tively) are high, and nearly equally so, during the initial intervals. The RNA phosphorus would appear to move as follows: Sp → DS → RNP.

The evidence on the manner in which ribonucleic acid participates in protein synthesis is still fragmentary. If such a participation requires the rapid renewal of the nucleic acid, our observations would suggest that the ribonucleic acid of the RNP particles of the microsomes is not directly involved in the formation of microsomal protein, though the protein precursors, activated in the Sp fraction, may be assembled in the microsomes to form the final polymer. It is perhaps significant that the cytoplasmic supernatant fraction, in which the enzymic activation of amino acids[5] has been shown to occur[6,7], also contained the ribonucleic acid exhibiting the highest initial uptake of ^{32}P.

We shall submit more detailed information on these studies in due course. This work, which benefited from the technical assistance of Miss EDITH A. LAWRENCE, has been supported by research grants from the U.S. Public Health Service, the National Science Foundation and the Rockefeller Foundation.

Cell Chemistry Laboratory, Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, N.Y. (U.S.A.)

HAROLD T. SHIGEURA
ERWIN CHARGAFF

[1] E. CHARGAFF, D. ELSON AND H. T. SHIGEURA, *Nature*, 178 (1956) 682.
[2] H. T. SHIGEURA AND E. CHARGAFF, *Federation Proc.*, 16 (1957) 246.
[3] J. W. LITTLEFIELD, E. B. KELLER, J. GROSS AND P. C. ZAMECNIK, *J. Biol. Chem.*, 217 (1955) 111.
[4] J. L. SIMKIN AND T. S. WORK, *Biochem. J.*, 65 (1957) 307.
[5] H. BORSOOK, *3ème Congr. intern. biochim., Bruxelles, 1955, Conf. et Rapp.*, p. 92.
[6] M. B. HOAGLAND, E. B. KELLER AND P. C. ZAMECNIK, *J. Biol. Chem.*, 218 (1956) 345.
[7] E. W. DAVIE, V. V. KONINGSBERGER AND F. LIPMANN, *Arch. Biochem. Biophys.*, 65 (1956) 21.

Received March 18th, 1957

The distribution of protease activities on liver cell fractions

Using hemoglobin as a substrate, DE DUVE and co-workers[1,2] found the highest cathepsin activity in the light mitochondria fraction of liver homogenates. Earlier MAVER AND GRECO[3] had found that the total mitochondria fraction contains the highest percentage of the total protease activity of liver homogenates not only when hemoglobin, but also when benzoyl-*l*-argininamide was used as a substrate.

As cathepsin is the collective name for the tissue proteinases and other proteases, *e.g.* dipeptidases and a carboxypeptidase, also occur in the tissues we thought it would be interesting to study the protease activities of the liver cell fractions with synthetic substrates, specific for various proteases.

1:5 rat liver homogenates were prepared in 0.25 *M* sucrose. The homogenate was layered on 0.35 *M* sucrose and centrifuged for 5.5 min at 900 × *g* in order to sediment the nuclear fraction. The supernatant containing the other subcellular components was centrifuged for 12 min at 18,000 × *g*. The total mitochondria sedimented in this way could be separated into the light and the heavy fraction by resuspending them in 0.25 *M* sucrose, centrifuging for 8 min at 11,000 × *g*, and resuspending the slightly pink light mitochondria layer (easily distinguishable from the dark yellow heavy mitochondria layer) by causing a slow rotatory movement in the supernatant by means of a small pestle fitted into the centrifuge tube. The suspension obtained in this way was then transferred to another centrifuge tube and the light mitochondria spun down by centrifuging for 12 min at 18,000 × *g*. The whole procedure was carried out at about 2° C. The final supernatant obtained by sedimenting the total mitochondria was not fractionated further; thus it still contained the microsomes. In order to avoid damage as much as possible the sedimented fractions were not washed.

With most substrates the protease activities of the fractions were determined by means of the recently described micro-modification of Sørensen's formaldehyde method[4]. In the case of benzoyl-*l*-argininamide the activity was measured by determination of the liberated ammonia[5]. The rate of hydrolysis of hemoglobin by the fractions, studied in order to check our fractionation technique as compared with that employed by DE DUVE, was determined by means of the ANSON method[6,7].

All homogenates and suspensions of fractions were studied in the presence of Triton-X-100*, added a few minutes before they were added to the other components of the reaction mixture*. As micro-methods were used for the determination of the rate of hydrolysis of the peptide bonds, the total volumes of the reaction mixtures could be kept as low as a few ml, containing amounts of fractions corresponding to a few hundred mg liver, while the amounts of the various substrates ranged from 3 to 15 mg. The pH's corresponded to the pH-optima of the hydrolysis of the substrates.

The results can be summarized as follows: The glycylglycine dipeptidase (determined in the presence of Co-ions) and the triglycine peptidase activity (determined in the absence of Co-ions) appeared to be concentrated for 100% in the supernatant. On the other hand, cathepsin A activity, measured with cbz-*l*-glutamyl-*l*-tyrosine, was completely absent from the supernatant. Most was found in the mitochondria, the ratio between the amounts found in the light and the heavy fraction being about 6:4. The latter result somewhat resembles the results obtained with hemoglobin as substrate. In our experiments the ratio for light and heavy mitochondria was about 5:1. According to DE DUVE[2] the ratio is about 2:1 in purified parenchymatous cells. We found a higher activity, expressed as percentage of the activity of the homogenate, in the nuclear fraction than DE DUVE and a lower in the supernatant. However, in our experiments the nuclear fractions were not washed, while in DE DUVE's experiments they were washed. It is remarkable that with hemoglobin as a substrate, we as well as DE DUVE found a considerable activity in the supernatant while with cbz-*l*-glutamyl-*l*-tyrosine as a substrate we were unable to detect any activity even in a very concentrated supernatant, *viz.* the supernatant of a 1:1.5 homogenate in 0.25*M* sucrose. Cathepsin B activity measured with benzoyl-*l*-argininamide as a substrate was distributed in all fractions. The amount in the supernatant was only a little lower than in the total mitochondria fraction, while the ratio between the light and heavy mitochondria was on the average about 5:4. Again, different results concerning the carboxypeptidase activity were obtained with cbz-glycyl-*l*-phenylalanine as a substrate. No activity could be detected in the supernatant (even from a 1:1.5 homogenate), while 80 to 90% of the total activity of the homogenate was present in the mitochondria. In this case, the heavy mitochondria fraction was much more active than the light fraction: the ratio was on the average about 10:1.

With the exception of the glycylglycine dipeptidase and triglycine peptidase activities, some activity was always found in the nuclear fraction. This may be caused by the contamination of these unwashed fractions with mitochondria, though it is not excluded that the nucleus also contains the enzymes studied.

These observations raised many questions that cannot be discussed in this note.

W. RADEMAKER
Laboratory for Physiological Chemistry, The University, Utrecht (The Netherlands) J. B. J. SOONS

1 C. DE DUVE, B. C. PRESSMAN, R. GIANETTO, R. WATTIAU AND F. APPELMANS, *Biochem. J.*, 60 (1955) 604.
2 R. WATTIAU, P. BAUDHUIN, A. M. BERLEUR AND C. DE DUVE, *Biochem. J.*, 63 (1956) 608.
3 M. MAVER AND A. GRECO, *J. Natl. Cancer Inst.*, 12 (1951) 37.
4 W. RADEMAKER AND J. B. J. SOONS, *Biochim. Biophys. Acta*, 24 (1957) 209.
5 J. P. GREENSTEIN AND F. M. LEUTHARDT, *J. Natl. Cancer Inst.*, 6 (1946) 303.
6 M. L. ANSON, *J. Gen. Physiol.*, 20 (1937) 565.
7 R. GIANETTO AND C. DE DUVE, *Biochem. J.*, 59 (1955) 433.
8 R. WATTIAU AND C. DE DUVE, *Biochem. J.*, 63 (1956) 606.

Received February 22nd, 1957

* We are very much indebted to Prof. C. DE DUVE for a sample of Triton-X-100.