Storage of Colloidal Carbon in Liver and Spleen after Thymectomy

IT has been shown that mice thymectomized just after birth develop hyperplasia of the reticuloendothelial cells and increased phagocytic activity as measured by the clearance of colloidal carbon from the blood[1–3]. Experiments carried out with repeated injections of large doses of carbon also showed increased granulopectic activity in thymectomized rats[4].

It is known that in normal animals about 90 per cent of the injected colloids are stored in the liver and spleen[5,6]. From the evidence provided by histological findings Miller and Howard[3] concluded that hyperplasia of Kupffer cells accounts for the marked increase of phagocytosis in thymectomized mice, whereas in the spleen hyperplasia of the reticuloendothelial system results in extensive haemopoiesis.

The present communication describes a quantitative investigation of the distribution of colloidal carbon which was carried out in thymectomized rats in order to estimate the relative importance of the reticuloendothelial system of liver and spleen in the phagocytic response to thymectomy.

Wistar albino rats 2 days old were thymectomized according to the method of Miller[7]. At 5–6 weeks of age, 15 mg of colloidal carbon per 100 g body weight were injected intravenously and the rate of removal from the blood was measured using the method of Halpern *et al.*[5]. In a number of animals, 5 or 6 h before the injection of carbon, 10 mg of iron (as saccharated iron oxide) per 100 g body weight were injected intravenously. The animals were killed 4 h after the injection of carbon. The liver and spleen were weighed and hydrolysed in 8 ml./g of boiling 30 per cent potassium hydroxide. The samples were diluted to 100 ml./g with water, and a few drops of hydrogen peroxide were added in order to reduce the variable pigmentation which might interfere with the absorption of carbon in the spectrophotometer. The concentration of carbon was measured at 570 mμ by comparison with a standard curve. The accuracy and consistency of the results were checked by mixing known amounts of carbon with organ hydrolysates. The results are summarized in Table 1.

Table 1. DISTRIBUTION OF INJECTED CARBON IN THE RETICULOENDOTHELIAL SYSTEM

	Percentage of recovery in the liver	mg recovered/g of liver	Percentage of recovery in the spleen	mg recovered/g of spleen	Total percentage of recovery	K
Normal rats without blockade	89·0 (±0·92)	2·9	6·0 (±0·77)	1·3	95·0 (±2·56)	0·018 (±0·0018)
Normal rats after blockade	74·7 (±2·20)	2·3	10·8 (±1·41)	3·0	85·5 (±2·66)	0·010 (±0·0010)
Thymectomized rats without blockade	84·5 (±1·29)	2·7	6·5 (±1·20)	2·6	91·0 (±4·16)	0·015 (±0·0009)
Thymectomized rats after blockade	81·1 (±2·20)	2·8	17·9 (±2·35)	7·0	98·0 (±1·22)	0·008 (±0·0002)

All values represent the average of six observations. The figures in brackets are standard errors.

The observations in normal animals injected only with carbon are in good agreement with the results of Halpern, Bonacerraf *et al.*[5,6]: almost all the carbon was recovered in the liver and spleen, the amount in the liver being about fifteen times the amount in the spleen. An injection of saccharated iron oxide was able to cause blockade of the reticuloendothelial system as shown by the reduction in the granulopectic index after a subsequent injection of carbon. In this case a somewhat smaller proportion of carbon was recovered in the liver and spleen; the amount stored in the liver was much reduced compared with the amount stored in the spleen.

In the thymectomized rats after one injection of carbon, a much larger recovery in the spleen (per unit weight) was observed in comparison with the normal animals. The differences were more striking after blockade with saccharated iron oxide. More than a fifth of the carbon was stored in the spleen: the recovery per unit weight was more than twice that in the control animals.

The present results seem to indicate that in thymectomized rats the phagocytic response is greater in the spleen than in the liver. The potential phagocytic activity of the spleen appears to be very great after thymectomy and is well seen under extreme conditions after blockade with saccharated iron oxide.

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Received November 8, 1966.

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BIOCHEMISTRY

Effect of Puromycin on Insulin-stimulated Amino-acid Transport in Muscle

INSULIN stimulates both the transport[1,2] of amino-acids and their incorporation[3] into protein in skeletal muscle. Previous attempts to associate these two effects have failed[4–7]. That is, inhibition of new protein synthesis by puromycin did not impair the stimulatory effect of insulin on amino-acid transport. It was therefore concluded that the stimulation of amino-acid transport by insulin did not depend on the synthesis of new protein. Recent studies have shown that the transport of neutral amino-acids in embryonic bone[8], foetal calvarium (G. A. Finerman, S. J. Downing and L. E. Rosenberg, unpublished results), diaphragm[9] and kidney cortex[10] was impaired by puromycin or *cyclo*heximide when 2 h of incubation[8] or preincubation[9,10] with these antibiotics preceded the uptake study. Because previous experiments with insulin and puromycin in diaphragm muscle did not measure amino-acid uptake after such intervals of preincubation with puromycin, we carried out experiments to reinvestigate the effect of prolonged inhibition of protein synthesis on the stimulation by insulin of amino-acid transport in muscle.

Normal male Sprague–Dawley rats on a full diet and weighing 60–90 g were killed by stunning and decapitation. Intact whole diaphragms were prepared by the technique of Kipnis and Cori[11]. These "cage" diaphragm preparations were then placed in 20 ml. of Krebs bicarbonate buffer (*p*H 7·4), gassed with 95 per cent oxygen–5 per cent carbon dioxide and preincubated in a Dubnoff metabolic shaker with or without puromycin dihydrochloride (0·55 millimolar) for 180 min. At the end of the preincubation period, the cage preparations were transferred to 20 ml. fresh buffer containing 0·1 millimolar α-amino-isobutyric acid-1-^{14}C (AIB-^{14}C), a model amino-acid which is not incorporated into protein. Bovine insulin (Lilly, *PJ*–4609, 23·8 U/mg) free from glucagon and at a concentration of 0·4 U/ml. was added to the flasks during the final incubation period as indicated in Table 1. The