several hundred to 1000 international units (IU)/mg. It is obtained by a procedure that may also extract the gonadotropin-inhibitory substance (II) reported previously (1–3). In the present work, the occurrence of II was studied in various commercially available brands of I and in highly purified preparations. Preliminary results were reported previously (4).

EXPERIMENTAL

Five preparations of human chorionic gonadotropin were studied: partially purified preparations Ia[1], Ib[2], and Ic[3] and highly purified preparations Id[4] and Ie[5], with an activity of 13,000 and 10,000 IU/mg, respectively. These materials were dissolved in distilled water (500 IU/ml), pH 5–6, and boiled under reflux in a water bath for 1 hr to inactivate the gonadotropin activity (3). The final volume was adjusted to the desired concentration by adding water.

Biological assays were done in immature mice, 7.5–10.0 g, of the Balb-c strain. Different doses of boiled I preparations were injected, alone or with unboiled I, at different sites or mixed in the same syringe. The total dose of boiled and unboiled I was given in five subcutaneous injections, 0.2 ml each, for 3 days. Necropsy was performed on the 4th day, 24 hr after the last injection.

The mice were killed with ether. The uteri were dissected clean of surrounding tissue, dried by blotting on filter paper, and weighed on a precision balance to the nearest hundredth of a milligram. The Student *t* test was used to compare the mean weights of the uteri obtained from the different experimental groups.

RESULTS AND DISCUSSION

The less pure preparations of I, with 1000 IU/mg, inactivated by boiling, inhibited the vaginal opening and uterine growth induced with unboiled I in immature mice (Table I). This effect appeared when both preparations were injected together or separately in different subcutaneous sites. No signs of toxicity were evidenced, and the growth of the treated and untreated mice was similar. The heat-inactivated I also in-

[1] Apoidine, Parke-Davis.
[2] APL, Ayerst Laboratories.
[3] Profasi, Serono, Italy.
[4] Lot E 231 TEBZ, Serono, Italy.
[5] Donated by Professor C. H. Li.

Table I—Antagonism between Boiled and Unboiled I on Uterine Growth in Immature Mice

Treatment	Open Vagina, %	Uterine Weight, Mean ± *SE*, mg	*n*	*p*
Unboiled I*a*, 0.125 I	100	19.05 ± 1.69	7	NS[a]
Unboiled I*a*, 0.125 IU, plus boiled I*a*, 0.125 IU	83	17.40 ± 2.34	6	
Unboiled I*a*, 0.25 I	100	26.67 ± 2.45	12	= 0.01
Unboiled I*a*, 0.25 IU, plus boiled I*a*, 0.25 IU	55	15.11 ± 2.28	9	
Unboiled I*a*, 0.50 I	100	41.50 ± 1.61	8	<0.01
Unboiled I*a*, 0.50 IU, plus boiled I*a*, 0.50 IU	50	22.50 ± 4.12	6	

[a] NS = not significant.

hibited spontaneous uterine growth (Table II). The more purified preparations of I, with 10,000 and 13,000 IU/mg, failed to produce this inhibition after inactivation by boiling.

According to these results, various preparations of I for clinical purposes contain a gonadotropin-inhibitory substance, apparently similar to that found in human urine previously (1–3). Previous investigators also reported an inhibition of the exogenous hormonal stimulation. In the present experiments, the material contained in different preparations of I also inhibited spontaneous sexual maturation, as shown by a delay in uterine growth.

The possibility that this gonadotropin-inhibitory substance might be generated from gonadotropin by the inactivation procedure was disproved by the fact that the more purified preparations showed no inhibitory activity after inactivation by heating. It seems more possible to relate II to some of the molecules contaminating I in the partially purified commercial preparations (5). One study (6) supported this contention. The urine of a patient with choriocarcinoma was concentrated by pressure dialysis, and a fourfold increase in the total amount of human chorionic gonadotropin activity of the sample was obtained, suggesting that the dialysis procedure eliminated an inhibitory contaminant of I.

The effect of II in humans is still unknown, but it may account for the variability of the biological activity of human chorionic gonadotropin preparations. Knowledge of an inhibiting impurity contaminating preparations of I might be an incentive to produce purer preparations.

Table II—Inhibitory Effect of Boiled Preparations of I on Uterine Growth in Immature Mice

Treatment, IU of Boiled I	Uterine Weight, Mean ± *SE*, mg	*n*	*p versus* Saline
Saline	6.03 ± 0.28	12	
I*a*, 0.125	5.42 ± 0.18	9	NS[a]
I*a*, 0.250	4.42 ± 0.25	8	<0.001
I*a*, 0.500	3.10 ± 0.43	8	<0.001
Saline	8.64 ± 0.98	19	
I*b*, 1.0	7.37 ± 0.55	12	NS
I*b*, 2.0	6.54 ± 0.94	6	NS
I*b*, 5.0	5.75 ± 0.47	6	<0.01
Saline	8.60 ± 0.53	6	
I*c*, 1.0	7.17 ± 0.49	7	NS
I*c*, 2.0	5.91 ± 0.44	7	<0.01
Saline	7.45 ± 0.40	10	
I*d*, 1.0	7.18 ± 0.94	5	NS
I*d*, 2.0	7.91 ± 0.90	5	NS
I*d*, 10.0	7.93 ± 0.94	6	NS
Saline	9.44 ± 0.90	10	
I*e*, 1.0	8.56 ± 0.66	6	NS
I*e*, 2.0	7.50 ± 0.53	6	NS
I*e*, 10.0	8.30 ± 0.58	6	NS

[a] NS = not significant.

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