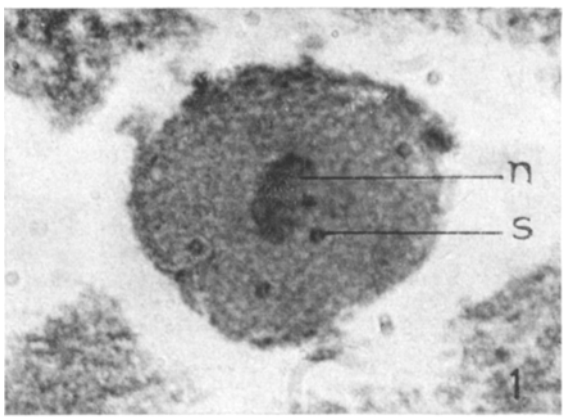
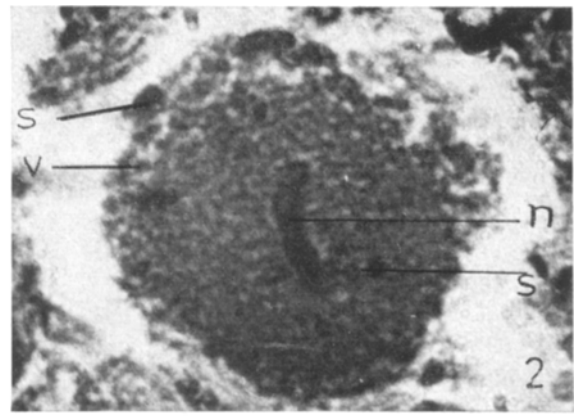


Fig. 1. Horizontal section of the thoracic ganglion of *Potamon magnum magnum* (Pretzman) showing giant cell. n = nucleus; s = secretory droplet, Gömöri.



25 μ

Fig. 2. Horizontal section of the thoracic ganglion of *Potamon magnum magnum* (Pretzman) showing giant cell in secretory phase. n = nucleus; s = secretory droplet; v = vacuole, Gömöri.

granules which appear in the vicinity of the modified nucleus spread over the entire cytoplasm. Many small vacuoles containing the neurosecretory substance appear in the cytoplasm and later migrate towards the periphery of the cell. During these secretory cycles, the nucleus shows changes in shape, i.e. a round nucleus gradually becomes flat or crescent-shaped, later becomes invisible and then expands and regains its shape. There is a direct relationship between the granules discharged and the nucleus, and it appears that the nucleus and cytoplasm of giant cells have secretory activities.

The existence of chromatophorotropins in the thoracic ganglion of crustaceans has been demonstrated by SMITH[8], ENAMI[2], and BROWN[1,9], but the physiological significance of the giant cells in the thoracic ganglion of *Potamon* is as yet unknown. Work on these lines is in progress and a detailed account and the results of further related observations will be published elsewhere[10].

Zusammenfassung. Im Thorax-Ganglion von *Potamon magnum magnum* wurden neurosekretorische Riesenzellen mit oder ohne Axone nachgewiesen. Diese zeigen während des sekretorischen Zyklus eine extreme Modifikation der Kerngrösse. Die Neurosekret-Granula wandern in das Perikaryon oder in die Axone ein. Sowohl Kern wie Cytoplasma zeigen sekretorische Aktivität.

I. C. BAID, R. A. HAFIDH, and S. DABAGH

Department of Zoology, College of Science, University of Baghdad, Mosul (Iraq), March 31, 1966.

[8] R. I. SMITH, Biol. Bull. *95*, 169 (1948).
[9] F. A. BROWN JR., Biol. Bull. *98*, 218 (1950).
[10] We wish to thank Professor A. A. HASWAN, Dean of the College of Science, Mosul, for the help he gave us in this work and Dr. I. GORDON, British Museum, London, for identification of the crab.

Triglyceride Behaviour in Liver and Serum of Rabbits Treated with Diphtheria Toxin

In liver diseases due to different hepatotoxic agents, such as CCl_4 and ethionine, or to nutritional causes, such as a diet deficient in choline, a marked and early increase in the triglyceride content of the liver has been demonstrated[1-3]. Many hypotheses have been formed about this fat accumulation[4-9]. These hypotheses demonstrate that there are many kinds of liver injuries; it remains to demonstrate whether for all kinds the same mechanism can be applied and whether it depends upon the same causes. For this reason we thought it interesting to study the damage produced by diphtheria toxin on triglyceride content both of liver and serum of rabbits. It is well known that such agents produce the so-called cloudy swelling accompanied by biochemical damage, such as fall in total high energy bond phosphorus and then fat liver[10,11].

Materials and methods. Rabbits of the same strain and breed, maintained on laboratory chow and weighing about 2.5 kg, were used. A group of rabbits was used as control: such rabbits were starved during the 21 h before killing. Another group of rabbits was injected i.p. with diphtheria toxin (kindly supplied by the Istituto Sieroterapico Toscano Sclavo) at a dosage of 1 d.m.m. per 250 g of body weight, and was starved for 6 h before treatment and throughout the subsequent experimental

period of 15 h. The animals were killed by bleeding. Blood was withdrawn from the heart and left to clot at room temperature for about $1^1/_2$ h, then centrifuged at room temperature for 10 min at about 9000 *g* to remove the clots and cell debris, and immediately used to perform the triglyceride analysis. The control sera were collected also from the same rabbits used for the treatment by toxin, just a week before the injection. The liver was immediately removed, washed with saline solution and extracted with chloroform-methanol, 2:1 (v/v), 4 times in a Potter-Elvenhjem homogenizer. The pooled extracts were washed by the method of FOLCH[12], dried in a flask evaporator and redissolved in chloroform. All the techniques were made under highly purified nitrogen at a temperature not higher than 20 °C. The triglyceride test was made on such extracts. In order to separate the phospholipids from the neutral lipids a suitable aliquot was placed in a test tube containing 2 g of silicic acid Mallinckrodt AR-100 mesh, which had been activated by heating for 5 h at 120 °C, with 5 ml of chloroform added. At this point the method used has practically that of VAN HANDEL and ZILVERSMIT[13]. In determinations made with quantitative thin layer chromatography, the recovery of the triglycerides standard was lower than with such technique, therefore we used the above method for our data. As concerns serum, the test was made directly, without any extraction, using the above method, with Mallinckrodt silicic acid instead of Doucil. As standard, tristearine (Merck) was used, the solvent being the product Analar (BDH). The nitrogen was estimated by the method of microkieldahl.

Results. In the Table results are reported on the triglyceride content of the liver and serum of rabbits treated with a high quantity of diphtheria toxin. From these results it is evident that after 15 h of treatment the triglyceride content of the liver markedly increases while the serum content decreases.

To explain the fat accumulation in the liver, it is suitable to find out the injury in the triglyceride cycle. During fasting the triglycerides of adipose tissue are hydrolysed into free fatty acids, the free fatty acids are constantly mobilized from adipose tissue and pass through the blood stream together with plasma albumin in great quantity (about 40%) to the liver. Here they can be either oxidized or resynthesized to triglycerides and complexed with proteins to form lipoproteins[14]. It is in this form that the liver pours the triglycerides into the blood stream, from which they come back to the depot fats. Many researchers have demonstrated a decrease of protein synthesis in liver of animals treated with ethionine and CCl_4, and a decrease of lipoproteins both in the liver and in the serum of these animals[2,6,7,15,16]. Therefore they conclude that the reason for fat accumulation is a deficiency of the proteic medium for the formation of lipoproteins that constitute a vehicle of neutral fat. Also after treatment with diphtheria toxin, KATO and PAPPENHEIMER[17] demonstrated a reduced rate of incorporation of orotic acid and methionine into RNA by cultures of normal human kidney cells. We can guess that in the diphtheria toxin treated animals the increase of triglycerides in the liver, coordinated with a decrease of triglycerides in the serum, is due to a diminished quantity of lipoproteins responsible for fat transport. And we hope to be able to prove this by assays that we have already started on this subject[18].

Triglyceride content of liver and serum of normal and diphtheria toxin treated rabbits

	No. of experiments	m*M* of triglycerides / %g of wet liver
Normal rabbit liver	8	1.702 ± 0.269
Liver of rabbits treated with diphtheria toxin	10	3.403 ± 1.467
		$P < 0.02$
		μM of triglycerides / g proteins
Normal rabbit serum	12	11.38 ± 0.593
Serum of rabbits treated with diphtheria toxin	12	6.64 ± 3.87
		$P < 0.01$

Riassunto. È stato osservato un notevole aumento di trigliceridi nel fegato di conigli trattati con tossina difterica, accompagnato da diminuzione dei trigliceridi del siero, dopo 15 h dal trattamento. Questo comportamento è attribuito, almeno in parte, a diminuito trasporto dei grassi da parte delle lipoproteine.

ANNA CASU, R. MONACELLI, and VALERIA PALA

Istituto di Patologia Generale della Università di Genova (Italy), February 22, 1966.

[1] D. S. ROBINSON and P. M. HARRIS, Biochem. J. *80*, 352 (1961).
[2] M. U. DIANZANI, G. Biochim. *2*, 180 (1953).
[3] B. LOMBARDI, G. UGAZIO, and A. RAICK, Fed. Proc. Am. Socs. exp. Biol. *23*, 126 (1964).
[4] R. O. RECKNAGEL and B. LOMBARDI, J. biol. Chem. *236*, 564 (1961).
[5] R. O. RECKNAGEL, B. LOMBARDI, and M. C. SCHOTZ, Proc. Soc. expt. Biol. Med. *104*, 608 (1960).
[6] D. S. ROBINSON and A. SEAKINS, Biochem. J. *82*, 9P (1962).
[7] G. UGAZIO and B. LOMBARDI, Lab. Invest. *14*, 711 (1965).
[8] B. LOMBARDI, G. UGAZIO, and A. RAICK, Am. J. Physiol. *209* (1966), in print.
[9] K. R. REES and V. L. SHOTLANDER, Proc. R. Soc. B. *157*, 517 (1963).
[10] A. FONNESU and C. SEVERI, Boll. Soc. ital. Biol. sper. *28*, 1123 (1952).
[11] A. FONNESU and C. SEVERI, Ital. J. Biochem. *2*, 326 (1953).
[12] E. FOLCH, M. LEES, and G. H. SLOANE STANLEY, J. biol. Chem. *226*, 497 (1957).
[13] E. VAN HANDEL and D. B. ZILVERSMIT, J. Lab. clin. Med. *50*, 152 (1957).
[14] B. BORGSTROM and T. OLIVECRONA, J. Lipid Res. *2*, 263 (1961).
[15] D. S. ROBINSON and A. SEAKINS, Biochem. biophys. Acta *62*, 163 (1962).
[16] B. LOMBARDI and G. UGAZIO, J. Lipid Res. *6*, 498 (1965).
[17] I. KATO and A. M. PAPPENHEIMER, J. exp. Med. *112*, 329 (1960).
[18] The present research has been supported by a grant from the C.N.R. Roma (Italy).