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The Effect of Ultrasonic Vibrations on the Protein-Synthesizing Activity of Microsome Preparations from Rat Liver

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One approach for the preparation of cell fragments with biological activity is the application of ultrasonic vibrations. In studies on protein synthesis this method has been used on mouse-ascites-tumour cells (Martin, Malec, Coote & Work, 1961) and on calf-heart mitochondria by Kalf & Simpson (1959) and Kroon (1963). In previous work on the synthesis of serum albumin by liver microsomes it was noticed that a marked clearing of a suspension of liver microsomes could be achieved by the application of ultrasonic vibrations. From this work it seemed possible that such treatment of liver microsomes might provide a particulate fraction, active in protein synthesis but possessing a simpler morphological structure. It was also possible that an active soluble preparation might be produced.

The present paper shows that treatment of liver microsomes by ultrasonic vibrations has an inhibitory effect on their ability to incorporate ^{14}C -labelled amino acids into protein. Similar treatment of ribonucleoprotein particles has a less marked effect and seems to be mainly associated with the release of a ribonucleic acid fraction important for protein synthesis.

A preliminary report of some aspects of this work has been given by Decken & Campbell (1961). Ogata, Watanabe, Morita & Sugano (1962) have confirmed these findings and have produced evidence for the metabolic specificity of ribonucleic acid released from liver ribonucleoprotein particles by ultrasonic treatment (Sugano, Ogata, Mirota, Tominaga & Watanabe, 1962).

Some aspects of the present work have been reported in a preliminary form (Campbell & Cooper, 1963).

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MATERIALS AND METHODS

Chemicals. The dipotassium salt of ATP, the sodium salt of GTP, and NADH and NADP⁺ were obtained from the Sigma Chemical Co., St Louis, Mo., U.S.A.

Pyruvate kinase, the silver barium salt of PEP,† the barium salt of glucose 6-phosphate and glucose 6-phosphate dehydrogenase were obtained from Boehringer und Soehne G.m.b.H., Mannheim, Germany.

Solutions of the free acid of PEP were prepared before use and adjusted to pH 7.4 (glass electrode) with N-KOH. Solutions of the free acid of glucose 6-phosphate were prepared and adjusted to pH 6.5 (glass electrode) with N-KOH. Tris and GSH were obtained from the British Drug Houses Ltd., Poole, Dorset. The sodium salt of deoxycholic acid was obtained from Merck A.-G., Darmstadt, Germany, the non-ionic detergent Lubrol W from Imperial Chemical Industries Ltd., Manchester, and polyU from Miles Laboratories, Stoke Poges, Slough, Bucks.

Radioactive amino acids, uniformly labelled L- ^{14}C -leucine (45.6 mc/m-mole) and L- ^{14}C -phenylalanine (61.9 mc/m-mole), were obtained from The Radiochemical Centre, Amersham, Bucks., and ^3H -polyU was kindly given by Dr T. Hultin.

Animals. Rats (150–200 g. body wt.) were Wistar albino strain highly inbred at this Institute. Regenerating liver was obtained from rats 2 days after partial hepatectomy by the method of Higgins & Anderson (1931). The rats were kept without food for approx. 18 hr. before being killed by a blow on the head followed by decapitation.

Tissue preparations. Rat liver was homogenized in medium A containing MgCl_2 (10 mM), tris buffer (35 mM) (pH 7.8 at 25°), KCl (25 mM) and sucrose (0.15 M). Microsomes, RNP particles, cell sap and pH 5 fraction were prepared as described by Decken & Campbell (1962). When necessary, 'washed' microsomes were prepared by resuspending the pellet from 4 g. of liver in medium A (11 ml.)

† The abbreviations used are: PEP, phosphoenolpyruvic acid; polyU, polyuridylic acid; RNP, ribonucleoprotein.

by gentle homogenization followed by centrifugation at 105 000g for 35 min.

Treatment with ultrasonic vibrations. This was performed as described by Campbell & Kernot (1962).

Method of incubation. The pellets were suspended in medium A and incubated at 37° with gentle shaking in a water bath under the conditions given in the Tables.

Extraction of protein for radioactive assay. After incubation of the various preparations the protein was precipitated with 5% (w/v) trichloroacetic acid. The precipitate, after being heated in 5% trichloroacetic acid at 90° for 20 min., was washed successively with cold 5% trichloroacetic acid, ethanol, ethanol-ether-chloroform (2:2:1, by vol.), acetone and ether, and finally gently dried.

Determination of glucose 6-phosphatase activity. The enzyme activity was determined by a method that followed closely that of de Duve, Pressman, Gianetto, Wattiaux & Appelmans (1955). The microsomal preparations were diluted with 0.15M-sucrose containing EDTA (2 mM), pH 6.5, to give a protein content of 0.2–0.5 mg. of protein/ml. The assay was performed on sufficient microsomal suspension to give 100–300 µg. of protein. The incubation mixture contained, in a final volume of 1 ml., glucose 6-phosphate (0.04M), EDTA (1 mM), citrate buffer (7 mM), sucrose (0.15M) and microsomal suspension. The pH of all solutions added was 6.5 (at the pH meter). Two different controls, one in the absence of glucose 6-phosphate and one in the absence of the microsomal suspension, were used. After 10 min. at 37° 1 ml. of 10% (w/v) trichloroacetic acid was added. The suspension was centrifuged and the phosphate of the soluble fraction assayed by the method of King, Abul-Fadl & Walker (1951). The proteins of the microsomal suspensions were determined according to the method of Lowry, Rosebrough, Farr & Randall (1951).

Determination of phosphodiesterase activity. The enzyme activity was measured by the method of Spahr & Schlesinger (1963).

Measurement of radioactivity. The protein was either plated on 0.28 cm.² Perspex disks and counted at infinite thickness, or was dissolved in formic acid (AnalaR) and transferred to 5 cm.² aluminium disks, dried under an infrared lamp and counted at infinite thinness. In the latter case the weight of protein was determined by weighing and the results expressed as counts/min./mg. of protein. In either case the radioactivity was determined in a thin-window gas-flow counter (Nuclear-Chicago Corp.). A standard planchet of 1 cm.² containing 1 µC of ¹⁴C/g. gave approx. 1800 counts/min. under these conditions.

Tritium was measured in a Packard Tri-Carb scintillation spectrophotometer at an operating voltage of 1180 v and a discriminator setting of 10–100 v. The scintillation liquid contained 0.5% (w/v) of 2,5-diphenyloxazole, 0.015% (w/v) of 1,4-di-(5-phenyloxazol-2-yl)benzene, 6% (v/v) of ethanol, 50% (v/v) of toluene, 40% (v/v) of dioxan and 3% (w/v) of SiO₂. The ethanol-soluble material was added directly to the scintillation liquid and counted. The ethanol-insoluble material was dissolved in 0.1 ml. of 10% (v/v) KOH and transferred to the counting vials by repeated additions of toluene. The gel formed after the addition of the scintillation liquid was mixed in a Vortex mixer (Scientific Industries Inc., Springfield, Mass., U.S.A.) and counted. Quenching was monitored with internal standards.

Determinations. Protein was determined by the Folin-Ciocalteu method of Lowry *et al.* (1951) with crystalline bovine serum albumin (Cohn fraction V) as standard. RNA was determined by the orcinol method of Mejbaum (1939), with hydrolysed yeast RNA as standard.

RESULTS

Effect of ultrasonic vibrations on the ability of microsomal preparations to incorporate amino acid into protein. The results presented in Table 1

Table 1. *Effect of ultrasonic vibrations on the ability of microsomal preparations to incorporate amino acid into protein*

The 'washed' microsome pellet from 8 g. of liver was suspended in 2.4 ml. of medium A (see the text) and subjected to ultrasonic vibrations for 1 min. The suspension was either used for incubation at this point or was centrifuged for 60 min. at 90 000g, the supernatant was decanted and the pellet was suspended in 2.4 ml. of medium A. The suspension (0.4 ml.) was incubated with 0.2 ml. of pH 5 fraction, 15 µmoles of PEP, 2 µmoles of ATP, 50 µg. of pyruvate kinase, 0.25 µmole of GTP and 0.25 µC of [¹⁴C]leucine in a total volume of 1 ml. In the 'no-energy' control tubes ATP, GTP, PEP and pyruvate kinase were omitted. Incubation was for 30 min. at 37°, after which 5% trichloroacetic acid was added, and the proteins were extracted and the radioactivity was determined at infinite thinness. The results are the averages of duplicate determinations.

Source of microsomes	Treatment		Sp. radioactivity of protein (counts/min./mg.)		Inhibition (%)
			'No-energy' control	Energy source present	
Normal liver	Ultrasonic treatment but no subsequent centrifugation	Control	6	293	—
		Experimental	4	55	81
	Ultrasonic treatment followed by centrifugation	Control	7	338	—
		Experimental	11	85	75
Regenerating liver	Ultrasonic treatment but no subsequent centrifugation	Control	7	614	—
		Experimental	4	124	79
	Ultrasonic treatment followed by centrifugation	Control	4	558	—
		Experimental	2	139	75

indicate that with normal liver the incorporation of amino acids by the microsome fraction is inhibited by about 80 % as the result of ultrasonic vibrations. Although the regenerating liver microsomes are more active than those from normal liver the percentage inhibition is very similar in the two cases. When the soluble fraction obtained by ultrasonic vibrations was removed by centrifugation the specific radioactivity of the microsomes was greater (85 counts/min./mg. compared with 55). This increase was probably mainly due to removal of protein which is not active for the incorporation of amino acid and also of free amino acids which would affect the radioactivity of the ^{14}C -labelled amino acid in the incubation medium. The failure to restore the activity of the microsomes by the removal of supernatant suggests that the inhibitory effect is not due to the release of an easily-removed soluble inhibitory substance.

The soluble fraction from the treated microsomes did not show a significant incorporation of amino acid into protein.

Effect of ultrasonic vibrations on the ability of ribonucleoprotein particles to incorporate amino acid into protein. When RNP particles were treated with ultrasonic vibrations under conditions similar to those used for the microsome preparations the inhibitory effect on amino acid incorporation was variable. The results of three typical experiments are shown in Table 2. Unlike the microsomes the inhibition always increased when the soluble fraction was removed after treatment, suggesting the removal of a substance active in protein synthesis. The percentage inhibition after ultrasonic vibrations and centrifuging varied between 74 and 32 %, but in general was much less than with microsomes.

Protein and nucleic acid contents of the material released by ultrasonic vibrations. The application of ultrasonic vibrations causes the release of both protein and RNA from the various preparations. The amount of protein released both in the control experiments in which the preparations were merely suspended by gentle homogenization and in those in which ultrasonic vibrations were applied was very variable. The variation was much less when the release of RNA was studied. The variable results with protein release may be due to differences in the amount of soluble cytoplasm associated with the microsomes and RNP particles on isolation. The low RNA:protein ratio of the soluble cytoplasm means that the major contribution of the latter would be to the amount of soluble protein.

The results shown in Table 3 indicate that the RNA:protein ratio of the preparations is not significantly affected by the ultrasonic treatment, so that the inhibitory effect is not to be attributed to a general decrease in the amount of RNA relative to protein. The amount of RNA released from RNP particles is much greater than from microsomes, and this is reflected in the much higher RNA:protein ratio of the supernatant from RNP particles than that from microsomes.

Nature of the inhibitory effect of ultrasonic vibrations. The possibility that the ultrasonic vibrations were inactivating an enzyme required for protein synthesis could not be tested directly, but since all the enzymes known to play a role in protein synthesis are in the soluble fraction of a cell homogenate the effect of treatment of this fraction was studied. The activity of normal liver microsomes for the incorporation of [^{14}C]leucine was tested with increasing amounts of pH 5 fraction

Table 2. *Effect of ultrasonic vibrations on ribonucleoprotein particles and their response to polyuridylic acid*

Ribonucleoprotein particles from 48 g. of liver were suspended in 5.4 ml. of medium A (see the text). The suspension was divided into three equal parts which were treated as follows: (a) kept at 0°; (b) treated with ultrasonic vibrations for 1 min. and kept at 0°; (c) treated with ultrasonic vibrations for 1 min. and then centrifuged at 90 000g for 60 min. The supernatant was removed and the particles were suspended in 1.8 ml. of medium A. The particles were then incubated for 1 hr. in air at 37°. Each tube contained, in a total volume of 1 ml., 0.25 μmole of GTP, 15 μmoles of PEP, 2 μmoles of ATP, 50 $\mu\text{g.}$ of pyruvate kinase, 0.1 ml. of pH 5 fraction, 0.25 μC of [^{14}C]phenylalanine and, where indicated, 50 $\mu\text{g.}$ of polyU. The MgCl_2 concentration was adjusted to 10 mM (cf. Campbell & Cooper, 1963). After incubation protein was precipitated by the addition of 5% trichloroacetic acid, the proteins were extracted and the radioactivity was determined. Each experiment represents the average of duplicate determinations.

Expt. no.	Conditions	Sp. radioactivity of protein (counts/min./mg.)		
		(a) Control (untreated)	(b) Ultrasonically treated	(c) Ultrasonically treated and centrifuged
I	Control	1033	492	268
	PolyU added	1218	1488	1905
II	Control	659	519	301
	PolyU added	1285	1050	1195
III	Control	555	415	373
	PolyU added	1179	1241	1918

Table 3. *Release of protein and ribonucleic acid from microsomes and ribonucleoprotein particles by ultrasonic vibrations*

The particulate preparations were resuspended by gentle homogenization, treated with ultrasonic vibrations for 1 min. and then centrifuged for 60 min. at 105 000g. The methods and concentrations of the suspension were as given in Tables 1 and 2. The protein and RNA contents of the pellet and supernatant fraction were determined by the Lowry *et al.* (1951) and Mejsbaum (1939) methods. Similar determinations were performed also on identical samples which were not treated with ultrasonic vibrations.

Preparation	Control (untreated) preparations			Ultrasonically treated preparations			Percentage release of RNA due to treatment
	RNA: protein ratio of pellet	RNA: protein ratio of supernatant	Percentage release of RNA	RNA: protein ratio of pellet	RNA: protein ratio of supernatant	Percentage release of RNA	
Normal microsomes	0.27	0.018	0.6	0.24	0.024	5.8	5.2
Regenerating-liver microsomes	0.35	0.053	0.7	0.39	0.041	2.5	1.8
RNP particles	1.0	0.05	1.6	1.0	0.7	19	18

and the activity was compared with that obtained in the presence of pH 5 fraction that had been treated with ultrasonic vibrations. No inhibitory effect on the activity of the pH 5 fraction was found.

When the length of treatment of the microsomes with ultrasonic vibrations was varied the effect was to decrease their capacity for protein synthesis from about 75 % inhibition at 30 sec. to about 95 % inhibition at 2 min.

It is known that the presence of reducing agents has a beneficial effect on the preservation of amino acid-activating enzymes (Allen, Glassman & Schweet, 1960). Microsomes were treated, therefore, with ultrasonic vibrations in the presence of reducing agents such as 10 mM- or 1 mM-GSH, 1 mM-NADH or approx. 1 mM-NADPH (derived from glucose 6-phosphate, glucose 6-phosphate dehydrogenase and NADP⁺). No positive effect was observed on the activity of the treated microsomes. Similarly, the addition of reducing agents to the incubation medium did not restore the activity of the treated microsomes.

Effect of polyuridylic acid on ribonucleoprotein particles subjected to ultrasonic vibrations. The effect of ultrasonic vibrations on the ability of RNP particles to incorporate amino acids into protein has already been referred to. C. Cooper & P. N. Campbell (unpublished work) have shown the effect of polyU on the incorporation of [¹⁴C]-phenylalanine by such a preparation. Table 2 shows that the stimulatory effect of polyU is variable, but that under the same conditions the activity of the RNP particles is brought to the same level. Similarly, after treatment with ultrasonic vibrations, with or without subsequent centrifugation, the activity is restored with polyU and in some cases bettered.

Effect of polyuridylic acid on microsomes treated with ultrasonic vibrations. Having shown that

Table 4. *Effect of polyuridylic acid on microsomes treated with ultrasonic vibrations*

'Washed' microsomes were prepared from normal rat liver. The pellets from 32 g. of liver were suspended in 9.6 ml. of medium A (see the text) and three 2 ml. samples were taken. Sample (a) was untreated, sample (b) was treated for 1 min. with ultrasonic vibrations, and sample (c) after ultrasonic treatment was centrifuged for 60 min. at 105 000g and the pellet was resuspended in 2 ml. of medium A. The incubation conditions were as described for RNP particles (Table 2) except that the incubation time was 30 min.

Preparation of microsomes	Sp. radioactivity of protein (counts/min./mg.)
(a) Control (untreated)	414
PolyU added	1878
(b) Ultrasonically treated	36
PolyU added	48
(c) Ultrasonically treated and centrifuged	67
PolyU added	97

polyU could restore the activity of RNP particles treated with ultrasonic vibrations, we did a similar experiment with microsomes from normal liver. Table 4 shows that the untreated microsomes were stimulated by the addition of polyU but that the activity of the treated preparations was not restored by the addition of polyU. Moreover, the stimulatory effect of the polyU on the residual activity of the treated preparations was decreased.

Effect of ultrasonic vibrations on the degradation of [³H]polyuridylic acid by microsomes and ribonucleoprotein particles. The failure of polyU to restore the amino acid-incorporating activity of treated microsomes suggested an increased breakdown of the added polynucleotide. By following the experiments of Spahr & Schlessinger (1963), this possibility was tested with [³H]polyU. Fig. 1 shows that

Fig. 1. Effect of ultrasonic vibrations on the degradation of $[^3\text{H}]\text{polyU}$ by microsomal preparations. The reaction mixture contained, in a final volume of 0.5 ml., $[^3\text{H}]\text{polyU}$ (7.4 μg ; 5100 counts/min.), KCl (0.1 M), MgCl_2 (0.5 mM), tris buffer, pH 7.5 at 25° (0.02 M), and microsomal preparations as follows: untreated microsomes (●) (0.16 mg. of protein), treated microsomes (○) (0.12 mg. of protein), soluble fraction released from microsomes either by washing (▲) (0.07 mg. of protein) or by ultrasonic vibrations (Δ) (0.14 mg. of protein). After incubation for the period indicated the reaction was stopped by the addition of 0.1 ml. of a yeast RNA solution (10 mg./ml. in 0.6 M-NaCl) followed immediately by 1 ml. of cold ethanol. The resulting suspension was kept at -15° for 1 hr. and then centrifuged. The clear supernatant was decanted and the precipitate washed once in 70% (v/v) ethanol-0.2 M-NaCl. Both the soluble fraction and the precipitate were counted in a scintillation spectrophotometer. The period of incubation is plotted against the total radioactivity found in the ethanol-NaCl soluble fraction. PolyU was completely stable when incubated in the absence of the microsomal fractions or when precipitated at zero time in the presence of these fractions.

there is a marked increase in the degradation of $[^3\text{H}]\text{polyU}$ by the soluble fraction and the insoluble fraction obtained after ultrasonic treatment of microsomes as compared with untreated microsomes. The breakdown products of $[^3\text{H}]\text{polyU}$ were characterized by paper chromatography as described by Spahr & Schlessinger (1963). On incubation of treated microsomes or soluble fraction a considerable increase of 5'-UMP was obtained, indicating the presence of phosphodiesterase in the microsomal preparations as one of the enzymes that degraded $[^3\text{H}]\text{polyU}$. Experiments made with treated and untreated RNP particles did not show significant differences in the breakdown of $[^3\text{H}]\text{polyU}$. When 7.5 μg . of $[^3\text{H}]\text{polyU}$ was incubated for 12 min. at 37° with 0.5 mg. of ribonucleoprotein about 5% of the polynucleotide was broken down by the particles.

Table 5. Release of glucose 6-phosphatase from liver microsomes by treatment with ultrasonic vibrations

Each incubation tube contained, in a final volume of 1 ml., glucose 6-phosphate (0.04 M), EDTA (1 mM), citrate buffer, pH 6.5 (7 mM), sucrose (0.15 M) and a microsomal suspension (containing 40–100 μg . of protein) or the soluble fraction derived from microsomes by washing or treatment with ultrasonic vibrations (containing 30–60 μg . and 100–200 μg . of protein respectively). The tubes were run in duplicates with two concentrations of the preparations to be tested. After incubation in air at 37° the proteins were precipitated in 5% (w/v) trichloroacetic acid. The amount of phosphate in solution was determined. The specific activity of glucose 6-phosphatase is expressed as μmoles of phosphate obtained/10 min./mg. of protein at 37°.

	Control (untreated) preparations		Ultrasonically treated preparations	
	Pellet	Soluble fraction	Pellet	Soluble fraction
Sp. activity of glucose 6-phosphatase	6.45	0.49	7.35	1.26
Percentage release of protein	—	4	—	23.5
Percentage release of total glucose 6-phosphatase activity	—	0.31	—	5.3

Effect of ultrasonic vibrations on the morphology of the components of the microsome fraction. Electron micrographs of the various microsome preparations and RNP particles were prepared before and after treatment with ultrasonic vibrations, but no clear change in morphology could be attributed to the treatment. The microsome preparations still showed the presence of membranes after treatment and the appearance of the RNP particles did not change.

Since glucose 6-phosphatase is known to be associated with the microsome fraction of rat liver the distribution of the enzyme between the soluble and insoluble fractions after ultrasonic treatment was studied. The results shown in Table 5 indicate that only about 5% of the enzyme is released by the ultrasonic treatment compared with 24% of the protein.

DISCUSSION

The protein-synthesizing activity of all the microsomal preparations studied was inhibited by treatment with ultrasonic vibrations. Both electron microscopy and estimations of the distribution of glucose 6-phosphatase activity suggest that the action of ultrasonic vibrations is to disrupt but not to render soluble the membrane of the vesicles present in the isolated microsome fraction. With RNP particles that contain no detectable membrane component no change in morphological

structure has been observed, although more detailed studies with the electron microscope might reveal some changes. Since the effect of the treatment of RNP particles seems to be more easily interpreted it is considered first.

Ogata *et al.* (1962) showed that the extent of the decrease in the activity of RNP particles on treatment with ultrasonic vibrations was paralleled by the amount of RNP released in a soluble form. The ultracentrifugal pattern of the particles was not affected by the treatment. Sugano *et al.* (1962) later showed that the released RNA was metabolically active and that it had some of the properties attributed to the so-called 'messenger' RNA. The present experiments are consistent with this concept. As shown in Table 2 the particles as isolated have variable activity for the incorporation of amino acid, but in the presence of polyU the activity rises to a uniform level. If RNP particles normally exist in the cell associated with a 'messenger'-like RNA (m-RNA), then it is possible that under the rigorous methods used for the isolation of the particles variable amounts of the m-RNA remain attached. Particles isolated with small amounts of m-RNA might then be stimulated relatively more than particles saturated with m-RNA by synthetic polynucleotides such as polyU. From Table 2 it would appear that ultrasonic vibrations separate m-RNA from ribosomal RNA, so that in the absence of added polyU the activity is very similar irrespective of the initial activity. The addition of polyU after treatment always restores the activity at least to its former level and often greatly enhances it. This could be because the treatment has removed natural m-RNA which codes for all amino acids and replaced it with a synthetic m-RNA which codes only for phenylalanine. This finding is in accordance with the work of Fessenden, Cairncross & Moldave (1963), who studied the effect of polyU on the transfer of phenylalanine from amino-acyl-'transfer' RNA to protein by a system containing rat-liver RNP particles. They found that the stimulation by polyU was much greater if the particles were first incubated with pH 5 supernatant.

The present results are consistent, therefore, with the idea that the effect of ultrasonic vibrations on RNP particles is to be attributed to the release of RNA. This conclusion would be firmer if it were possible to add back a purified RNA to the treated RNP particles and restore the activity. So far it has not been possible to demonstrate this effect.

The effect of ultrasonic vibrations on the microsome fraction is usually greater than that with RNP particles, and is not so simply explained. It is probable that the most active morphological constituent of the microsome fraction in protein syn-

thesis consists of elements of the rough-surfaced endoplasmic reticulum. This consists of a membrane studded with RNP particles or ribosomes. Presumably these particles lose RNA on ultrasonic treatment just as they do when they are treated after detachment from the membranes by detergents. Table 3 shows that, although the percentage release of RNA from the microsomes is less than from the RNP particles, the RNA:protein ratio of the microsome pellets is much less than that of the RNP particles. The effect of ultrasonic treatment of the microsomes is not, however, merely to be attributed to its effect on the RNP particles attached to membrane, for in this case it should be possible to restore the activity of the treated microsomes by the addition of polyU. As is shown in Table 4, this is not so. It cannot be argued that RNP particles attached to membrane are unable to be activated by polyU for, as shown by Campbell & Cooper (1963), microsomes are in general more susceptible to polyU than are RNP particles.

A more likely explanation of the difference between microsomes and RNP particles with respect to ultrasonic vibrations is related to the phosphodiesterase activity of the preparations. The enhanced activity of this enzyme in microsomes treated with ultrasonic vibrations indicates a rapid degradation of added polyU. The presence of this active enzyme excludes the possibility of restoration of the original amino acid-incorporating activity of ultrasonically treated microsomes by the addition of polyU. The results of these experiments do not necessarily imply that the phosphodiesterase attacks the m-RNA bound to the microsomal particles; but such an interpretation would be in accordance with the greater effect of ultrasonic treatment on microsomes than RNP particles, for the enzyme activity is not increased in the latter preparation by ultrasonic treatment.

SUMMARY

1. The effect of ultrasonic vibrations on microsomes from normal and regenerating liver and on ribonucleoprotein particles prepared from microsomes by treatment with detergents and potassium chloride was investigated. The amino acid-incorporating activity was diminished after treatment with ultrasonic vibrations both in the presence and in the absence of reducing agents. The enzymes present in the soluble fraction of the cell and necessary for protein synthesis were not affected.

2. Protein and ribonucleic acid were released from the various preparations after treatment with ultrasonic vibrations.

3. Polyuridylic acid restored the ability of ultrasonically treated ribonucleoprotein particles to incorporate phenylalanine into protein.

4. The activity of ultrasonically treated microsomes could not be restored by polyuridylic acid.

5. The application of ultrasonic vibrations on microsomes caused a several-fold increase in phosphodiesterase activity as measured by the breakdown of [³H]polyuridylic acid. As a breakdown product, an increased amount of 5'-UMP was obtained. There was no significant effect on the breakdown by ultrasonically treated ribonucleoprotein particles.

6. Electron microscopy showed that on treatment by ultrasonic vibrations the microsomal fraction retained membranes and particles. No apparent change in the structure of isolated ribonucleoprotein particles was observed. The membrane-bound enzyme glucose 6-phosphatase was not destroyed by treatment with ultrasonic vibrations for 1 min.

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The Influence of Post-Mortem Conditions on the Solubilities of Muscle Proteins

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Of all animal tissues, muscle undergoes possibly the most marked biochemical and physicochemical changes after death. For this reason it is often essential to prepare homogenates or extracts as soon as is feasible after killing the animal, in order that neither the process of rigor mortis nor the lowering of muscle pH by conversion of glycogen into lactic acid adversely affects the factors being studied. Both the rigor-mortis process (Bate-Smith & Bendall, 1947) and the rate of post-mortem pH fall (Bendall, 1960; Marsh, 1954) have been subjects of considerable study both in this Laboratory and others; the present investigation sets out

to determine the biochemical changes that occur as a result of these processes, in particular the solubility characteristics of myofibrillar and sarcoplasmic proteins in various induced and simulated post-mortem circumstances.

METHODS

Preparation of myofibrils. Muscle (100 g.) was homogenized in 300 ml. of 30 mM-sodium β -glycerophosphate (adjusted to pH 6.5 with N-HCl) in a high-speed blender. The homogenate was centrifuged at 1000g for 15 min., and the residue was resuspended in β -glycerophosphate buffer