behaviour or in the surface of particles from different tissues, may influence the response to hypotonic conditions or to lytic enzymes activated at pH 5 and 37 °C. The possibility that volume and not structural differences are concerned in the observed phenomena has been checked by submitting liver and hepatoma lysosomes (L fractions) to phospholipase A (phosphatide acyl-hydrolase, EC. 3.1.1.4), digitonin and trypsin. The response to these agents may be influenced by extra lysosomal components or lysosomal volume, so that apparent resistance is dissimulated. Increasing amounts of added agents, however, may overcome lysosomal apparent resistance. In the absence of this overcoming effect, the role of lysosomal membrane properties must be taken into account.

When liver and tumour L fractions are submitted to phospholipase A (Figure 2A), the β-GNase activation in tumour L fraction proceeds more slowly but, practically, to the same extent as in liver lysosomes. Digitonin, also, is able to liberate all the β-GNase activity in hepatoma lysosomes, when added in 3 times the amount which induces almost complete activation of liver lysosomes enzymic activity (Figure 2B). Different patterns are observed when hepatoma and liver lysosomes are treated with trypsin. Preincubation for 30 min at 37 °C of lysosomes from both tissues with 100 μg of trypsin per mg protein fails to induce enzyme maximum activation (Figure 3A). Once again tumour lysosomes are less susceptible to the damaging agent. It appears that after 10 min incubation only a slight increase in activity is obtained, although Triton is still able to induce enzyme release up to 98%. Figure 3B shows that further activation up to 87% of liver lysosomes β-GNase is obtained with 400 μg of trypsin per mg protein. This activity can no longer be activated by Triton, probably because of β-GNase partial inhibition. In the case of hepatoma L fraction, 40% maximum activation is induced by trypsin and no additional increase of activity occurs between 100 μg and 1200 μg of trypsin per mg protein. Amounts of trypsin higher than 200 μg per mg protein induce some β-GNase inhibition. This inhibition accounts for the difference between free activity calculated as percent of total activity, or of that released by Triton after trypsin treatment.

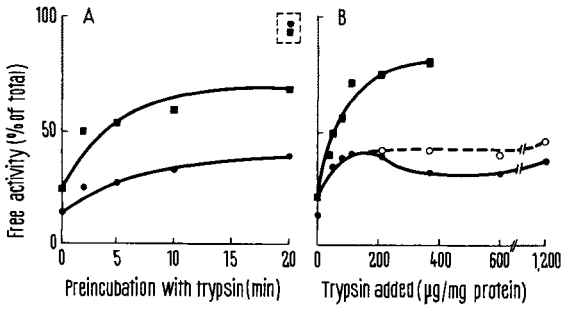

Fig. 3. Activation by trypsin of lysosomes (L fraction) of normal liver and Yoshida ascites hepatoma. (A), aliquots of L fractions (0.7–1 mg protein) were preincubated at 37 °C for varying perdiods with trypsin (Sigma, type III, 100 μg/mg), and β-GNase was determined. (B), incubation with different amounts of trypsin was made 20 min at 37 °C. Other conditions as in (A). Free activity is calculated as percent of total activity in Triton treated samples. Each point represents an average value of 5 and 6 experiments, respectively, in (A) and (B), for liver (■) and hepatoma (●) lysosomes. Symbols in dotted square indicate Triton-induced activation after incubation with trypsin. Dotted line refers to free activity calculated as percent of Triton-induced release after preincubation with trypsin.

The slower response of tumour lysosomes to digitonin and phospholipase A may depend on quantitative differences of membrane constituents attacked by these agents, as well as of the whole L fraction chemical composition. The latter mechanism does not explain the inability of increasing amounts of trypsin to induce activation of β-GNase of hepatoma lysosomes. Differences in molecular organization or in accessibility to trypsin of protein in lysosomal membranes could be concerned in the phenomenon. In this connection it is of interest that also the damaging effect of endogenous lysosomal enzymes, activated by incubation at pH 5 and 37 °C, reaches a saturation point when it can no longer be activated by prolonging the incubation time. These patterns could account for different structural organization in lysosome membranes of hepatoma and liver particles[8].

Riassunto. Omogenati e frazioni L di epatoma ascite di Yoshida AH-130 sono più resistenti di quelli di fegato all'attivazione termica a pH 5, al trattamento ipotonico ed alla digestione con tripsina.

F. FEO and G. BONELLI

Istituto di Patologia generale dell'Università di Torino, Corso Raffaello 30, I-10125 Torino (Italy), 27 July 1970.

[8] Acknowledgment. The present investigation has been aided by a grant from the National Research Council of Italy.

Release of Growth Hormone from Somatotropin Producing Cells of Hepatectomized Mice Without the Participation of the Golgi Complex[1]

Somatotropin producing cells of hepatectomized mice present striking changes suggesting intense hormone synthesis and release, some hours before the first DNA synthesis peak in the regenerating liver[2,3].

We have not found in these cells the ultrastructural picture of secretion previously described for somatotropin producing (STH) cells in different situations[4–6], in which the outstanding mechanism is that of exocytosis[7]. Alternatively, images such as those described by SCHARRER[8] for neurosecretion in Blattarian insects, were frequently found in our hepatectomy STH cells.

STH cells of hepatectomized mice present strong cartographic dilatation of the endoplasmic reticulum, containing dense material[2]. The dilated cisternae were frequently found contacting with the plasmalemma in zones of increased electron density (Figures 1 and 2).

These images suggest a direct release of the hormone synthesized by the endoplasmic reticulum, bypassing the

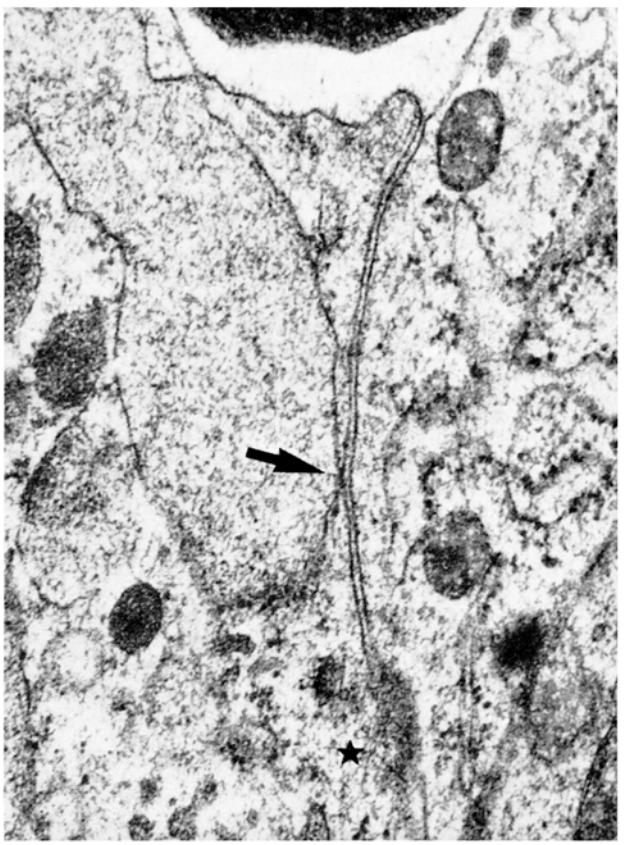

Fig. 1. A zone of contact between a dilated cisternae of the endoplasmic reticulum of a hepatectomy STH cell and the plasmalemma is indicated by the arrow. The star indicates a microvesicles aggregate in relation to a zone of increased density in the plasmalemma. ×35,000.

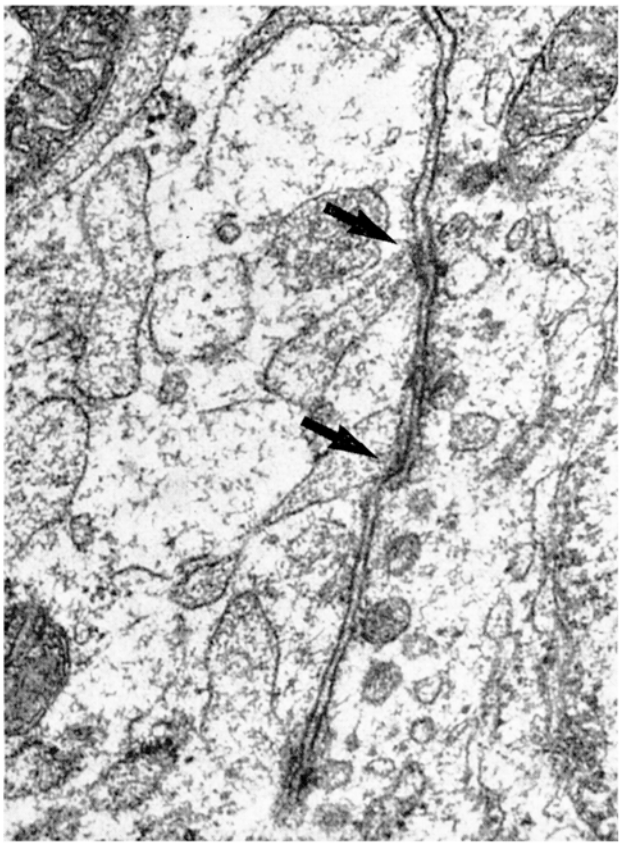

Fig. 2. The 2 arrows indicate zones of contact between the plasmalemma and 2 cisternae of the dilated endoplasmic reticulum. Increased density of the plasmalemma and dense material in the intersticial space are seen in these zones. ×35,000.

Golgi complex which, otherwise, is not inactive but greatly hypertrophied in hepatectomy STH cells[2], and contains many immature granules within the Golgi zone.

It is suggested that the deep metabolic changes appearing after hepatectomy, trigger, in some way yet unknown, this mechanism of short circuit direct release.

Resumen. El reticulo endoplasmico de las celulas somatotropas del raton hepatectomizado se encuentra muy dilatado y contiene material denso en el interior de sus cisternas. Estas se observan frecuentemente en contacto con la membrana celular en zonas en que ésta y el intersticio presentan un aumento de densidad. Se sugiere un mecanismo directo de secreción de la hormona sintetizada en exceso en el reticulo endoplasmico, sin pasar previamente por el complejo de Golgi.

J. M. Echave Llanos and C. L. Gómez Dumm

Instituto de Embriologia, Biologia e Histologia, Facultad de Ciencias Medicas, Universidad Nacional de La Plata, 60 y 120 La Plata (Argentina), 29 July 1970.

[1] This investigation was supported by a grant from the Comision de Investigacion Cientifica de la Universidad Nacional de La Plata. Thanks are due to the members of the technical staff of the Institute for their technical assistance.

[2] J. M. Echave Llanos, C. L. Gomez Dumm and A. C. Nessi, Z. Zellforsch., in press (1970).

[3] J. M. Echave Llanos, C. L. Gomez Dumm and J. M. Surur, Experientia, in press (1970).

[4] G. De Virgilis, J. Meldolesi and F. Clementi, Endocrinology *83*, 1278 (1968).

[5] E. F. Couch, A. Arimura, A. V. Schally, M. Saito and S. Sawano, Endocrinology *85*, 1084 (1969).

[6] C. Girod et P. Dubois, C. r. Acad. Sci., Paris *266*, 2361 (1969).

[7] M. G. Farquhar, Trans. N.Y. Acad. Sci. *23*, 346 (1961).

[8] B. Scharrer, Z. Zellforsch. *89*, 1 (1968).

On the Mechanism of Modification of Radiation Effect by Dimethyl Sulfoxide

The effect of dimethyl sulfoxide (DMSO) on radiation response involves a dichotomy-protection in some instances and sensitization in others. It has been shown that DMSO is an effective radioprotective substance at the molecular[1], cellular[2] and animal[3] levels. DMSO is the initial topically effective protector against radiation cataract[4,5]. Moreover, concomitant sensitization of the irradiated cornea was observed with high concentrations