Phospholipid Alterations in Liver and Serum of Rabbits Treated with Diphtheria Toxin

In previous research[1] we found a triglyceride increase in the liver and a triglyceride decrease in the serum of rabbits treated with diphtheria toxin. To find out what leads to these alterations after treatment with such hepatotoxic agents, we thought it interesting to study in liver and serum the behaviour of phospholipids that are found in all biological membranes and are components with triglycerides of serum lipoproteins, which are poured from the liver into the blood.

From the literature it appears that diphtheria toxin acts both on the structure and synthesis of proteins. MONTANARO and SPERTI[2] demonstrated that diphtheria toxin is capable of binding the structural protein of Green: this protein participates, together with cytochrome *c* and phospholipid[3], to build the subcellular structure; KATO and PAPPENHEIMER[4,5] demonstrated that diphtheria toxin acts on the synthesis of cytochrome *c*. Lipoproteins and protein synthesis are reported to be decreased in fatty liver due to many hepatotoxic agents such as CCl_4, yellow P, and ethionine[6–13].

We then thought that the behaviour of phospholipids, components of the lipoproteins, might be a new factor for understanding the mechanism of diphtheria toxin injury.

Materials and methods. Rabbits weighing about 2.5 kg were injected i.p. with diphtheria toxin at a dosage of 1 d.m.m./250 g body weight for 15 h, as already described in a companion paper[1]. Phospholipid extraction from the serum was carried out by adding .3 ml of serum drop by drop and mixing with 45 ml $CHCl_3/CH_3OH$ 1:1 (v/v); the mixture was kept at room temperature for about 15 min with occasional mixing, filtered, and the residue reextracted twice with $CHCl_3/CH_3OH$ 2:1 (v/v) in Potter-Elvenhjem homogenizer for 2 min. The pooled extracts were washed by the method of FOLCH et al.[14]. Further extraction on the residue done with more polar solvents did not show any measurable amount of P. The residue was then dried in a flask evaporator and redissolved in $CHCl_3/CH_3OH$ 2:1 (v/v). The extraction of liver phospholipids was made as already described[1]. Quantitative analysis of phospholipids was done as reported in a previous paper[15]. The phospholipid amount was determined by estimating the phospholipidic phosphorus according to the method of CHEN, TORIBARA, and WARNER[16]. A factor of 25 was used to convert the inorganic phosphorus to phospholipids. The nitrogen was estimated by the method of microkjeldahl; the reagent being the product Analar BDH.

Results and discussion. The data reported in the Table show that treatment with diphtheria toxin causes a decrease of P content in the serum (94.05 ± 15.60 µg P/ml of normal rabbit serum, 68.89 ± 10.09 µg P after treatment, $t = 2.87$ for 10 experiments). The P decrease in the serum is in great part explicable by quantitative alterations of phospholipids (42.06 ± 6.02 µg phospholipidic-P/ml normal serum, 25.12 ± 12.20 µg/ml serum after treatment).

Of the phospholipid, the fraction which mainly decreases is lecithin, then follows phosphatidilethanolamine (see Table). As for hepatic phospholipids, there is an increase, though not significant, of phospholipids in rabbits treated with diphtheria toxin in comparison with normal ones: 1302 ± 150 µg phospholipidic-P/g of wet liver in normal rabbits, and 1485 ± 91 µg in treated rabbits.

Phospholipid and triglyceride behaviour, that is the decrease of phospholipids and triglycerides in the serum of animals treated with diphtheria toxin and triglyceride accumulation in the liver, could be interpreted either as a decreased lipid synthesis (but this would not explain the triglyceride accumulation in the liver) or as a decreased mobilization in the form of lipoproteins. In fact it is known that plasma lipids are almost totally in the form of lipoproteins[17]. The latter hypothesis might depend either on a decrease in the proteic medium by impaired synthesis, or a disarrangement of the system which combines proteins and lipids to form lipoproteins. But to demonstrate unequivocally which of these mechanisms is responsible for the liver damage produced by diphtheria toxin, further experiments are needed, even if we are inclined to accept the latter hypothesis from the data in our hands[18].

Serum phospholipids of starved rabbits treated with diphtheria toxin for 15 h

Fractions	Treatment	mg phospholipids %ml serum
Lisolecithin	Control rabbits	12.668 ± 0.559
	Rabbits treated with diphtheria toxin	8.655 ± 0.624
		$0.01 < P < 0.02$
Sphingomyelin	Control rabbits	12.387 ± 0.437
	Rabbits treated with diphtheria toxin	9.690 ± 0.381
		$0.01 < P < 0.02$
Lecithin	Control rabbits	72.600 ± 1.373
	Rabbits treated with diphtheria toxin	40.431 ± 0.707
		$P < 0.01$
Phosphatidil-ethanolamine	Control rabbits	7.431 ± 0.534
	Rabbits treated with diphtheria toxin	3.956 ± 0.186
		$P < 0.01$

The averages are calculated from the data of 10 experiments.

Riassunto. I fosfolipidi del siero di conigli a digiuno, trattati con tossina difterica per 15 ore, diminuiscono: questa diminuzione è a carico sorpattutto della frazione lecitinica. Le cause di questo comportamento sono brevemente discusse.

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