

ELECTRON MICROSCOPIC RADIOAUTOGRAPHIC DETECTION OF SITES OF PROTEIN SYNTHESIS AND MIGRATION IN LIVER

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ABSTRACT

The sites of synthesis of proteins and their subsequent migration in rat liver have been studied during a 75 min period after labeling of liver-slice proteins by exposure to leucine- H^3 for 2 min. Incorporation of the label into protein began after 1 min and was maximal by 4 min. Electron microscopic radioautography showed that synthesis of proteins in hepatocytes occurs mainly on ribosomes, particularly those in rough endoplasmic reticulum and, to some extent, in nuclei and mitochondria. Most of the newly formed proteins leave the endoplasmic reticulum in the course of 40 min, and concurrently labeled proteins appear in Golgi bodies, smooth membranes, microbodies, and lysosomes. A likely pathway for the secretion of some or all plasma proteins is from typical rough endoplasmic reticulum to a zone of reticulum which is partially coated with ribosomes, to the Golgi apparatus, and thence to the cell periphery. The formation of protein by reticuloendothelial cells was measured and found to be about 5% of the total protein formed by the liver.

INTRODUCTION

The liver is one of the organs which retains a high level of protein synthesis in the adult animal (35). It produces proteins for secretion and for replacement of its own substance in about equal amounts (18, 51). The use of conventional cell fractionation techniques has established that most liver proteins are formed on ribosomes (30, 31), and that at least some of the proteins produced for secretion to the plasma move from a region of granular membranes (isolated as rough-surfaced microsomes) through a region of agranular membranes (isolated as smooth-surfaced microsomes) and thence to the circulation (28, 41). It has not been possible, however, to identify this smooth-surfaced fraction more specifically as Golgi bodies, microvilli or smooth vesicles of the endoplasmic reticulum (e.r.). Nor has it been possible to define the rela-

tive rates of protein formation in hepatocytes and reticuloendothelial (R. E.) cells.

We have used radioautography at the electron microscopic level after a brief exposure to leucine- H^3 to identify more precisely the sites of protein synthesis and the pathways of intracellular transport of proteins. For such studies to be reliable, the assumptions must be valid that the observed silver grains represent protein which has been newly formed, and that artefactual loss of labeled protein from the tissue is minimal. An inhibitor of protein synthesis, puromycin, was used to stop protein synthesis without preventing uptake of free leucine- H^3 in cells as proof that the technique of formaldehyde fixation and washing was adequate to remove leucine not bound in large molecules. The need to avoid bifunctional fixatives such as

glutaraldehyde in studies of this type has been demonstrated previously (44). Free leucine taken up by liver is not catabolized into other low molecular weight compounds to a significant extent (15, 18), probably due to the lack of the necessary transaminases (10), and is utilized primarily for protein synthesis (15). Hence, bound tritium atoms can be equated with newly formed protein, rather than with other substances such as lipids into which catabolites of leucine might have been incorporated. Loss of labeled protein in the formaldehyde fixative has been considered in a recent publication (45) and found to be negligible.

The experiments were conducted with small slices of liver rather than in the intact animal in order to permit more direct control of environmental conditions and to minimize cost of isotope. Liver slices can secrete specific proteins such as albumin (7, 27, 32, 39, 40, 60), α -glycoprotein (56), high- and low-density lipoproteins (33, 48), transferrin (34), fibrinogen (59), prothrombin, and Factor VII (46), and produce specific intracellular proteins such as ferritin (62), so that protein synthesis in slices appears to be qualitatively if not quantitatively normal.

This paper describes the distribution of label in liver cell constituents during a 75 min period after a 2 min exposure to leucine- H^3 . Two emulsions of different grain sizes, Ilford L-4 and Kodak NTE, were employed to evaluate their relative usefulness in radioautography. In parallel experiments the total incorporation of leucine- C^{14} into liver protein at various times under the conditions of the radioautographic experiments was also measured. Brief reports of some of these findings have appeared (2, 42, 43).

METHODS

Materials

Leucine- H^3 was L-leucine-4,5- H^3 , 8–35 c/mmole (TRK-170, Nuclear-Chicago Corporation, Des Plaines, Ill.) Leucine- C^{14} was L-leucine-U- C^{14} , 6.8 mc/mmole (Calbiochem, Los Angeles, Calif.). Incubation medium (Krebs-Ringer-bicarbonate with 5% CO_2-O_2 as gas phase), 4% formaldehyde fixative (freshly prepared from paraformaldehyde), washing buffer, and puromycin were as described (44).

Incubations

Slices 0.3–0.5 mm thick were prepared from livers of male Sprague-Dawley rats of 100–250 g as in the previous study (44). For radioautography, 3×4 mm

rectangles were cut from these slices and equilibrated in the incubation medium for 30 min at 38°C. In a typical experiment, six such liver-slice specimens were then pulse-labeled in a 23×55 mm vial containing 0.6 ml of medium with 1–3 mc of leucine- H^3 for 2 min. Concentration of leucine- H^3 added was 0.03–0.14 mM, which is within the range reported for normal rat plasma (21). The vial was then filled with warm medium containing nonlabeled leucine at 5 mM, or 35–170 times the level of the labeled leucine. This medium was changed 4 times in the next 10 min. In some instances specimens were transferred to medium containing 2 mM puromycin at 4 min after addition of label (2 min after transfer to non-labeled medium), a time when incorporation into protein was maximal. When longer incubation times were desired, incubation was continued in the 5 mM leucine medium in 10 ml Erlenmeyer flasks. In separate experiments not reported here, the increased concentration of leucine has been found not to affect formation of proteins. Liver slices generally are not sensitive to the level of free amino acids in the incubation medium (57, 63), and a similar insensitivity has been noted for slices of pancreas (23).

At appropriate times slices were removed and placed in formaldehyde fixative on a shaker at room temperature. The fixative was changed 10 times during the next 2 hr, and was followed by 4 changes of washing buffer over the next 2 hr. The first change of washing buffer contained 10 mM leucine. The specimens were then treated with OsO_4 , dehydrated, and embedded in Epon with a vertical orientation as described (44).

In parallel experiments designed for evaluation of total uptake of labeled leucine in slices, slices of the same thickness but of approximately 50 mg wet weight were incubated at 38°C in 1 ml of medium containing 1 μ c of leucine- C^{14} in 10 ml Erlenmeyer flasks. Concentration of leucine- C^{14} added was 0.15 mM. After 2 min the leucine- C^{14} was replaced with warm nonlabeled leucine, 5 mM (35 times the level of labeled leucine), which was changed several times as before. At the desired times specimens were blotted and homogenized in 5% trichloroacetic acid at 0°C. These were washed, plated, and counted as described (44).

Control specimens to test the completeness of washout of free leucine were preincubated for 30 min in 2 mM puromycin before addition of label. The number of silver grains per unit area in these puromycin-inhibited specimens, counted on electron microscopic radioautographs as described below, was at most only 3% of that in experimental sections.

Radioautography

Sections of Epon-embedded specimens were placed on collodion-covered slides and stained with uranyl



FIGURE 1 Arrangement used for quantitation of radioautographs. Negative micrographs were studied with a dissecting microscope. Also shown are the two types of template, one with guide lines for counting silver grains, the other with crosses for locating points to allow estimation of the relative area occupied by the various cell components in the same micrograph.

acetate and lead citrate. Only a limited number of slides were stained at one time, so that staining could proceed quickly from the uranyl acetate to the lead citrate. If slides were allowed to dry between the stains, carbon dioxide apparently was taken up by the Epon and caused precipitation of lead carbonate. Carbon was evaporated on the sections, and Ilford L-4 or Kodak NTE emulsion was applied by dipping. Otherwise, the methods were those of Salpeter and Bachmann (52). Exposure times were 3–20 weeks for L-4 and 10–30 weeks for NTE emulsions.

The NTE emulsion was developed according to Salpeter and Bachmann (52), and the L-4 as described (44).

Quantitation

Four experiments were conducted: in one, L-4 emulsion was used; in one, both L-4 and NTE; and in two, NTE alone. Each specimen was a liver slice removed and fixed at a specific time interval after incubation with labeled leucine, then processed and coated with emulsion. For each of 32 specimens 9 micrographs were taken on $3\frac{1}{4} \times 4$ inch plates at $5000 \times$ magnification, with an RCA 3G electron microscope operated at 100 kv with a 200μ condenser aperture and a 35μ objective aperture. Some selectivity was required in order to obtain areas with sig-

nificant numbers of silver grains since active cells in slices are restricted to the oxygenated peripheral zone. The background was determined for each specimen by taking micrographs of an area of comparable size but lacking tissue. Background levels were generally less than 4% of the levels over tissue in individual specimens.

Identification of the cell component falling under the central point of each silver grain was made by examining the negative micrographs directly with a dissecting microscope. The plates were placed on top of a template with horizontal lines which was taped on a freely movable X-ray viewbox (Fig. 1). In our experience this technique was more accurate than examination of prints, in which case some sacrifice of either contrast or detail was commonly encountered. Direct examination of the negatives was also faster and less expensive than study of enlarged prints. The most probable point of origin of a beta particle was considered to be the center of the resulting grain, which was located visually as the center of an imaginary circle encompassing the whole grain (53). Thus, for example, if the central point fell on ribosomes not attached to membranes and without organization, even though typical rough e.r. was found nearby, the grain was scored as over free ribosomes. Identification of the underlying component was recorded by

verbal dictation. The total number of grains counted was 16,872, for an average of 527 grains per specimen.

The distribution of cytological components in each specimen was then determined in a similar manner by using a second template containing 50 evenly spaced crosses (Fig. 1). A total of 14,000 points was thus located, an average of 450 for each specimen. Grains and points appearing over dirt or artifacts were ignored.

In order to permit comparison of the results among specimens, since the exposure times and isotope levels were not constant, the grain counts for each cell component were scaled to make the total grain count for a specimen equal to the total incorporation observed in slice protein at the corresponding incubation time in the parallel experiments (Fig. 2). The maximum (4 min) value for total incorporation was set at 100. Grain counts for individual cell components hence represent per cent of maximal observed grain count. Results from specimens with the same incubation times were averaged to give the data points of Figs. 6-8.

RESULTS

Incorporation into Liver Slices

The pulse-labeling of the free leucine in liver and the resulting label in liver protein are shown in Fig. 2, which is a composite curve of the incubations with L-leucine- C^{14} conducted in parallel with the incubations with leucine- H^3 for radioautography. At 2 min the label in free leucine in the slice had attained about two-thirds of the maximal value it would have reached on continuing incubation (see Fig. 1 in ref. 45). Upon replacement of the labeled leucine in the incubation medium with unlabeled leucine, at 2 min the radioactivity in free leucine in the liver slice fell abruptly. Exchange of free leucine between cell and medium apparently occurs by diffusion rather than by a specific energy-requiring mechanism. The rapidity of leucine interchange has also been noted for ascites cells (50), red blood cells, and several other tissues (8). The rise and fall of label in the intracellular free leucine obtained in the liver slice system is sharper than that obtainable by the administration of the labeled compound *in vivo* (23).

The label appeared in liver protein after about 1 min and rose rapidly to a maximum at about 4 min, lagging 1-1.5 min after the rise and fall of label in free leucine. Thereafter the activity in protein fell slowly over the next 75 min. The 4 min peak value of 45 disintegrations per min per mg in protein amounted to incorporation of about 0.1% of the 1 μ c of leucine- C^{14} added to the incubation of a 50 μ g slice.

Puromycin, 2 mM, does not affect either the uptake or the release of free leucine by liver slices (44).

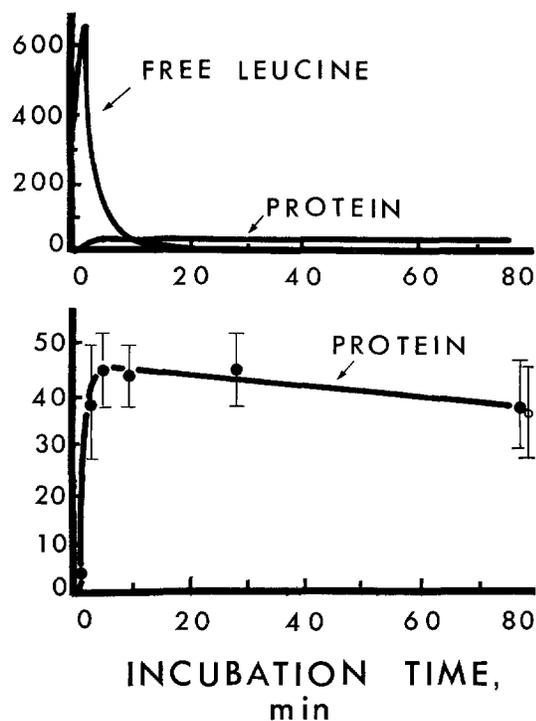


FIGURE 2 *Upper graph:* time course of the pulse of free leucine- C^{14} and the resulting incorporation into protein in liver slices, as evaluated in incubations companion to the radioautographic studies. *Lower graph:* the incorporation into protein on a larger scale. The mean and standard deviation of 5-6 specimens at each time point are shown. The ordinates are disintegrations per min per mg wet weight of liver. Duration of exposure to leucine- C^{14} was 2 min. When 2 mM puromycin was added at 4 min, the C^{14} -level in liver protein at 75 min (open circle) was the same as in the absence of puromycin (closed circles).

Riggs (50) obtained a similar result with ascites cells. When present at the start of incubation, puromycin completely blocks incorporation into protein (44, 45). When added at 4 min in the present experiment, after incorporation of labeled leucine into protein was complete, puromycin had no effect on the amount of label remaining at 75 min (Fig. 2, open circle).

Radioautographic Studies

DISTRIBUTION OF LABELED PROTEIN IN SLICES: Active cells are not homogeneously distributed in liver slices but occur in a zone which reflects the effective penetration of oxygen—about 0.15 mm for 95% O_2 as employed here. Cells of the outermost layer are inactive due to damage during slicing. Deane et al. (12) demonstrated this

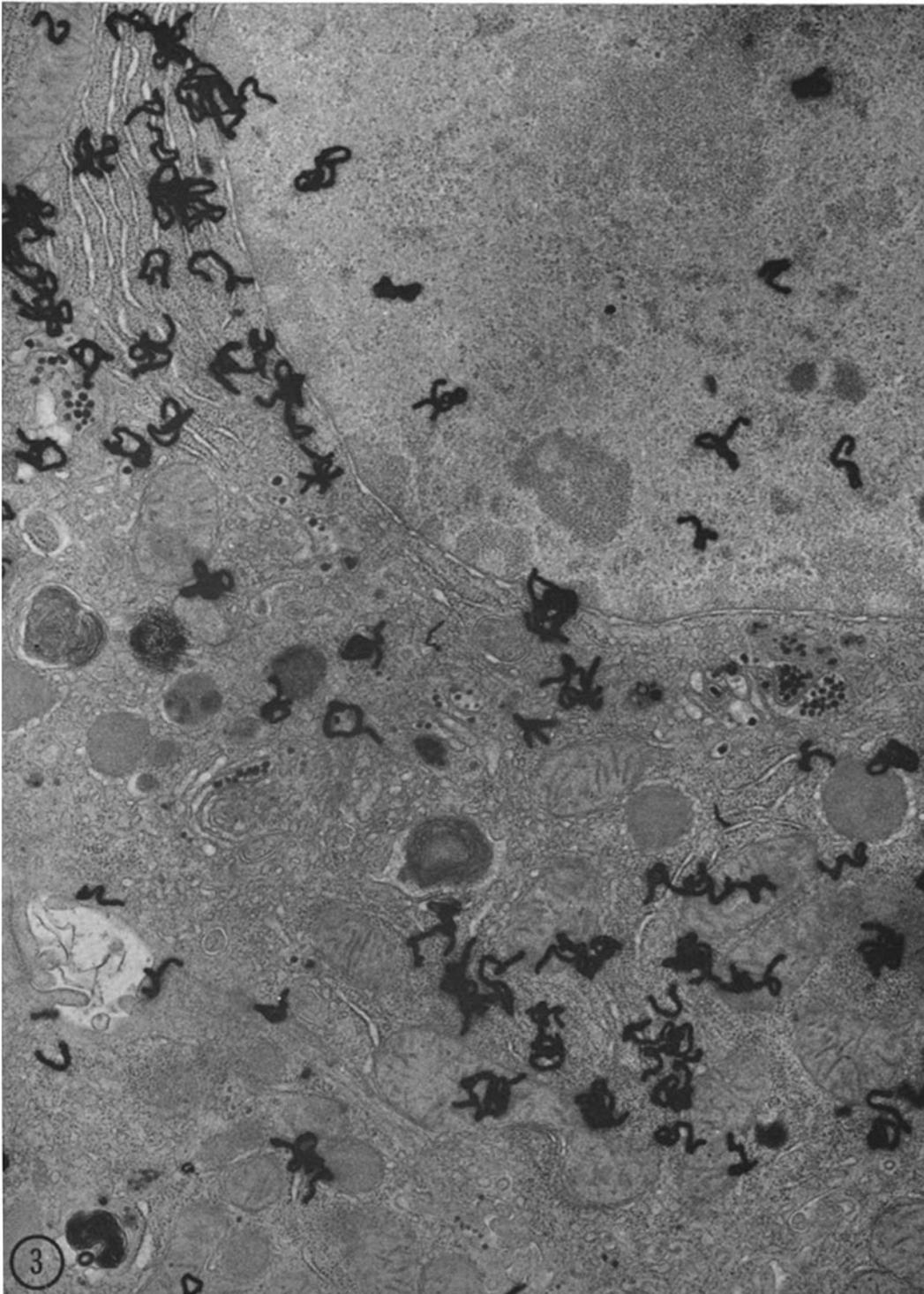


FIGURE 3 Radioautograph of rat liver slice fixed with formaldehyde immediately after 2 min pulse of leucine-³H. Postfixed in osmium tetroxide; uranyl acetate and lead citrate stain; exposed 11 weeks to Ilford L-4 emulsion. Many grains are over the rough e.r. in the left upper corner and on free ribosomes toward the bottom. Few grains are over the Golgi complexes near the center. $\times 18,000$.

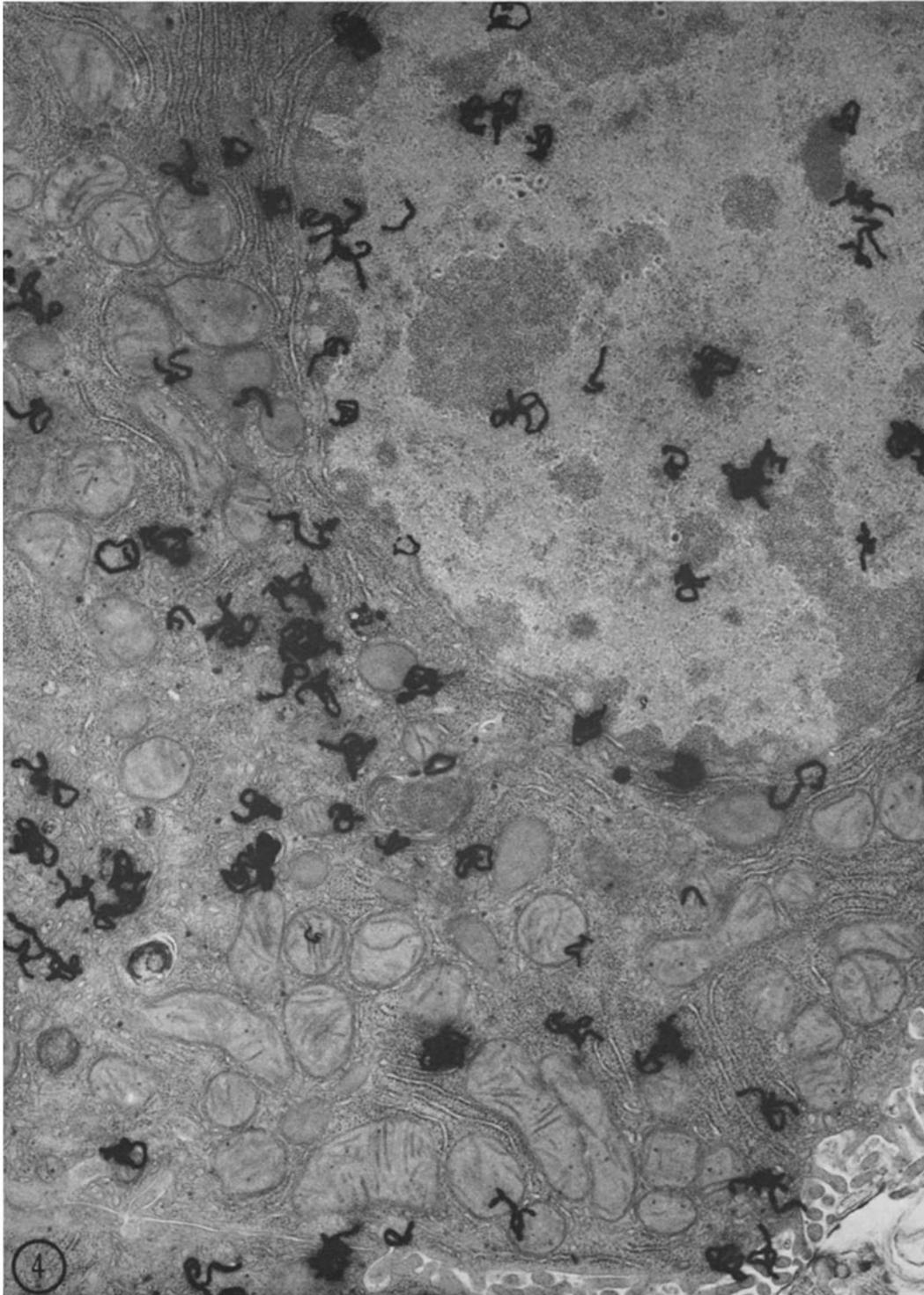


FIGURE 4 Radioautograph of rat liver slice incubated for 38 min after a 2 min pulse of leucine-³H. Fixed and stained as for Fig. 3. Exposed 24 weeks to Ilford L-4 emulsion. At this time grains are concentrated over the Golgi complex in the center and to the left. Few grains are over the rough e.r. and the free ribosomes. $\times 18,000$.

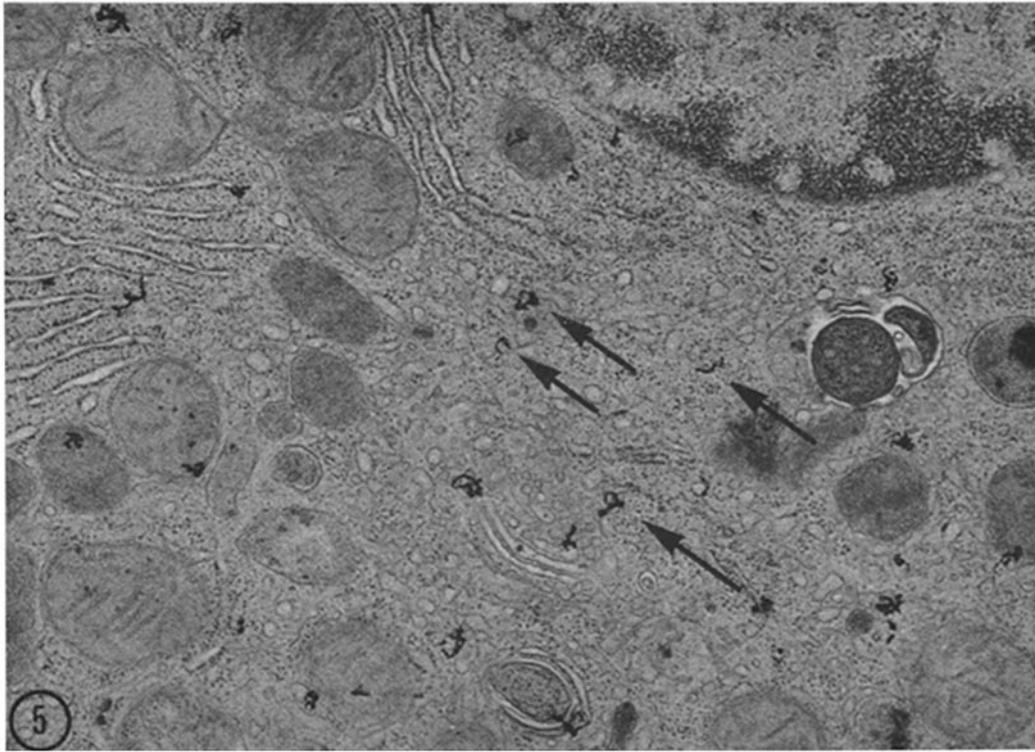


FIGURE 5 Radioautograph of rat liver slice incubated for 23 min after a 2 min pulse of leucine- ^3H . Fixed and stained as for Fig. 3. Exposed 19 weeks to Kodak NTE emulsion. The grains near the nucleus lie over free ribosomes. Those marked by arrows were considered to lie over mixed endoplasmic reticulum. Comparison with Figs. 3 and 4 shows the increased resolution of the NTE emulsion. $\times 21,000$.

zone of active cells in slices by the ability to form glycogen from glucose, as did Moyson (37) by radioautography after incubation of 1 mm cylinders of liver in glycine- C^{14} . In light microscopic radioautographs of liver slices after a 2 min pulse of leucine- H^3 as in the present study, the expected zonal distribution of silver grains was also observed (45). Few grains were found over cells in the damaged external layer or in the central half of the slice.

COMPARISON OF ILFORD L-4 AND KODAK NTE EMULSIONS: Resolution with the Ilford L-4 emulsion (Figs. 3, 4) is less precise than with the finer grained Kodak NTE emulsion (Fig. 5). Salpeter et al. (53), using techniques similar to those employed in this study, have estimated that the half-distance of resolution of a source of radioactivity is 0.165μ for L-4 and 0.10μ for NTE. Despite the greater resolution attainable with NTE, similar results were obtained when experi-

ments with NTE and L-4 were evaluated separately and compared (Fig. 6). The lower sensitivity of NTE required exposures about 3 times as long.

QUANTITATION; RELATIVE AREAS: Percentages of the points falling over various cell components in the radioautographic specimens are given in Table I. The categories of cell components are those in common usage, with the possible exception of "mixed e.r." and "cell margin." "Free ribosomes" were defined by the criteria of Bruni and Porter (5) as particles not attached to membranes and showing no tendency to a regular organized configuration (Fig. 5). "Mixed e.r." consists of membranes to which some ribosomes are attached but in which the architectural pattern lacks the regular "stacked" orientation of the more typical rough endoplasmic reticulum (Fig. 5) (see also page 703 and Fig. 7 of ref. 5). The "cell margin" was arbitrarily defined as the zone beneath the cell membrane comparable in width

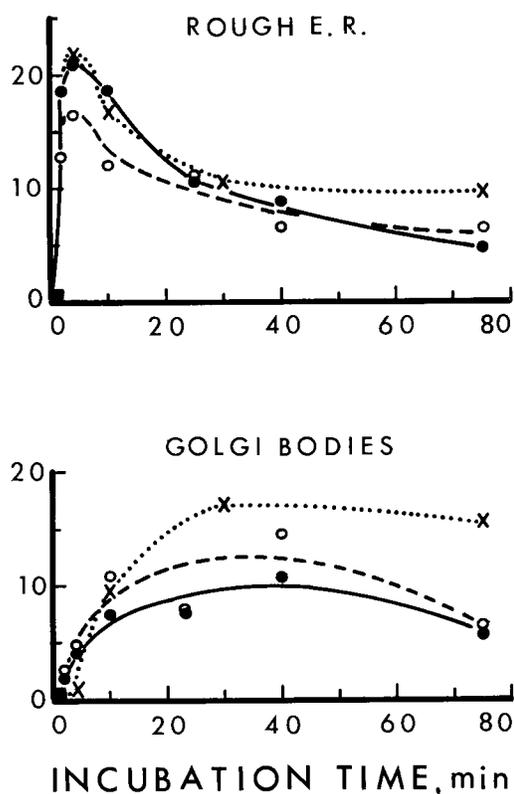


FIGURE 6 Comparison of the levels of silver grains over rough e.r. and Golgi bodies observed with Ilford L-4 and Kodak NTE emulsions, and when the experimental animal was fasted for 18–24 hr or fed ad lib. Liver slices were exposed to leucine- H^3 for 2 min (dark rectangles). The ordinates are per cent of maximal incorporation, because the total grains per specimen were made proportional to the total incorporation in a companion liver slice at that time (Fig. 2) as described in the Methods section. ●—●, L-4 emulsion, fasted rat, 2 experiments. ○—○, NTE emulsion, fasted rats, 2 experiments. X.....X, NTE emulsion, rat fed ad lib, single experiment.

to a developed L-4 grain, about 0.15μ , plus the microvilli.

Hepatocytes occupied about 90% and R. E. cells about 6% of the total area. In one of the four experiments liver from a rat fed ad lib. was employed. The glycogen area was excluded from the calculations of this experiment, as was the relatively low count of silver grains over glycogen. The time curves for rough e.r. and Golgi bodies in this experiment were not materially different from those of experiments with livers from rats fasted

18 hr (Fig. 6). The rationale behind the exclusion of the glycogen area is that cell size (11) and liver weight (47) increase with increasing glycogen content in liver cells, the accumulated glycogen apparently causing expansion of the cytoplasmic volume rather than reduction in amounts of the constituents already present.

QUANTITATION; SILVER GRAINS: Figs. 7 and 8 show the locations of grains with increasing time after the 2 min pulse of leucine- H^3 . The data are the composites of result on all specimens of four experiments. Since total incorporation was maximal by 4 min, and washing of the labeled free leucine from the cells was nearly complete at this time (Fig. 2), it appears probably that the grains over individual components at 4 min correspond to the presence of protein formed in situ, and that changes in grain counts occurring after this time indicate migration of newly formed proteins within the cell.

In free ribosomes and rough e.r. (Fig. 7) the grain counts rose rapidly, the highest level at 4 min being that of the rough e.r. The activity in the rough e.r. also declined the most rapidly thereafter. The curve for mixed e.r. rose at first sharply but then more gradually to a maximum at about 20 min. Levels in smooth e.r. and Golgi bodies increased more slowly to broad maxima between 20 and 40 min. The radioactivity in the cell margin rose rapidly, and remained at a relatively constant level for the duration of the experiment. The fall in radioactivity in the rough e.r. and the rise in the Golgi bodies between 4 and 40 min were each significant to a probability of < 0.01 when compared by student's *t* test.

The grain counts over nuclei (Fig. 8) reached a plateau at 4 min and later rose slightly. Mitochondria were highly labeled by 4 min but not maximally until 10–20 min. The activity in microbodies and lysosomes was low but tended to peak at roughly 40 min. Reticuloendothelial cell protein was labeled promptly and then remained constant at 4–6% of the total radioactivity in liver. The grain counts over extracellular space were low, and would not be expected to increase much since secreted proteins would have been washed from the liver slices. Table I lists grain concentrations, i.e. per cent of grains per unit area, for cell components at 4 min and at the time of maximal label.

If synthesis of protein was stopped by addition of puromycin at the time when total label was maximal (4 min), the data points shown as broken

TABLE I
Distribution of Cell Components and Radioautographic Silver Grains (Excluding Glycogen)

Component	Per cent of total observed area	Per cent of area of hepatic cell cytoplasm	Grain concentration		
			at 4 min	at time of maximal label	Time of maximal label
			% grains/% area		min
Nuclei	7.7 ± 3.3	—	0.56	0.97	75
Mitochondria	25.2 ± 3.8	30.7	0.67	0.72	10-20
Free ribosomes	10.0 ± 4.0	12.2	1.22	1.22	4
Rough e.r.	10.4 ± 3.8	12.7	1.87	1.87	4
Mixed e.r.	12.4 ± 4.2	15.4	1.05	1.66	20-25
Smooth e.r.	1.7 ± 1.6	2.1	2.4	4.37	25
Golgi bodies	4.5 ± 3.6	5.5	0.82	2.45	30-40
Cell margin	16.2 ± 4.0	19.8	0.83	1.09	75
Microbodies	1.1 ± 0.8	1.3	0.9	2.1	(40)
Lysosomes	0.5 ± 0.4	0.6	0.2	2.2	(40)
R. E. cells	5.7 ± 2.4		0.74	0.93	(75)
Red blood cells	0.6 ± 0.9		—	—	—
Extracellular	3.7 ± 2.3		0.2	0.7	40-75
Total	100		(1.0)		(4)
Total hepatic cell	89.7				
Glycogen area, rat fed <i>ad lib.</i>	27.5 ± 3.8				

lines in Figs. 7 and 8 were obtained. The results for most organelles were changed but little from the findings without puromycin, except for the lower values for smooth e.r. There was an even more pronounced fall in the grain count over the rough e.r. and free ribosomes, and a more prominent rise over the Golgi bodies and mixed e.r.

DISCUSSION

Results of this radioautographic study, as given in Figs. 7 and 8 and Table I, are consistent with current concepts of protein synthesis. The early peak of activity in free ribosomes and rough e.r. confirms the generally accepted role of ribosomes as the site of origin of most cytoplasmic proteins. Other sites of protein synthesis in hepatocytes, as judged by the grain counts at 4 min (Figs. 7 and 8), are nuclei, mitochondria, mixed e.r., and the cell margin. The lower activity in free ribosomes than in bound ribosomes, expressed either as total grains (Fig. 7) or grain concentration (Table I), is in agreement with several studies with isolated cell fractions (19, 20). The activity at the cell margin

is of lower intensity (Table I) and can probably be explained by the presence of rough e.r. in this heterogeneous component. The relatively high grain concentration computed for smooth e.r. at 4 min is of doubtful significance, considering the small volume of this component.

Nuclei (1) and mitochondria (55, 61) possess recognized mechanisms for production of proteins. When expressed as grain concentration per unit area (Table I), the 4 min values for nuclei and mitochondria are below the average for liver, in accord with the lower incorporation of labeled amino acids into nuclei and mitochondria found when these components were isolated after labeling (14, 36, 54).

Transport of newly formed proteins from one area to another appears in Figs. 7 and 8 as a change in grain count after 4 min. The fall in grain count is greatest in the rough e.r., less in the free ribosomes, and is present but somewhat delayed in the mixed e.r. The gradual rise in nuclear labeling suggests a slow inward movement of a small amount of protein of cytoplasmic origin, and the

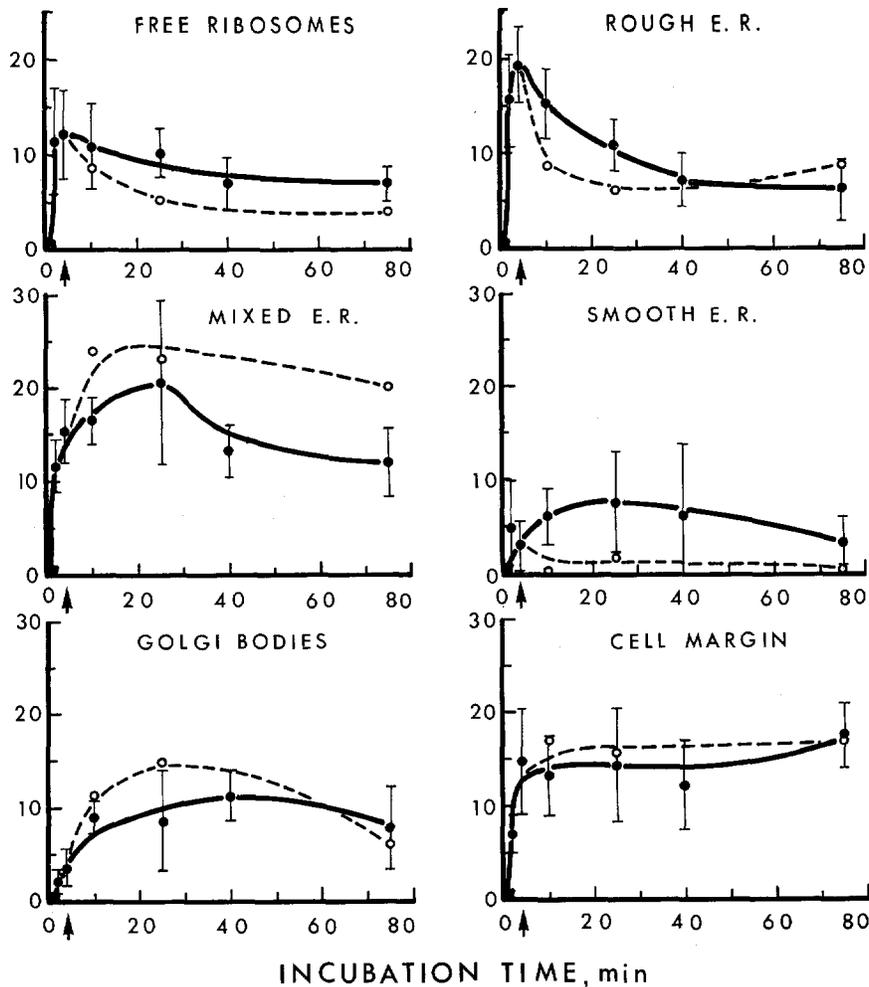


FIGURE 7 Distribution of silver grains over cytological components of liver slices possibly involved in secretion of newly formed proteins at various times after a 2 min pulse of leucine-³H (shown as dark rectangles). Ordinates are per cent of maximal incorporation as described for Fig. 6. Mean values and standard deviations for 4-5 specimens at each time point are plotted. The open circles (O) connected by broken lines refer to single specimens to which 2 mM puromycin was added at 4 min (arrows).

continued rise in mitochondrial labeling after 4 min may reflect similar migration into mitochondria. Transfer of protein into mitochondria has been reported by Beattie (4) and Kadenbach (29) in studies with cell fractionation.

Essner (17) proposed, from cytological evidence, that microbodies arise from the rough e.r., and Higashi and Peters (22) have shown that catalase, an enzyme later found to be characteristic of microbodies (3), originates in rough e.r. Similarly Dallner et al. (9) and Manganiello and Phillips (31) have published tracer studies suggesting that the rough e.r. produces the membranous portion of the smooth e.r. One could presume, from the

increases in label after 4 min in the present study, that most of the proteins of the microbodies, smooth e.r., Golgi bodies, and lysosomes are not made in situ, but one would be unable to offer a precise origin except to say that the rough e.r. seems the most likely source. Formation of microbodies (5) or lysosomes (16) as outcroppings of Golgi saccules, however, can not be excluded.

Plasma proteins have been calculated to make up one third to one half of the total protein synthesized by liver (18, 41, 51). Their secretion occurs between 20 and 40 min after their synthesis (15). Even though the silver grains do not distinguish between labeled liver proteins and proteins

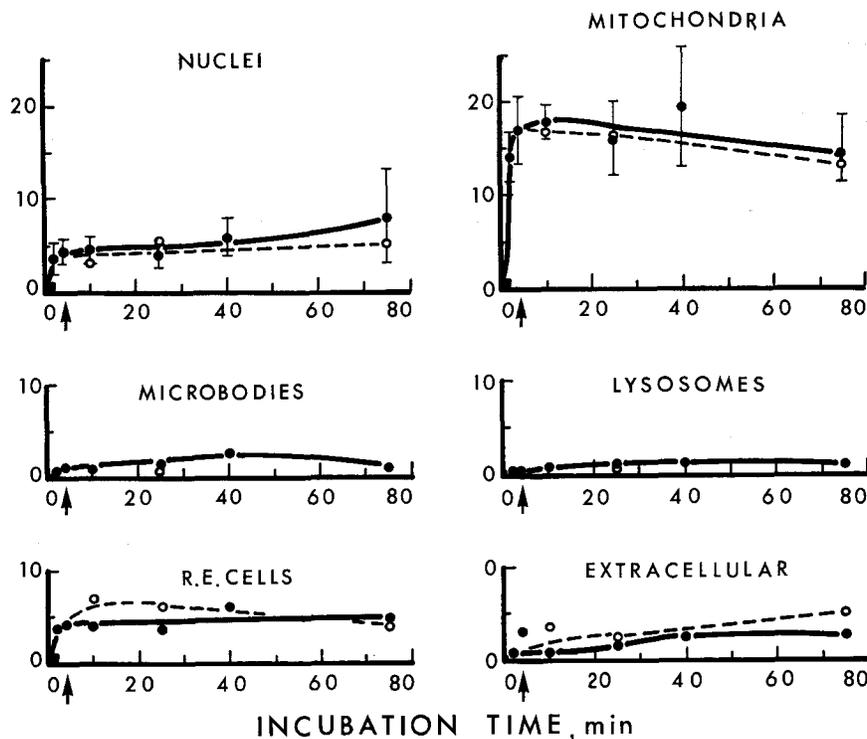


FIGURE 8 Distribution of silver grains over various cytoplasmic components of rat liver slices. Details are the same as in Fig. 7.

which are in the process of secretion into the plasma, such a large component of labeled protein must influence the level of labels in intracellular constituents. The sequence of peaks in the curves of Fig. 7 and Table I suggests that the secretion pathway is from rough e.r., through mixed e.r., then through the Golgi apparatus to the cell margin. This pathway is in accord with earlier findings with isolated cell fractions, and is consistent with the radioautographic study of Droz and Lacassagne (13). Inclusion of the smooth e.r. in the pathway is a possibility, but the results with this component were variable.

Passage of plasma glycoproteins and lipoproteins through the Golgi apparatus is made likely by the findings of Neutra and Leblond (38) using radioautography and of Rambourg et al. (49) using specific staining that glycoproteins acquire carbohydrate constituents in the Golgi complex, and by the reports of Stein and Stein (58) and Jones et al. (26) that lipids are coupled to lipoproteins in the same site. In the pancreas the Golgi apparatus functions to package proteins into secretory granules (23), but whether albumin is similarly

packaged in liver, as proposed by Bruni and Porter (5), has not been answered by this study and must await means of specific detection of albumin within Golgi saccules.

The results with puromycin agree with those found in the pancreas by Jamieson and Palade with cycloheximide or puromycin (24), namely, that further synthesis of protein is not required in order for newly formed proteins to move from one cell component to another. Puromycin acts similarly on the perfused rat liver since the output of labeled albumin ceases only after a half an hour, approximately the time required for secretion of the albumin already formed when the inhibitor was added (6, 25). The increased sharpness of the peaks of the free ribosomes and rough e.r. after puromycin addition may have been caused by rapid permeation of the inhibitor to the sites of protein synthesis, resulting in cessation of further incorporation of the small amount of labeled leucine remaining at 4 min (Fig. 2) and thus sharpening the pulse-label. One interpretation of the effect of puromycin in blocking the appearance of label in the smooth e.r. is that the formation of

the smooth vesicles requires coordination of synthesis of membrane and other protein constituents in a prescribed time sequence, the later stage of which has been blocked by puromycin.

Use of a small slice of tissue as an experimental system for electron microscopic radioautography offers certain obvious advantages. Cost of isotope is lowered, since the amount of tissue labeled can be kept to a minimum. Environmental conditions can be more varied, can contain inhibitors toxic to the intact animal, and can be more rapidly changed, e.g., exposure to label can be terminated more abruptly than is possible in the intact animal. Jamieson and Palade (23) have recently presented similar arguments for the use of pancreas slices.

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