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LABELED ASIALO–HUMAN CHORIONIC GONADOTROPIN AS A LIVER-SCANNING AGENT

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Iodine-131-asialo–human chorionic gonadotropin was injected into rats to determine its usefulness as a liver-scanning agent. Thirty minutes after injection, 86% of the desialylated hormone is found in the liver. The specific clearance reaction of this asialo glycoprotein by hepatic parenchymal cells suggests that it can be applied as an agent for liver scanning in man.

The observation by Morell and his colleagues (*1*) that some desialylated glycoproteins are rapidly cleared from plasma by the liver has led us to postulate that the uptake of such derivatives could serve as an effective carrier of radioactivity for liver scanning. Because the binding sites for asialo glycoproteins appear to reside on the membranes of liver parenchymal cells and not on membranes of cells of other organs (*2*), delineation of metastatic lesions in the liver should be possible. Moreover, because the binding is to the plasma membrane and may not be dependent upon bile excretion or other liver function, it seems possible that successful scanning may be achieved in the presence of biliary obstruction or advanced liver failure.

In order to demonstrate the feasibility of this approach to liver scanning, human chorionic gonadotropin (hCG) was desialylated, iodinated, and injected into rats. A placental-produced glycoprotein hormone with a molecular weight of 39,000, hCG consists of two nonidentical subunits the primary structures of which have been determined (*3–5*).

EXPERIMENTAL

Preparation of desialylated hCG. Crude hCG was purchased from Organon (Netherlands) and purified by methods described earlier (*6*). Purified hCG (135 mg at 20 mg/ml) was dissolved in 0.5 *M* sodium acetate, pH 5.0, and digested with 0.75 mg of Clostridium perfringens neuraminidase (Worthington Biochemical Corp.) for 1 hr at 37°C. The method employed was adapted from that described originally by Van Hall, et al (*7*). The mixture was dialyzed 16 hr at 4°C against 0.025 *M* sodium acetate, pH 5.0, after which the digestion was repeated with 0.75 mg of enzyme and then dialyzed against 1% acetic acid and lyophilized. The product was assayed for sialic acid by the thiobarbituric acid method of Warren (*8*) with the finding that over 98% of the original sialic acid had been removed.

Radioiodination of asialo–hCG. The asialo–hCG was labeled by a modification of the method of Greenwood and Hunter (*9*). The following reagents were added sequentially to the reaction vessel: (A) 10 μl asialo–hCG (3.0 mg/ml), (B) 20 μl 0.3 *M* sodium phosphate, pH 7.4, and (C) 20 μl ^{131}I (10 mCi) obtained from Isoserve (Cambridge Nuclear) and 10 μl chloramine T (14 mg/ml). Thirty seconds following the addition of the chloramine T, 20 μl sodium metabisulfite (24 mg/ml) were added followed by two drops of a 5% solution of bovine serum albumin (BSA) in 0.3 *M* sodium phosphate, pH 7.4. The unreacted iodine was removed by passage of the reaction mixture through a G-50 Sephadex column (15 × 0.7 cm) equilibrated with and eluted with 0.01 *M* sodium phosphate–0.1% BSA, pH 7.4. Fractions containing the labeled protein were pooled and used in the following experiments.

Animal experiments. Female albino rats (250 gm) were injected through the tail vein with doses ranging between 8.5 and 9.4 μCi of the ^{131}I-asialo-hCG preparation. The animals were sacrificed by cervical dislocation 30 min after injection. One rat was se-

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lected at random for scanning using a Picker Magnascanner III, 2806F, with the following settings: scan speed, 45 cm/min; line spacing, 0.2 cm; background cutoff, 0%; maximum counting rate, 15,000 cpm; dot factor, 16; density, 25; time constant, 0.04; range differential, 0.90; window, 310–410 keV. Another four animals injected with the same preparation were dissected and portions of liver, spleen, ovary, and kidney were weighed and the ^{131}I contents determined with a Packard Auto Gamma, Model 578. A rat bearing a carcinogen-induced hepatoma (Morris-tumor 16) transplanted subcutaneously in the left rear leg (*10*) was also injected with ^{131}I-asialo-hCG and scanned alongside a normal rat (Fig. 1). Scans were repeated with several different batches of ^{131}I-asialo-hCG with results identical to those shown in the illustration.

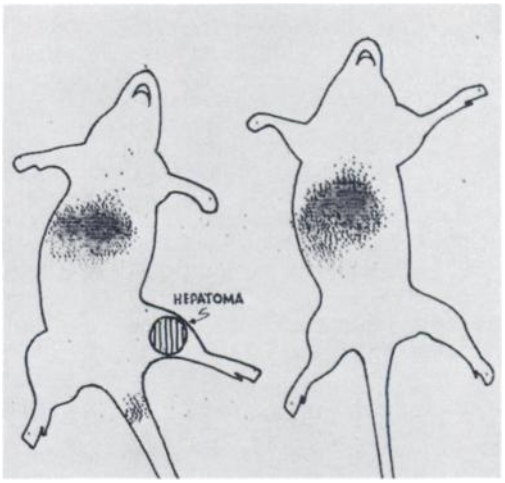

FIG. 1. Total-body scans of ventral surfaces of two female albino rats sacrificed 30 min after tail vein injection of ^{131}I-asialo-hCG. Rat on left had transplanted hepatoma in left hind leg. Radioactivity localized in tail region was residual material from injection at that site. Rat on right was normal. Figure is black-and-white reproduction of color scan.

RESULTS AND DISCUSSION

The results of the scanning study are shown in Fig. 1. The normal rat and the rat bearing a transplantable hepatoma in the left rear leg concentrated the injected ^{131}I-asialo-hCG only in the liver. There was no evidence of specific uptake by any other tissue nor uptake by the hepatoma nodule.

The results listed in Table 1 indicate that about 86% of the administered dose of ^{131}I was bound in the liver at 30 min. A moderate amount of radioactivity (10%) was also found in the kidney presumably as the result of glomerular filtration. Although hCG has a molecular weight in the order of 39,000, the presence of hydrated carbohydrate side chains makes its Stokes' radius approximately that of albumin (*11*). However, removal of the sialic acid residues may have produced sufficient reduction in molecular diameter to result in loss through glomerular filtration.

The uptake of labeled asialo–hCG by ovary was negligible. It has been known for many years that hCG loses virtually all of its biologic activity after desialylation but only recently has it been shown that this is an in vivo effect resulting from rapid clearance of the desialylated molecule by the liver (*1,7,12*).

These data appear to confirm the hypothesis that asialo–hCG can serve as an effective carrier of radioactivity for liver scanning and the results are consistent with those of Rajaniemi and Vanha-Perttula (*13*), who showed that labeled native hCG was slightly concentrated by Kupffer cells whereas labeled asialo–hCG was localized to a greater degree by parenchymal cells. Morell, et al (*14*) have postulated that this parenchymal cell localization is the result of a specific reaction with galactose residues that are exposed by removal of sialic acid, and the results of Tsuruhara, et al with asialo–agalacto–hCG (*15*) are consistent with this hypothesis.

Nonhepatic tissue, e.g., metastases, should exhibit essentially no uptake of asialo–hCG. Liver clearance of asialo–hCG is rapid but it is not known whether extensive liver failure or biliary obstruction will affect this uptake. Biliary concentration of the agent was not measured in these experiments. Although the carcinogen-induced hepatoma in the rat did not bind labeled hormone derivative, some well-differentiated hepatomas in man may retain this capacity and in human use radiolabeled asialo–hCG uptake might serve to distinguish these tumors from others that metastasize to liver. We believe that these questions should be tested with clinical studies in man.

Although no effort was made to eliminate the small quantity of neuraminidase from the asialo–hCG employed in these animal experiments, it would be

TABLE 1. DISTRIBUTION OF ^{131}I-ASIALO-hCG IN RAT

Organ	Percent uptake ± s.d.*	(Spec. act. liver tissue) / (Spec. act. other tissue)
Liver	86.00 ± 8.07	1.0
Spleen	0.30 ± 0.08	18.8
Kidneys	10.00 ± 1.23	1.4
Ovaries	0.07 ± 0.05	15.2
Muscle	†	53.4

* A sample of each tissue was taken and specific activity determined. Data given are corrected to show percent uptake of injected dose by entire organ.

† Gastrocnemius muscle was sampled for specific activity; total weight of muscle mass in animal was unknown.

desirable to use an insoluble neuraminidase to prepare asialo–hCG for clinical studies. It would also be desirable to prepare ^{99m}Tc- or ^{123}I-labeled asialo–hCG for such studies.

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