TABLE I. Thyroidal Organic Iodine at the End of 24 Hr (% of I-131 Present in Cells).

Thyroid No.	Precipitation with TCA	Radio-chromatography
6	22.6	24.5
8	25.1	28.7
11	21.7	26.4

trating ability were noted between those cells incubated with or without methimazole. The cell suspensions containing methimazole reflect the maximal iodide-concentrating ability of these cells. Addition of sodium perchlorate to the cell suspensions was followed by marked decreases in the cell-to-medium I-131 concentration ratios, thus further substantiating the fact that these cells had concentrated I-131.

Radiochromatograms of the "unblocked" cells revealed radioactivity only at the origin and in the iodide area. Isolated normal human thyroid cells can incorporate I-131 into iodinated proteins. The amount of organically-bound iodine after 24 hours' incubation with I-131 is given in Table I. The slight differences between the results obtained by the two methods is probably due to small amounts of adsorbed inorganic iodine.

It is apparent from these data that normal human thyroid cells are able to concentrate iodide and to incorporate the iodine into organically bound iodine in the absence of intact thyroid follicles and colloid. Microscopic examination of the cell suspensions obtained by the technique used in these studies have revealed the presence mainly of isolated thyroid follicular cells and absence of intact follicles(4,5). This work confirms previously published reports on the ability of isolated pathologic human thyroid cells and sheep thyroid cells to concentrate iodide and to incorporate it into organic form.

Summary. Normal human thyroid cells were dispersed by trypsinization and incubated in tissue-culture medium 199. When I-131 was added to the culture medium, the isolated cells were shown to retain their ability to concentrate iodide. This indicates that intact thyroid follicles and colloid are not essential for iodide-concentration and organic binding of the trapped iodine by normal human thyroid cells.

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Role of Cell Proliferation in Hepatic Carcinogenesis.* (28919)

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Hepatocellular carcinoma develops through stages of nodular regeneration as described in rats following the feeding of Aramite(1). By contrast it was demonstrated that during the induction of cirrhosis and hepatic carcinoma by an azo-dye, cell proliferation in the non-carcinomatous portions of the liver is not increased, while the tumor takes up more tritiated thymidine(2) and has a high mitotic index(3). This was taken to indicate that hepatic carcinoma does not develop over in-

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creasingly uncontrolled regeneration but arises *de novo*. In view of these findings, regeneration in the presence of primary hepatic carcinoma arising in a non-cirrhotic liver was studied. The carcinogen chosen was diethylnitrosamine, a substance previously shown to produce a high yield of hepatic carcinomas, without accompanying fibrosis or cirrhosis (4). The parameters studied were: 1) relative rates of cell proliferation in neoplastic and non-neoplastic liver, 2) relation of mesenchymal cell proliferation to carcinoma cell proliferation, and 3) effect of cytoplasmic alterations upon the rate of liver cell proliferation.

Materials and methods. Six male Sprague-Dawley rats, with an average initial weight of 150 g were fed a synthetic diet(5) and given 0.005% diethylnitrosamine in their drinking water (about 1.5 mg per day per rat). Two rats served as controls. All rats were sacrificed after 240 days. Each animal was given tritiated thymidine, 0.5 μc per gram body weight, intraperitoneally 2 hours before sacrifice. Sections of liver were fixed in 10% neutral formalin, after which 4 μ thick paraffin sections were stained with hematoxylin and eosin. Autoradiographs were prepared by coating sections with Kodak NTB-3 nuclear track emulsion. These were exposed for 30 days at 4°C, processed in Kodak D19 developer, and stained with Harris hematoxylin. Cell counts were performed by the method previously described(5).

Results. All animals approximately doubled their weight during the experiment. Livers of all animals fed diethylnitrosamine revealed 3 distinct features.

1) *Carcinomas.* These were grossly visible tumors, some of which were necrotic and hemorrhagic. Microscopically, the tumors were hepatocellular carcinomas of varying degrees of differentiation (Fig. 1). Some were trabecular carcinomas, with formation of liver plates many cells in thickness. Others were more anaplastic, bearing little resemblance to normal liver. Several tumor nodules were extremely vascular.

2) *Hyperplastic nodules.* (Fig. 2). These microscopic nodules were scattered throughout the liver, without definite pattern. They varied from several cells up to 50 cells in diameter. The liver cell plates were 2 to 3 cells thick and irregularly arranged. They merged in some hyperplastic nodules with surrounding parenchyma, while in others they ran parallel to the periphery of the nodule and compressed surrounding tissue, as evidenced by thinning of the neighboring liver cell plates. Occasionally mature bile ducts were found in these nodules.

TABLE I. Percentage of Labeled Cells in Autoradiographs of Livers of Control Rats and of Rats Given Diethylnitrosamine for 240 Days. Figures represent averages of all animals.

	Hepatocytes	Mesenchymal cells
Control	.16	.44
DEN treated "Normal" areas	.25	.90
Nodular cytoplasmic alteration	.20	.78
Hyperplastic nodules	8.0	7.5
Carcinoma	8.8	7.7

3) *Nodular cytoplasmic alterations.* In these foci, which were about the same size as hyperplastic nodules, the cytoplasm of the hepatocytes was somewhat larger than in the surrounding parenchyma, and showed diffuse vacuolization. The number of cells was not increased, nor was the arrangement of the liver cell plates altered.

The architecture of the liver outside these 3 types of foci was maintained, without fibrosis or cirrhosis.

In autoradiographs of control animals, few labeled hepatocytes or mesenchymal cells were seen (Table I). In the livers of rats fed diethylnitrosamine, the areas of unaltered parenchyma showed the same or only a slightly higher percentage of labeled hepatocytes than the control. Areas of nodular cytoplasmic alteration showed no increased labeling. In hyperplastic nodules (Fig. 3), the proportion of labeled hepatocytes was about 30 times that found in normal areas. Labeled mesenchymal cells in hyperplastic nodules were also increased. All carcinomatous areas, regardless of degree of differentiation, showed a striking increase in labeled hepatocytes and mesenchymal cells (Fig. 4) of about the same magnitude as in hyperplastic nodules.

Discussion. The various stages of nodule

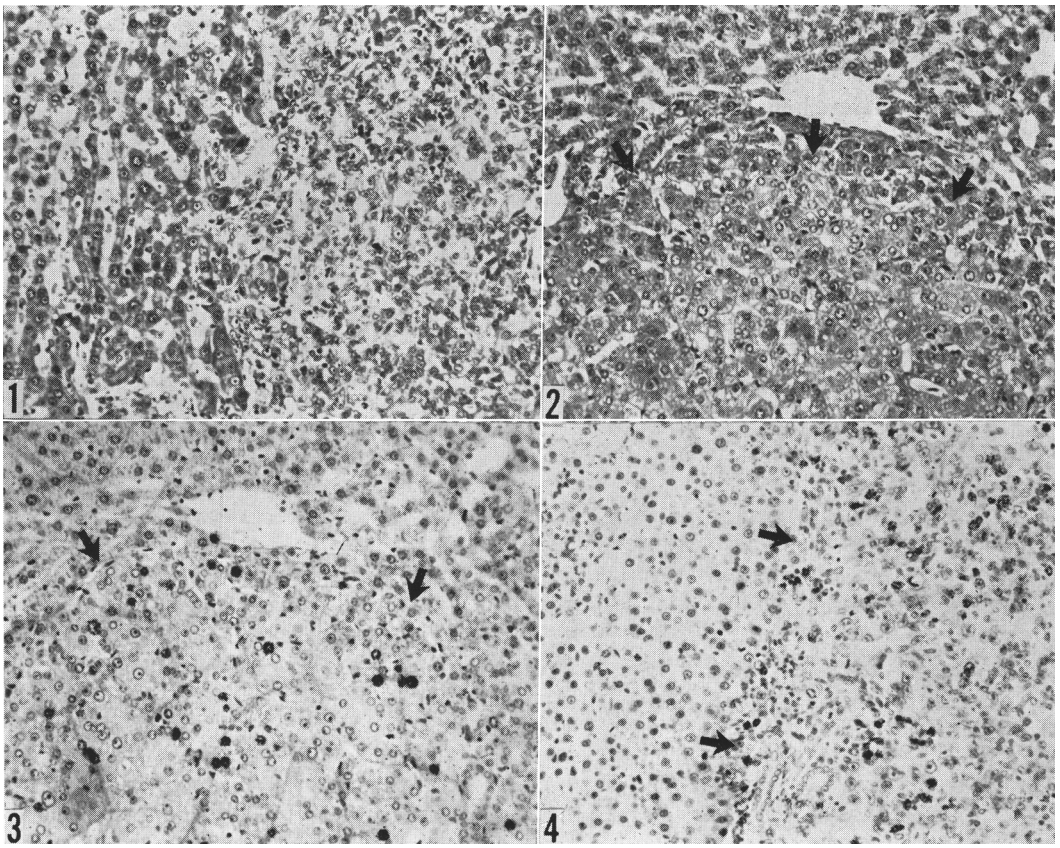

FIG. 1. Liver of rat fed diethylnitrosamine for 240 days. On left is histologically normal liver. On right is poorly differentiated hepatocellular carcinoma. H & E (×90).

FIG. 2. Liver of rat fed diethylnitrosamine for 240 days. Above is histologically normal parenchyma. Below is hyperplastic nodule. Transitional zone is sharp (arrows) and hyperplastic cell plates merge with normal ones. H & E (×90).

FIG. 3. Autoradiograph of area in Fig. 2. Arrows indicate demarcation of hyperplastic nodule. Note large number of labeled cells in nodule as compared with normal area. Hematoxylin (×90).

FIG. 4. Autoradiograph of area in Fig. 1. Arrows point to margin of carcinoma. Note numerous labeled tumor cells. Hematoxylin (×90).

formation correspond to those described after feeding of Aramite(1) and the nomenclature used in that model applies here. Both normal areas and foci of cytoplasmic alteration, in which the hepatocytes do not appear increased in number, reveal normal or slightly increased DNA synthesis in parenchymal and mesenchymal cells, while the hyperplastic nodules exhibit considerably increased DNA synthesis. There is no reason to consider the hyperplastic nodules to be carcinoma because: 1) they exhibit gradual transition to normal liver; 2) they contain mature bile duct elements; 3) they occur in old rats on normal diets(1); and 4) they cannot be transplanted, in contrast to carcinoma(5). These nodules correspond to the benign nodular regenerative hyperplasia in man(6). Although these nodules are not carcinomatous, their rate of DNA synthesis is considerably greater than in the morphologically normal areas, and equals that in carcinoma. It thus appears that primary hepatic carcinoma in the non-cirrhotic liver may arise through transitional stages, involving increased nodular regeneration, as postulated in Aramite intoxication(1). It deserves emphasis that DNA synthesis in mesenchymal cells in both hyperplastic and carcinomatous nodules is also greatly increased, suggesting generalized in-

creased growth stimulation.

Summary. Primary hepatic carcinoma, without accompanying cirrhosis or fibrosis of the liver, was induced in rats with diethylnitrosamine. Hyperplastic nodules and nodular cytoplasmic alterations were found in the same livers. Autoradiographs after administration of tritiated thymidine showed no increase in DNA synthesis in morphologically normal areas or in foci of cytoplasmic alterations, but a striking increase in both hyperplastic nodules and carcinoma. It is concluded that primary hepatic carcinoma in the non-cirrhotic liver may arise through stages of increased nodular regeneration.

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Coating of Vascular Surfaces and Cells. A New Concept in Prevention of Intravascular Thrombosis.* (28920)

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Thromboses, arterial and venous, are major clinical problems still inadequately resolved by modern methods of treatment. Among potential anti-hemostatics, macromolecular plasma volume expanders have been known to interfere with clotting(1-4) since 1918, although by mechanisms that are still obscure(5-8). Recently dextran, the most widely used, has been shown to have an antithrombic effect *in vivo*(9,10,11), and *in vitro* it adheres to red blood cells and platelets (14). Infused dextran changes the thrombelastogram as if a defect in platelet function existed, although platelet concentration is not significantly diminished(12,13).

In the dog subjected to standardized arterial trauma by intimectomy of small (femoral) arteries 2.0-3.0 mm I.D., infusion of commercially supplied 6% dextran-saline in amounts equivalent to 10 mg dextran per kg body weight has prevented clotting at the sites of arterial injury(9-11). The anti-hemostatic effect is attributable in part to changes in circulating coagulation mechanisms. It may depend also on changes in luminal vascular surface, particularly at sites of damage. The present data show that C-14 labelled dextran adheres *in vivo* to circulating cells. It also adheres to luminal surfaces of arterial walls especially preferentially at sites of injury.

Procedure. C-14 labelled dextran (characterized in Table I) was used as was commercial dextran in previous experiments(9,11). Dogs were exsanguinated into ACD-solution 30-60 minutes after infusion and, in Experiments B and C, infusions were given just before release of clamps occluding sites of femoral arterial intimectomy.

TABLE I. Radioactive Dextran—Specifications.

Concentration B 512 in saline	6.06 g/100 ml
Molecular wt (light scattering)	82,600 avg
Intrinsic viscosity 25°C	.20
Specific activity (combustion carbon)	6.36 μC/g
Molecules of dextran/disintegration/min	10^{12}
Tagged molecules	1 out of 260
Size—Approx. radial diam.	90 angstroms
" axial "	3,700 "
Counting efficiency	20.1%
Dose injected	1% body wt

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