two antigenic determinants, both of the egg high-density-lipoprotein type, one of which is common to the very-low-density lipoprotein. In native high-density lipoprotein only the unique determinant is sufficiently exposed to react. On delipidation high-density lipoprotein also reacts with anti-(very-low-density lipoprotein). The difference between very-low-density lipoprotein and high-density lipoprotein is apparently due to the lipid moiety of the yolk high-density lipoprotein.

We thank Dr S. Mookerjee for helpful discussion. This work was supported by the Ontario Heart Foundation and the Medical Research Council of Canada.

- Bernardi, G. & Cook, W. H. (1960). *Biochim. biophys. Acta*, **44**, 105.
 McCully, K. A., Maw, W. A. & Common, R. H. (1959). *Can. J. Biochem. Physiol.* **37**, 1457.
 Schjeide, O. A. (1963). In *Progress in the Chemistry of Fats and other Lipids*, vol. 6, p. 251. Ed. by Holman, R. T., Lundberg, W. O. & Malkin, T. New York: Pergamon Press Inc.
 Schjeide, O. A. & Urist, M. R. (1959). *Exptl Cell Res.* **17**, 84.
 Schjeide, O. A., Wilkens, M., McCandless, R. G. Munn, R., Peterson, M. & Carlsen, E. (1963). *Am. Zool.* **3**, 167.

An Alkaline Proteinase in Muscle Homogenates

By D. HOLMES, M. E. PARSONS, D. C. PARK and R. J. PENNINGTON. (*Regional Neurological Centre, General Hospital, Newcastle upon Tyne NE4 6BE, U.K.*)

Homogenates of mouse or rat skeletal muscle in 50mM-potassium chloride degraded casein to perchloric-acid-soluble products at pH 8.3. The products were measured by extinction at 224nm, and at 260nm to correct for nucleic acid breakdown (which was relatively small). After centrifuging of the homogenates at 100000g for 30min nearly all of this proteolytic activity was usually found in the sediment fraction. The sedimented activity, when measured over the range pH 7.5–12.1, was maximum at pH 11; it was increased by 0.3M-sodium chloride, slightly at pH 10 but four- to five-fold at pH 7.5. The activity was lost when the fraction was heated at 80°C for 10min. Soya-bean trypsin inhibitor (1µg/0.3ml) produced a 77% inhibition of activity; there was no inhibition by hen-ovomucoid trypsin inhibitor (1µg) or by the supernatant (0.05ml) obtained by centrifuging a dialysed muscle homogenate at 100000g for 30min. Mouse serum (diluted 1:200; 0.05ml) caused a slight (10%) inhibition.

Attempts were made to solubilize the enzyme by extracting the fraction for 15min at room

temperature with various reagents followed by centrifuging at 30000g for 30min. Little or no enzyme was extracted with tricine buffer, pH 8.0 (30mM), acetate buffer, pH 5.0 (30mM), Triton X-100 (0.3%) or urea (8M)+2-mercaptoethanol (10mM). Sodium dodecyl sulphate (10mM) or guanidine hydrochloride (5M)+2-mercaptoethanol (10mM) completely inactivated the enzyme. The enzyme was extracted with 0.75M-sodium chloride or -potassium thiocyanate after removal of a large part of the inactive protein (mainly myofibril protein) with 8M-urea. The enzyme was slowly precipitated on standing in the cold and could be redissolved in 0.75M-potassium thiocyanate resulting in a tenfold purification (460-fold compared with the original homogenate). The extract hydrolysed *N*-benzyloxycarbonyl-L-tyrosine *p*-nitrophenyl ester but not *N*-toluene-*p*-sulphonyl-L-arginine methyl ester.

Huston & Krebs (1968) purified a Ca²⁺-dependent proteolytic enzyme from muscle extracts, with maximum activity at pH 7.5. Brush & Cheng (1968) purified an insulin-degrading protein from rat muscle, also with optimum activity at pH 7.5. Neither of these enzymes appears to be identical with the one studied here. The autolytic breakdown of a muscle myofibril fraction reported by Noguchi & Kandatsu (1969, 1970) also appears to be due to a different enzyme, since it is strongly inhibited by the supernatant fraction.

This work was supported by the Medical Research Council and the Muscular Dystrophy Group.

- Brush, J. S. & Cheng, E. W. K. (1968). *Fedn Proc. Fedn Am. Soc. exp. Biol.* **27**, 495.
 Huston, R. B. & Krebs, E. G. (1968). *Biochemistry, Easton*, **7**, 2116.
 Noguchi, T. & Kandatsu, M. (1969). *Agric. biol. Chem., Tokyo*, **33**, 1226.
 Noguchi, T. & Kandatsu, M. (1970). *Agric. biol. Chem., Tokyo*, **34**, 390.

The Effect of Chlorambucil on Nuclear Protein Synthesis in Yoshida Ascites-Sarcoma Cells

By PAMELA G. RICHES and K. R. HARRAP. (*Department of Applied Biochemistry, Chester Beatty Research Institute, Fulham Road, London SW3 6JB, U.K.*)

Increases have been reported in the nucleic acids and protein content of a drug-sensitive strain of Yoshida ascites cells after treatment of the host animals with alkylating drugs; comparable changes have not been observed in a drug-resistant strain of the tumour (Harrap & Hill, 1969a,b). After the administration of a therapeuti-

cally effective dose of chlorambucil (Leukeran) (8mg/kg body wt.) an increased incorporation of thymidine into the DNA of sensitive cells was observed. The rates of RNA and total protein biosynthesis were not affected by drug treatment (Hill & Harrap, 1970).

In the present studies the effects of chlorambucil on the nuclear proteins of Yoshida cells have been investigated. Cells were harvested from animals at various time-intervals after treatment with chlorambucil (8mg/kg body wt.). Histone was extracted in 0.25M-hydrochloric acid from chromatin obtained after saline washing of isolated nuclei. The remaining protein, after histone extraction, is referred to as non-histone protein. Changes in the histone/DNA and non-histone protein/DNA ratios were seen only in the drug-sensitive cells: the histone/DNA ratio fell, whereas the non-histone protein/DNA ratio increased. Electron micrographs of sensitive treated cells have shown that these changes were accompanied by 'giant-cell' formation and an altered appearance of the chromatin. Dense material (heterochromatin), seen in untreated sensitive cells, disappeared after treatment, becoming more diffuse (probably euchromatin). No alteration in the chromatin of resistant cells was observed after treatment.

The rate of incorporation of [^{14}C]leucine into histone and non-histone protein has shown an inhibition of synthesis of both proteins, after treatment, in the drug-sensitive cells. This observation, together with a concurrent increase in DNA synthesis, would account for the low histone/DNA ratio. The increase in non-histone protein/DNA ratio, however, cannot be accounted for in this way, and might therefore suggest a reorganization of previously synthesized material.

Since total protein synthesis was not affected under these conditions, chlorambucil appears to have a specific effect in inhibiting nuclear protein synthesis.

We thank Mr M. S. C. Birbeck for preparing the electron micrographs.

Harrap, K. R. & Hill, B. T. (1969a). *Br. J. Cancer*, **23**, 210.
Harrap, K. R. & Hill, B. T. (1969b). *Br. J. Cancer*, **23**, 227.
Hill, B. T. & Harrap, K. R. (1970). *Neoplasia*, **17**, 5.

Isolation of Messenger-Like Ribonucleic Acid from Immunochemically Precipitated Polyribosomes

By S. L. BOYD,* T. L. DELOVITCH,† G. HOLME and A. H. SEHON. (*Department of Immunology, University of Manitoba, Winnipeg 3, Manitoba, Canada*)

Specific antibodies to a given protein have been used to precipitate polyribosomes synthesizing that protein. For example, the technique was used for the identification of polyribosomes synthesizing the heavy chains and light chains of immunoglobulin G (Williamson & Askonas, 1967) and for the isolation of mRNA for α -glucosidase (Hartlief & Koningsberger, 1968). However, this study showed that precipitation of intact polyribosomes by whole antibodies was not specific, owing to binding of immunoglobulin G through the Fc portion to ribosomes.

Polyribosomes of MOPC 149 plasmacytoma, which synthesized an immunoglobulin light chain, were labelled *in vivo* with sodium [^{32}P]phosphate and their nascent peptides incorporated ^{14}C -labelled amino acids during incubation of the tumour cells *in vitro*. Cell disruption and polyribosome fractionation occurred in one step on a sucrose density gradient (Davis, Delovitch & Sehon, 1969). Fractions from the gradient, or suspensions of the total polyribosomes, were treated with either rabbit antiserum to MOPC 149 light chain (specific) or normal rabbit serum (control). The optimum amount of sheep antiserum to rabbit immunoglobulin G was then added to cause complete precipitation.

Specific and control antisera precipitated similar amounts of ^{32}P -labelled components from each polyribosome fraction when precipitation occurred in TMK buffer [10mM-tris-HCl buffer (pH 7.3)–3mM-magnesium chloride–15mM-potassium chloride). Removal of nascent peptides with puromycin had no effect on the precipitation of ^{32}P -labelled components. Both antisera precipitated similar amounts of ^{32}P - and ^{14}C -labelled components from a suspension of total polyribosomes in TMK buffer. In contrast, in phosphate-buffered saline [20mM-phosphate buffer (pH 7.4)–0.14M-sodium chloride] the ^{14}C -labelled components in the control precipitates were decreased. However, in phosphate-buffered saline ^{32}P -labelled components were absent from both precipitates, indicating degradation of ribosomes.

* Present address: MRC Neuroendocrinology Unit, Department of Human Anatomy, University of Oxford, Oxford OX1 3QX, U.K.

† Present address: Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass. 02139, U.S.A.