very similar composition for the hormones from the two species. The recovery of residues of amino acids together with the hexose and hexosamine accounts for 70 % of the weight of sample; with beef material recoveries of 80–85 % were obtained (corrected for moisture and ash[1]). Although the low recoveries may be attributed to losses during hydrolysis the possibility of an unrecognized component in the thyrotropins exists. Analysis of other samples of sheep thyrotropins for sialic acids and tryptophan indicates that the two are not constituents of the molecule.

GRÖSCHEL AND LI[8] have recently reported on the carbohydrates of ovine follicle-stimulating hormone and interstitial-cell-stimulating hormone. In comparing the carbohydrate content of ovine thyrotropin with these hormones, a difference in hexoses is found in that the two gonadotropins contain galactose in addition to mannose; galactose is absent in the best preparations of sheep thyrotropin analysed. The 2.44 % sialic acid found in the follicle-stimulating hormone[8] is consistent with the differences in its electrophoretic behavior as compared to thyrotropin[2].

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Purification of chorionic gonadotropin from the urine of patients with trophoblastic tumors

Human chorionic gonadotropin can be detected in the blood and urine of pregnant women and also, in much larger amounts, in the blood and urine of patients* with such trophoblastic tumors as choriocarcinoma, hydatid mole, chorioadenoma destruens, and syncytial endometritis[1]. The gonadotropin titer of these patients can be used as an index of tumor growth or regression. Whereas the hormone from pregnancy urine has been purified and characterized[2], it has not been concentrated or purified thus far from the urine of patients with malignant tumors. We wish to report here

Abbreviations: Tris, tris(hydroxymethyl)aminomethane; DEAE-, diethylaminoethyl-.
* These patients are referred to collectively in this paper as "tumor patients".

studies on the purification of the hormone from the latter source, and some properties of the product.

Crude chorionic gonadotropin was obtained from the pooled urine of tumor patients by the kaolin adsorption method of ALBERT[3], and the concentrates were thoroughly dialyzed against distilled water and lyophilized. 1 g of this crude material, which gave an assay value of 150–200 international unite (I.U.)/mg by the mouse-uterine- weight method[4], was dissolved in 40 ml Tris–phosphate buffer, pH 8.6 (0.005 *M* phosphate, 0.04 *M* Tris) and placed on a DEAE-cellulose column (10 × 4 cm) which had been equilibrated with the same buffer. The applied material was fractionated by stepwise elution, keeping the pH constant while gradually increasing the salt concentration of the eluant with NaCl. The elution schedule and effluent diagram are shown in Fig. 1. Eluate was collected in 7-ml fractions at 3-min time intervals. Protein concentration of the effluent fractions was estimated by measuring their

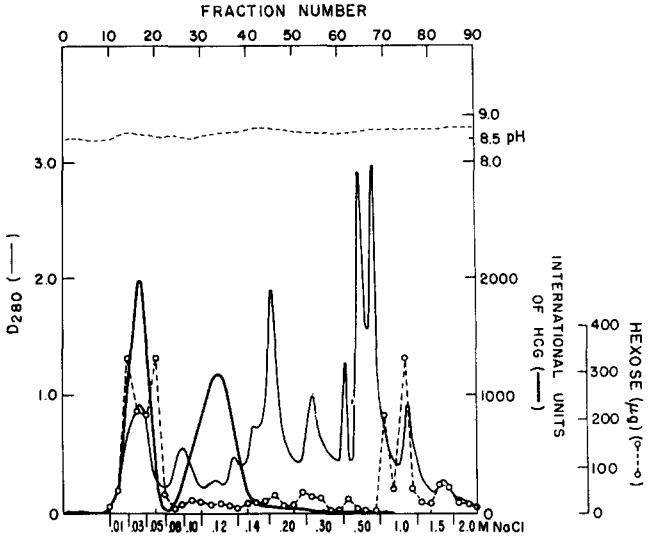

Fig. 1. Fractionation of crude chorionic gonadotropin by stepwise elution on DEAE-cellulose at pH 8.6.

absorption at 280 mμ. Hexose content was determined by the method of SHETLAR[5]. Gonadotropic activity was estimated by the ovarian hyperemia response in immature, female Sprague-Dawley rats[6].

Fig. 1 illustrates that most of the activity can be eluted from the column at a NaCl concentration ranging from 0.01 to 0.12 *M*. The bulk of inert proteins is thus separated from the active material. The effluent containing the hormone was practically colorless, whereas fractions eluted with 0.14 *M* NaCl and up showed a tan to dark-brown color. The appearance of color thus serves almost as a demarcation line. The effluent fractions containing most of the chorionic gonatropin activity were dialyzed and lyophilized. From 1.0 g of crude product (150–200 I.U./mg), we obtained 0.1 g of material with an activity of 1500 I.U./mg. Thus 90 % of inactive material was removed while most of the biological activity was retained. We called this preparation "A".

Preparation "A" (0.5 g) was dissolved in 10 ml 0.005 *M* phosphate buffer, pH 6.0,

and the solution placed on a DEAE-cellulose column (21 × 2.5 cm). The material was eluted with a straight-line gradient, keeping the pH constant while varying the NaCl concentration. This gradient was obtained by using two chambers of a nine-chamber multiple-gradient device described by PETERSON AND SOBER[7]. The first chamber contained 200 ml 0.005 *M* phosphate buffer, pH 6.0, 0.08 *M* NaCl and the second chamber 200 ml of the same buffer containing 0.3 *M* NaCl.

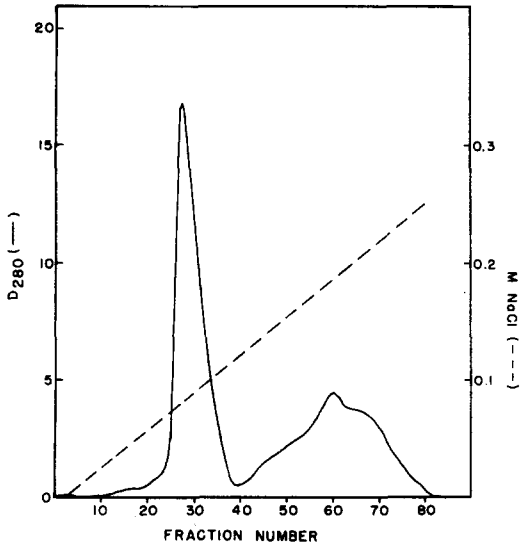

Fig. 2. Fractionation of purified hormone (Preparation "A") by gradient elution on DEAE-cellulose at pH 6.0.

Fig. 2 shows the effluent diagram obtained, the dotted line indicating the salt gradient. Eluate was collected in 5-ml fractions at 3-min time intervals. The bulk of activity was found between fractions 25 and 35. The potency of the product was enhanced about 10-fold. From 0.5 g of preparation "A", we obtained 0.05 g of preparation "A-1". Biological activity data, as determined by the mouse-uterine-weight assay, are summarized in Table I.

We also used a procedure which LEGAULT *et. al.*[8], applied successfully to purify pregnant-mare-serum gonadotropin[8]. The procedure is essentially an adsorption of inactive proteins on $BaCO_3$. The gonadotropin remains in the supernatant, which is then suitably treated to remove barium. Applying this procedure to preparation "A", we were able to increase the biological activity from 1500 I.U./mg to 10–12,000 I.U./mg. However, when this material was examined by moving-boundary electrophoresis it was heterogeneous, exhibiting three distinct components.

TABLE I

BIOLOGICAL ACTIVITY OF CHORIONIC GONADOTROPIN PREPARATIONS

Preparation	*Activity I.U./mg*	*Purification*
Crude	150–200	
"A"	1500	× 10
"A-1"	15000	× 100

Preparation "A-1" was examined by moving-boundary electrophoresis, applying a current of 20 mA and 150 V at pH's 8.5, 6.5, 4.0 and 2.0. After running for 120 min, we observed essentially one symmetrical peak. However, after 180 and 240 min, there was a pronounced skewing effect indicating heterogeneity. The isoelectric point of preparation "A-1", as estimated from electrophoretic mobility data, is between pH 3.8 and 4.0. Hexose content was determined by the cysteine–H_2SO_4

TABLE II

CHARACTERISTICS OF CHORIONIC GONADOTROPIN

	Trophoblastic tumors	*Pregnancy urine*
Activity I.U./mg	15,000	10,–12,000
N %	11.2	10.5
Hexoses %	16.2	11.0
Hexosamine %	6.4	8.7
Hexoses/hexosamine	2.5	1.26
Isoelectric point	3.8–4.0	2.95

method of DISCHE[9] and by the tryptophane–H_2SO_4 method of SHETLAR[5]. Hexosamine was determined by the ELSON–MORGAN procedure[10] and by the indole–HCl method of DISCHE[11]. Table II summarizes the data obtained and lists as a comparison results obtained by GOT[12] for chorionic gonadotropin isolated from pregnancy urine.

The data presented show the feasibility of obtaining highly purified chorionic gonadotropin from the urine of tumor patients by simple methods of DEAE-cellulose chromatography. A comparison of some of the physical-chemical properties of the hormone obtained from the pregnancy source and from the trophoblastic tumor source suggests a possible difference between the hormones obtained from the two sources. This conclusion must remain tentative, however, pending isolation of a preparation of established homogeneity from the tumor sources.

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