4-position of D-ribitol, have been established by periodate oxidation, oxidation and hydrolysis to D-glyceric acid and from optical-rotation studies.

5. Studies with β -N-acetylglucosaminidase confirm the presence of α and β linkages in the acetylglucosaminides (III) and (IV), and it is concluded that both types of glycosidic linkage occur in teichoic acid preparations from some batches of the organism.

6. The proportion of α and β linkages in the teichoic acid has varied in samples prepared from different batches of organism, and batches produced recently yield a teichoic acid which apparently does not contain α linkages.

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REFERENCES

- Archibald, A. R., Armstrong, J. J., Baddiley, J. & Hay, J. B. (1961a). *Nature, Lond.*, **191**, 570.
- Archibald, A. R., Baddiley, J. & Buchanan, J. G. (1961b). *Biochem. J.* **81**, 124.
- Armstrong, J. J., Baddiley, J. & Buchanan, J. G. (1960). *Biochem. J.* **76**, 610.
- Armstrong, J. J., Baddiley, J. & Buchanan, J. G. (1961). *Biochem. J.* **80**, 254.
- Armstrong, J. J., Baddiley, J., Buchanan, J. G., Carss, B. & Greenberg, G. R. (1958). *J. chem. Soc.* p. 4344.
- Baddiley, J., Buchanan, J. G., Handschumacher, R. E. & Prescott, J. F. (1956). *J. chem. Soc.* p. 2818.
- Baddiley, J. & Davison, A. L. (1961). *J. gen. Microbiol.* **24**, 295.
- Chen, P. S., Toribara, T. Y. & Warner, H. (1956). *Analyt. Chem.* **28**, 1756.
- Consden, R. & Gordon, A. H. (1948). *Nature, Lond.*, **162**, 180.
- Dixon, J. S. & Lipkin, D. (1954). *Analyt. Chem.* **26**, 1092.
- Fischer, F. G. & Nebel, H. G. (1955). *Hoppe-Seyl. Z.* **302**, 10.
- Foster, A. B., Horton, D. & Stacey, M. (1957). *J. chem. Soc.* p. 81.
- Foster, A. B., Martlew, E. F. & Stacey, M. (1953). *Chem. & Ind.* p. 825.
- Hanes, C. S. & Isherwood, F. A. (1949). *Nature, Lond.*, **164**, 1107.
- Haukenes, G., Ellwood, D. C., Baddiley, J. & Oeding, P. (1961). *Biochim. biophys. Acta*, **53**, 425.
- Hough, L., Powell, D. B. & Woods, B. M. (1956). *J. chem. Soc.* p. 4799.
- Hough, L. & Taha, M. I. (1956). *J. chem. Soc.* p. 2042.
- Jeanes, A., Wise, C. S. & Dimler, R. J. (1951). *Analyt. Chem.* **23**, 415.
- Janczura, E., Perkins, H. R. & Rogers, H. J. (1961). *Biochem. J.* **80**, 82.
- Kelemen, M. V. & Baddiley, J. (1961). *Biochem. J.* **80**, 246.
- Leaback, D. H. & Walker, P. G. (1957). *J. chem. Soc.* p. 4754.
- Mitchell, P. & Moyle, J. (1951). *J. gen. Microbiol.* **6**, 981.
- Park, J. T. & Strominger, J. L. (1957). *Science*, **125**, 99.
- Partridge, S. M. (1949). *Nature, Lond.*, **164**, 443.
- Rosen, H. (1957). *Arch. Biochem. Biophys.* **67**, 10.
- Salton, M. R. J. & Horne, R. W. (1951). *Biochim. biophys. Acta*, **7**, 177.
- Schmidt, G. (1955). In *Methods in Enzymology*, vol. 2, p. 523. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Shockman, G. D., Kolb, J. J. & Toennies, G. (1957). *Biochim. biophys. Acta*, **24**, 203.
- Smith, J. D. & Wyatt, G. R. (1951). *Biochem. J.* **49**, 144.
- Strominger, J. L., Park, J. T. & Thompson, R. E. (1959). *J. biol. Chem.* **234**, 3263.
- Trevelyan, W. E., Procter, D. P. & Harrison, J. S. (1950). *Nature, Lond.*, **166**, 444.
- Viscontini, M., Hoch, D. & Karrer, P. (1955). *Helv. chim. acta*, **38**, 642.

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Amino Acid Transfer from Aminoacyl-Ribonucleic Acid to Serum Albumin by the Microsome Fraction from Rat Liver

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In 1958 it was reported that the soluble cytoplasm of rat liver contained a ribonucleic acid fraction, to which amino acids could be bound in the presence of adenosine triphosphate (Hoagland, Stephenson, Scott, Hecht & Zamecnik, 1958). Amino acids attached to this nucleic acid were transferred to protein when rat-liver microsomes

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were incubated in the presence of an energy-generating system. The ribonucleic acid fraction involved in these reactions has been designated soluble or transfer ribonucleic acid. It is now known that the carboxyl group of the amino acid is linked to the 2',3'-position of the terminal ribose of the nucleic acid (Zachau, Acs & Lipmann, 1958; Preis, Berg, Ofengand, Bergmann & Dieckmann, 1959). Hence the complex is an aminoacyl-ribonucleic acid.

In the experiments of Hoagland *et al.* (1958) the transfer of amino acid from transfer ribonucleic acid to the uncharacterized proteins precipitated by trichloroacetic acid was studied. Since it has been shown that in this process the nucleic acid becomes attached to the nucleic acid of the microsomes (Decken & Hultin, 1958; Hoagland, 1958a, b; Hoagland & Comly, 1960) it seemed important to determine whether the amino acid transferred was incorporated into a specific soluble protein synthesized by the microsome preparation. Evidence for the synthesis of serum albumin by such microsome preparations from rat liver has been provided (Campbell, Greengard & Kernot, 1960). The object of the present work was therefore to determine whether amino acid attached to transfer ribonucleic acid was utilized for the synthesis of serum albumin by the isolated microsome system.

A preliminary report of some aspects of this work has appeared (Decken & Campbell, 1961).

MATERIALS AND METHODS

Abbreviations. Phosphoenolpyruvate, PEP.

Chemicals. The dipotassium salt of ATP, and the sodium salt of GTP and of CTP, were obtained from the Sigma Chemical Co., St Louis, Mo., U.S.A. The sodium salt of PEP was kindly given by Dr V. M. Clarke. Pyruvate kinase was obtained from C. F. Boehringer und Soehne, Mannheim, Germany. Ovalbumin was obtained from Armour Laboratories, Eastbourne.

Radioactive amino acids. Uniformly labelled L-[¹⁴C]-leucine with a specific radioactivity of 90 mc/m-mole was obtained from the Institut Pasteur, Paris, France, and with that of 6 mc/m-mole from The Radiochemical Centre, Amersham, Bucks. These preparations will be referred to as either Pasteur or Amersham respectively.

The radiochemical purity of the Amersham [¹⁴C]leucine was not studied but owing to the high specific radioactivity the amino acid from the Institut Pasteur was examined. A small sample was added to a mixture of amino acids, which were then subjected to two-dimensional paper chromatography. This showed that not all the radioactivity was confined to leucine, but the other contaminants, which appeared to represent less than 10% of the total radioactivity, did not correspond with known amino acids. Since control experiments with liver microsomes incubated with the [¹⁴C]leucine in the absence of energy showed that the radioactive contaminants did not affect the radioactivity of the microsomal protein the Pasteur amino acid was not further purified.

Animals. Rats were Wistar albinos highly inbred at this Institute. Partial hepatectomy was performed by the method of Higgins & Anderson (1931).

Rat serum albumin. This was prepared as described by Campbell *et al.* (1960).

Antisera against rat serum albumin and ovalbumin. Rabbits were injected with alum-precipitated albumin as described by Campbell (1955). The amount of antibody in the rabbit serum was estimated by serial dilution to be about 10 mg. of precipitable protein/ml. of serum.

Microsomes. Microsomal preparations were made by a method which followed closely that described by Zamecnik & Keller (1954). Normal liver from rats weighing 80–100 g. was used, or regenerating liver from rats 44–48 hr. after partial hepatectomy. The rats were starved for about 18 hr. before being killed by a blow on the head followed by decapitation. The liver was homogenized in 2.5 times its weight of a medium containing 0.15 M-sucrose, 25 mM-KCl, 10 mM-MgCl₂ and 35 mM-tris buffer, pH 7.8 (Rendi & Hultin, 1960). The tissue suspension was centrifuged for 15 min. at 12 000g. The supernatant obtained was centrifuged in a Spinco model L preparative centrifuge (no. 40 rotor) for 55 min. at 105 000g. The clear supernatant (cell sap) was siphoned off with a syringe and the remainder was rejected. The pellet was rinsed with medium and suspended in 0.4 ml. of medium/g. of original liver by gentle homogenization in a small all-glass Potter-type homogenizer.

pH 5 Fraction. The cell sap (10 ml.) was titrated to pH 5.2 as described by Hoagland, Keller & Zamecnik (1956) with the modification that 0.01 N-HCl was used. The precipitate was collected by centrifuging for 5 min. at 12 000g, suspended in 1 ml. of 0.4 M-tris buffer, pH 7.8, by gentle homogenization in a small all-glass Potter-type homogenizer and diluted to 5 ml. with medium.

Preincubated pH 5 fraction (cf. Hecht, Stephenson & Zamecnik, 1958). Cell sap was adjusted to pH 5.2 by addition of 1 M-acetic acid. The precipitate was collected by centrifuging and suspended in the original volume of 1% EDTA, pH 8.0, in 0.25 M-sucrose. The pH was adjusted to 8.0 and the mixture incubated for 30 min. at 37° to free the pH 5 fraction from attached amino acids. The mixture was diluted after incubation with 1 vol. of ice-cold glass-distilled water, and brought to pH 5.2 with 1 M-acetic acid. The precipitate formed was suspended again in sucrose-EDTA and incubated for 20 min. at 37°. This precipitation-incubation cycle was repeated once more. The final pH 5.2 precipitate was suspended in 0.4 M-tris buffer (1 ml./30 ml. of original cell sap) and diluted with medium (4 ml./30 ml. of original cell sap). The suspension was centrifuged for 10 min. at 12 000g. The supernatant after this centrifuging, which was not completely clear, was designated 'pre-incubated pH 5 fraction'.

Transfer RNA. Transfer RNA was isolated from cell sap by the method of Kirby (1956), with slight modifications as described by Hoagland *et al.* (1958).

Labelling of transfer RNA with L-[¹⁴C]leucine. Three methods were used to obtain a transfer RNA with a high specific radioactivity. The methods are slight modifications of those described by Hecht *et al.* (1958) and Zamecnik, Stephenson & Scott (1960).

(1) The isolated transfer RNA was incubated at 37° for 1 hr. in 0.1 M-Na₂CO₃ buffer, pH 9.0, to free the RNA from attached amino acids. The preparation was dialysed in the cold against distilled water for 18 hr. with two changes of the water. The amount of RNA was determined by measuring the extinction at 260 mμ, an extinction coefficient of 34.2 mg.⁻¹ cm.² being used (Hoagland *et al.* 1958). The transfer RNA (4 mg.) was then incubated with 0.5 ml. of pH 5 fraction, 20 μmoles of ATP-MgCl₂, pH 7.8, and 40 μC of L-[¹⁴C]leucine (Pasteur) in a final volume of 5 ml. After 15 min. at 37° an equal volume of freshly distilled phenol saturated with water at 4° was added and the mixture homogenized at room temperature in an all-glass Potter-type homogenizer for 5 min. The water phase was

separated from the phenol phase by centrifuging in the cold, collected and the RNA precipitated by addition of 0.1 vol. of 20% potassium acetate, pH 5.0, together with 2.5 vol. of ethanol. After 15 hr. at -15° the collected precipitate was dissolved and dialysed against water in the cold with two changes of water.

(2) The incubation of the transfer RNA in Na_2CO_3 buffer was carried out as under (1) but at pH 10. The dialysed transfer RNA (5 mg.) was then incubated in a final volume of 8 ml. in the presence of 3 ml. of preincubated pH 5 fraction, 100 μmoles of ATP-MgCl₂, pH 7.8, 4 μmoles of CTP and 10 μC of L-[¹⁴C]leucine (Pasteur) for 10 min. at 37°. Transfer RNA was isolated as described under (1).

(3) Preincubated pH 5 fraction (4 ml.) was incubated with 50 μmoles of ATP-MgCl₂, pH 7.8, 4 μmoles of CTP and 10 μC of L-[¹⁴C]leucine (Pasteur) for 10 min. at 37° in a final volume of 7 ml. The transfer RNA was isolated as described under (1).

During preparations (2) and (3) non-radioactive transfer RNA was prepared in the same way but [¹⁴C]leucine was replaced by 15 $\mu\text{g.}$ of [¹⁴C]leucine.

Incubation of microsomes. The incubation mixture contained 0.4 ml. of microsomal suspension, 0.15 ml. of pH 5 fraction, 1 μmole of ATP, 10 μmoles of PEP, 0.25 μmole of GTP, 50 $\mu\text{g.}$ of pyruvate kinase and [¹⁴C]leucine- or [¹²C]leucine-labelled transfer RNA or free [¹⁴C]leucine in amounts as indicated. Before use the transfer RNA was freeze-dried in the incubation tubes. After incubation for 10 or 30 min. at 37°, the microsomes were isolated from the incubation mixture by centrifuging at 90 000g for 55 min.

Isolation of proteins

Serum albumin. The microsome fraction was treated with ultrasonic vibrations as described by Campbell *et al.* (1960). The albumin in the clear extracts of either the microsomes or the incubation medium was isolated as an antibody-antigen complex by the addition of a specific antiserum as described by Campbell & Stone (1957). Before the antiserum to rat albumin was added the tissue extracts were 'cleared' by the addition of ovalbumin, which was removed by the addition of ovalbumin antiserum as previously described.

Proteins other than albumin. After removal of albumin from the soluble fraction an equal volume of 10% (w/v) trichloroacetic acid was added. The particulate fraction which remained after treatment with ultrasonics was also treated in this way. The precipitates were treated with hot trichloroacetic acid and organic solvents according to a modified Schneider procedure (Zamecnik, Loftfield, Stephenson & Steele, 1951).

Measurement of radioactivity

Radioactivity was measured with a thin mica end-window Geiger-Müller counter. With ¹⁴C at infinite thickness this counter gave 1000 counts/min. for 1 $\mu\text{C/g./cm.}^2$ Proteins extracted (mixed proteins) with trichloroacetic acid were compressed on to 0.28 cm.^2 Perspex disks and counted at infinite thickness.

Albumin. The method was that described by Campbell *et al.* (1960).

[¹⁴C]Leucine transfer RNA. About 50 $\mu\text{g.}$ of the RNA in 0.2 ml. of water was transferred to polythene disks and dried in a vacuum desiccator. When dry the disks could be

counted at infinite thinness. The amount of RNA was determined by dissolving the RNA in water and measuring the extinction at 260 $\text{m}\mu$.

RESULTS

Attachment of [¹⁴C]leucine to transfer RNA

Three preparations of [¹⁴C]leucine transfer RNA were used. In preparation (1) transfer RNA was isolated from normal rat liver and after incubation at pH 9.0 it was incubated with ATP, pH 5 fraction, and a relatively large amount of [¹⁴C]leucine (40 μC for 4 mg. of RNA). This preparation had a specific radioactivity of 14 300 counts/mg. of RNA.

Although the specific radioactivity of preparation (1) was sufficiently high for the subsequent incorporation experiments the amount of [¹⁴C]leucine used was too large for an extensive series of experiments. A study was made therefore to determine the optimum conditions for the attachment of [¹⁴C]leucine to transfer RNA. As suggested by Hecht *et al.* (1958), the pH 5 fraction used to catalyse the attachment first was incubated at pH 8.0 and reprecipitated three times to remove free amino acids which otherwise lower the specific radioactivity of the [¹⁴C]leucine added to the incubation medium. After determination of the optimum amount of ATP in the medium the radioactivity attached to the transfer RNA of the pH 5 fraction and of the pH 5 fraction plus added transfer RNA was determined. The results obtained are shown in Fig. 1. It will be observed that with the small amount of transfer RNA in the pH 5 fraction alone saturation was soon reached but with the amount of isolated transfer RNA used the saturation point was not reached. Preparation (2) was obtained by incubating preincubated pH 5 fraction and transfer RNA (5 mg.) with 10 μC of [¹⁴C]leucine. The specific radioactivity was 7300 counts/min./mg. of RNA.

In order to obtain a preparation with an even higher specific radioactivity a large amount of preincubated pH 5 fraction was incubated in the absence of added transfer RNA. Preparation (3) had an activity of 17 700 counts/min./mg. of RNA.

Effect of transfer RNA on the incorporation of free [¹⁴C]leucine

Since the object of the present experiments was to compare the transfer of [¹⁴C]leucine to protein in the isolated microsome system when the amino acid was presented either free or attached to transfer RNA, the conditions for the controls were of importance. It seemed possible that transfer RNA itself might influence the incorporation of [¹⁴C]leucine into microsomal protein. This possibility was tested by incubating liver microsomes

with free [^{14}C]leucine in the presence of approximately the amount of transfer RNA used in the experiments to be described. The results of such an experiment are shown in Table 1. It is seen that transfer RNA markedly inhibited the incorporation of [^{14}C]leucine. This effect could have been due to

Fig. 1. Incubation tubes contained 10 μmoles of ATP-MgCl₂, increasing amounts of [^{14}C]leucine and 0.2 ml. of preincubated pH 5 fraction (●) or 0.2 ml. of pH 5 fraction plus 100 $\mu\text{g.}$ of preincubated transfer RNA (O). Tubes were incubated for 15 min. at 37°. A control tube containing 0.25 μc of [^{14}C]leucine was kept in the ice bath. After incubation an equal volume of cold 10% trichloroacetic acid was added. The precipitate was washed four times with cold 5% trichloroacetic acid and once with ethanol. The precipitate was dissolved in aq. 5N-NH₃ soln., the solution was transferred to polythene disks and these were dried in a vacuum desiccator and counted at infinite thinness. The radioactivity of the protein from the control tube was 13 counts/min.

Table 1. *Effect of transfer RNA on the incorporation of free [^{14}C]leucine into microsomal protein*

Each tube contained the microsomes from 1 g. of wet liver suspended in the tris buffer medium, together with 0.1 ml. of pH 5 fraction, [^{14}C]leucine (Pasteur, 0.06 μc), 0.25 μmole of GTP, 1 μmole of ATP, pyruvate kinase (50 $\mu\text{g.}$) and 10 μmoles of PEP in a total volume of 1 ml. When added the transfer RNA was freeze-dried in the incubation tubes (700 $\mu\text{g./tube}$). Incubation was in air for 10 min. at 37°. Trichloroacetic acid was then added to bring the final concentration to 5% (w/v). The protein was extracted and the radioactivity determined at infinite thickness on 0.28 cm.² disks. The results are the average of two experiments.

	Radioactivity (counts/min.)
Without transfer RNA	339
With transfer RNA	105
With preincubated transfer RNA	169

a reduction of the specific radioactivity of the free [^{14}C]leucine by the amino acids attached to transfer RNA. This was checked by using preincubated transfer RNA, from which the amino acids would have been removed, in place of freshly isolated transfer RNA. It is seen that the inhibitory effect of transfer RNA is thereby reduced but is still marked.

As a result of the above experiment, in most of the experiments to be described, the control tubes contained transfer RNA which had been incubated with [^{14}C]leucine.

Transfer of [^{14}C]leucine from transfer RNA to protein

(1) By microsomes from regenerating liver together with [^{14}C]leucine transfer RNA prepared by method (1). The results of this experiment are shown in Table 2. The total transfer of radioactivity from incubation medium to protein was 14% with transfer RNA and 7% with free amino acid. These figures were calculated in terms of μmc by comparing the radioactivity of the protein at infinite thickness with that of a known standard and by using the weight of protein extracted. Where the radioactivity measurements were made at infinite thinness, i.e. albumin precipitates, free [^{14}C]leucine and [^{14}C]leucine-labelled transfer RNA, the calculation was made on the basis that 1 μmc is equivalent to 167 counts/min. under the conditions of counting used. The radioactivity of the individual samples of microsomal serum albumin were, for free amino acid, 15 (7) and 11 (6) counts/min., and for transfer RNA, 22 (8) and 21 (7) counts/min., the radioactivity of the ovalbumin precipitate being given in parenthesis. These results provide clear evidence of incorporation of amino acid into serum albumin, for the count of the rat albumin precipitate always exceeded by a wide margin that of the ovalbumin. In the incubation medium there was no evidence of synthesis. Transfer RNA here provided a more efficient source of amino acid for protein synthesis but no RNA was added to the control tubes.

(2) By normal-liver microsomes with [^{14}C]leucine transfer RNA prepared by method (2). The microsomes for this experiment were obtained from young rats with a body weight of 80–100 g. which had been starved for 18 hr. Incubation was extended to 30 min. The results obtained are shown in Table 3 (a). Compared with that shown in Table 2, the difference between the two sources of [^{14}C]leucine is now more marked. This is due to the inhibitory effect of the transfer RNA added to the control tubes. The synthesis of microsomal albumin in the presence of [^{14}C]leucine transfer RNA is clear-cut since the ovalbumin precipitate had only a very low radioactivity. The transfer of radioactivity was 17%.

Table 2. *Transfer of [¹⁴C]leucine from transfer RNA to protein with isolated microsomes from regenerating liver*

Each tube contained the microsomes from 1 g. of wet liver from an animal 48 hr. after partial hepatectomy. Incubation tubes contained, in a total volume of 1 ml.: 0.25 μ mole of GTP, 1 μ mole of ATP, 10 μ moles of PEP, pyruvate kinase (50 μ g.) and 0.1 ml. of the pH 5 fraction prepared also from regenerating liver. Tubes contained, as indicated, 880 μ g. of [¹⁴C]leucine transfer RNA (preparation 1), which contained 12 000 counts/min. of radioactivity or [¹⁴C]leucine (Amersham, 0.06 μ C, 10 000 counts/min.). After incubation for 10 min. at 37° the microsomes were recovered by centrifuging the incubation mixture at 90 000g for 55 min. The soluble fraction was decanted and the microsomes were subjected to ultrasonic vibrations, frozen and again centrifuged. To the soluble fractions obtained both from the incubation medium and the microsomes, ovalbumin (60 μ g.) was added and removed by the addition of an antiserum to ovalbumin. The rat albumin present was then precipitated by the addition of an antiserum to rat albumin. Other non-albumin proteins both from the soluble fractions and from the insoluble particulate protein were treated with trichloroacetic acid (5%, w/v) and extracted. Radioactivity of the albumin-antibody precipitate was determined at infinite thinness. Total counts of the ovalbumin precipitates are given in parenthesis. Radioactivity of the trichloroacetic acid-precipitated proteins was determined at infinite thinness. Results are the averages of two experiments.

[¹⁴ C]Leucine present as ...	Free amino acid			Transfer RNA		
	Specific activity		Total count (counts/min.)	Specific activity		Total count (counts/min.)
	Infinite thickness*	Infinite thinness†		Infinite thickness*	Infinite thinness†	
Microsomal protein						
Insoluble	202	—	—	437	—	—
Soluble: Non-albumin	20	—	—	39	—	—
Albumin	—	364	13 (6)	—	560	21 (7)
Incubation medium						
Soluble: Non-albumin	18	—	—	28	—	—
Albumin	—	107	5 (7)	—	174	7 (11)

* Counts/min./0.28 cm.².

† Counts/min./mg.

Table 3. *Transfer of [¹⁴C]leucine from transfer RNA to protein with isolated microsomes from normal liver*

Conditions of incubation were in general as described for Table 2. (a) Experimental tubes contained 1 mg. of transfer RNA (preparation 2) with a radioactivity of 7300 counts/min. Control tubes contained the same amount of transfer RNA prepared in an identical manner except that [¹⁴C]leucine replaced the [¹⁴C]leucine. In addition, sufficient free [¹⁴C]leucine was added to provide 7300 counts/min. (b) Experimental tubes contained 1 mg. of transfer RNA (preparation 3) with a radioactivity of 17 700 counts/min. Control tubes contained [¹⁴C]leucine transfer RNA and free [¹⁴C]leucine (see a). Incubation was for 30 min. at 37° in air. Results are expressed as described for Table 2.

[¹⁴ C]Leucine present as ...	Free amino acid			Transfer RNA		
	Specific activity		Total count (counts/min.)	Specific activity		Total count (counts/min.)
	Infinite thickness*	Infinite thinness†		Infinite thickness*	Infinite thinness†	
(a) Microsomal protein						
Insoluble	69	—	—	265	—	—
Soluble: Non-albumin	6	—	—	23	—	—
Albumin	—	20	2 (1)	—	208	15 (2)
Incubation medium						
Soluble: Non-albumin	1	—	—	5	—	—
Albumin	—	47	4 (0)	—	170	9 (2)
(b) Microsomal protein						
Insoluble	43	—	—	373	—	—
Soluble: Albumin	—	18	2 (0)	—	159	16 (2)

* Counts/min./0.28 cm.².

† Counts/min./mg.

When this experiment was repeated with twice as much transfer RNA, radioactive microsomal serum albumin was obtained, but the transfer of radioactivity was reduced by half. Although the radioactivity of the rat albumin was the same as in the last experiment, the degree of contamination, as shown by the radioactivity of the ovalbumin, was higher.

(3) By normal liver microsomes with [^{14}C]leucine transfer RNA prepared by method (3). The conditions for this experiment were the same as those described under (2) but the radioactivity of the RNA preparation was twice as high. The results are presented in Table 3 (b). Again there is good evidence of microsomal albumin synthesis and as expected the contamination as indicated by the ovalbumin activity is low.

DISCUSSION

The greatest practical problem in conducting these experiments has been the preparation of [^{14}C]leucine transfer RNA with a sufficiently high radioactivity. This is necessary if the radioactivity incorporated into the microsomal serum albumin is to be accurately determined. The results clearly indicate that [^{14}C]leucine is transferred from transfer RNA. The control experiments indicate that the transfer of amino acid is through an intermediate other than free amino acid. The design of such control experiments was complicated by the finding that transfer RNA inhibited the incorporation of free [^{14}C]leucine amino acid. As has been suggested by Webster (1961), the polypeptide-bound amino acids of the microsomal ribonucleoprotein can be transferred back to transfer RNA. By adding high amounts of this RNA fraction to the incubation system the amino acids otherwise available for incorporation into protein are diverted to transfer RNA. This may also explain the fact that by increasing the amount of [^{14}C]leucine transfer RNA to more than 1 mg. of RNA/5 mg. of microsomal protein the radioactivity of the proteins after incubation is not significantly increased. The percentage transfer of radioactivity decreases in proportion to the increasing concentration of labelled transfer RNA added. Many other explanations for these results are possible. It might merely be that the capacity of the microsomes to accept amino acid has reached a saturation point.

The present results support the concept that transfer RNA is an intermediate in the synthesis of those proteins which are synthesized by liver microsomes. However, these experiments do not prove that the transfer RNA is an essential intermediate for the pathway of amino acids to protein. Other alternatives for the incorporation of free

amino acids into microsomal proteins and the role of transfer RNA in protein synthesis have been discussed by Campbell (1960).

SUMMARY

1. Methods were investigated for the preparation of rat-liver transfer ribonucleic acid with [^{14}C]leucine attached, the object being to obtain a preparation of high specific radioactivity.

2. When rat-liver microsomes were incubated with free [^{14}C]leucine the incorporation of radioactivity into protein was inhibited by the addition of non-radioactive transfer ribonucleic acid.

3. When the microsome fraction from either normal or regenerating liver was incubated under suitable conditions with transfer ribonucleic acid labelled with [^{14}C]leucine there was a transfer of [^{14}C]leucine to the albumin released from the microsome particles by ultrasonic vibrations.

4. The incorporation of [^{14}C]leucine into serum albumin under the above conditions was compared with that when the microsomes were incubated in the presence of the same amount of radioactivity in the form of free [^{14}C]leucine and non-radioactive transfer ribonucleic acid. Under these conditions the albumin was not significantly radioactive.

5. It is concluded that [^{14}C]leucine is transferred from transfer ribonucleic acid to microsomal serum albumin in a form other than free amino acid.

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REFERENCES

- Campbell, P. N. (1955). *Biochem. J.* **61**, 496.
- Campbell, P. N. (1960). *Biol. Rev.* **35**, 413.
- Campbell, P. N., Greengard, O. & Kernot, B. A. (1960). *Biochem. J.* **74**, 107.
- Campbell, P. N. & Stone, N. E. (1957). *Biochem. J.* **66**, 19.
- Decken, A. von der & Campbell, P. N. (1961). *Biochem. J.* **80**, 38.
- Decken, A. von der & Hultin, T. (1958). *Exp. Cell Res.* **15**, 254.
- Hecht, L. I., Stephenson, M. L. & Zamecnik, P. C. (1958). *Biochim. biophys. Acta*, **29**, 460.
- Higgins, G. M. & Anderson, R. M. (1931). *Arch. Path.* **12**, 186.
- Hoagland, M. B. (1958a). *Rec. trav. chim. Pays-Bas*, **77**, 623.
- Hoagland, M. B. (1958b). *Proc. 4th int. Congr. Biochem., Vienna*, **8**, 199.
- Hoagland, M. B. & Comly, L. T. (1960). *Proc. nat. Acad. Sci., Wash.*, **46**, 1554.

- Hoagland, M. B., Keller, E. B. & Zamecnik, P. C. (1956). *J. biol. Chem.* **218**, 345.
- Hoagland, M. B., Stephenson, M. L., Scott, J. F., Hecht, L. I. & Zamecnik, P. C. (1958). *J. biol. Chem.* **231**, 241.
- Kirby, K. S. (1956). *Biochem. J.* **64**, 405.
- Preis, J., Berg, P., Ofengand, E. J., Bergmann, F. H. & Dieckmann, M. (1959). *Proc. nat. Acad. Sci., Wash.*, **45**, 319.
- Rendi, R. & Hultin, T. (1960). *Exp. Cell Res.* **19**, 253.
- Webster, G. (1961). *Fed. Proc.* **20**, 387.
- Zachau, H. G., Acs, G. & Lipmann, F. (1958). *Proc. nat. Acad. Sci., Wash.*, **44**, 885.
- Zamecnik, P. C. & Keller, E. B. (1954). *J. biol. Chem.* **209**, 337.
- Zamecnik, P. C., Loftfield, R. B., Stephenson, M. L. & Steele, J. M. (1951). *Cancer Res.* **11**, 592.
- Zamecnik, P. C., Stephenson, M. L. & Scott, J. F. (1960). *Proc. nat. Acad. Sci., Wash.*, **46**, 811.

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The Intracellular Distribution of Dolichol in Pig Liver

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Rowland, Latimer & Giles (1956) reported the isolation from tobacco of solanesol, a long-chain isoprenoid alcohol. Solanesol was originally thought to be a primary alcohol, $C_{60}H_{81}OH$, consisting of ten isoprenoid residues, but it has since been shown to have the formula $C_{45}H_{73}OH$ (Erickson, Shunk, Trenner, Arison & Folkers, 1959; Rüegg *et al.* 1960). When searching for solanesol in animal tissue, Hemming, Morton & Pennock (1960) found a compound which showed no selective absorption in the ultraviolet region but had an infrared spectrum closely resembling that of solanesol.

Pennock, Hemming & Morton (1960) showed that the new compound was a long-chain isoprenoid alcohol with 20 isoprene residues and a primary alcohol group. The complete chemical structure has not yet been established, but the compound was shown to be $C_{100}H_{161}OH$, and the name dolichol was proposed. Dolichol has been found in several animal tissues in different quantities and small amounts have been found in plants. Dolichol-like material has been found in baker's yeast (J. Burgos, unpublished work) and dolichol was also isolated from *Aspergillus fumigatus* (N. M. Packter & J. Glover, unpublished work). The physiological function of dolichol is still unknown, and it was thought that its distribution among different animal and plant tissues and in the various cell fractions would be a great help in any attempt to establish its role.

The present paper deals with the intracellular distribution of dolichol in pig liver, which contains the alcohol in relatively high concentration.

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EXPERIMENTAL

Cell fractionation. Pig liver was obtained from the Stanley Abattoir, Liverpool, as soon as possible after slaughter of the animal, and was transported in an ice-cold Thermos flask. After removal of fat and connective tissue the liver was minced with scissors and forced through a stainless-steel sieve (20 mesh) and the pulp was collected. A portion of the pulp was taken as a control and the remainder was homogenized and submitted to a scheme of differential centrifuging based on that of Schneider & Hogeboom (1950). During these procedures the temperature was kept between 0° and 5°.

The sieved pulp was disrupted in a Potter-Elvehjem homogenizer in 5 g. batches, 10 ml. of 0.25 M-sucrose being used each time. Each batch was homogenized for about 90 sec. at full speed, the pestle being moved up and down five times during this period. The cell debris and nuclei were sedimented in a refrigerated centrifuge (Measuring and Scientific Equipment Ltd.) at 900g for 15 min. The precipitate was resuspended in sucrose and rehomogenized in the Potter-Elvehjem apparatus and then centrifuged as before. This process was then repeated with a less rigorous homogenization. The supernatant fractions were bulked together.

The mitochondria were sedimented at 7500g for 20 min. and the precipitate was resuspended in sucrose solution and centrifuged once more. The microsome precipitate was obtained by centrifuging at 20 000g for 20 min. The temperature was kept between 0° and 3° during these operations. The supernatant fraction was heated in a boiling-water bath for about 15 min., the protein being denatured and separated by centrifuging at speed 10 (3500g) in an International centrifuge for 30 min. to give a 'groundplasm' precipitate and another supernatant fraction.

Protein estimation. A small portion of each cell fraction was used for protein determination. The method adopted was the biuret quantitative reaction (Cleland & Slater, 1953) and the extinction at 540 mμ was measured in a