NOTE

Insulin Transport by Thoracic Duct of Male Rabbits with CCl₄-induced Liver Cirrhosis

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Synopsis

Insulin concentration in the peripheral blood and the thoracic duct lymph from male rabbits with CCl₄-induced liver cirrhosis was measured using a radio-immunoassay technique. Although insulin concentration in the lymph was lower in the cirrhotic animals than in the control ones, the hourly transport of the hormone by the lymphatic vessel of the cirrhotic animals markedly increased in company with the higher flow rate of the lymph.

It is well known that hormones are carried to general circulation in venous blood from within the endocrine gland itself. In addition, it has been shown that the lymphatic system plays a part in transporting a number of hormones (Lindner, 1963; Lindner *et al.*, 1964; Daniel *et al.*, 1967 a, b; Daniel and Henderson, 1966, 1967; Kotani *et al.*, 1968; Seiki *et al.*, 1968). The lymph draining from the pancreas is of particular interest with respect to its secretion, insulin, probably by-passing the liver during its course to the thoracic duct. The present study was attempted to demonstrate the presence of insulin in the thoracic duct lymph from rabbits with CCl₄-induced liver cirrhosis.

Materials and Methods

Twenty male albino rabbits, weighing about 2 kg, were used. They were maintained on a pellet diet (RC5, Oriental Yeast Mfg., Tokyo), with water *ad libitum* throughout the experiment. Ten animals were given the intramuscular injection of CCl₄-olive

oil (1:4, 0.5 ml/kg body wt.) 50-55 times at 4 day intervals. Another 10 animals received the injection of olive oil only (0.5 ml/kg body wt.) as many times at 4 day intervals. Three days after the last injection, all the animals were made to fast for 24 hrs, water being allowed *ad libitum*. Thoracic duct lymph was then collected by cannulation with polyethylene tubing in the neck under sodium pentobarbital anesthesia (40 mg/kg body wt., i.v.). Powdered heparin was added to the lymph to prevent clotting. Immediately after collection of the lymph for 1.5-2.0 hrs, blood was withdrawn from the carotid artery. Blocks of the liver were excised and fixed in 10% formalin, and the sections were stained with hematoxylin-eosin.

Total protein levels in the blood and lymph plasma were measured, using a refractometer (Hitachi Seisakusho Co., Tokyo). To examine fractionated pattern of protein in the blood and lymph plasma, zone electrophoresis was carried out on a cellulose acetate filter paper in veronal buffer for 1 hr. Before assay of insulin the blood and lymph plasma were diluted 10 times with distilled water (Rasio *et al.*, 1966), and a double antibody method for insulin radioimmunoassay was carried out according to the method of Hales and Randle (1963), using the insulin immunoassay kit from the Radiochemical Centre (Amersham, England). Highly purified beef insulin (25.5 U/mg; Shimizu Seiyaku Co., Tokyo) was used as the reference standard. Radioactivity of the insulin-antibody precipitate was measured using a well-type scintillation counter (Aloka Co., Tokyo). Each sample was assayed in duplicate, the agreement being usually within 5%.

Results

The liver from CCl₄-treated rabbits showed the gross and microscopic changes characteristic of nodular cirrhosis as described by Kotani *et al.* (1967). On the other hand, the liver from the control animals did not show any histological change. The rate of lymph flow in the thoracic duct of cirrhotic animals (12.6 ± 1.6 ml/hr) was 3 or more times higher than in the control ones (3.1 ± 0.3 ml/hr). As shown in Table 1, the level of total proteins was increased significantly both in the blood and lymph plasma from cirrhotic animals, as compared with that in the control ones. The fractionated pattern of proteins in the blood and lymph plasma was changed after CCl₄ injection in company with the relative decrease in albumin, resulting in a low A/G ratio. But the absolute content of albumin, and globulin too, increased in the lymph from cirrhotic animals, whereas that in the blood plasma did not change even after CCl₄ treatment.

As shown in Table 1, insulin concentration in the blood and lymph plasma from cirrhotic rabbits was lower than that from the controls. But, due to the higher rate of lymph flow the total amount of the hormone transported in the lymph of CCl₄-treated animals ($391 \mu\text{U/hr}$) was 2 or more times larger than that in the control animals.

Discussion

The present study clearly demonstrated the existence of a fairly amount of insulin in both the blood and lymph plasma from cirrhotic and control animals, indicating that the hormone secreted from the pancreas is carried to general circulation not only through the blood vessels but also through the lymphatic vessel. Daniel and Henderson (1966, 1967), who found a considerable amount of insulin in the thoracic duct lymph of rabbits, suggested that the hormone might enter the lymphatic vessel directly from the pancreas. However, there is until today no strong evidence to show that the islets of Langerhans contain lymphatic capillaries within them (Rusznayák *et al.*, 1967), although lymphatic capillaries surrounding the islets have been demonstrated (Jdanov, 1960). It is, therefore, at the present time supposed that insulin secreted from the islets preferably enters the portal vein rather than the lymphatics, and reaches the liver where a fairly amount of the hormone is degraded or inactivated by the hepatic cells. The rest of the hormone is then partitioned between the lymph and venous blood, and finally transported from the liver through the thoracic duct to general circulation.

The present study revealed that insulin concentration in the thoracic duct lymph

Table 1. Concentrations of protein and insulin in plasma of blood and thoracic duct lymph from 10 control and 10 CCl₄-induced cirrhotic rabbits

Sample	Animals	Total Protein (g%)	Albumin (%)	Globulin (%)			A/G Ratio	Insulin ($\mu\text{U/ml}$)
				α	β	γ		
Blood plasma	Control	$5.1 \pm 0.2^\#$	63.7	19.5	8.4	8.4	1.81 ± 0.1	63 ± 10
	Cirrhotic	6.1 ± 0.2	52.8	24.5	11.8	11.0	1.19 ± 0.1	51 ± 7
Lymph plasma	Control	3.0 ± 0.2	68.8	15.2	7.6	8.4	2.28 ± 0.2	54 ± 9
	Cirrhotic	5.1 ± 0.2	60.3	19.6	7.8	12.3	1.51 ± 0.2	31 ± 3

$^\#$ Mean $\pm$ standard error.

strikingly decreased after CCl_4 treatment, although the total protein content in the lymph increased. There is an evidence that some of the circulating insulin is bound to larger proteins (Antoniades, 1961), and variations in binding may have important physiologic consequences. From this point view, the present results are understood that the properties of proteins circulating in the lymph are altered after CCl_4 treatment, resulting in the diminution of binding capacity of the proteins to insulin.

The present study also revealed that the hourly transport of insulin in the thoracic duct was greater in the cirrhotic condition than that in the normal one, although the rate of lymph flow strikingly increased in company with the remarkable decrease in insulin concentration. In the liver cirrhosis, the formation of the hepatic lymph is promoted (Kotani *et al.*, 1967; Nix *et al.*, 1951a, b) probably as the result of an increase in production and retention of tissue fluid leaking through blood capillaries within the liver. The increased volume of the hepatic lymph may not only lower the insulin concentration in the lymph but also carry much insulin to the lymphatics. In addition, CCl_4 treatment severely affects the hepatic cells to degrade or inactivate the hormone, resulting in the transport of more insulin in the lymphatic vessels.

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References

- Antoniades, H. N. (1961). *Endocrinology*, **68**, 7.
- Daniel, P. M. and J. R. Henderson (1966). *J. Physiol.* **184**, 36.
- Daniel, P. M. and J. R. Henderson (1967). *Lancet* **1** 1256.
- Daniel, P. M., O. E. Pratt, I. M. Roitt and G. Torrigiani (1967a). *Quart. J. exp. Physiol.* **52**, 184.
- Daniel, P. M., O. E. Pratt, I. M. Roitt and G. Torrigiani (1967b). *Immunology* **12**, 489.
- Hales, C. N. and P. J. Randle (1963). *Biochem. J.* **88**, 137.
- Jdanov, D. A. (1960). *Acta Anat.* **41**, 240.
- Kotani, M., K. Seiki, A. Yamashita, A. Takashima, T. Nakagawa and I. Horii (1967). *J. Lipid Res.* **8**, 181.
- Kotani, M., K. Seiki, M. Higashida, Y. Imanishi, A. Yamashita, M. Miyamoto and I. Horii (1968). *Endocrinology* **82**, 1047.
- Lindner, H. R. (1963). *J. Endocrinol.* **25**, 483.
- Lindner, H. R., M. B. Sass and B. Morris (1964). *Ibid.* **30**, 361.
- Nix, J. T., E. V. Flock and J. L. Bollman (1951a). *Am. J. Physiol.* **164**, 117.
- Nix, J. T., F. C. Mann, J. L. Bollman, J. H. Grindlay and E. V. Flock (1951b). *Ibid.* **164**, 119.
- Rasio, E. A., J. S. Soeldner and J. Steinke (1966). *Proc. Soc. Exp. Biol. Med.*, **123**, 701.
- Rusznayák, I., M. Földi and G. Szabo (1967). *Lymphatics and Lymph Circulation*, 2nd ed., Pergamon Press, New York.
- Seiki, K., M. Kotani, A. Yamashita, M. Miyamoto and I. Horii (1968). *J. Endocrinol.* **42**, 157.