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Incorporation of Amino Acids into some Subcellular Fractions of Hepatic Tissues of the Rat

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After the injection of an isotopically labelled amino acid there is a marked difference in the rate at which the protein of the various subcellular constituents of the liver cells becomes labelled. This was first shown by Hultin (1950) with chicks, then by Borsook, Deasy, Haagen-Smit, Keighley & Lowy (1950) for guinea pigs, and later by Keller, Zamecnik & Loftfield (1954) for rats. When slices of rat liver are incubated with a labelled amino acid and subsequently homogenized, the distribution of radioactivity in the different subcellular fractions is very similar to that in the experiment *in vivo* (Campbell, Greengard & Jones, 1957). If homogenates of liver are incubated with a radioactive amino acid and a suitable energy source, the distribution of radioactivity is still similar to that in the intact cell (Siekevitz, 1952). Thus a certain characteristic of the amino acid-incorporating system is maintained in the disrupted cells, sug-

gesting that it might be possible to study the regulation of protein synthesis in subcellular preparations.

In view of the above considerations it was naturally of interest to continue our experiments, designed to elucidate differences between protein synthesis in the normal and malignant liver cell, on cell-free systems. As in our previous studies, liver tumours have been induced by feeding 4-dimethyl-aminoazobenzene to rats. In order to make comparisons with a rapidly growing normal tissue, preparations from regenerating liver have also been used.

Since the microsomal fraction of the liver cell incorporates amino acids more rapidly than any of the other subcellular fractions so far studied, emphasis, in the present experiments, has been placed on this fraction. Zamecnik & Keller (1954) were the first to demonstrate the incorporation of

amino acids into the protein of isolated microsomes. Their system, in which the level of adenosine triphosphate is maintained by an added source, has the advantage that differences due to the ability of the different tissues to generate energy-rich phosphate are eliminated. Such microsomal preparations have been used therefore in the present experiments. The effect of the various subcellular fractions on the ability of each to incorporate amino acids has also been studied.

A preliminary report of some aspects of this work has already appeared (Campbell & Greengard, 1957).

MATERIALS AND METHODS

Chemicals. The dipotassium salt of adenosine triphosphoric acid (ATP) was obtained from the Sigma Chemical Co., St Louis, Mo., U.S.A. and neutralized with KOH. The barium silver salt of phosphoenolpyruvic acid (PEP) was prepared by the method of Ohlmeyer (1951). A solution of the free acid was prepared shortly before use and was adjusted to pH 7. Glutathione was obtained from The Distillers Co. (Biochemicals) Ltd., Liverpool, and used in a freshly prepared solution (adjusted to pH 7 with KOH).

Radioactive amino acids. Uniformly labelled [^{14}C]-glycine ($100\ \mu\text{C} \equiv 1.16\ \text{mg.}$), L-[^{14}C]leucine ($100\ \mu\text{C} \equiv 1.49\ \text{mg.}$) and L-[^{14}C]lysine ($100\ \mu\text{C} \equiv 1.13\ \text{mg.}$) were supplied by The Radiochemical Centre, Amersham, Bucks.

Tissues. (1) Liver tumours. Tumours were induced in rats by feeding them on a diet containing 0.06% of 4-dimethylaminoazobenzene as described by Campbell (1955). All rats bearing tumours were kept on a normal diet free of the carcinogen for at least 4 weeks before use. (2) Regenerating liver. Rats (150 g. body wt.) were partially hepatectomized by the method of Higgins & Anderson (1931). The regenerating livers were used 44–48 hr. after the operation. (3) Normal liver. This was obtained from rats (150 g. body wt.) fed on the stock diet.

Tissue preparations. In all cases centrifuging took place at approx. 1° . (1) Microsomes. The method used followed closely that described by Zamecnik & Keller (1954). The tissue was homogenized in 2.5 times its weight of a medium containing phosphate buffer (0.01M-MgCl₂, 0.02M-potassium phosphate (pH 7.8), 0.03M-KHCO₃, 0.025M-KCl and 0.35M-sucrose). The tissue suspension was centrifuged at 12 000 g for 5 min. The supernatant from this centrifuging was then centrifuged in a Model L Spinco preparative centrifuge (no. 40 rotor) for 50 min. at 105 000 g. The surface of the microsomal pellet was washed with 1 ml. of medium and suspended, in 0.4 ml. of medium/g. of original liver, by gentle homogenization in a small all-glass Potter homogenizer. (2) Mitochondria. The method used was based on that of Schneider & Hogeboom (1950). The tissue was homogenized in a Potter-Elvehjem type glass homogenizer in nine times its weight of 0.25M-sucrose. The debris and nuclei were removed by centrifuging at 500 g for 30 min. The supernatant was then centrifuged at 12 000 g for 10 min. The supernatant, together with the fluffy layer, was decanted, and the sediment homogenized in a small volume of 0.25M-sucrose and spun down at 12 000 g for 10 min. Before incubation the mitochondria were suspended in medium as described for the microsomes.

(3) Nuclei. The tissues were homogenized in an equal weight of 1.45M-sucrose, followed by a further homogenization in ten times the tissue weight of 0.88M-sucrose (Palade, 1951). The sediment after centrifuging at 600 g for 10 min. was discarded and the supernatant was spun again at this force for 20 min. The sedimented nuclei were stirred with a rod in 0.88M-sucrose and respun. Before incubation the pellet was suspended in medium as described for the microsomes.

Method of incubation. The above cell fractions, individually or in combination, were incubated under a continuous stream of N₂ + CO₂ (95:5) at 37° in a total volume of 1 ml. The incubation mixture contained 2 μ moles of ATP, labelled amino acid, 10 μ moles of PEP, 0.3 ml. of cell sap (i.e. supernatant after centrifuging at 105 000 g) and 0.4 ml. of the suspension of the particles in the medium containing phosphate buffer.

The amount of microsomal proteins in each tube was 6–7 mg. The wet weight of tissues yielding this amount of microsomal protein was 1 g. of liver and regenerating liver and 1.5 g. of tumour. The amount of mitochondrial protein per tube was 8–9 mg., obtained from 1.2 g. wet wt. of either normal or regenerating liver or 2.5 g. of tumour. The amount of nuclear protein per tube was 5–6 mg., obtained from 2 g. of either normal or regenerating liver or tumour.

Isolation of protein. If, after incubation, it was necessary to separate the different particulate fractions, this was effected by the fractionation procedure previously described. The proteins were precipitated in aqueous 5% (approx.) trichloroacetic acid. They were then treated with hot trichloroacetic acid and organic solvents according to a modified Schneider procedure (Zamecnik, Loftfield, Stephenson & Steele, 1951).

In order to determine whether the radioactivity was due to radioactive peptides attached to the protein by disulphide bonds, the normal washing procedure was in some cases followed by treatment of the protein with thioglycollic acid, as suggested by Siekevitz (1952). The protein (10 mg.) was suspended by homogenization in 1.5 ml. of an aqueous solution of 6% (w/v) potassium thioglycolate at pH 8.0. After 2 hr. at room temperature 1.5 ml. of 10% (approx.) trichloroacetic acid was added and the sediment was washed with hot (90°) 5% trichloroacetic acid, cold 5% trichloroacetic acid, ethanol and acetone.

Measurement of radioactivity. The proteins were counted at infinite thickness in one of two ways. In the first, the radioactivity was determined on 0.28 cm.² Perspex disks in a Geiger-Müller counter with a mica end-window (2.3 mg./cm.²). In the second, stainless-steel disks of similar dimensions were used in a Tracerlab SC-16 windowless flow counter. In this case the protein was compressed on the disk with a stainless-steel pestle, as described by Busch Simbonis, Anderson & Greene (1956). In a given time a radioactive sample gave four times as many counts in the flow counter as in the mica end-window counter. The background count in the flow counter was also four times that in the window counter. Provided that the counting rate exceeded 50 counts/min. above background a total of at least 1000 counts was collected, in which case the coefficient of variation of the count was less than 10%. If the radioactivity of the sample fell below 50 counts/min. above background the samples were counted for four periods of 5 min. interspersed with background counts. Under these circumstances, samples having a count exceeding 20/min. had a coefficient of variation of the

Table 1. *Comparison of the incorporation of [¹⁴C]glycine into microsome preparations of normal and regenerating liver*

Microsomes and cell sap (in amounts described under Methods) were incubated anaerobically for 50 min. with 2 μ moles of ATP and 2 μ C of [¹⁴C]glycine. An equal volume of 10% trichloroacetic acid was then added. The radioactivity of the protein (microsomal + cell sap) is expressed as counts/min. at infinite thickness/0.28 cm.² with the mica end-window counter.

Phosphoenolpyruvate (10 μ moles)	-	+	-	+	+	+
Normal-liver microsomes	+	+	-	-	+	-
Normal-liver cell sap	+	+	-	-	-	+
Regenerating-liver microsomes	-	-	+	+	-	+
Regenerating-liver cell sap	-	-	+	+	+	-
Radioactivity of protein	5	65	34	236	110	135

Table 2. *Comparison of the incorporation of [¹⁴C]glycine into microsome preparations of normal liver and liver tumour*

For experimental conditions and expression of results see Table 1.

Phosphoenolpyruvate (10 μ moles)	-	+	-	+	+	+
Normal-liver microsomes	+	+	-	-	-	+
Normal-liver cell sap	+	+	-	-	+	-
Liver-tumour microsomes	-	-	+	+	+	-
Liver-tumour cell sap	-	-	+	+	-	+
Radioactivity of protein	8	77	12	18	18	42

count of less than 15% and samples with a count below 20/min. had a coefficient of variation of the count of less than 20%.

RESULTS

Incorporation of amino acid into microsomal protein

When microsome preparations of normal and regenerating liver are compared under the same conditions, those from the regenerating tissue are found to have an enhanced ability to incorporate amino acid. These results are shown in Table 1.

The control value, i.e. the radioactivity of proteins incubated in the absence of PEP, was established in each case and was considered as being due to a non-specific adsorption, although it may partly represent a real incorporation process, utilizing the residual endogenous energy source. It can be seen that this control value is higher for regenerating liver and, since the same amount of protein was incubated with the same quantity of radioactive amino acid, this consistent result cannot have been due to a difference in non-specific adsorption of radioactive amino acid. The significance of this finding, however, is diminished by the fact that when amino acids other than glycine were used and the microsomal protein was separated from the cell sap after incubation, the values for the control, without PEP, were as low as those of normal liver. The table also shows that when microsomes of regenerating liver were incubated with normal cell sap, or normal microsomes incubated in regenerating cell sap, the incorporation of radioactivity, in both cases, was higher than with normal constituents alone. Thus both fractions of the regenerating liver are superior, in

Table 3. *Incorporation of [¹⁴C]lysine and [¹⁴C]leucine into microsome preparations of liver and liver tumour*

Microsomes and cell sap (in quantities described under Methods) were incubated anaerobically for 50 min. with 2 μ moles of ATP and 1 μ C of [¹⁴C]amino acid. The microsomes were separated from the cell sap immediately after incubation. The specific activity of the microsomal protein is expressed as counts/min. at infinite thickness/0.28 cm.², in the windowless flow counter.

	Liver		Liver tumour	
	Control	With PEP (10 μ moles)	Control	With PEP (10 μ moles)
[¹⁴ C]Leucine	15	204	20	83
[¹⁴ C]Lysine	33	256	44	109

this respect, to that of normal liver. Similar results have been obtained independently by Hultin & von der Decken (1957).

The microsomes isolated from liver-tumour slices after incubation with [¹⁴C]glycine are as active as those isolated from normal liver incubated under similar conditions (Campbell *et al.* 1957). Microsome preparations of liver tumour, however, were only slightly active (Table 2). The combination of tumour microsomes with normal cell fluid did not lead to a higher incorporation. The tumour-cell sap does not lack the ability to stimulate incorporation since, when used with normal microsomes, a significant incorporation can be obtained.

In all experiments of the type reported in Table 2, the energy-dependent uptake of glycine by tumour preparations was consistent, but so small that it could be doubted whether it represented a real

Table 4. *Effect of glutathione on the incorporation of [14 C]lysine by normal microsome preparations of liver and liver tumour*

For details of incubation and measurement of radioactivity see Table 3. Glutathione, when used, was added at the beginning of the incubation.

	Normal liver			Liver tumour			
Phosphoenolpyruvate (10 μ moles)	-	+	+	-	+	+	+
Glutathione (μ M)	-	-	2.5	-	-	2.5	1.0
Radioactivity of microsomal protein	20	357	250	41	63	97	93

Table 5. *Effect of the nuclear fraction on the incorporation of [14 C]amino acids by liver-microsome preparations*

Microsomal preparations were incubated anaerobically for 50 min. with 2 μ moles of ATP, 10 μ moles of PEP and 2 μ C of [14 C]glycine or 1 μ C of [14 C]leucine, in the presence and absence of the nuclear fraction in quantities described under Methods. The nuclear fractions were isolated by centrifuging. When glycine was used the microsomal and cell-sap proteins were precipitated together and their radioactivity is expressed as counts/min./0.28 cm.², at infinite thickness with the mica end-window counter. When leucine was used, the microsomes were separated from the cell sap and the radioactivity of the microsomal protein is expressed as counts/min./0.28 cm.², at infinite thickness in the windowless flow counter.

Amino acid	Microsomal fraction of	Nuclear fraction present	Radioactivity of protein
[14 C]Glycine	Normal liver	None	30
	Normal liver	Liver	11
	Normal liver	Tumour	13
	Regenerating liver	None	136
	Regenerating liver	Regenerating liver	21
	Regenerating liver	Tumour	8
[14 C]Leucine	Normal liver	None	294
	Normal liver	Liver	78

incorporation process. It can be seen, however, from Table 3 that by using amino acids which are taken up to a greater extent, by improving the method of counting and by separating the microsomes from the less radioactive cell-sap proteins after incubation, a considerable incorporation into tumour microsomal proteins may be demonstrated.

It can be seen from Table 4 that a 30% increase in lysine incorporation could be obtained by adding glutathione to the incubation medium, whereas the incorporation by normal liver microsomes is inhibited by it. When [14 C]leucine was used, instead of lysine, the stimulation by glutathione was smaller and more variable.

Effect of the nuclear fraction on incorporation of amino acid into microsomes

In preliminary experiments it was observed that, when whole homogenates of liver were incubated with a labelled amino acid, the incorporation into the microsomes was much lower than in the above type of microsomal preparation. The possibility that one of the fractions is inhibitory was tested. The nuclear fractions of all the three hepatic tissues under study were not able to incorporate amino acids to any significant extent into their proteins. When the nuclear fractions were mixed with microsomes, in a ratio similar to that in the cell, a nearly complete inhibition of microsomal

incorporation resulted (Table 5). When smaller amounts of the nuclear fraction were used, the inhibition was less marked. Since an inhibition of the already very low tumour microsomal incorporation could not be easily detected, the inhibitory action of the tumour nuclear fraction was tested on normal and regenerating liver microsomes and was found to be as marked as that of the other nuclear fractions (Table 5).

Incorporation of amino acid into mitochondria and their effect on microsomal incorporation

The purity of the mitochondrial fraction used in these studies has not been tested; it is probable that it contains small quantities of microsomes. Simpson, McLean, Cohn & Brandt (1957) found that, after repeated washing, the ribonucleic acid content of liver mitochondria was 1 mg./100 mg. of protein and it is not known to what extent this represents contamination by microsomes.

The results given in Table 6 show that when mitochondria, prepared from normal liver, are incubated with [14 C]leucine or [14 C]glycine together with some cell sap, there is a considerable incorporation of radioactivity. For leucine the activity of the mitochondrial preparations is less than that of the microsomes under similar conditions, whereas for glycine the reverse tends to be true. Moreover, whereas the incorporation of glycine into the

microsomes requires PEP, that into the mitochondria is not significantly effected by it. If the radioactivities of the thioglycollate-treated proteins are compared it will be seen that the results are qualitatively similar for [^{14}C]glycine and [^{14}C]leucine. The reason for this is probably that, in the presence of mitochondria, glutathione containing [^{14}C]glycine is formed which, through an -S-S- linkage, becomes attached to the protein but can be removed by the reducing agent (Peterson & Greenberg, 1952). The radioactivity of the [^{14}C]leucine-labelled proteins and that of the [^{14}C]glycine-labelled microsomal proteins is not much changed after thioglycollate treatment. This suggests that the synthesis of glutathione is largely confined to the mitochondrial fractions.

In order to investigate the effect of mitochondria and microsomes on each other, they have been incubated together in the presence of [^{14}C]leucine. This presents the problem of separating the two fractions after incubation. The mitochondrial fraction isolated by the usual differential centrifuging after incubation contains microsomes which have aggregated during incubation and thus sediment at a low centrifugal force. To test the extent of this contamination, microsomes were incubated

with [^{14}C]leucine under the usual conditions and then mixed with mitochondria which had been incubated in the absence of radioactive amino acid. The two fractions were then separated. From the amount of protein in the two fractions and their respective specific activities, it was calculated that 15% of the microsomes sedimented together with the mitochondria. The values given in Table 7 for mitochondrial incorporation in the presence of microsomes were corrected on this basis, i.e. the radioactivity due to the co-sedimented microsomes was subtracted from the measured activity of the mitochondrial protein. The contamination of microsomes by mitochondria is unlikely because of the tendency for particulate fractions, after incubation, to sediment at lower centrifugal forces. In any case this factor has been eliminated in the experiment, the results of which are given in Table 7.

Table 7 thus shows that the mitochondrial incorporation is greater in the presence of microsomes, whereas that of the microsomes is considerably diminished in the presence of mitochondria. It can also be seen from Table 7 that the PEP-free control value for microsomal protein radioactivity is higher in the presence of mitochondria, the

Table 6. *Comparison of the incorporation of amino acids by mitochondria and microsome preparations from normal liver and the effect of treatment with thioglycollic acid on the radioactivity of the proteins*

For details of incubation and isolation of proteins see Table 1. The treatment with thioglycollic acid is explained under methods. The results are given as counts/min. at infinite thickness/0.28 cm.² with the mica end-window counter.

		Radioactivity of proteins			
		Control		With PEP (10 μ moles)	
Amino acid	Fraction	Untreated	Thioglycollate treated	Untreated	Thioglycollate treated
[^{14}C]Leucine	Microsomal	15	14	207	177
	Mitochondrial	15	15	74	64
[^{14}C]Glycine	Microsomal	13	6	50	35
	Mitochondrial	73	15	69	20

Table 7. *Incorporation of [^{14}C]leucine by liver microsome and mitochondria preparations*

Liver microsomes and mitochondria were incubated anaerobically for 50 min. with cell sap (in quantities given under Methods), 2 μ moles of ATP and 1 μC of [^{14}C]leucine. When microsomes were incubated alone, mitochondria which had been incubated in a non-radioactive medium were added to the microsomes immediately after incubation. In order to determine the radioactivity of the different cell fractions, the mitochondria and the microsomes were isolated from the cell sap by differential centrifuging. Results are expressed as counts/min. at infinite thickness/0.28 cm.² in the windowless flow counter.

		Radioactivity of the protein fractions isolated after incubation			
		Microsomal		Mitochondrial	
		Control	With PEP (10 μ moles)	Control	With PEP (10 μ moles)
Fractions incubated					
Liver microsomal		12	594	—	—
Liver microsomal + mitochondrial		37	285	4	69
Liver mitochondrial		—	—	11	34
Tumour mitochondrial		—	—	17	44

reason probably being that the mitochondrial metabolism, with endogenous substrates, is able to maintain the supply of ATP needed for the process of amino acid incorporation.

The result obtained with liver-tumour mitochondria is also included in Table 7, showing that the labelling of their protein by incubation with [^{14}C]leucine is similar to that of normal liver mitochondria, in contrast with the results obtained with microsomes.

DISCUSSION

It has been shown by Zamecnik, Keller, Littlefield, Hoagland & Lofffield (1956) that the incorporation of amino acid, which takes place when liver microsomes are incubated under the conditions used in the present experiments, involves peptide bonds. Since, however, it is not at present possible to demonstrate the net synthesis of a specific protein in such systems it may be doubted whether *de novo* protein synthesis is being studied. It has been demonstrated recently that in such a system the amino acids are incorporated into a substance having the immunological characteristics of rat serum albumin (Campbell, Greengard & Kernot, 1958). Such systems therefore offer the best prospects at present available of studying protein synthesis in subcellular particles.

One way of determining the extent to which amino acid incorporation in the disrupted cell is related to protein synthesis in the whole cell is to compare the amino acid incorporation under different metabolic conditions. For this purpose a comparison of normal and regenerating liver has much to commend it. Eliasson, Hammarsten, Reichard, Åqvist, Thorell & Ehrensward (1951) found that the incorporation of [^{15}N]glycine into the proteins of regenerating rat liver in the whole animal was highest 56 hr. after hepatectomy.

It has previously been shown that when slices of liver and regenerating liver are incubated with [^{14}C]glycine there is a much greater degree of incorporation of radioactivity into the proteins of the regenerating liver than the normal liver, but that the pattern of intracellular incorporation is similar in both tissues (Campbell *et al.* 1957). Since the concentration of amino acids in the regenerating liver is greater than that in the normal liver (Christensen, Rothwell, Sears & Streicher, 1948) this result is unlikely to be due to the difference in the radioactivity of the precursor amino acids. This difference between the ability of the microsomes present in the whole cells, as slices of tissue, is also shown when these microsomes are isolated and then incubated. In the present experiments the microsomes of regenerating liver showed approximately three times the ability of the microsomes from normal liver to incorporate amino acids. Thus

it should be possible to use this subcellular preparation to investigate the factors underlying the difference between this fast-growing tissue and its normal counterpart. Such studies have been undertaken in greater detail by Hultin & von der Decken (1957) and von der Decken & Hultin (1958).

Such a parallelism between slices and subcellular preparations can, however, only occur if the process of cellular disruption damages the microsomes from the two types of tissue to the same extent. This may account for the failure to detect such a correlation when microsomes from normal liver are compared with those from liver tumour. Thus whereas liver-tumour microsomes in slices are somewhat more active than those of normal liver, they lose much of their activity when homogenized. In this connexion it is of interest that microsomes from the Ehrlich ascites tumour are less active than those from liver, although the reverse is true if the incorporation into intact cells is studied (Littlefield & Keller, 1957).

Zamecnik & Stephenson (1949) showed, by an enzymic method, that the concentration of glutathione in liver tumour was very low compared with that of the normal liver. This was confirmed by Campbell & Halliday (1957), who studied the composition of tissue extracts by ion-exchange chromatography. Sachs (1957), using a freeze-dried preparation of liver microsomes, showed that glutathione stimulated the incorporation of radioactive leucine. It seemed possible therefore that the low activity of the tumour microsomes could have been due to the low level of glutathione. That this is at least in part true is shown by the stimulation of amino acid incorporation when glutathione is added to tumour microsomes. The failure in the present experiments to obtain any effect on the incorporation into normal-liver microsomes by glutathione is possibly due to the use of a crude cell-sap fraction rather than the purified one used by Sachs. He also showed that there was an optimum concentration of glutathione, higher concentrations being inhibitory.

The nuclear fraction isolated from liver-tumour slices which were incubated with [^{14}C]glycine was much more active than that of normal liver (Campbell *et al.* 1957). In the present studies no difference between the nuclear fraction of the tumour and the liver could be detected. All three types of nuclear fraction inhibited the incorporation of amino acid into microsomes. Although the reason for this inhibition of the incorporation in one subcellular fraction by that of another is not understood at present, a practical point does emerge. If, when the whole homogenate of a tissue is incubated with a radioactive amino acid, the incorporation is very low, it is still possible that the incubation of

the individual subcellular fractions might produce more rewarding results.

In liver and in some other tissues, after the injection of radioactive amino acid, it is the microsomal protein which becomes highly labelled before the other fractions. It was suggested (Littlefield, Keller, Gross & Zamecnik, 1955) that this fraction is the primary site of protein synthesis in the cell. However, the possibility that organelles other than microsomes possess the ability to synthesize protein has not been excluded. Allfrey, Mirsky & Osawa (1957) have provided evidence that thymus nuclei are able to synthesize proteins without the contribution of other fractions. Bates, Craddock & Simpson (1958) have recently demonstrated the incorporation of [14 C]valine into cytochrome *c* by isolated rat-liver mitochondria, suggesting that mitochondria, also, are able to synthesize proteins. Although in the present studies the hepatic nuclei were inactive, the mitochondria had considerable activity. No attempt has been made to find the optimum conditions for the incorporation of amino acids into mitochondria, for the object was to study the effect of mitochondria on the incorporation into microsomes. The lowering of the microsome incorporation by mitochondria indicates that the two processes are not independent, but further studies have to decide whether a competition between the two synthesizing sites takes place or whether mitochondria exert a direct inhibition on microsomal incorporation. Elucidation of such problems may contribute to the understanding of the ways in which the characteristic subcellular distribution of amino acid incorporation, *in vivo* and *in vitro*, may be regulated.

SUMMARY

1. Microsomal preparations of regenerating liver incorporate three to four times as much [14 C]-glycine into their proteins as do those of normal liver, during a 50 min. incubation period.

2. Liver-tumour microsomal preparations have the ability to incorporate 14 C-labelled glycine, leucine and lysine into their proteins, in the presence of phosphoenolpyruvate. The extent of this incorporation, however, is much smaller than in preparations from normal liver.

3. Glutathione stimulates the incorporation of [14 C]lysine by preparations of liver-tumour microsomes.

4. The presence of the nuclear fraction from normal liver, liver tumour or regenerating liver greatly inhibits the incorporation of amino acids into the proteins of microsomal preparations.

5. Mitochondria of normal liver and of liver tumour are able to incorporate [14 C]leucine in the absence of microsomes, and this process is energy-

dependent. When liver microsomes and mitochondria are incubated together, the incorporation into the microsomes is reduced and that into the mitochondria is increased.

6. The relevance of these studies, on the incorporation of amino acids into subcellular preparations, to the problem of the mechanism of protein synthesis, is discussed.

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