UPTAKE OF RADIOIODINATED HUMAN CHORIONIC GONADOTROPIC HORMONE BY OVARIAN CARCINOMA

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Labeling of human chorionic gonadotrophin (HCG) with 1–2 atoms of radioactive iodine does not affect the biological, immunological, and physiochemical behavior of the hormone (*1*). In the rodent the ovary has been demonstrated to concentrate HCG more than any other organ. Labeled albumin or ^{131}I are not concentrated by the ovary even when animals are stimulated simultaneously with unlabeled HCG (*1,2*). When increasing doses of ^{131}I-HCG, ^{131}I-albumin, and ^{131}I-sodium are injected, a concomitant increase in ovarian radioactivity is observed only in the animals receiving the labeled HCG (*3*).

When antiserum to HCG is injected 5 min before ^{131}I-HCG, the labeled hormone becomes bound to the antiserum as a hormone-antihormone complex and is not concentrated by the ovary. Autoradiography of histological sections of ovary after ^{131}I-labeled HCG administration in vivo reveals that the radioactivity is confined mainly to the follicular envelope, theca cells, and struma (*4*).

We wish to report that ^{125}I-labeled HCG concentrates as well in theca cell carcinoma of the ovary as in the normal ovary. These findings have stimulated us to investigate the possible use of ^{131}I-HCG for diagnosis of this lesion by in vivo scanning techniques.

MATERIALS AND METHODS

Hormone procurement. A highly purified preparation of HCG (Antuitrin-S*, 1,700 IU/mg) was employed in all phases of the present study. In addition, human growth hormone† (HGH) was used as a protein and trophic hormone control.

Radioiodination procedures. Both HCG and HGH were labeled with ^{125}I by the method of Hunter and Greenwood (*5*)*. The labeled hormones were freed from unbound iodine by gel filtration on Sephadex G-100. The ^{125}I-HCG was subjected to an additional filtration on a G-200 column to insure homogeneity of the radiopreparation. Radioactivity counts were measured in an ionization chamber. Pooled fractions of ^{125}I-HCG contained a specific activity of 78.42 μCi/μg and a protein concentration of 3.35 μg/ml, while the ^{125}I-HGH had a specific activity of 86.64 μCi/μg and a protein content of 1.94 μg/ml. The radiohormones were suspended in 0.05 *M* phosphate buffer stabilized with 1.0% bovine serum albumin. The free iodine (^{125}I) content of the stored radiopreparations was determined periodically by thin-layer radiochromatography.

Antibody production. The production of rabbit antibodies to HCG by the present investigators has already been described (*6,7*). In brief, HCG (500 IU/ml) was mixed with equal parts of Freund's complete adjuvant for antigen immunization. Mature male New Zealand rabbits were immunized with biweekly subcutaneous injections (0.5 ml of antigen-adjuvant mixture) for an 8-week period. The animals were bled and the serum obtained 14 days after the last injection.

Hemagglutination inhibition (HI). Sheep red blood cells preserved in Alsever's solution, were tanned with pyruvaldehyde for use in the hemagglutination inhibition (HI) method of Butt (*8*). The pyruvaldehyde-tanned red cells were sensitized with 200 μg of purified HCG for 1 hr at 50°C. Rabbit anti-HCG serum was first titrated in micro-Takasky plates to obtain a hemagglutination (HA) titer. Once the

* Purified HCG was kindly supplied to us by Merritt R. Callantine, Endocrinology Section, Parke-Davis, Ann Arbor, Mich.

† HGH was supplied through the courtesy of the Abbott Pharmaceutical Co., North Chicago, Ill.

Received July 1, 1971; revision accepted Sept. 10, 1971.

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* Radiolabeling and isolation procedures were performed by B. J. Green and Fred Jefferies, under the supervision of Jonathan Miller, Abbott Radiopharmaceuticals, North Chicago, Ill.

antiserum HA titer was achieved, known amounts of purified HCG were added to constant amounts of antiserum to obtain a HI titer. In this fashion the radiohormone preparations were analyzed for HCG concentrations.

Biologic activity. The ^{125}I-HCG was tested for biologic activity by the methods of Albert and Berkson (*9*) and Delfs (*10*). In the former method, ^{125}I-HCG (1.5 IU units) was injected i.p. into six immature female Wistar rats and the ovaries inspected for hyperemia 6 hr later. The Delfs procedure is based on the increase in uterine weight of immature female rats over a 72-hr period. In this method, ^{125}I-HCG in six equally divided doses was administered i.p. to six immature rats over a 48-hr period. The entire uterus of each animal was removed and weighed 24 hr after the last injection. The concentration of HCG in IU was obtained from a standardized graph. The two assay procedures were repeated using six rats, each injected with comparable amounts of ^{125}I-HGH.

Animal studies. Normal and tumor-bearing mice (both sexes) of the A7-(C57 × A)* strain, weighing 20–30 gm, were employed for radioisotope tissue-distribution studies. The tumor-bearing mice possessed either a granulosal or thecal cell tumor which had been implanted subcutaneously 1–3 months previous to the study. The mice received 30–40 μCi (0.5–1.0 μg protein) of either ^{125}I-HCG or ^{125}I-HGH intravenously at various time intervals from 48 to 1 hr before death to determine the time of optimum localization. At the time of sacrifice, 19 tissues were removed, weighed, and assayed for radioactivity in an automatic gamma well-counter (Nuclear-Chicago). Radioactivity of tissue samples was compared with that in an equal weight of blood taken from the same animal. Counts were accumulated for a period of time to insure less than 5% statistical counting error, and corrections were made for radioactive decay and for counting efficiency of the machine. Standards of the original solutions were made and counted identically to the tissues. Results were expressed as tissue-to-blood (T/B) ratios.

In addition to the studies in mice, normal young adult *Macacus mulatta* rhesus female monkeys (10–20 lb) were employed for similar studies. The monkeys were injected intravenously with 70–100 μCi of ^{125}I-HCG and sacrificed with an overdose of sodium pentothal 1–5 hr postinjection. Tissue samples were removed for autogamma counting as described for mice.

* The mice-bearing ovarian tumors were kindly supplied to us by W. U. Gardner, Department of Anatomy, Yale University.

Autoradiographic techniques (*11*) were applied to fixed sections of various tissues from the normal and tumor-bearing mice. The ovaries, tumors, liver, and kidney were fixed in Bouin's solution and the tissue sections cut at 4–6 microns in thickness. Tissue sections for autoradiography were stained in hematoxylin and eosin.

RESULTS

Radioiodination of HCG. Labeling of HCG with ^{125}I with subsequent passage through Sephadex G-100 resulted in the multiphasic curve seen in Fig. 1A. A shoulder (A) and two major peaks (B and C) were obtained in addition to the free iodine (^{125}I) peak. Fraction B, which possessed the greatest biologic activity, was filtered a second time through G-200; this yielded a product with one uniform peak which was called fraction B′ (Fig. 1B). All studies described below employed the B′ fraction of HCG.

Immunologic and biologic activities. The immunologic activity of ^{125}I-HCG was evaluated by the method of hemagglutination inhibition. With this assay method, the radiolabeled HCG concentration was found to be 30 IU/ml. Correspondingly, the biologic activity of ^{125}I-HCG as determined by the Delfs uterine weight assay was 24 IU/ml. Thus there was a close correlation between the immunologic

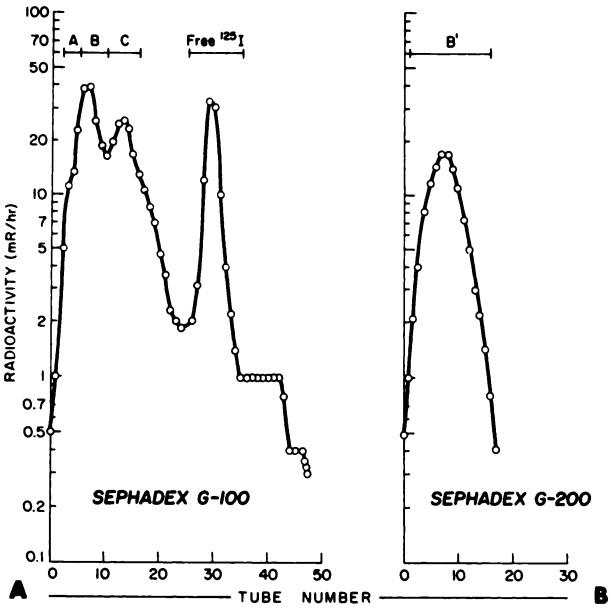

FIG. 1. In A antuitrin-S HCG (Parke Davis) was radiolabeled with ^{125}I and filtered through sepadex G-100. Three components (A, B, and C) in addition to free radioiodine were isolated as seen from plot of radioactivity versus effluent samples. Radioactivity (mR/hr was measured in ionization chamber. In B ^{125}I-HCG was subjected to additional filtration on G-200 to ensure homogeneity of radiohormone. One uniform peak resulted from second filtration.

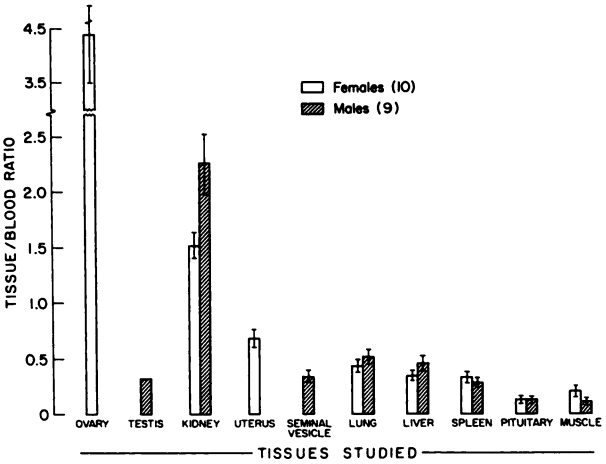

FIG. 2. Uptake of ^{125}I-HCG is demonstrated in various organs of normal male and female mice. Radioiodinated HCG is not concentrated by any tissue of either sex except ovary. Number of animals sampled is indicated in parenthesis.

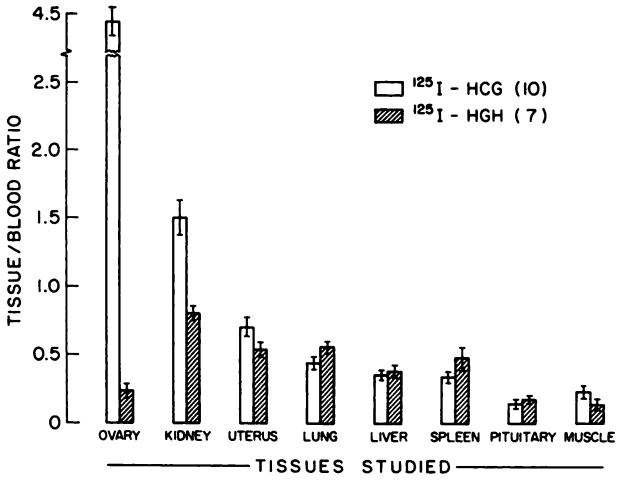

FIG. 3. Uptake of ^{125}I-HCG as function of ^{125}I-HGH is compared in normal female mice. Number of animals sampled is indicated in parenthesis. HGH is not specific for mouse ovary or other female tissues studied.

and biologic activity (immunologic/biologic activity ratio = 1.25) of the radioiodinated HCG. In the qualitative measurement of ^{125}I-HCG biologic activity, the gonadotrophic function of the radiohormone was consistently demonstrated by ovarian hyperemia in immature rats. It can also be noted that ovarian hyperemia could be completely suppressed by incubation of the ^{125}I-HCG with whole rabbit anti-HCG serum prior to injection into rats. The amount of serum required for hyperemic suppression was estimated from the results of the HI assay.

Tissue distribution in animals: Mouse studies. The optimum time of assay for the tissue radioactivity distribution was found to be 3.0 hr postinjection, and this time period was used for all normal and tumor-bearing mouse studies. The ^{125}I-HCG uptake in normal male and female mice is shown in Fig. 2. The ovary showed a higher concentration of radioactivity than any other organ. The ovary concentration at 3 hr postinjection was 4.5 times that of blood (mean value) and 8–12 times greater than the concentration of radioiodine by any other tissue studied except the kidney. The high concentration of radioactivity in the kidney presumably was related to the excretory function of this organ for ^{125}I-HCG and free iodine. The increased kidney levels in the male might be related to the absence of competing ovarian uptake in males. The use of ^{125}I human growth hormone (HGH) as a radiohormone control was compared with ^{125}I-HCG in Fig. 3. Human growth hormone is not specific for the mouse ovary or any of the other female tissues studied.

The ovarian thecal cell tumors approached the same range of concentration of ^{125}I from HCG as the normal ovaries (Fig. 4). The thecal tumors concentrated ^{125}I from HCG as much as 2.5 (mean value) times the blood and 6–10 times that of non-renal and nonovarian tissues. However, the granulosa cell tumors did not concentrate ^{125}I from HCG significantly more than did nonovarian tissues. On the other hand, when the control substance ^{125}I-HGH was given to mice-bearing thecal cell tumors, the radio-uptake in the tumor was not significant. The ^{125}I-HCG levels in the ovaries of mice with granulosa cell tumors was higher than in mice with theca cell tumors.

Tissue distribution in animals: Monkey studies. The concentration of the ^{125}I-HCG from 1 to 5 hr by various tissues of the rhesus monkey is shown in Fig. 5. The tissue distribution levels of ^{125}I-HCG in the monkey are similar to those of the rodent. However, the radio-uptake level in monkey ovary is less than in mouse ovary. Optimum radio-uptake was found at 2.0 hr. Uptake levels in the ovary were 1.2 times blood and 2.4–12 times that of nonovarian and renal tissues. The radioiodine uptake in the breast of the lactating female sacrificed at 5 hr was impressive. This may be attributed to the prolactin-like function of HCG.

Finally, the high pituitary uptake of ^{125}I-HCG in the monkey at 4 hr may be similar to that previously observed in the dog (*19*).

Tissue autoradiography. Autoradiographic analysis of the fixed tissue sections of the normal mouse ovary served to confirm the earlier observations of Eshkol and Lunenfeld (*3*). The radioisotope was more heavily concentrated in the thecal layers of the follicles and in the interstitium. In the theca cell tumors, granules were detected in heavy amounts overlying the thecal cell cytoplasm (Fig. 6). In con-

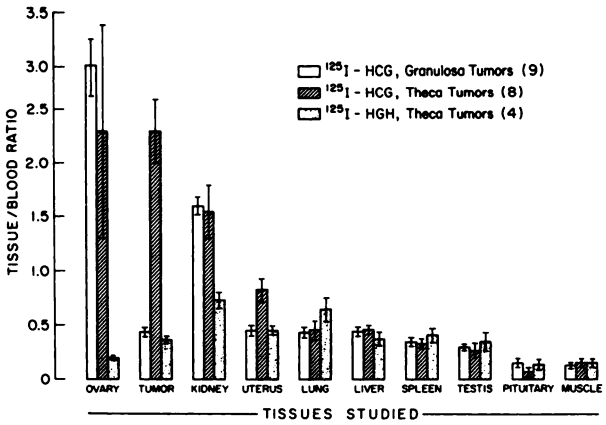

FIG. 4. Histogram displaying radioisotope concentration of ^{125}I-HCG by mouse ovarian thecal cell tumor. Radiolabeled HCG is not taken up by granulosal cell tumors. Uptake level seen in thecal cell tumors in mice given ^{125}I-HCG is comparable with that of ovarian level. Number of animals sampled is indicated in parentheses.

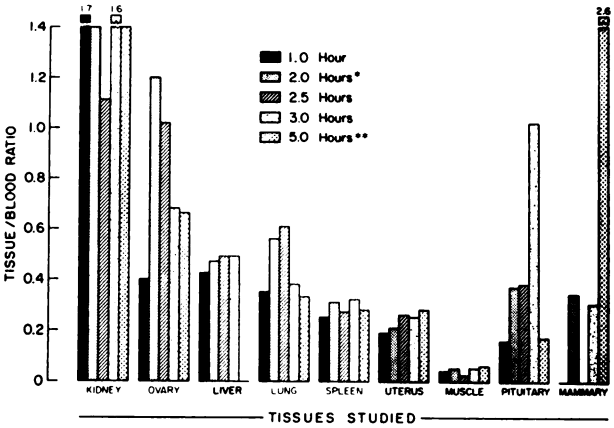

FIG. 5. Time study histogram demonstrating tissue radioactivity levels of ^{125}I-HCG in female rhesus monkey. One asterisk denotes monkey in menstruation; two asterisks signify lactating female. Each bar of histogram indicates one monkey study.

trast, the granulosa cell tumors displayed little isotopic concentration except in sinusoids and blood vessels (Fig. 7). The concentration of isotopic granules in the livers of mice and monkeys was unremarkable.

DISCUSSION

The observation of Eshkol and Lunenfeld (*1,3*) that radiolabeled HCG concentrates specifically in the ovary is important, although not surprising. Aschheim (*12*) made a similar, although indirect, observation in 1930 and since then HCG has been employed in various tests for pregnancy. However, the cell type in the ovary which is responsible for HCG concentration is not well established. Previous workers (*4,13*) have suggested that the thecal interstial or the stromal cell is the site of the ovarian response to HCG. These cells are comparable to the cells of Leydig in the male which are known to respond to HCG stimulation (*14,15*). In the present study, both granulosal and thecal cell tumors were challenged by exogenous radiolabeled HCG in the mouse. The thecal tumors concentrated ^{125}I HCG at levels approaching those of the normal mouse ovary while the granulosal tumors did not. Thus the present report provides direct evidence that the thecal cell is the target of HCG in the rodent ovary.

The mechanism of HCG cell stimulation is still somewhat obscured. The ultimate result of gonadotrophic stimulation is an increased production of specific steroid hormones. During pregnancy, HCG stimulates the ovary to produce certain hormones that the young placenta is incapable of synthesizing. HCG, similar to LH, is thought to be the first messenger of a three-messenger system (*16,17*). This gonadotrophin presumably activates adenyl cyclase in the target cell membrane which then produces a second messenger—cyclic 3′, 5′-adenosinemonophosphate (AMP). Cyclic AMP then somehow participates in the biosynthesis of a specific steroid or group of steroids—the third messenger. HCG is used in the cell for only a short period and is excreted rapidly in the monkey and in the mouse. The biological half-life of HCG has been studied in humans by Midgley and Jaffe using the radioimmunoassay technique. It has two linear components, the first corresponding to a half-life of 8.9 hr and the second to a half-life of 27.2 hr (*18*).

The concentration of ^{125}I from ^{125}I-HCG in thecal cell carcinoma of the ovary in mice has encouraged us to label HCG with ^{131}I and give it to women with metastatic thecal cell carcinoma of the ovary. The specificity of the uptake of this labeled stimulating hormone for its target organ is relatively unique. The

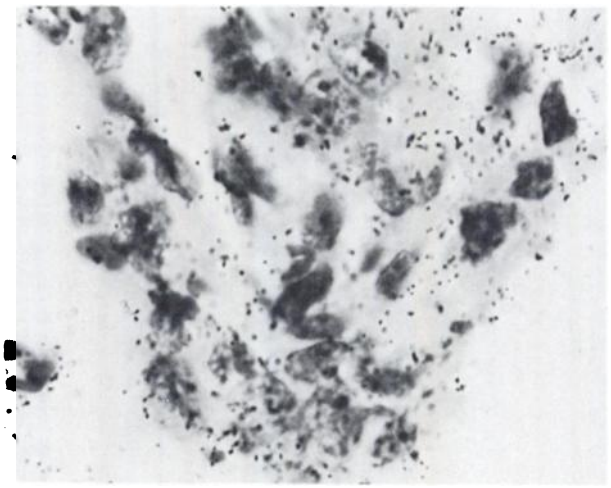

FIG. 6. Tissue autoradiogram displaying dense isotopic granule distribution overlying mouse theca cell tumor, 400X, oil.

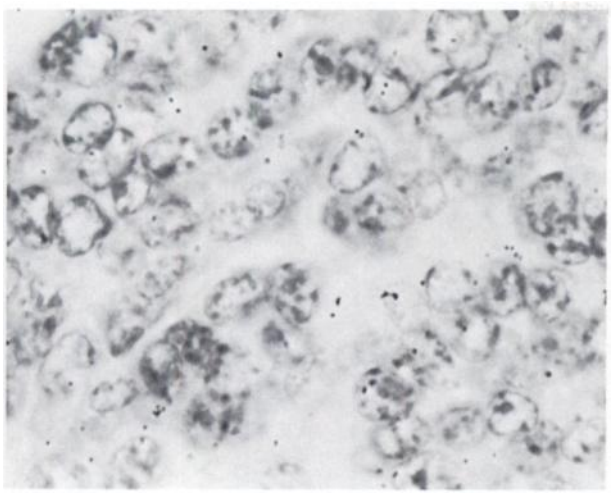

FIG. 7. Tissue autoradiogram demonstrating scant isotopic granule distribution overlying mouse granulosa cell tumor, 400X, oil.

possible target-to-nontarget ratio of ovary (12:1 for blood; 24:1 for liver) as compared to nonrenal and nonthyroidal tissues should be adequate to insure diagnostic scanning. It is of interest that ovarian scans were obtained in a dog when ^{131}I-HCG was employed (*19*).

The mouse ovarian tumor types employed in the present study have been previously characterized according to growth pattern and histology by W. U. Gardner (*20*). The granulosa (Types I and III) and theca tumors (Type II) described by Gardner were usually composed of at least 70–80% of the particular cell type designated. In addition, the tumors were functional, having been shown to produce estrogen, and to a lesser extent, androgen. It was noted by Li and Gardner (*21,22*) that the ovaries of tumor-bearing mice treated with gonadotrophin (pregnant mare serum) showed androgenic effects. The ovarian suppression by the theca cell tumors noted in the present study further attest to the androgenic effects produced by this tumor type. Thus the histological and endocrine aspects of these tumors are well established.

Estrogen-producing human ovarian cancers probably arise from cells of the early ovarian mesoderm which differentiate with both granulosa and thecal cells (*23*). The tumors arising from this mother tissue may therefore assume the characteristics of either granulosa cell or thecal cell cancers. Further evidence to substantiate this theory comes from the finding of both morphological types in some cancers. Both tumors are subject to luteinization, and under this circumstance the cells strongly resemble the thecal tumor cells of the ovary. The granulosa cell is by far the more common of the two and comprises about 10% of all solid malignant tumors of the ovary.

SUMMARY

Iodine-125-HCG was administered to ten female and nine male mice and tissue-to-blood ratios were studied 3 hr later at sacrifice. The mean ovary-to-blood ratio was 4.5. The ovarian concentration of ^{125}I was 8–12 times greater than that in all other tissues studied, except the kidney. The kidney-to-blood ratio of 1.5–2.5 presumably was related to the excretory function of this organ. The ^{125}I-HGH given to seven female mice showed no concentration in the ovary at 3 hr. Ovarian theca cell tumors in eight mice concentrated ^{125}I-HCG in similar amounts to that of normal ovaries. Ovarian granulosa cell tumors in nine mice did not concentrate ^{125}I-HCG. The ^{125}I-HGH did not concentrate in ovarian theca cell tumors in four mice.

Female monkeys given ^{125}I-HCG and sacrificed one each at 1, 2, 2.5, 3, and 5 hr, showed a maximum uptake of ^{125}I at 2 hr with an ovary-to-blood ratio of 1.2, 2–12 times greater than the uptake in nonovarian and renal tissues. Autoradiography showed the radioactivity to be concentrated in the theca layers of the follicles and in the interstitium, and in the theca cell cytoplasm of theca tumors.

ACKNOWLEDGMENT

This work was supported in part by the United States Atomic Energy Contract AT(11-1) 2031 and the Nuclear Medicine Research Fund.

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