Effect of Ethanol on Rat-Liver Ribonucleoprotein Previously Exposed to Cold Trichloroacetic Acid

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The separation of liver ribonucleic acid and deoxyribonucleic acid from the associated protein material without hydrolysis can be accomplished by a salt-extraction procedure described by Barnum & Huseby (1950). This method, based on ribose recovery (Lowe & Rand, 1956; C. U. Lowe & P. R. Venkataraman, in preparation), yields 80 % of the total ribonucleic acid of normal liver homogenate or cell fractions. When ribonucleic acid was isolated by a salt-extraction procedure (described in the text) comparable with that of Tyner, Heidelberger & LePage (1953), which utilizes an initial treatment of the liver homogenate or cytoplasmic fraction with trichloroacetic acid followed by extraction with ethanol and ethanol-ether (3:1, v/v), the yield, as measured by ultraviolet spectrophotometry and content of ribose and total phosphorus, was reduced to 62–53 % of the total content. The apparent discrepancy between these two procedures was more obvious in the cytoplasmic fraction, and these observations motivated a detailed investigation to trace the possible source of loss of nucleic acid by the latter method. It was found that the first ethanol extract obtained after exposure of nucleoprotein to cold trichloroacetic acid contained appreciable amounts of ribonucleic acid-like material. If, after exposure of the nucleoprotein to cold trichloroacetic acid, ethanol treatment was omitted, extraction of the precipitated nucleoprotein with hot 10 % sodium chloride solution, after neutralization of the excess of trichloroacetic acid, restored the yield of ribonucleic acid to a level of 80 % of the total content. The results reported here appear to emphasize that ribonucleoprotein previously exposed to cold trichloroacetic acid is solubilized by ethanol even in the cold. In contrast, deoxyribonucleic acid protein appears to be unaffected.

EXPERIMENTAL

Animals. Male rats of the Wistar strain, 6–7 weeks old, fed *ad libitum* on Lab-Blox R diet (Allied Mills, Peoria, Ill., U.S.A.), were used in this study. All animals were fasted for 24 hr. before being killed.

Cellular disruption. At the time of killing, the animals were lightly anaesthetized with ether, the hepatic vein was

severed and the liver excised. The livers were rinsed in the suspending sucrose medium, blotted dry and placed in a chilled steel meat press. The expressed liver pulp from groups of three to four animals was then rapidly weighed on a chilled watch glass and transferred to a chilled Potter-Elvehjem-type, plastic-plug homogenizer. A 10 % homogenate was prepared in cold 0.25 M-sucrose containing 0.18 mM-calcium chloride (Hogeboom, Schneider & Striebig, 1952; Takagi, Hecht & Potter, 1956). Unless otherwise stated, all further operations were carried out in a cold room maintained at 1–2°.

Preparation of cytoplasmic fraction. The homogenate was distributed in 18 ml. plastic tubes and spun in a centrifuge (International, model CM) with a horizontal yoke (no. 240) at 600 g for 10 min.; the supernatant was carefully separated from the debris with a bulb pipette and reconstituted with the homogenizing medium to maintain a 10 % suspension of the liver pulp.

Chemical methods

Total nucleic acid. Portions of the homogenate or cytoplasmic fractions (2 ml.) were mixed with 5 ml. of 5 % (w/v) trichloroacetic acid, stirred well and centrifuged immediately for 10 min. at 800 g. The resulting precipitate was washed four times with 5 ml. amounts of cold 5 % trichloroacetic acid, and the residue was extracted with hot trichloroacetic acid by the method of Schneider (1945) without prior removal of the phospholipids (Webb & Levy, 1955). This extract was analysed for ribose as described by Brown (1946) and deoxyribose by the diphenylamine reaction (Dische, 1930).

Isolation of sodium nucleate after removal of acid-soluble substances and phospholipids (procedure A). Portions (5 ml.) of homogenate or cytoplasmic fraction were pipetted into 5 ml. of cold 5 % (w/v) trichloroacetic acid, stirred well and centrifuged immediately at 800 g for 10 min. The supernatant was discarded. The residue was washed four times with 7–8 ml. amounts of 5 % cold trichloroacetic acid, resedimented and the washings were discarded. The fourth acid wash was free of ultraviolet-absorbing material. The acid-washed residue was cooled in an ice bath, stirred vigorously with 7–8 ml. of absolute ethanol and allowed to stand in the ice bath for 10 min. The ethanol extract, which was transparent and dull brown, was separated by centrifuging. The residue was once more suspended in 7–8 ml. of cold absolute ethanol, stirred and centrifuged immediately. This was followed by a similar treatment of the residue with absolute ethanol at room temperature. The ethanol-treated residue was now heated twice with 8–10 ml. of 95 % ethanol-ether (3:1, v/v), at 45–50° for 1 min., the super-

nant being discarded each time after centrifuging. The phospholipid-free residue was now stirred with 7–8 ml. of cold 0.9% sodium chloride solution containing 3 mM-sodium bicarbonate (pH 7.8) and was centrifuged immediately. After a second wash with 7–8 ml. of the cold buffered 0.9% sodium chloride solution, the nucleoprotein residue was suspended in 5 ml. of 10% (w/v) sodium chloride solution. The pH was adjusted to 7.0 by the addition of dilute sodium hydroxide, phenol red being used as an internal indicator. Throughout extraction at 100° for 30 min. the pH range was maintained at 7.0–7.4 by the addition of dilute sodium hydroxide solution. The mixture was chilled immediately in ice-water and centrifuged to recover the extract. The precipitate was re-extracted twice with 2.0–2.5 ml. of 10% (w/v) sodium chloride solution at 100° for 5 min. each time. The sodium nucleate was precipitated from the combined extracts by the addition of 2.5 vol. of absolute ethanol. This was allowed to stand at 30° for 18–22 hr. The precipitate was collected by centrifuging, and washed three times with 7–10 ml. of a mixture of 2.5 vol. of absolute ethanol and 1 vol. of 10% (w/v) sodium chloride solution. The pH of the buffered 0.9% sodium chloride washings ranged between 6.5 and 7.2, and they were free of ultraviolet-absorbing material.

Isolation of sodium nucleate after removal of acid-soluble substances but without removal of phospholipids (procedure B). Portions (5 ml.) of homogenates or cytoplasmic fractions were treated with 5% (w/v) trichloroacetic acid and washed free of acid-soluble materials as in procedure A. To the residue, a cold solution of 0.9% sodium chloride containing 0.1 M-sodium bicarbonate (pH 7.95) was added, 1–2 ml. at a time, with vigorous stirring. A total of 7–8 ml. of this solution was added with stirring. Four drops of octan-2-ol were added, the mixture was sedimented at 800 g and the clear supernatant was separated. The residue was suspended in 5 ml. of 10% (w/v) sodium chloride solution, and the pH was adjusted to 7.0 with dilute sodium hydroxide, with phenol red as an internal indicator. The isolation of sodium nucleate was carried out exactly as in procedure A. The pH of 0.1 M-sodium bicarbonate–0.9% sodium chloride washings ranged between 5.7 and 5.9. Although the yield of sodium nucleate obtained by ethanolic precipitation at 30° was not significantly different from that obtained at 0°, the speed of precipitation appeared to be enhanced at the lower temperature.

Analysis of sodium nucleate. The washed sodium nucleate obtained by procedure A or B was dissolved in 8 ml. of cold water and freed of any insoluble residue by centrifuging at 800 g for 10 min. To 1 ml. of the nucleate solution was added 5 ml. of 3% (w/v) perchloric acid. This was heated at 80° for 30 min., cooled, centrifuged and diluted to 10 ml. Ribose and extinction were determined after suitable dilution. All measurements of extinction were made at 260 m μ unless otherwise specified.

Isolation of 'free' nucleic acid. To 6 ml. of the chilled sodium nucleate solution, obtained as in previous steps and derived from approx. 360 mg. of tissue, 2 ml. of 12% (w/v) cold perchloric acid was added with stirring, and the mixture was allowed to stand in an ice bath for 1 hr. The 'free' nucleic acid was sedimented by centrifuging (800 g) for 10 min. and washed twice with 2 ml. of cold 3% (w/v) perchloric acid. The sediment was hydrolysed with 5 ml. of 3% (w/v) perchloric acid at 80° for 30 min., and the residue, if any remained, was hydrolysed after centrifuging for a

further period of 5 min. at 80°. Any residue remaining was washed twice with 1 ml. amounts of 3% perchloric acid. The combined hydrolysate and washings were diluted to 10 ml. with 3% perchloric acid. Ribose, deoxyribose, total phosphorus (Fiske & Subbarow, 1925) and extinction were determined on appropriate portions. Deoxyribose estimation on the 'free' ribonucleic acid (RNA) from the cytoplasmic fraction was carried out only as a means of checking the effectiveness of the initial centrifugal separation of the homogenate into nuclear and cytoplasmic fractions.

Examination of the first ethanol extract from procedure A. The first ethanol extract, obtained from homogenate or cytoplasmic fraction immediately after precipitation and washing with trichloroacetic acid was evaporated to dryness *in vacuo* in a rotary Rinco Evaporator (The George Borg Corp., Wis., U.S.A.) at room temperature. The residue was washed three times, first with ether and then with light petroleum, 20 ml. of the solvent being used for each wash. The residue in the evaporator was then steamed for 5 min. after addition of 5–7 ml. of 3% (w/v) perchloric acid. When the film of material peeled off in a jelly-like mass it was transferred quantitatively, together with the perchloric acid to a 12 ml. centrifuge tube and heated at 80° for 30 min. and centrifuged. The residue was extracted a second time with 2 ml. of 3% (w/v) perchloric acid at 80° for 5 min. The pooled hydrolysate was diluted to 10 ml. Samples were used for ribose analysis and the determination of extinction.

In a separate experiment, the first ethanol-extract residue remaining after ether and light petroleum washing was treated with either water or 3% (w/v) perchloric acid at room temperature. Only an insignificant amount of the ultraviolet-absorbing material passed into the aqueous phase. The residue remaining after hydrolysis by 3% (w/v) perchloric acid at 80° gave a positive biuret reaction.

Evaluation of nucleic acid phosphorus from ultraviolet-absorption data. Measurements of extinction at 260 m μ were made with a Beckman DK-1 recording spectrophotometer adjusted to 100% transmission at 300 m μ . Hydrolysed samples of sodium nucleate or 'free' nucleic acid were prepared for absorption measurements by heating at 100° in 3% (w/v) perchloric acid for 15 min. to abolish any ϵ_F anomaly. The extinction at 260 m μ was converted into nucleic acid phosphorus by reference to $E_{1\%}^{1\text{cm}}$ values obtained under similar conditions from an independent study (C. U. Lowe & P. R. Venkataraman, in preparation).

Determination of nucleotide composition of the first ethanol extract from cytoplasmic fraction by paper chromatography. A portion (20 ml.) of the first ethanol extract derived from 400 mg. of fresh liver, obtained as in procedure A, was concentrated *in vacuo* and hydrolysed for 1 hr. at 100° with 10 ml. of N-HCl. After the hydrolysate was centrifuged, the residue was washed with 1 ml. of N-HCl and the combined supernatants were diluted to 12 ml., portions of 5 ml. were transferred to short wide-mouthed weighing bottles and evaporated *in vacuo* to dryness over sodium hydroxide pellets. The residue was dissolved in 0.2–0.3 ml. of 0.1 N-HCl and portions (50 μ l.) were applied to Whatman paper no. 1, 17 cm. $\times$ 56 cm. Separation of the components was effected by descending chromatography with a 70% *tert.*-butanol–water mixture which was 0.8 N with respect to HCl (Smith & Markham, 1950). The amount of base or nucleotide eluted from the

chromatogram was determined by the procedure of Knight (1952). The molar extinction coefficients of the standards, as taken from chromatograms, agreed with the values quoted by Knight (1952).

RESULTS

The results are presented in Tables 1-4. Determination of the total deoxyribonucleic acid (DNA) and RNA content of the homogenate and RNA content of the cytoplasmic fraction was carried out according to Schneider (1945), after removal of acid-soluble substances, but without the removal of phospholipids (Webb & Levy, 1955). The values quoted in the present study (Table 1) are in general accord both with those of other laboratories (Barnum, Nash, Jennings, Nygaard & Vermund, 1950; Schneider, Hogeboom & Ross, 1950) and those of a previous publication from this Laboratory (Lowe & Rand, 1956). No correction was applied for the interference of deoxyribose in the estimation of ribose, as this source of error has been assumed to be constant in the present experiments. It is apparent from Tables 1 and 2 that 83% of total DNA of the homogenate could be recovered as 'free' nucleic acid, whether procedure A or B was used. The small difference noted in the DNA content of the 'free' nucleic acid between the two procedures is not significant ($0.05 < P < 0.1$, by Student's *t* test). In contrast, the percentage of the total nucleic acid ribose found as sodium nucleate or 'free' nucleic acid obtained by procedure A is only 62 and 53 for the homogenate and cytoplasmic fractions respectively. In the method which did not utilize ethanol (procedure B) 80% of total nucleic acid ribose could be recovered from either

the homogenate or cytoplasmic fraction. The nucleic acid ribose values of either sodium nucleates or 'free' nucleic acids, obtained from homogenate and cytoplasmic fraction by procedure A, are respectively 31 and 38% lower than those obtained by procedure B. These differences are statistically significant ($P < 0.001$). The differences in the ribose values between those of sodium nucleate and 'free' nucleic acid for any one sample did not vary, on an average, by more than 6%. The action of ethanol on nucleoprotein samples previously exposed to cold trichloroacetic acid involves a concomitant loss of nucleic acid phosphorus and ribose. Thus for whole homogenate and cytoplasm, the total phosphorus content of sodium nucleate and 'free' nucleic acids are respectively 24 and 41% lower than those of the samples in which ethanol treatment had been eliminated. These differences in the phosphorus values are also significant ($P < 0.001$). For homogenate, the loss of nucleic acid phosphorus can be ascribed entirely to loss of RNA, since DNA remains virtually unaltered. The values for phosphorus of sodium nucleates and 'free' nucleic acid generally agree, on average, within 5% of those calculated from the ultraviolet-absorption data.

Extinction units and ribose content of the first ethanol extract from procedure A are presented in Table 3. Irrespective of whether the homogenate or cytoplasmic fraction is used, the same quantity of ultraviolet-absorbing material and ribose is found in the first ethanol extract. This fact indicates that the material soluble in ethanol is of cytoplasmic origin. Treatment of cytoplasmic nucleoprotein as in procedure A with 95% ethanol releases into the ethanolic phase amounts of ultraviolet-absorbing material and ribose identical with those obtained with absolute ethanol. However, when 80% (v/v) ethanol was used the amount of nucleic acid material dissolved was reduced by 50%. It is readily apparent from Tables 2 and 3 that the quantity of ribose found in the first ethanol extract is approximately equal to the nucleic acid ribose that failed to appear as sodium nucleate or 'free' nucleic acid. Nucleic acid phosphorus estimation on the perchloric acid hydrolysate of the first ethanol-extract residue could not be carried out because of interferences by phospholipid phosphorus, but it is evidence from results presented in Table 2 as well as from the chromatographic analysis of the first ethanol extract that nucleic acid phosphorus was present. The second and third ethanol extract obtained under these conditions contained only 15 and 7% respectively of the total extinction units and ribose of the first ethanol extract. The ethanol-ether washings were virtually free of ultraviolet-absorbing material and ribose.

Table 1. *Nucleic acid content of rat-liver homogenate and cytoplasm*

In Tables 1-3, values given are mean and standard error of the mean. Figures in parentheses indicate the number of observations per experiment. Each observation is a mean of duplicate or triplicate analysis. Figures in brackets represent the number of separate experiments.

	Concn. ($\mu\text{g./g.}$ of fresh liver)
Homogenate	
Deoxyribose*	1200 ± 42 (10) [5]
Ribose†	4411 ± 70 (10) [5]
Cytoplasm	
Ribose	3629 ± 72 (10) [5]

* Deoxyribose as determined by the diphenylamine reaction, and hence a measure of DNA.

† The estimated value for ribose has been doubled to allow for the non-reaction of ribose bound to pyrimidines; it is assumed that purine and pyrimidine ribosides are present in equimolar amounts.

The result of chromatographic analysis of the hydrochloric acid hydrolysate of the first ethanol-extract residue derived from a cytoplasmic fraction as in procedure A is presented in Table 4. No corrections were made for the small amount of hydrolysis of pyrimidine nucleotides, as suggested by Markham & Smith (1951). The methods adopted do not take into account the different extinctions of the 'a' and 'b' isomers of nucleotides which are present. Since hydrolysis with hydrochloric acid in the present case was carried out on crude material rather than on purified polynucleotides, the values quoted for nucleotide composition in Table 4 must be viewed with some reserve. Despite limitations, the results of chromatographic analysis afford evidence which helps to correlate other observations presented in this paper. It is apparent that the first ethanol-soluble fraction of cytoplasmic origin contains the four ribonucleotides usually present in a cytoplasmic polynucleotide. In the two experiments reported here an average of 4.3 μ moles of ribonucleotides/g. of liver is found in the first ethanol fraction. This figure is in reasonable agreement with the approximately 5.2 μ moles of ribonucleotide/g. of liver calculated from the data in Table 3.

DISCUSSION

The term 'free' nucleic acid referred to in this paper signifies the polynucleotide obtained by precipitation of sodium nucleate from water with 3% (w/v) perchloric acid at 2-4°. To facilitate the analysis of 'free' nucleic acid, it is generally made soluble with 3% (w/v) perchloric acid at 80°.

Results obtained in the present study suggest that cytoplasmic ribonucleoprotein of liver, previously exposed to cold trichloroacetic acid, is dissolved by ethanol in the presence of acid with appearance of material which contains ribose, and shows characteristic ultraviolet absorption, in the ethanolic phase. Chromatographic evidence is presented to show that the ultraviolet-absorbing material and ribose in the ethanolic phase reflect the presence of the four common ribonucleotides of adenine, guanine, uracil and cytosine. Further studies are necessary to describe completely the chemical nature of ethanol-soluble nucleotides.

Preliminary observation indicates that the four nucleotides appear to be firmly bound to what is probably a lipoprotein. In procedure B, in which the use of ethanol after exposure to trichloroacetic acid has been omitted, the ethanol-soluble nucleotides appear in association with 'free' RNA.

The precise manner by which ethanol in the presence of acid exerts a solublizing effect on liver ribonucleoprotein is obscure. The stability of deoxyribonucleoprotein under the experimental

Table 2. Effect of ethanol on the amount of sodium nucleate and 'free' nucleic acid obtained from rat-liver homogenate and cytoplasm by two different procedures

See notes in Table 1. All values are given as μ g./g. of fresh liver. Procedure A: nucleoprotein precipitated by trichloroacetic acid and washed immediately with ethanol; procedure B: nucleoprotein precipitated by trichloroacetic acid and ethanol wash omitted.

	Procedure A				Procedure B			
	Total phosphorus		Total phosphorus		Total phosphorus		Total phosphorus	
	Found	Calc.*	Found	Calc.*	Found	Calc.*	Found	Calc.*
Homogenate								
Sodium nucleate	—	696 $\pm$ 19 (14) [4]	—	3906 $\pm$ 48 (8) [2]	—	906 $\pm$ 13 (8) [2]	—	906 $\pm$ 13 (8) [2]
'Free' nucleic acid	1021 $\pm$ 26 (12) [3]	2693 $\pm$ 52 (14) [4]	714 $\pm$ 19 (6) [2]	3683 $\pm$ 44 (8) [2]	937 $\pm$ 24 (6) [2]	897 $\pm$ 11 (8) [2]	223	204
Mean difference† between A and B for 'free' nucleic acid	—	—	—	990	45	—	—	—
Cytoplasm								
Sodium nucleate	—	402 $\pm$ 10 (16) [4]	—	3196 $\pm$ 84 (8) [2]	—	594 $\pm$ 15 (8) [2]	—	594 $\pm$ 15 (8) [2]
'Free' nucleic acid	—	1884 $\pm$ 47 (15) [4]	354 $\pm$ 12 (8) [2]	2927 $\pm$ 70 (8) [2]	596 $\pm$ 16 (8) [2]	552 $\pm$ 7 (8) [2]	242	166
Mean difference† between A and B for 'free' nucleic acid	—	—	—	1043	—	—	—	—

* Calculated from $E_{260}^{1\%}$, at 260 m μ , obtained from an independent study (C. U. Lowe & P. R. Venkataraman, in preparation).
† All mean differences are significant, $P < 0.001$, except for DNA, where it is $0.05 < P < 0.10$.

Table 3. *Effect of various concentrations of ethanol on the amount of ribose and ultraviolet-absorbing material in the first ethanol extract obtained immediately after exposure of liver nucleoprotein to cold trichloroacetic acid*

See notes in Table 1.

Extinction units = $\frac{\text{observed extinction at } 260 \text{ m}\mu}{\text{g. of fresh liver}}$		
	Ribose ($\mu\text{g./g. of}$ fresh liver)	Extinction units
Homogenate		
Absolute ethanol	875 $\pm$ 51 (16) [4]	49 $\pm$ 2.7 (16) [4]
Cytoplasm		
Absolute ethanol	905 $\pm$ 69 (16) [4]	52 $\pm$ 2.0 (16) [4]
95 % (v/v) Ethanol	980 $\pm$ 83 (12) [5]	51 $\pm$ 3.6 (12) [5]
80 % (v/v) Ethanol	472 $\pm$ 32 (10) [4]	28 $\pm$ 2.0 (9) [4]

Table 4. *Nucleotide composition of the first absolute ethanol extract of liver cytoplasm obtained after precipitation of nucleoprotein with cold trichloroacetic acid*

Values are means, from duplicate chromatograms, calculated by assuming the molecular weight of the respective nucleotide.

	Expt. 1 ($\mu\text{moles/g. of fresh liver}$)	Expt. 2
Adenylic acid	1.12	0.86
Guanylic acid	1.23	1.00
Cytidylic acid	1.13	0.85
Uridylic acid	1.27	1.21

conditions of this study is consistent with the observations of Sacks, Hurley & Young (1955). Whether cold ethanol in the presence of acid exerts a solubilizing action on the ribonucleoproteins of other rat tissues has not been examined.

Methods comparable in several respects with procedure A have been widely used in nucleic acid-tracer studies (Davidson & Smellie, 1952; Tyner *et al.* 1953; Deluca, Rossiter & Strickland, 1953), where emphasis has been placed more on isolation, in contradistinction to estimation, procedures. In these isolation procedures it has hitherto been assumed that the treatment of trichloroacetic acid- or perchloric acid-precipitated nucleoprotein with 95 % ethanol involved no loss of RNA. It was pointed out by Herbert, Potter & Hecht (1957) that the use of 95 % ethanol, immediately after the perchloric acid precipitation of liver nucleoprotein, involved a loss in the yield of nucleic acid isolated by subsequent extraction with hot 10 % (w/v) sodium chloride solution. Consequently, Schneider & Potter (1958) eliminated the use of ethanol after

dilute perchloric acid treatment of liver-cell fractions, but instead extracted the acid-precipitated nucleoprotein with 10 % (w/v) sodium chloride solution after neutralization with potassium hydroxide. The modified procedure of Schneider & Potter (1958) would be comparable with procedure B of this paper. However, these investigators do not appear to have considered a solubilizing effect of ethanol on ribonucleoprotein previously exposed to acid.

SUMMARY

1. Nucleoprotein was obtained from a homogenate or cytoplasmic fraction of rat liver by precipitating with dilute trichloroacetic acid. When extracted either with cold absolute ethanol or 95 % (v/v) ethanol partial solubilization of ribonucleic acid protein occurred in the presence of acid with simultaneous appearance of what seemed to be a lipoprotein-polynucleotide complex in the ethanol phase.

2. In contrast, deoxyribonucleic acid protein under similar conditions is not apparently affected.

3. Evidence is presented to suggest that the ribonucleic acid protein susceptible to the solubilizing action of ethanol in the presence of acid is of cytoplasmic origin.

4. Chromatographic analysis of the hydrolysate of the first ethanol extract of ribonucleoprotein precipitated with trichloroacetic acid indicates the presence of adenine, guanine, uridylic acid and cytidylic acid.

5. When the liver nucleoprotein, obtained by precipitation and washing with dilute trichloroacetic acid, is extracted with cold absolute ethanol, the yield of ribonucleic acid obtained by subsequent extraction with 10 % (w/v) sodium chloride solution is significantly reduced.

6. The loss in the yield of ribonucleic acid can be avoided by eliminating the extraction of ribonucleoprotein with absolute ethanol after precipitation with trichloroacetic acid.

7. These observations have obvious implications in nucleic acid-tracer studies.

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

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

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

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

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

MATERIALS AND METHODS

Chemicals. Adenosine triphosphate (ATP; dipotassium salt) and guanosine triphosphate (GTP; sodium salt) were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. Solutions were brought to pH 7.8 (indicator papers) with KOH. The barium-silver salt of phosphoenolpyruvic acid (PEP) was prepared by the method of Ohlmeyer (1951). A solution of the free acid was prepared shortly before use and was adjusted to pH 7.8 (indicator papers). Glutathione (GSH) was obtained from The Distillers (Biochemicals) Co.

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