Effect of Lipids, in particular Cholesteryl 14-Methylhexadecanoate, on the Incorporation of Labelled Amino Acids into Transfer Ribonucleic Acid *in vitro*

By J. HRADEC AND Z. DUŠEK

Department of Biochemistry, Oncological Institute, Prague 8, Czechoslovakia

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1. Rat liver pH5 enzymes and cell sap extracted with various organic solvents showed a variable decreased incorporation of labelled amino acids into s-RNA ('soluble' or transfer RNA) *in vitro*. 2. The original enzymic activity could be fully restored, though at different rates, by the addition of lipid extracts in quantities corresponding to those originally present. 3. Of the main lipid groups separated from the extract, only free cholesterol and cholesteryl esters were able to reactivate the extracted pH5 enzymes in the same way as the whole lipid extract. 4. Addition of pure cholesteryl 14-methylhexadecanoate also fully restored the enzymic activity. 5. There was no energy-dependent incorporation of labelled amino acids into ribosomal protein in the presence of extracted cell sap. Addition of cholesteryl 14-methylhexadecanoate fully restored the activity of the cell sap to incorporate labelled leucine and lysine into ribosomal protein and enhanced the incorporation of labelled protein hydrolysate and phenylalanine over the level found with the corresponding non-extracted preparations. 6. It is concluded that lipids play an important role in the synthesis of aminoacyl-s-RNA complexes and that cholesteryl 14-methylhexadecanoate may be the active lipid in this respect.

The cellular fraction of pH5 enzymes contains all constituents for the initial steps of protein synthesis. These include, in particular, aminoacyl-s-RNA* synthetases (Hoagland, Keller & Zamecnik, 1956) and s-RNA (Hoagland, Zamecnik & Stephenson, 1957). In addition, several other enzymes are probably present in this fraction since more than 20% of the cell-sap nitrogen is precipitated at pH5 (Anderson & Green, 1967). The cellular supernatant fraction contains various groups of enzymes (Roodyn, 1965) and its composition is fairly well established (Anderson & Green, 1967). However, no systematic studies have been reported on the detailed composition of the pH5 enzyme fraction.

We found in preliminary experiments that rat liver pH5 enzymes contain lipids that may be extracted from the freeze-dried enzyme preparation without extensive protein denaturation (Hradec, 1964). Relatively high quantities of carcinolipin, a lipid showing carcinogenic activity (Hradec & Kruml, 1960), are present in this fraction. Carcinolipin has also been isolated in our Laboratory from other biological materials (Hradec & Štroufová, 1960). We have presented some evidence that cholesteryl (+)-14-methylhexadecanoate is the

active component of this lipid (Hradec & Sommerau, 1968; Hradec & Menšík, 1968; Hradec & Dolejš, 1968).

It was considered desirable for the elucidation of the mode of action of this lipid to study the possible significance of the fact that a substance stimulating protein synthesis (Hradec, 1961) is apparently a normal cell constituent. Since extracted pH5 enzymes showed a decreased ability to activate amino acids (Hradec, 1964), a study of the possible role of lipids in the early steps of protein synthesis as well as the possible connexion between these 'active' lipids and cholesteryl 14-methylhexadecanoate seemed to be desirable.

MATERIALS AND METHODS

Animals. Wistar rats of both sexes, weighing 150–200 g., bred in this Laboratory and kept on a mixed diet, were used.

Chemicals. Phosphoenolpyruvic acid (silver-barium salt), pyruvate kinase and GTP (sodium salt) were products of Koch-Light Laboratories Ltd., Colnbrook, Bucks. Natural L-amino acids were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A. ATP (sodium salt) was a product of Reanal, Budapest, Hungary, and was purified as described by Hradec (1967). Silicic acid for chromatography (Silica gel CH) was obtained from Spolana, Velvary, Czechoslovakia. All solvents were redistilled before use.

* Abbreviation: s-RNA, 'soluble' or transfer RNA.

Light petroleum was the fraction boiling between 35° and 50°. Cholesteryl (+)-14-methylhexadecanoate was synthesized (Hradec & Menšík, 1968; Hradec & Dolejš, 1968).

Radioactive compounds. U-¹⁴C-labelled algal-protein hydrolysate (769mc/g.), L-[U-¹⁴C]leucine (72mc/m-mole), L-[U-¹⁴C]phenylalanine (66mc/m-mole), L-[U-¹⁴C]valine (51mc/m-mole), L-[U-¹⁴C]lysine (82mc/m-mole) and L-[U-¹⁴C]alanine (72mc/m-mole) were products of the Institute for Research, Production and Utilization of Radioisotopes, Prague, Czechoslovakia.

Preparation of subcellular fractions. Rat liver pH5 enzymes and cell sap (S-105 fractions) were isolated as described by Hradec (1967). After the second acid precipitation pH5 enzymes were resuspended in ice-cold water by homogenization and immediately frozen on Petri dishes at -80°. Cell sap was dialysed against water at 0-5° overnight and then also frozen at -80°. Both preparations were freeze-dried in an apparatus of Deutsche Vakuum-apparate, Sangerhausen, Germany, and the resulting product was stored at -50°.

Extraction of subcellular fractions. A weighed quantity (0.5-1 g.) of freeze-dried pH5 enzymes or the S-105 fraction was extracted with 150ml. of the solvent in a Soxhlet apparatus for 4hr. Extracted enzyme preparations were immediately dried *in vacuo* (0.05mm. Hg) for 4-5hr. and then stored at -50°. When necessary, freeze-dried enzyme preparations were suspended in a buffered medium by homogenization to give a protein concentration of 50-100mg./ml. These suspensions were divided into smaller portions, each sufficient for one set of experiments (to avoid repeated thawing and freezing), and stored at -50°. Extracts were evaporated to dryness under a stream of nitrogen and the residue was dissolved in a small volume of chloroform. This solution was chromatographed on silicic acid developed with a mixture of *n*-hexane-diethyl ether-acetic acid (85:15:1, by vol.). Standards of cholesteryl oleate, triolein, oleic acid and cholesterol were run simultaneously on the same plate. After development and drying individual lipid fractions were detected by their fluorescence in ultraviolet light. The portions of the silicic acid containing single lipid groups were sucked off and eluted with diethyl ether (or acetone for material remaining on the starting line). Fractions containing cholesteryl esters, triglycerides, fatty acids, cholesterol and phospholipids with mono- and di-glycerides were evaporated to dryness under a stream of nitrogen. For enzyme reactivation experiments the necessary volume of polyethylene glycol (mol.wt. 600) was added to an ether solution of the lipid and the solvent was evaporated by agitating the flask under hot water. The last traces of the solvent were eliminated with a stream of nitrogen (Hradec, 1967).

Incubation procedures. When the incorporation of labelled amino acids into s-RNA was studied, incubation mixtures contained 2ml. of medium A (Littlefield & Keller, 1957), 0.1ml. of the pH5 enzyme suspension (5-10mg. of protein, exactly the same quantity in extracted as in control preparations), 0.1ml. of the lipid suspension (or 0.1ml. of polyethyleneglycol in control mixtures), 10 μ moles of ATP and 7-8 μ moles of labelled amino acid or 0.6 μ g. of algal-protein hydrolysate in a total volume of 2.4ml. The time-course of the incorporation at 37.5° with extracted enzyme preparations and lipid extracts added was significantly different from that obtained with non-extracted enzyme preparations or extracted enzyme preparations alone (Fig.

Fig. 1. Time-course of the labelling of s-RNA with algal-protein hydrolysate in the presence of a non-extracted pH5 enzyme preparation (○), the same enzyme preparation extracted with ether (●) and the extracted enzyme preparation with the cholesteryl ester fraction of the extract added (△).

1). A maximum incorporation appeared to occur after 10min. and this time was used throughout the experiments. The systems for the incorporation of labelled amino acids into ribosomal protein were prepared essentially as described by Weisberger, Armentrout & Wolfe (1963) and consisted of 100 μ moles of tris-HCl buffer, pH 7.60, 5 μ moles of MgCl₂, 50 μ moles of KCl, 1 μ mole of ATP, 0.25 μ mole of GTP, 10 μ moles of phosphoenolpyruvate, 40 μ g. of pyruvate kinase, a mixture of 20 natural amino acids (0.04 μ mole of each) without that used in radioactive form (the amino acid mixture was omitted in experiments with labelled protein hydrolysate), 0.1ml. of cholesteryl 14-methylhexadecanoate suspension (or 0.1ml. of polyethylene glycol in control mixtures), 0.1ml. of S-105 fraction (8-10mg. of protein, exactly the same quantity in extracted as in control preparations), 0.1ml. of ribosome suspension (3-5mg. of protein) and 7-8 μ moles of labelled amino acid or 0.6 μ g. of labelled protein hydrolysate in a total volume of 1.0ml. Mixtures were incubated for 60min. at 37.5°.

Radioactive assay. Protein was precipitated and washed after incubation as described by Hradec (1967). The perchloric acid extract of s-RNA (0.1ml.) was mixed with 6ml. of SLS-31 scintillation mixture (Spolana, Neratovice, Czechoslovakia). Proteins from incubation mixtures containing ribosomes and the cell sap were dissolved after washing and drying in 0.2ml. of 0.5N-NaOH and added to 6ml. of the SLS-31 scintillation mixture. All samples were counted in the NZB315 coincidence liquid-scintillation

Table 1. *Incorporation of labelled amino acids into s-RNA in the presence of extracted pH 5 enzymes*

The actual incorporation into s-RNA in control mixtures was 220–329 counts/min./mg. of s-RNA for algal-protein hydrolysate, 1385–1755 counts/min./mg. of s-RNA for L-lysine and 1693–2287 counts/min./mg. of s-RNA for L-leucine. Three different batches of pH 5 enzymes were used. Results are given as mean values (with numbers of experiments in parentheses) and ranges.

pH 5 enzymes extracted with ...	Radioactivity incorporated (% of that with non-extracted preparation)							
	Ether		Chloroform		Light petroleum		Benzene	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Algal-protein hydrolysate	39 (8)	36–44	22 (10)	14–25	61 (8)	54–66	64 (6)	59–70
L-Lysine	41 (3)	38–45	37 (3)	31–40	58 (3)	55–60	63 (3)	55–65
L-Leucine	60 (2)	54–65	50 (3)	40–52	94 (2)	93–95	94 (2)	90–97

counter (Tesla, Přemyslení, nr. Prague, Czechoslovakia). The counting efficiency was 65% for RNA and 76% for protein. Samples were counted for sufficient periods to decrease the probable error to less than $\pm 2\%$ and corrected for quenching when necessary. The quenching was determined by the use of internal standards (^{14}C -labelled toluene). Protein and RNA were determined as described by Hradec (1967).

RESULTS

Labelling of s-RNA in the presence of extracted pH 5 enzymes. A decreased incorporation of labelled algal-protein hydrolysate, L-lysine and L-leucine into s-RNA was found in the presence of all batches of extracted pH 5 enzymes. Extraction with different solvents decreased the incorporation to different extents (Table 1). In addition, there was no energy-dependent incorporation of either L-alanine or L-valine into s-RNA in the presence of any extracted pH 5 enzymes. This was true for three different batches of pH 5 enzymes, each of them extracted with ether, light petroleum, chloroform and benzene. In control mixtures s-RNA was labelled with 560–757 counts/min./mg. of s-RNA in the presence of alanine (nine experiments) and with 700–1100 counts/min./mg. of s-RNA when valine was used (nine experiments).

Reactivation of extracted pH 5 enzymes with lipid extracts. When lipids extracted from freeze-dried pH 5 enzymes were added into incubation mixtures in quantities corresponding to those that were originally present, the enzymic activity of the extracted pH 5 enzymes was restored to the level found with the corresponding non-extracted pH 5 enzymes.

Incorporation of labelled algal-protein hydrolysate into s-RNA of extracted pH 5 enzymes with lipids added did not differ from that with control samples. This was true for all three batches of pH 5 enzymes tested, each of which was extracted with ether, light petroleum, chloroform and ben-

Fig. 2. Reactivation of an extracted preparation of pH 5 enzymes (preparation C extracted with ether; Table 3) with the lipid extract (○) or cholesteryl 14-methylhexadecanoate (●). A 0.1-ml. portion of the lipid extract contained the quantity of either lipid present in the original enzyme. The radioactivity incorporated with the control non-extracted pH 5 enzyme preparation was 305 counts/min./mg. of s-RNA.

zene. Addition of smaller amounts of lipids than those that were originally present in a given enzyme preparation resulted in an increase of enzymic activity when compared with that of the extracted enzyme preparation only. However, the incorporation was lower than in control mixtures. Addition of greater quantities of lipids than those

extracted from a particular preparation of pH5 enzymes partially inhibited the incorporation. Thus only quantities of lipids corresponding exactly to those originally present in the preparation were able to induce a complete reactivation of the extracted pH5 enzyme preparation (Fig. 2). Similar results were found in experiments in which labelled lysine was used instead of the protein hydrolysate. A complete reactivation of extracted pH5 enzymes was obtained with all enzymic preparations to which appropriate quantities of extracted lipids were added.

When labelled leucine was used extracted lipids reactivated completely only five out of nine extracted enzyme preparations tested. In the others increased incorporation was found in the presence of lipids when compared with individual preparations of extracted pH5 enzymes only, but complete restoration of the enzymic activity was not obtained. This effect was not dependent on the batch of pH5 enzymes and was found with preparations extracted with ether or chloroform. The same preparations showed the usual response to lipids when protein hydrolysate or lysine was used.

Incorporation of L-valine into s-RNA of extracted pH5 enzymes was not affected by the addition of lipid extracts in three enzymic preparations out of nine tested. These were the same as those that could not be reactivated by extracted lipids when labelled leucine was used. Three further preparations showed the same activity as control non-extracted pH5 enzymes after the addition of lipids. With the three remaining preparations, however, the presence of extracted lipids in amounts exactly corresponding to those that had been extracted from the preparation of pH5 enzymes induced an enhanced incorporation of labelled valine into s-RNA and values corresponding to 178, 258 and 860% of the control mixture were found. These preparations were of three different batches of pH5 enzymes and were extracted with ether, chloroform and benzene. They showed the same response to added lipids as the other batches of pH enzymes when labelled algal-protein hydrolysate or lysine was used as described above.

When the incorporation of labelled alanine into s-RNA was studied in the presence of extracted pH5 enzymes, no response to the addition of lipids was obtained with two enzymic preparations, the same that gave no response when leucine and valine were used. The remaining six preparations of pH5 enzymes were reactivated to levels found with corresponding non-extracted preparations in the presence of appropriate amounts of extracted lipids.

Effect of individual lipid fractions on the incorporation of labelled protein hydrolysate into s-RNA of extracted pH5 enzymes. Only the addition of

Table 2. *Incorporation of labelled algal-protein hydrolysate into s-RNA in the presence of extracted pH5 enzymes and individual lipid fractions*

Rat liver pH5 enzymes were isolated and extracted with ether. Lipid fractions that had been separated from the extract by thin-layer chromatography were added to the incubation mixtures in quantities corresponding to those originally present. The same quantity of lipids was added to non-extracted enzymes.

Lipid fraction added	Radioactivity incorporated (counts/min./mg. of s-RNA)	
	Non-extracted pH5 enzymes	Extracted pH5 enzymes
None	311	124
Phospholipids	239	91
Cholesterol	261	295
Fatty acids	259	85
Triglycerides	255	81
Cholesteryl esters	270	308

cholesterol or cholesteryl esters to the extracted pH5 enzymes restored the level of labelling of s-RNA to that of the corresponding non-extracted pH5 enzymes. Similar results were also found when authentic cholesterol was used. All other lipid fractions separated from the extract of pH5 enzymes induced a partial inhibition of the incorporation of labelled protein hydrolysate into s-RNA of extracted enzymes. This was true when lipids were added in quantities equal to those originally present in the pH5 enzyme preparation (Table 2). The time-course of the labelling of s-RNA with algal-protein hydrolysate in the presence of extracted pH5 enzymes with cholesterol or cholesteryl esters added was quite dissimilar to that obtained with extracted enzyme alone (Fig. 1). When the same amounts of individual lipids as those that were necessary for the reactivation of extracted pH5 enzymes were added into incubation mixtures containing non-extracted pH5 enzymes, a significantly decreased incorporation compared with that found in control mixtures was obtained (Table 2).

Cholesteryl 14-methylhexadecanoate contents in extracts of pH5 enzymes and the cell sap. The quantities of the cholesteryl ester extracted from three different batches of freeze-dried rat liver pH5 enzymes are given in Table 3. Chloroform extracted slightly greater amounts than ether, whereas light petroleum and benzene extracted less than ether. Chloroform-methanol (2:1, v/v) mixture extracted the same quantity as chloroform, and iso-octane or toluene roughly the same amount as benzene. The quantity of the cholesteryl ester extracted was roughly proportional to the decrease of the enzymic

Table 3. *Quantities of cholesteryl 14-methylhexadecanoate extracted from different pH 5 enzyme preparations and the residual activity of such preparations*

Samples (2g.) of freeze-dried rat liver pH 5 enzyme preparation were extracted with 150 ml. of solvent for 4 hr. The activity (incorporation of labelled algal-protein hydrolysate into s-RNA) is expressed as a percentage of that with the non-extracted preparation. The incorporation found in control incubation mixtures was 265 counts/min./mg. of s-RNA for preparation A, 234 counts/min./mg. of s-RNA for preparation B and 305 counts/min./mg. of s-RNA for preparation C. Cholesteryl 14-methylhexadecanoate (CMH) was determined by the method of Hradec (1968), and is expressed as $\mu\text{g./g.}$ of protein.

pH 5 enzymes extracted with		Ether	Light petroleum	Chloroform	Benzene
Preparation					
A	CMH extracted ($\mu\text{g./g.}$)	67.8	43.7	81.7	43.7
	Activity (%)	41	62	20	70
B	CMH extracted ($\mu\text{g./g.}$)	59.3	42.8	74.4	42.0
	Activity (%)	44	66	23	69
C	CMH extracted ($\mu\text{g./g.}$)	75.1	48.0	88.2	49.3
	Activity (%)	36	55	14	59

Table 4. *Quantities of cholesteryl 14-methylhexadecanoate extracted from freeze-dried S-105 fraction of rat liver cell sap with different solvents*

		Cholesteryl 14-methylhexadecanoate ($\mu\text{g./g.}$ of protein)			
S-105 fraction extracted with		Ether	Light petroleum	Chloroform	Benzene
Preparation					
A		48.4	32.2	74.4	29.5
B		47.3	30.7	70.3	29.0
C		59.2	52.2	86.7	35.4

activity (Table 3). Less cholesteryl 14-methylhexadecanoate was extracted with the same solvents from the freeze-dried cell sap (Table 4).

Effect of cholesteryl 14-methylhexadecanoate on the activity of extracted pH 5 enzymes. The addition of the cholesteryl ester to mixtures containing extracted pH 5 enzymes enhanced the incorporation of labelled algal-protein hydrolysate into s-RNA. When quantities of this substance corresponding to those present in the original non-extracted enzyme preparation were used the same incorporation was obtained as in control mixtures with non-extracted pH 5 enzymes. As with the lipid extract, smaller and greater amounts of this cholesteryl ester were less active (Fig. 2). The time-course of incorporation in mixtures containing extracted pH 5 enzymes with cholesteryl 14-methylhexadecanoate added was identical with that obtained after the addition of the lipid extract or cholesteryl esters to these extracted pH 5 enzymes (Fig. 1).

Suitable quantities of cholesteryl 14-methylhexadecanoate fully reactivated the ability of all nine extracted enzymic preparations tested to incorporate labelled lysine into s-RNA.

As with the lipid extract, the same extracted pH 5 enzymes were not affected by the addition of the cholesteryl ester when labelled leucine was

used. However, the activity of the remaining four preparations was fully restored to the level of that of non-extracted pH 5 enzymes in the presence of this substance.

Cholesteryl 14-methylhexadecanoate also enhanced the incorporation of labelled valine into s-RNA of extracted pH 5 enzymes. With three preparations, values below the control value of the non-extracted pH 5 enzymes (14, 25 and 32% of the control value) were obtained when this substance was added. These were the same preparations of pH 5 enzymes that gave no response to the lipid extract (see above). With the other enzyme preparations, however, addition of the cholesteryl ester in the same quantities that were present in the enzyme preparation before the extraction yielded incorporations exceeding those found in control mixtures (average 440% of the control value for experiments with six enzyme preparations, range 263–820%).

When s-RNA was labelled with alanine in the presence of extracted pH 5 enzymes cholesteryl 14-methylhexadecanoate in suitable doses induced in eight out of nine preparations tested a reactivation of the enzyme preparation to the activity found in the control mixture. In the one remaining case the mixture with the cholesteryl ester added

showed only 48% of the incorporation found in the control sample. This preparation gave no response to lipid extracts, as described above.

Incorporation of labelled amino acids into ribosomal protein in the presence of extracted cell sap. There was no energy-dependent incorporation of labelled

algal-protein hydrolysate, L-phenylalanine, L-lysine or L-leucine into the ribosomal protein in the presence of extracted cell sap (postmicrosomal supernatant). This result was found with three different batches of rat liver cell sap, each of them extracted with ether, light petroleum, chloroform and benzene. Control experiments with non-extracted cell-sap preparations showed an average incorporation of 590 counts/min./mg. of ribosomal protein for algal-protein hydrolysate (five experiments, range 485–600 counts/min./mg.), 800 counts/min./mg. for L-leucine (four experiments, range 500–935 counts/min./mg.), 740 counts/min./mg. for L-lysine (four experiments, range 460–795 counts/min./mg.) and 610 counts/min./mg. for L-phenylalanine (four experiments, range 560–890 counts/min./mg.).

Reactivation of the cell sap with cholesteryl 14-methylhexadecanoate. Addition of the cholesteryl ester in the same quantities that were originally present in a given enzyme preparation into incubation mixtures containing ribosomes and extracted cell sap resulted in an energy-dependent incorporation of algal-protein hydrolysate into ribosomal protein. This incorporation was significantly higher than that in control mixtures and an average value 428% of the control sample (eight experiments, two different batches of cell sap extracted with ether and chloroform, range 240–640%) was obtained. Moreover, even additions of smaller quantities of the cholesteryl ester than those originally present in the supernatant fraction induced an enhanced incorporation when compared with control mixtures containing non-extracted cell sap (Fig. 3).

Very similar results were obtained with phenylalanine, additions of appropriate quantities of cholesteryl 14-methylhexadecanoate to extracted cell sap resulting in an enhanced incorporation of this amino acid into ribosomal protein (average

Fig. 3. Effect of cholesteryl 14-methylhexadecanoate on the incorporation of labelled algal-protein hydrolysate (○) and L-leucine (●) into ribosomal protein in the presence of extracted cell sap (preparation C extracted with chloroform; Table 4). The radioactivity incorporated with the control non-extracted preparations was 520 counts/min./mg. of ribosomal protein for the protein hydrolysate and 845 counts/min./mg. of ribosomal protein for L-leucine. Results of two experiments are given.

Table 5. *Effect of cholesteryl 14-methylhexadecanoate added to incubation mixtures with extracted cell sap on the incorporation of labelled amino acids into ribosomal protein*

Rat liver ribosomes and cell sap were isolated as described by Hradec (1967). Results of experiments with the same batch of cell sap are given. Absolute values of the incorporation in control mixtures were 523 counts/min./mg. of ribosomal protein for algal-protein hydrolysate, 748 counts/min./mg. of ribosomal protein for L-leucine, 534 counts/min./mg. of ribosomal protein for L-lysine and 705 counts/min./mg. of ribosomal protein for L-phenylalanine.

Cell sap extracted with ...	Radioactivity incorporated (% of that with non-extracted preparation)			
	Ether	Light petroleum	Chloroform	Benzene
Labelled amino acid				
Algal-protein hydrolysate	240	390	640	252
L-Leucine	97	110	112	94
L-Phenylalanine	162	220	396	130
L-Lysine	105	111	124	98

290% in eight experiments with two batches of cell sap extracted with ether, chloroform, light petroleum and benzene, range 130–396%). When either lysine or leucine was used, incubation mixtures containing extracted cell sap with suitable quantities of the cholesteryl ester added showed roughly the same incorporation as control mixtures (79–124% of the control value, 16 experiments) (Table 5).

DISCUSSION

There are several reports indicating that lipids play a role in protein synthesis (Hendler, 1959; Gaby & Silberman, 1960; Hunter & Goodsall, 1961). Labelled amino acids seem to form some intermediary complexes with lipids, most probably phospholipids (Hunter & James, 1963), and Gaby, Wolin & Zajac (1960) suggest that such complexes may be concerned in amino acid transport processes. However, no paper has described results demonstrating that cholesterol and its esters can stimulate protein synthesis.

Only these two lipid fractions restored the activity of extracted pH5 enzymes in our experiments. However, it seems improbable that free cholesterol could mediate directly in protein synthesis since it does not stimulate the incorporation of labelled amino acids into proteins of rat tissue homogenates *in vitro* (Hradec, 1959). Moreover, if the free sterol were so active, then all cholesteryl esters might be expected to give the same result as cholesteryl 14-methylhexadecanoate. However, many of them are ineffective in stimulating the incorporation of labelled algal-protein hydrolysate into rat liver s-RNA *in vitro* (Hradec & Sommerau, 1968). Only cholesteryl 14-methylhexadecanoate of all cholesteryl esters present in the calf liver showed a stimulating effect on protein synthesis (Hradec & Menšík, 1968). One possible explanation of these apparent contradictions is that cholesteryl 14-methylhexadecanoate could be synthesized from added free cholesterol and the 14-methylhexadecanoic acid present in the fatty acid pool of pH5 enzymes by cholesterol-esterifying enzymes in this cellular fraction. The presence of such enzymes in rat liver cell sap has been established by Swell & Law (1967).

Remarkable differences between doses of lipids necessary for the stimulating effect with native and extracted pH5 enzymes suggest that the restoration of the activity of extracted enzymes could hardly be regarded as a mere stimulation of their residual activity by added lipids. It seems more probable that these lipids may be present in pH5 enzymes as a functional constituent. Experiments on the time-course of the incorporation of labelled amino acids into s-RNA in the presence of extracted pH5 enzymes and lipids seem to support such an

opinion. The distinct lag phase found only in these mixtures may indicate that some time is necessary for the recombination of the added lipid with the extracted enzyme before the original enzymic activity is regained. Several enzymes have been described as containing lipids that in some cases seem to be essential for their activity. A complex of cytochrome c_1 with a lipoprotein relatively rich in lipids was isolated by Green, Järnefelt & Tisdale (1959). The ubiquinone (coenzyme Q)-lipoprotein complex is also supposed to play a fundamental role in the function of some respiratory enzymes (Järnefelt, Basford, Tisdale & Green, 1958). Nason & Lehman (1955) demonstrated that the activity of cytochrome c reductase is lost after the extraction of this enzyme with iso-octane and that the enzyme may be completely reactivated by the addition of the lipid residue.

It is clear from our results that lipids affect protein synthesis at its early stages. However, it can scarcely be concluded that they affect the enzymes directly involved in aminoacyl-s-RNA synthesis. The pH5 enzyme fraction undoubtedly contains several groups of different enzymes. On the other hand, significant differences in the ability of extracted pH5 enzymes to catalyse the incorporation of different labelled amino acids into s-RNA may indicate that a direct effect on aminoacyl-s-RNA synthetases is involved. These enzymes are known to be specific for individual amino acids (Moldave, 1963). Also, the failure to obtain a complete reactivation of some extracted pH5 enzyme preparations only with some amino acids (apparently as a result of denaturation) suggests that different enzymes may be affected by the extraction and that these show different sensitivities towards this type of denaturation.

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