be considered as 1-deoxyglucopyranose. Fresh solutions of glucono-1,5-lactone in 0.5 *M* citrate buffer, pH 5.5, caused a fairly strong inhibition of the activity with glucose as observed with the Tes-Tape, but no evidence of its significance could be obtained.

It is hoped that these observations, by preventing possible errors in the utilization of glucose oxidase, will contribute to its value as an analytical tool.

TABLE I

SUBSTRATES AND COMPETITIVE INHIBITORS OF GLUCOSE OXIDASE

*Compound**	*Relative rates***	*Michaelis constant**** *M*	*Oxidation coefficient*§
Glucose	100	0.02	1.00
2-Deoxyglucose	25	0.07	0.07
6-Deoxy-6-fluoroglucose	> 3		0.004
Mannose	> 2		0.003
Glucosamine§§	> 2		0.003
Xylose	> 0.4		0.0006
Maltose§§§	—		
N-Acetylglucosamine	—		
1,5-Sorbitan		—	
1,4-Sorbitan		—	

* Obtained as previously described[6,5] except the maltose, which was obtained from the Pfanstiehl Chemical Co. Solutions were allowed to equilibrate before use.

** The unqualified figures are maximal rates. Those preceded by > are the relative rates observed at 0.1 *M* concentration. A dash in this column indicates an undetectable rate at 0.1 *M* concentration (less than 0.05).

*** The last two compounds were tested as possible inhibitors at 0.5 *M* concentration with 0.005 *M* glucose as substrate, using the paper test.

§ Relative rates at substrate concentration (below 0.001 *M*) low enough for essentially first order kinetics even with the substrate of highest affinity. It is equivalent to the phosphorylation coefficient used in the evaluation of substrates for hexokinase[6].

§§ At pH 8.0, using glucose and mannose buffered at this pH as reference standards. No activity was observed with unneutralized glucosamine-HCl solutions, although its pH is within the range optimum for the enzyme.

§§§ In the presence of yeast hexokinase and ATP-Mg in sufficient excess to compete successfully with the glucose oxidase for the glucose liberated by a trace of maltase which is present in the preparations utilized. This contaminating maltase has no appreciable activity on methyl-α-glucoside.

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1 D. KEILIN AND E. F. HARTREE, *Biochem. J.*, 42 (1948) 221.
2 D. KEILIN AND E. F. HARTREE, *Biochem. J.*, 50 (1952) 331.
3 D. KEILIN AND E. F. HARTREE, *Biochem. J.*, 42 (1948) 230.
4 J. P. COMER, *Anal. Chem.*, 28 (1956) 1748.
5 A. SOLS, *Biochim. Biophys. Acta*, 20 (1956) 62.
6 A. SOLS AND R. K. CRANE, *J. Biol. Chem.*, 210 (1954) 581.
7 R. BENTLEY AND A. NEUBERGER, *Biochem. J.*, 45 (1949) 584.

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Ribonucleoprotein from rabbit appendix microsomes*

It is generally believed that microsomes are the main loci of cytoplasmic protein synthesis. Recent findings have shown that they are probably a complex of ribonucleoprotein and protein free of ribonucleic acid (RNA)[1,2,3]. It has also been supposed that it is the ribonucleoprotein moiety that plays the essential part in the synthesis of protein[1,3,4]. In order to analyze the mechanism whereby microsomal ribonucleoprotein participates in the synthesis of specific cytoplasmic

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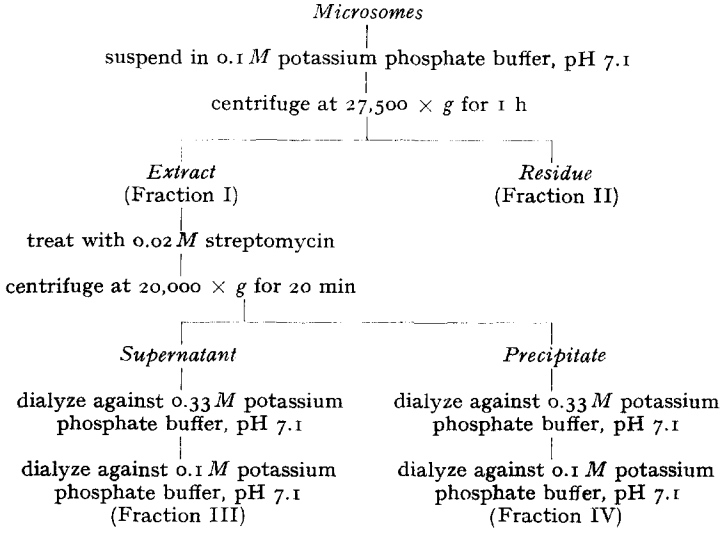
proteins, it is necessary to isolate pure ribonucleoprotein from microsomes. Attempts have been made to do this by the use of deoxycholate[1,2,4]. Ribonucleoproteins with a high RNA content (about 30 to 50%) have been obtained by this method.

In this note, a procedure for isolating an electrophoretically homogeneous sample of ribonucleoprotein from rabbit appendix microsomes will be reported. 100 g of appendix tissue excised from ten albino rabbits (1800 g) are minced well, and homogenized in 0.25 *M* sucrose-0.004 *M* $CaCl_2$. The homogenate is centrifuged at 10,000 × *g* for 20 min, and the sediment containing unbroken cells, nuclei and mitochondria discarded. Microsomes are then obtained by centrifuging the supernatant at 27,500 × *g* for 2 h in the International Refrigerated Centrifuge. The procedure of isolating the ribonucleoprotein from the microsomes is briefly summarized in the following scheme.



The essential part of this procedure involves the extraction from the microsomes with dilute phosphate buffer of some proteins containing a large part of ribonucleoprotein of the microsomes, followed by the differential precipitation of nucleoprotein with streptomycin[5–8]. About 40% of the RNA and 4.5% of the protein of the microsomal pellet are recovered in the final product. No deoxyribonucleic acid has been detected.

As shown in Fig. 1b, ribonucleoprotein thus isolated reveals considerable homogeneity in an electrophoretic field. In Fig. 1a, showing the electrophoretic pattern of the original phosphate extract of microsomes (Fraction I), two main components can be seen. The mobility of the streptomycin-precipitable ribonucleoprotein (Fraction IV) is very close to the fast component (A) of the original extract. The supernatant after streptomycin treatment (Fraction III) is almost completely devoid of Component A, and has a low RNA content. These facts strongly suggest that the ribonucleoprotein purified with streptomycin is the Component A originally present in the phosphate extract of microsomes; streptomycin selectively brings down the Component A. This suggests that the ribonucleoprotein we obtained is not an artifact produced by the streptomycin treatment. The ribonucleoprotein has very high RNA content, reaching about 65% by

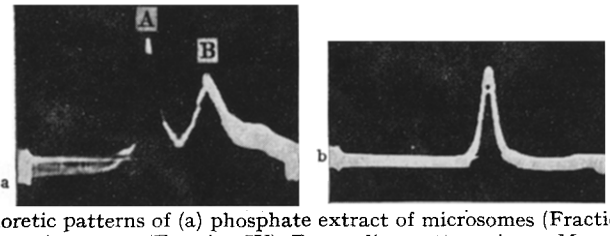


Fig. 1. Electrophoretic patterns of (a) phosphate extract of microsomes (Fraction I) and (b) ribonucleoprotein from microsomes (Fraction IV). Descending patterns in 0.1 *M* potassium phosphate buffer at pH 7.1 in a field of 7.3 volts/cm.

TABLE I

RIBONUCLEIC ACID CONTENT OF MICROSOMAL PROTEIN FRACTIONS OF ALBINO RABBIT APPENDIX

Fraction	μg RNA-P/mg protein N		% RNA	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
1. Microsomes	—	134	—	17.7
2. Phosphate extract of microsomes (Fraction I)	360	205	36.5	25.2
3. Residue of phosphate extract (Fraction II)	—	71	—	10.2
4. Supernatant of streptomycin-treated phosphate extract (Fraction III)	—	25	—	3.9
5. Precipitate of streptomycin-treated phosphate extract (Fraction IV)	1180	1100	66.0	63.7

weight (Table I). We believe that this is the ribonucleoprotein with the highest RNA content so far reported.

The physiological role and some physico-chemical properties of this ribonucleoprotein are now being investigated.

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1 J. W. LITTLEFIELD, E. B. KELLER, J. GROSS AND P. C. ZAMECNIK, *J. Biol. Chem.*, 217 (1955) 111.
2 G. E. PALADE AND P. SIEKEVITZ, *J. Biophys. & Biochem. Cytol.*, 2 (1956) 171.
3 M. RABINOVITZ AND M. E. OLSON, *Exptl. Cell Research*, 10 (1956) 747.
4 V. G. ALLFREY, M. M. DALY AND A. E. MIRSKY, *J. Gen. Physiol.*, 37 (1953) 157.
5 S. S. COHEN, *J. Biol. Chem.*, 168 (1947) 511.
6 R. J. KUTSKY, T. TRAUTMAN, M. LIEBERMAN AND R. M. CALLEAU, *Exptl. Cell Research*, 10 (1956) 48.
7 S. COHEN, R. LEVI-MONTALCINI AND V. HAMBURGER, *Proc. Natl. Acad. Sci. U.S.*, 40 (1954) 1014.
8 T. YAMADA AND K. TAKATA, *Embryologia*, 3 (1956) 69.

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A micro-method for the determination of amino acids, peptides and proteolytic activity; demonstration of the formation of a complex of glycylglycine dipeptidase and cobalt

In the last few years extensive use has been made in clinical laboratories of simple photometers for accurate micro-titrations. This means that the titrations are carried out in the cuvettes or tubes placed between an appropriate filter and the photo-cell; the end point of a titration is indicated by a certain extinction read on the instrument. We have made use of this procedure for the titration of amino acids and peptides, and for the determination of proteolytic activities according to Sørensen's formaldehyde method. Thus in a simple way very small amounts of NH_2-groups present in small volumes can be determined with great accuracy. Our procedure is thus very appropriate for use in studies of the proteolytic activity of tissue homogenates and cell fractions, and this is of particular importance in experiments with small amounts of tissue and expensive substrates.

We made use of an EEL photometer with filter 625. The titration of glycylglycine will be described as an example.

In the photometer tube were placed 1 ml 33% formaldehyde, 0.25 ml glycylglycine solution and 5 ml of a 0.001% solution of phenolphthalein in a 1:10 (v/v) mixture of ethanol and water. The titration was carried out with 0.01 *N* NaOH from a 5 ml burette, graduated in 0.01 ml. With the phenolphthalein concentration indicated above, the end point of the titration (pH 9.0) was reached when in our EEL photometer with filter 625 the extinction was 0.150.

The result of 20 titrations of 2440 γ glycylglycine was 2.529 $\pm$ 0.014 ml 0.01 *N* NaOH. As the absolute error does not increase with smaller amounts of NH_2-groups the amount of glycylglycine can still be considerably reduced before the error of the titration will make the method unreliable.