THE DISTRIBUTION OF ALBUMIN SYNTHESIS
THROUGHOUT THE LIVER LOBULE

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INTRODUCTION
The membrane-attached polysomes of the liver cells are assumed to be the intracellular site of albumin synthesis (for recent critical review see Campbell (1)). The histological localization of albumin by fluorescent antisera has been described repeatedly (3, 5–7). It is not possible, however, to decide whether albumin localized in the cell by this technique is newly synthesized, or taken up by the cells from the blood, or simply adsorbed during preparation.

In radioautographs we observed predominant labeling of the periportal area of the liver lobules after intraportal injection of leucine-3H, whereas intracaval injection led to uniform labeling throughout the lobule. The way of administration of the tracer thus permits a study of protein synthesis of selected regions of the liver lobule.

Recently, the isolation of radiochemically pure albumin from liver homogenates and a method to determine the proportion of albumin to total protein synthesis have been described (9).

In this report the ratio of albumin to total protein synthesis after intraportal injection was compared to that after intracaval injection. By combining these results with the radioautographic observations, it was possible to decide whether or not albumin synthesis was distributed uniformly throughout the liver lobule.

MATERIALS AND METHODS
Animals and biochemical methods have been described previously (8, 9).

For radioautography small pieces of liver were fixed in 0.75 M sodium phosphate buffer of pH 7.0 with 4% formaldehyde for 24 hr at 4°C, dehydrated in ethanol, and embedded in paraffin blocks. Slices of 5 µ and control slices from unlabeled livers were cut and transferred to the same microscopical slides. Paraffin was removed by alcohol, methylbenzoate, and xylene. The slices were washed twice for 20 min in 0.76 mm nonradioactive leucine and then dipped into Ilford K5 liquid emulsion (Ilford Ltd., Ilford, Essex, England). After 24 days' exposure at 18°C and after photographic development, radioautographs were stained through the emulsion with Mayer's hemalum, made transparent by 70% ethanol with 4 vol % of 1 N HCl, rinsed in running tap water, and dried.

The density of silver grains was determined by leading a measuring diaphragm of 552 µ² around the central and the periportal areas of the liver.
lobule and measuring the light reflected by the silver grains in a microscope equipped with a photomultiplier (4). The pulses of the photomultiplier were recorded as "working units" by a Knott analog digital counter. Two animals were measured after intraportal, and two after intracaval injection of the label. Almost identical values were obtained within each group. The obtained values were averaged after subtraction of the background, which was determined with unlabeled slices. After intraportal injection, 100 positions of the diaphragm were measured, each over the central area and the periportal area. After intracaval injection, 107 positions were counted over the periportal, and 110 over the central area. The number of background measurements was 43 for intraportal and 42 for intracaval injection, respectively. The density of the silver grains ranged from 0 to 70 grains per 10 $\mu^2$, after subtraction of the background. In this range, working units were increasing linearly with the number of grains per measured area. Average background density was 10.9 grains per 10 $\mu^2$.

RESULTS AND DISCUSSION

Distribution of Leucine in the Liver after Intracaval and Intraportal Injection

After opening the abdomen by a median incision, 0.2 mCi of L-leucine-4,5-3H, 100 g of body weight, 1 Ci/mmmole, dissolved in 0.9% NaCl in a concentration of 1 mCi/ml, was injected into the caval vein or into the portal vein of about 3-month old male rats of an average body weight of 250 g. The animals were kept under ether anesthesia during the experiments. Ether anesthesia does not influence the incorporation of amino acids into albumin and total liver protein (2). Livers were removed 10 min later, and the distribution of the label in protein was studied by radioautography. Radioactivity was found to be incorporated uniformly throughout the liver lobule, when leucine was injected into the caval vein (Fig. 1). The peripheral region of the liver lobule was predominantly labeled if leucine had been injected into the portal vein (Fig. 2). The results of a quantitative analysis of the distribution of the incorporated label are presented in Fig. 3. The number of measured areas was plotted against the density of silver grains found within the area. We cannot yet give an experiment-based explanation for the observed differences of the distribution of incorporated label.

Comparison of the Synthesis of Albumin and Total Protein

The incorporation of intravenously injected amino acids into liver protein is influenced by the

FIGURE 1 Uniform labeling throughout the liver lobule after intracaval injection of L-leucine-4,5-3H$_2$. A periportal tract can be seen in the center of the lower part of the picture; a central vein is shown at the right rim and another in the upper left corner. Unstained radioautograph. Magnification, 115 X.
The dark tone of the cells around the periportal fields is caused by small silver grains, which can be distinguished only at higher magnification. Unstained radioautograph. Magnification, 115 X.

When the injection of radioactive amino acid was given by the intracaval route, the distribution of radioactive protein was uniform throughout the liver lobule. Under these circumstances the

FIGURE 3  Quantitative analysis of the distribution of protein labeling throughout the liver lobule by radioautography.

3.5%, whereas after intraportal injection 2.8% of total protein radioactivity was found in albumin.

Distribution of Albumin Synthesis throughout the Liver Lobule

When the injection of radioactive amino acid was given by the intracaval route, the distribution of radioactive protein was uniform throughout the liver lobule. Under these circumstances the
proportion of radioactivity in the whole liver that was present in serum albumin, compared with total protein, was about 3%. Under conditions where the incorporation of radioactivity was confined virtually to the periphery, i.e. intraportal injection, the proportion of radioactivity in serum albumin, compared with total protein, was also about 3%. If the synthesis of serum albumin is greater in the periphery than in the interior of the liver lobule, the proportion of radioactive albumin to total protein in the periphery would have to exceed 3%. This was not the case. Hence, the periphery of the liver could not have been more active in the synthesis of serum albumin than the interior. Thus the capacity to synthesize albumin is distributed uniformly between the periphery and interior of the liver lobule.

**SUMMARY**

Injection of radioactive leucine into the caval vein of rats led to uniform labeling of protein throughout the liver lobule. Injection into the portal vein produced labeling of protein predominantly in the peripheral regions of the lobule. After both kinds of injection procedures, albumin was purified from liver homogenates to constant specific radioactivity. The ratio of the radioactivity incorporated into albumin to that incorporated into total protein was the same in both cases; hence, the capacity to synthesize albumin was distributed equally between the peripheral and the central regions of the liver lobule.

| Table I | Radioactivity in Albumin and Total Protein in the Livers of Eight Rats 10 Min after the Intracaval or Intraportal Injection of 0.016 mCi L-Leucine-1-14C per 100 g of Body Weight |
|---------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Albumin per ml of homogenate | Intracaval injection | Intraportal injection |
| Specific radioactivity of purified albumin | 1.257 mg | 1.038 mg |
| Radioactivity in albumin per ml homogenate | 7470 dpm/mg | 11350 dpm/mg |
| Radioactivity in total protein per ml homogenate | 9.39·10^4 dpm | 12·10^5 dpm |
| Radioactivity in albumin | 268·10^5 dpm | 429·10^5 dpm |
| Radioactivity in total protein | 0.035 | 0.028 |

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