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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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.

An example of the use of the procedure in the study of tissue proteases, which we believe to be of some interest, is the following:

1 g rat liver was homogenized in 6 ml water at 2° C in the Potter-Elvehjem apparatus. After centrifugation for 30 min at 1800 *g* at 2° C, the glycylglycine dipeptidase activity of the supernatant was determined in the presence of 0.001 *M* $CoCl_2$. The reaction mixtures contained 1.5 ml supernatant, and glycylglycine solution, phosphate buffer, pH 8.1, and $CoCl_2$ solution to a final volume of 3.0 ml (final concentrations: glycylglycine 0.037 *M*, phosphate 0.017 *M*, $CoCl_2$ 0.001 *M*).

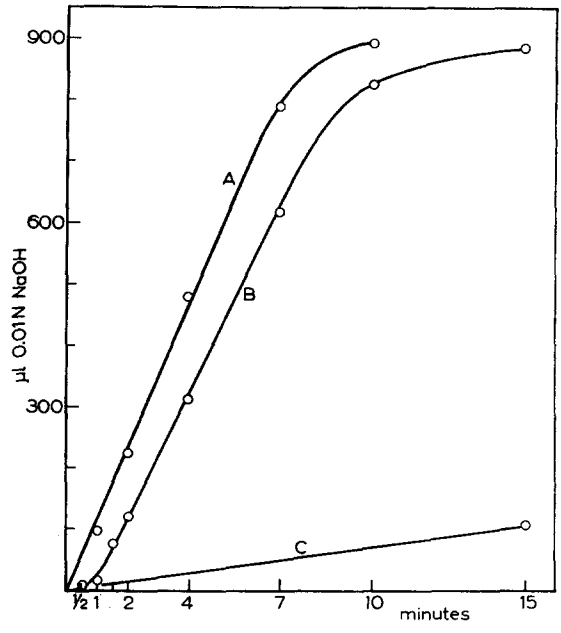

Fig. 1. Hydrolysis of glycylglycine by glycylglycine dipeptidase. A: $CoCl_2$ added 10 min before substrate; B: $CoCl_2$ added simultaneously with substrate; C: blank (water instead of $CoCl_2$).

The glycylglycine solution was added at zero time. In the case described by curve A, the $CoCl_2$ was added 10 min before the glycylglycine; in the case described by curve B it was added simultaneously with the glycylglycine. At the times plotted on the abscissa, 0.25 ml of the reaction mixtures was transferred to photometer tubes containing 1 ml 33% formaldehyde. The titrations were carried out as described above.

Upon considering Fig. 1 one observes that curve B demonstrates the occurrence of a lag-time in contrast to curve A, which indicates a constant reaction rate from zero time until the substrate has been utilized almost completely. With other methods (*cf.* SMITH[1], RABIN AND CROOK[2]) no lag-time in the hydrolysis of glycylglycine by glycylglycine dipeptidase upon adding cobalt could be detected. We believe that the fact that the lag-time is not observed when the cobalt is in contact with the enzyme a few minutes before the substrate is added is of considerable interest for the discussion of the kinetics of the cobalt-activated reaction, as it will now be necessary to assume that the reaction is initiated with the rapid formation of an enzyme-cobalt complex.

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