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The Role of Cytoplasmic Ribonucleic Acid in the Incorporation of Amino Acids into Microsomal Proteins

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An incorporation of labelled amino acids into microsomal proteins dependent on the continuous presence of adenosine triphosphate occurs *in vitro* in the presence of the soluble cytoplasm (cell sap) (Zamecnik & Keller, 1954). If the cell sap is brought to pH 5.2 a fraction (pH 5 fraction) is precipitated which can replace the whole cell sap, provided that guanosine triphosphate and an energy-generating system are present (Keller & Zamecnik, 1956). It has been shown by Hoagland, Keller & Zamecnik (1956) that this fraction contains enzymes which activate the carboxyl groups of the amino acids in the presence of adenosine triphosphate. More recently it has been found that the ribonucleic acid of the pH 5 fraction (pH 5 RNA) binds the labelled amino acids in the presence of adenosine triphosphate (Hoagland, Stephenson, Scott, Hecht & Zamecnik, 1958). If the pH 5 RNA labelled with the amino acid is isolated and incubated with microsomes, the labelled amino acid is transferred to the microsomal protein without its prior release. This reaction is catalysed by enzymes present in the pH 5 fraction which are called, therefore, transferring enzymes. In summary, it has been postulated that in the incorporation of amino acid into microsomal proteins the free amino acid is first activated, then bound to the pH 5 RNA and finally is transferred to the microsomes.

Sachs (1957) has fractionated the cell sap in order to determine the nature of the substances responsible for facilitating the incorporation of

amino acids into protein. He attempted to remove the nucleoproteins contained in the cell sap by the addition of protamine and obtained, after further purification, an active fraction designated S-protein. It was of interest to determine whether in this fraction too there was a metabolically active ribonucleic acid. It has been found that S-protein contains no more than trace amounts of ribonucleic acid. Liver microsomes in the presence of S-protein appear, therefore, to incorporate amino acids by a process which is independent of the presence of pH 5 RNA.

Recently it has been shown that the carboxyl-group activation of methionine is inhibited by the addition of ribonucleic acid to the incubation medium (Rendi, Di Milia & Fronticelli, 1958). Since the pH 5 fraction contains appreciable quantities of ribonucleic acid it seemed possible that this fraction might inhibit amino acid activation as a result of a binding between the pH 5 RNA and the enzymes. Evidence will be presented in support of this hypothesis.

MATERIALS AND METHODS

Chemicals. Adenosine triphosphate (ATP; dipotassium salt) and guanosine triphosphate (GTP; sodium salt) were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. Solutions were brought to pH 7.8 (indicator papers) 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 7.8 (indicator papers). Glutathione (GSH) was obtained from The Distillers (Biochemicals) Co.

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Ltd., Liverpool. The compounds were dissolved and adjusted to pH 7.8 (indicator papers) with KOH. PEP-kinase, obtained from C. F. Boehringer und Söhne, Mannheim, Germany, was dialysed against twice-distilled water and diluted so that the protein concentration was 1 mg./ml. Protamine sulphate (from herring) was obtained from L. Light and Co.

Radioactive amino acids. Uniformly labelled [^{14}C]glycine, L-[^{14}C]alanine and L-[^{14}C]leucine were obtained from The Radiochemical Centre, Amersham, Bucks.

Tissues. Spleen and liver were obtained from Wistar albino rats fed on stock diet, weighing 150 g. Rats bearing transplantable Murphy lymphosarcoma were obtained from the Imperial Cancer Research Fund Laboratories, Mill Hill, London, N.W. 7.

Tissue preparations. (1) Microsomes. Tissues were homogenized with 2.5 vol. of a medium containing 0.01 M-MgCl₂, 0.03 M-KHCO₃, 0.025 M-KCl, potassium phosphate (KH₂PO₄-K₂HPO₄; 1:9) buffer, 0.02 M at pH 7.8, and 0.35 M-sucrose, in an all-glass Potter homogenizer. The suspension was centrifuged for 10 min. at 12 000 g at 2°. The supernatant was centrifuged in a model L Spinco preparative centrifuge (no. 40 rotor) for 50 min. at 105 000 g_{av}. The surface of the resulting microsomal pellet was washed with 1 ml. of medium and suspended in 0.4 ml. of medium/g. of original tissue for liver and 0.4 ml./2 g. of original tissue for spleen, by gentle homogenization.

(2) pH 5 fraction. The supernatant from the microsome pellet (cell sap) was brought to pH 5.2 (glass electrode) by the dropwise addition of M-acetic acid. The precipitate was spun down and suspended in 0.5 ml. of medium/g. of original tissue.

(3) S-Protein. The S-protein was prepared from the cell sap by the method of Sachs (1957), involving protamine and ethanol precipitation and finally treatment with charcoal. The final precipitate was dissolved in 0.01 M-potassium phosphate buffer, pH 7.8. In some cases this was used without dialysis (non-dialysed), but usually the S-protein was dialysed for 4 hr. at 0° against 0.1 mM-GSH and 0.1 mM-ethylenediaminetetra-acetic acid (EDTA) as described by Sachs (1957).

(4) Ribonucleic acid (RNA). pH 5 RNA from liver and spleen pH 5 fractions were prepared by the phenol method as described by Hoagland *et al.* (1958). With the same method RNA was prepared from the deoxycholate-insoluble fraction of liver microsomes prepared by the method described by Littlefield, Keller, Gross & Zamecnik (1955).

Ribonucleic acid and protein determinations. pH 5 fraction and S-protein were precipitated with 5 vol. of 0.4 N-HClO₄, washed twice with 0.2 N-HClO₄, twice with CHCl₃-ethanol-ether (1:2:2), once with ether and dried. The RNA was then extracted with hot 0.4 N-HClO₄ and the amount determined by its absorption at 260 mμ as described by Hoagland *et al.* (1958). In some cases an alternative method for the determination of RNA was used. The HClO₄ precipitate after treatment with organic solvents was heated (90°) for 20 min. with 5% (w/v) trichloroacetic acid and the amount of RNA extracted estimated by the orcinol method of Mejbaum (1939). The residual protein was dried and weighed.

Method of incubation. To study the incorporation of amino acids into the cytoplasmic fractions alone, both kinds of preparations (pH 5 fraction, or S-protein) were

gently shaken for 10 min. in air at 37°. The incubation mixture contained 10 μmoles of ATP, labelled amino acids and medium to bring the total volume to 1 ml. For incorporation studies in the presence of microsomes the latter were incubated under a continuous stream of N₂+CO₂ (95:5) at 37° and gently shaken for 30 min. in a total volume of 1 ml. The incubation mixture contained 1 μmole of ATP, 10 μmoles of PEP, 0.25 μmole of GTP, labelled amino acids, PEP-kinase and pH 5 fraction or S-protein.

Preparation of material for determination of radioactivity of ribonucleic acid or protein

The radioactivity of the RNA was determined by the method of Hoagland (personal communication). The proteins and RNA were precipitated with 5 vol. of 0.4 N-HClO₄, washed three times with 0.2 N-HClO₄, twice with CHCl₃-ethanol-ether (1:2:2), once with ether and dried. The radioactivity of this material was determined. The RNA was then in some cases extracted with 0.4 N-HClO₄ at 90° for 20 min. The radioactivity of the residual protein was again determined.

If, after incubation, it was necessary to separate the microsomes, the suspension was centrifuged for 50 min. at 105 000 g_{av}. The supernatant was rejected and the proteins of the microsome pellet were precipitated with aqueous 5% (w/v) trichloroacetic acid. They were then treated with hot trichloroacetic acid and organic solvents according to the method of Zamecnik, Loftfield, Stephenson & Steele (1951).

Measurement of radioactivity. The proteins were counted at infinite thickness on 0.28 cm.² stainless-steel disks in a Tracerlab SC-16 windowless flow counter. The protein was compressed on the disk with a stainless-steel pestle as described by Busch, Simbonis, Anderson & Greene (1956). The counting procedure was as previously described (Campbell & Greengard, 1959).

RESULTS

Binding of labelled amino acids to ribonucleic acid of pH 5 fraction

An energy-dependent binding of labelled amino acids to the pH 5 fraction of liver, spleen and lymphosarcoma was found (Table 1). Since most of the radioactivity could be readily extracted by hot HClO₄ the amino acids were presumably bound to the RNA. It will be seen that alanine is incorporated to the same extent into liver or spleen pH 5 RNA, whereas the incorporation of leucine is twice as high in liver as in spleen. There are three possible reasons for this. First, the enzymic activity for leucine may be higher in the liver extract, secondly, the number of binding sites for leucine in liver pH 5 RNA may be higher than in spleen and, finally, the ratio of RNA to protein may be higher in liver pH 5 fraction than in spleen. The last explanation is excluded by the results of the determinations given in Table 2. It will be seen that the RNA/protein ratios for the liver and spleen are similar. The use of the term RNA should be explained. The figures in Table 2

Table 1. *Incorporation of labelled amino acids into ribonucleic acid of purified fractions of the cell sap of different tissues*

Samples (0.7 ml.) of the fractions were incubated with 1 μ C of labelled amino acids with and without 10 μ moles of ATP for 10 min. at 37°. The protein and RNA were precipitated with 5 vol. of 0.4 N-HClO₄ and treated as described in the Methods section. The radioactivity is expressed as counts/min. at infinite thickness/0.28 cm.² in the windowless flow counter. Values in parentheses are the radioactivities of the proteins after extraction of the RNA with 0.4 N-HClO₄ at 90° for 20 min.

pH 5 fraction	Glycine		Alanine		Leucine	
	Control	Plus ATP	Control	Plus ATP	Control	Plus ATP
Liver	12	55 (4)	14	90 (17)	16	553 (17)
Spleen	17	42	12	89 (16)	19	240 (13)
Lymphosarcoma	—	—	—	—	9	98 (4)

Table 2. *Protein and ribonucleic acid content of pH 5 fractions of the cell sap of different tissues*

pH 5 fraction	Protein (mg./g. of fresh tissue)	RNA	
		(mg./g. of fresh tissue)	(μ g./mg. of protein)
Liver	3.2	0.070	22
Spleen	2.6	0.060	23
Lymphosarcoma	5.25	0.093	18

Table 3. *Effect of dilution on the incorporation of [¹⁴C]glycine into liver pH 5 ribonucleic acid*

The pH 5 fraction was incubated for 10 min. at 37° with 1 μ C of [¹⁴C]glycine and ATP as indicated and the final volume brought to 1 ml. with medium. For expression of the results see Table 1.

Incubation conditions	Radioactivity
pH 5 Fraction (0.75 ml.)	40
pH 5 Fraction (0.75 ml.) + 10 μ moles of ATP	98
pH 5 Fraction (0.45 ml.) + 10 μ moles of ATP	141

are based on the absorption of the solutions at 260 m μ multiplied by a suitable factor given by Hoagland *et al.* (1958). These authors have justified their use of the term RNA and, since the same methods of extraction and estimation have been used in the present work, it has seemed convenient to use the same nomenclature.

Enhancement of incorporation into pH 5 ribonucleic acid by dilution

If liver pH 5 fraction is diluted with the buffer medium and incubated with the same amount of [¹⁴C]glycine and ATP the incorporation into pH 5 RNA is increased (Table 3). This may be due to the fact that more labelled amino acid is present for the same amount of RNA or to the presence of an inhibitor which is less effective in the diluted system. When the pH 5 fraction is incubated with increasing amounts of [¹⁴C]glycine (Fig. 1) it can be seen that the RNA in both the diluted and undiluted fractions becomes increasingly labelled as the concentration of [¹⁴C]glycine is increased. If the extent of labelling of the RNA in the two systems is compared when the ratio of the amounts

Fig. 1. Effect of the concentration of [¹⁴C]glycine on its incorporation into the RNA of the diluted (curve A) and undiluted (curve B) pH 5 fraction from rat liver. For curve A 0.4 ml., and for curve B 0.8 ml., of pH 5 fraction was incubated for 10 min. at 37° with various amounts of [¹⁴C]glycine and 10 μ moles of ATP, the volume of the mixture being brought to 1 ml. by the addition of medium in each case.

Table 4. *Effects of potassium fluoride and ethylenediaminetetra-acetic acid on the incorporation of labelled glycine into pH 5 ribonucleic acid of liver*

A sample (0.7 ml.) of pH 5 fraction of liver was incubated in a final volume of 1 ml. with the compounds cited in the table, and with 1 μ C of [¹⁴C]glycine, for 10 min. at 37°. For the expression of the results see Table 1.

Addition	Radioactivity
None	19
10 μ moles of ATP	73
10 μ moles of ATP + 25 μ moles of KF	57
10 μ moles of ATP + 0.5 μ mole of EDTA	59

of RNA to [¹⁴C]glycine present in the incubation mixture is the same, it will be seen that the RNA in the undiluted system (B) is less active than in the diluted system (A).

Since these results seemed to exclude the possibility that the enhancement of incorporation by dilution was due to a change in the ratio of RNA to

the concentration of [^{14}C]glycine, it seemed likely that an inhibitor was present. To determine whether this inhibitor was adenosine triphosphatase (ATPase) the effect of fluoride, which inhibits the activity of this enzyme but has no effect on amino acid activation (Rendi *et al.* 1958), was studied. As is seen in Table 4 the inclusion of fluoride in the medium did not enhance the incorporation. Higher concentrations of ATP were also without effect. These results suggest that ATPase is not the inhibitor involved. The failure of EDTA to enhance the incorporation suggests that the inhibitor is not a metal ion.

Requirements for amino acid incorporation into liver microsomes in the presence of S-protein

Sachs (1957) has shown that amino acids are incorporated into microsomal protein in the presence of a purified fraction of the cell sap (S-protein). The results in Table 5 are in agreement with those of Sachs and show an energy-dependent incorporation of [^{14}C]leucine. Sachs also reported that the presence of glutathione in the medium enhanced the incorporation in the presence of dialysed but not undialysed S-protein. These results were also confirmed.

When the RNA content of the liver S-protein was determined by the orcinol method it was found that it contained less than 2 μg . of RNA/mg. of protein. In view of this it was of interest to determine the effect of adding isolated pH 5 RNA to the system. As shown in Table 5 such an addition enhanced the incorporation, especially with the non-dialysed preparation. The amount of RNA added (40 μg .) was such that the RNA content of the medium was restored to about the same level as in the experiments with pH 5 fraction (Table 1).

Role of ribonucleic acid in systems containing S-protein

In view of the almost complete absence of RNA in S-protein it was not surprising that when preparations of S-protein were incubated with [^{14}C]leucine ATP was without effect on the incorporation of radioactivity into the HClO_4 precipitate (Table 6). Moreover, the slight incorporation that did take place under these conditions was not influenced by subsequent extraction of the precipitate with hot HClO_4 . It was then of interest to determine whether the [^{14}C]leucine would be incorporated into pH 5 RNA when a preparation isolated from the cell sap was incubated with S-protein and ATP. The results shown in Table 6 indicate that the [^{14}C]leucine was incorporated into the pH 5 RNA. The presence of glutathione increased the incorporation with dialysed S-protein, but had little effect with non-dialysed S-protein.

Effect of glutathione on incorporation in the presence of dialysed pH 5 fraction

In view of the effect of glutathione on the incorporation of leucine into systems involving dialysed S-protein the effect of glutathione with other fractions of the cell sap was studied. Campbell & Greengard (1959) have shown that glutathione does not enhance the incorporation of [^{14}C]leucine when liver microsomes are incubated with whole cell sap. Similar results were obtained when pH 5 fraction was used. It seemed possible that this was because the pH 5 fraction had not been dialysed. The pH 5 fraction was therefore dialysed under the same conditions as were used for the S-protein and the effect of glutathione on the incorporation of [^{14}C]leucine into liver microsomes incubated with this dialysed fraction was then studied. The results shown in Table 7 indicate that glutathione either has an inhibitory effect or is without effect. Dialysis of the pH 5 fraction against EDTA enhances its activity, but this activity was not further increased by dialysis against glutathione.

Table 5. *Incorporation of [^{14}C]leucine into liver microsomes in the presence of dialysed and non-dialysed S-protein*

Samples (0.4 ml.) of liver-microsome suspension (see Methods section) and 0.3 ml. of S-protein from liver were incubated with 1 μmole of ATP, 10 μmoles of PEP, 0.25 μmole of GTP, PEP-kinase and the additions cited, in a final volume of 1 ml. for 30 min. at 37° under an atmosphere of $\text{N}_2 + \text{CO}_2$ (95:5). In the control PEP and PEP-kinase were omitted. The proteins were precipitated with 5% trichloroacetic acid and washed and counted as described in the Methods section. For the expression of the results see Table 1.

Additions	S-Protein	
	Dialysed	Non-dialysed
Control	7	7
None	124	59
1.2 μmoles of GSH	205	28
1.2 μmoles of GSH + 40 μg . of pH 5 RNA	251	107

Table 6. *Incorporation of [^{14}C]leucine into ribonucleic acid in S-protein preparations*

Volumes (0.7 ml.) of S-protein (prepared as described in the Methods section) with 10 μmoles of ATP were incubated in a total volume of 1 ml. for 10 min. at 37°. In the control ATP was omitted. After incubation the protein and RNA were precipitated by the addition of 0.4N- HClO_4 . The precipitate was treated and its radioactivity determined as described for Table 1.

Additions	Dialysed S-protein	Non-dialysed S-protein
Control	28	15
None	26 (25)	14 (14)
40 μg . of pH 5 RNA	112	80
40 μg . of pH 5 RNA + 1.2 μmoles of GSH	169 (30)	91 (18)

Effect of ribonucleic acid on incorporation
into microsomes

Rendi & Campbell (1959) have shown that when spleen microsomes are incubated with pH 5 fraction the incorporation of leucine is greater when the pH 5 fraction is derived from liver (181 counts/min.) than from spleen (125 counts/

Table 7. Incorporation into liver microsomes of [¹⁴C]leucine in the presence of dialysed pH 5 fraction

Volumes (0.4 ml.) of liver microsomes were incubated with 0.3 ml. of the different pH 5 fractions, 1 μmole of ATP, 0.25 μmole of GTP, 10 μmoles of PEP, PEP-kinase and 1 μc of [¹⁴C]leucine with and without 1.2 μmoles of GSH. For the expression of the results see Table 1.

Preparations of pH 5 fraction	Control	Plus GSH
pH 5 fraction dialysed against phosphate	587	496
pH 5 fraction dialysed against phosphate and EDTA	677	531
pH 5 fraction dialysed against phosphate and EDTA and GSH	684	689

Table 8. Incorporation of [¹⁴C]leucine into spleen microsomes in the presence of pH 5 fraction from spleen and increasing amounts of spleen pH 5 ribonucleic acid

A suspension of spleen microsomes (0.4 ml.) and 0.3 ml. of pH 5 fraction was incubated together with various amounts of pH 5 RNA obtained from spleen, anaerobically for 30 min. at 37° with 0.5 μc of [¹⁴C]leucine, 1 μmole of ATP, 10 μmoles of PEP, 0.25 μmole of GTP and PEP-kinase. The total volume of the mixture was brought to 1 ml. with medium. For the expression of the results see Table 1.

Amount of pH 5 RNA present (μg.)	Radioactivity of microsomal proteins
40	207
133	227
200	256

Table 9. Effect of various ribonucleic acid fractions on the extent of incorporation of labelled leucine into liver microsomes in the presence of liver S-protein

Volumes (0.4 ml.) of microsome suspension and 0.3 ml. of the undialysed S-protein from liver (see Methods section) were incubated with 1 μmole of ATP, 0.25 μmole of GTP, 10 μmoles of PEP, PEP-kinase, and 1 μc of [¹⁴C]leucine with 40 μg. of the different RNA fractions. For the incubation and the expression of the results see Table 1.

Additions	Radioactivity of protein
None	171
Liver pH 5 RNA	232
Spleen pH 5 RNA	96
Liver microsome RNA	240

min.). It has been shown above that leucine incorporation into pH 5 RNA is twice as high when the pH 5 fraction is derived from liver as when it is derived from spleen. This difference could be due to a paucity in the leucine-binding sites on spleen pH 5 RNA, compared with liver pH 5 RNA.

If this were the reason for the enhanced effect of liver pH 5 fraction on leucine incorporation into spleen microsomes enrichment of spleen pH 5 fraction with spleen pH 5 RNA might be expected to increase the incorporation into microsomes. The results shown in Table 8 indicate that this is the case, although the increase is not proportional to the amount of pH 5 RNA added.

Tissue specificity of effect of ribonucleic
acid added to S-protein

It has already been shown (Table 5) that the addition of pH 5 RNA enhances the incorporation into liver microsomes in the presence of undialysed S-protein. To test the specificity of the activity of this fraction, the effect of liver and spleen pH 5 RNA on the incorporation of [¹⁴C]leucine into liver microsomes in the presence of undialysed liver S-protein has been studied. As may be seen in Table 9, whereas liver pH 5 RNA enhances the incorporation, spleen pH 5 RNA inhibits it. The effect of adding the RNA isolated from the deoxycholate-insoluble fraction of liver microsomes (Littlefield *et al.* 1955) to the system was also determined. It will be seen (Table 9) that this fraction replaces pH 5 RNA from liver. This effect may be due to contamination of microsomal RNA with pH 5 RNA.

DISCUSSION

Hoagland *et al.* (1958) were the first to demonstrate that the ribonucleic acid (RNA) contained in the fraction of the cell sap precipitated at pH 5.2 plays a role in the transfer of free amino acids to microsomal protein. This finding raised a number of points, some of which it has been the purpose of this paper to investigate.

First, it is of interest to know if the pH 5 RNA is a necessary intermediate in the transfer of amino acids to the microsomes, or whether it is merely a storage site for activated amino acids. Secondly, since the enzymes responsible for the carboxyl-group activation of the amino acids are present in the pH 5 fraction it is important to know if an association occurs between them and the pH 5 RNA. Finally, the tissue specificity of the pH 5 RNA has been studied.

The fraction of the cell sap (S-protein) which Sachs (1957) used in his studies on the incorporation of amino acids into microsomal proteins is prepared from a supernatant of the sap after the

addition of protamine. Since protamine precipitates nucleic acids the system microsomes plus S-protein appeared to present the opportunity of studying amino acid incorporation in the absence of pH 5 RNA. In fact the proportion of RNA to protein in S-protein is negligible compared with that of the pH 5 fraction.

As reported by Sachs it was possible to enhance the incorporation of [^{14}C]leucine into microsomes in the presence of S-protein by the addition of glutathione, but only when the S-protein was dialysed. Since S-protein catalysed the incorporation of leucine into added isolated pH 5 RNA (Table 6), S-protein apparently contains the leucine-activating enzyme. The effect of glutathione could therefore be due to the leucine-activating enzyme in liver, like the tyrosine-activating enzyme from yeast (van de Ven, Koningsberger & Overbeek, 1958), being a sulphhydryl enzyme.

It has been shown previously (Rendi *et al.* 1958) that yeast RNA inhibits the formation of methionine hydroxamate catalysed by the methionine-activating enzyme of rat liver. It seemed possible that the RNA in the pH 5 fraction might cause a similar inhibition. Evidence that the pH 5 fraction contains an inhibitor for the incorporation of amino acid into pH 5 RNA has been presented. However, there is no direct evidence at present that the inhibitor is RNA. In this connexion it is interesting that Lipmann (1958) has found that a 'polynucleotide factor' isolated from the pancreas inhibits the incorporation of tryptophan into the nucleotide bound to tryptophan-activating enzyme.

There is one further observation which concerns the nature of the binding between the activated amino acids and RNA. It has been shown that S-protein contains the amino acid-activating enzymes, for this fraction catalyses the incorporation of amino acids into pH 5 RNA. However, after the treatment of the cell sap with protamine, adjustment of the supernatant to pH 5.2 does not lead to the formation of a precipitate. This, of course, is in contrast with the situation with whole cell sap from which the enzymes are precipitated at pH 5.2. It is tempting to postulate that under these latter conditions the precipitate contains amino acid-activating enzymes bound to pH 5 RNA.

The above-mentioned results, together with those of Hoagland *et al.* (1958), suggest that amino acid incorporation into microsomal protein can follow two different routes. These may be represented as shown in Scheme 1.

In the presence of pH 5 fraction the incorporation occurs through an RNA intermediate which is bound to the activating enzymes, whereas in the

presence of S-protein the incorporation appears to occur by a direct route, glutathione being required. Although as much cell sap as possible was removed from the microsomal pellets used in the experiments with S-protein it is still possible that some pH 5 RNA is present in the microsomes and that this permits incorporation through an RNA-bound intermediate. The existence of such a mechanism seems improbable on the basis of the results with glutathione, since this substance inhibits the incorporation in the presence of the pH 5 fraction but enhances it when S-protein is present. The results of recent unpublished experiments with subfractions of the microsome pellet confirm that incorporation in the absence of an RNA-bound intermediate does occur.

To explain the observed differences in the extent of amino acid incorporation into liver and spleen preparations it was previously suggested (Rendi & Campbell, 1959) that the cell sap contains different amounts of enzymes and cofactors specific for the different amino acids. In the present experiments the incorporation of both alanine and leucine into the pH 5 RNA from liver and spleen has been studied. The incorporation of leucine into spleen pH 5 RNA was found to be only 50 % of that into liver pH 5 RNA. This correlates well with the previous finding that the incorporation of leucine into liver microsomes was reduced by half if liver pH 5 fraction was replaced by spleen pH 5 fraction. Thus it appears that the step which accounts for the more active incorporation of leucine into liver microsomes by liver pH 5 fraction is the activation of leucine and its binding to the pH 5 RNA. With alanine the extent of incorporation into pH 5 RNA is the same in spleen and liver preparations (Table 1). On the other hand, alanine is incorporated into both liver and spleen microsomes to a much smaller extent by spleen pH 5 fraction than by liver pH 5 fraction. It seems probable, therefore, that the transferring enzyme of alanine is less

Scheme 1

concentrated in the pH 5 fraction of spleen than in the pH 5 fraction of liver. This would be in accord with the loss, during precipitation of the spleen cell sap at pH 5.2, of an active component for the incorporation of alanine into microsomal proteins (Rendi & Campbell, 1959). That adjustment to pH 5.2 does not precipitate all the enzymes concerned in the incorporation of amino acids has been demonstrated for the leucine-activating and -transferring enzymes by Zamecnik, Stephenson & Hecht (1958).

In systems in which the pH 5 RNA acts as an intermediate the differences in the extent of incorporation of amino acid into microsomal protein can be envisaged as occurring either by differences in the number of binding sites for amino acids on the pH 5 RNA or by differences in the rate of the enzymic reactions involved. Rendi (1959), using preparations of normal and regenerating liver, has shown that whereas the number of amino acid-binding sites in the pH 5 RNA of the liver preparations is similar, the incorporation into the microsomes is much greater in regenerating liver than in normal liver. The present results, which show that an increase in the amount of pH 5 RNA only slightly increases the incorporation into the microsomes (Table 8), support the idea that the rate of the enzymic reactions is the predominant factor controlling the extent of incorporation into the microsomes.

One of the aims of these experiments was to determine whether the factors in the cell sap of different tissues had some degree of specificity for the incorporation of amino acids. The fact that the cell sap from spleen and liver could be used for the incorporation of amino acid into microsomes of both tissues (Rendi & Campbell, 1959) suggested little tissue specificity. However, the inhibition by spleen pH 5 RNA of the incorporation of leucine into liver microsomes (Table 9), which may be explained by a trapping of the activated amino acids by the heterologous pH 5 RNA, suggests some degree of specificity. The influence of the tissue-specific components in the cell sap on the synthesis of well-characterized proteins in cell-free systems is at present being investigated.

SUMMARY

1. The incorporation of amino acids into the ribonucleic acid (RNA) of the fraction of the cell sap precipitated at pH 5.2 (pH 5 fraction) of liver, spleen and lymphosarcoma has been studied. Alanine is incorporated to the same extent into liver and spleen preparations, whereas leucine is more actively incorporated into liver pH 5 RNA.

2. Dilution of the pH 5 fraction enhances the incorporation of amino acid into pH 5 RNA.

3. If the cell sap is fractionated by the method of Sachs, which involves the removal of a precipitate formed by the addition of protamine, a fraction is obtained (S-protein) which promotes the incorporation of amino acid into microsomal protein. S-Protein contains no more than traces of RNA.

4. When pH 5 RNA is added to S-protein the ability of this fraction to promote the incorporation of amino acid into microsomal protein is enhanced. Under these conditions amino acids are incorporated into pH 5 RNA.

5. Incorporation of leucine into microsomal protein in the presence of non-dialysed S-protein was not enhanced by glutathione. The reverse is true if dialysed S-protein is used.

6. Although the addition of liver pH 5 RNA to liver S-protein enhanced the incorporation of amino acids into liver microsomes, spleen pH 5 RNA had an inhibitory effect.

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