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INFLUENCE OF PORTAL BLOOD FLOW AND OF PARTIAL HEPATECTOMY ON THE GRANULOPECTIC ACTIVITY OF THE RETICULO-ENDOTHELIAL SYSTEM

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In previous studies (Halpern, Benacerraf & Biozzi, 1953; Biozzi, Benacerraf & Halpern, 1953) a method was described for investigating in the rat the granulopectic activity of the reticulo-endothelial system (R.E.S.) by measuring the rate of clearance from the blood of carbon particles of known size (about 250Å). The liver and the spleen were seen to be the main organs engaged in the activity when the carbon suspensions were injected intravenously. Within our experimental conditions, the clearance of carbon from the blood followed an exponential equation $C = C_0 10^{-Kt}$. The constant, K, of the equation was called the granulopectic index (G.I.) and reflects the granulopectic activity of the R.E. cells of the animal for a given dose of carbon. The granulopectic index for a given dose of carbon varied with the cube of the relative weight of the organs, liver and spleen, principally involved in the phagocytic activity according to the following equation:

$$\alpha = \frac{W}{W_{ls}} \sqrt[3]{K},$$

where W is the weight of the individual animal and W_{ls} the weight of the liver and spleen. α was called the 'corrected granulopectic index' (C.G.I.) and showed much smaller individual variations than the 'granulopectic index', K, itself.

No immediate explanation could be found for the relationship involving the cube of the weight of the organs responsible for the phagocytic activity. It seemed, however, probable that one factor should be the number of cells, a value which is directly related to the weight of the organs, and another factor the blood flow through the organ which is also directly related to its weight. In order to investigate the influence of these two factors upon the granulopectic activity of the R.E.S., experiments were performed in which changes in the

portal blood flow and in the quantity of liver tissue irrigated were carried out independently.

The variations in the granulopectic index, K, the corrected granulopectic index, α, and the ratio of body weight to the combined weights of liver and spleen, W/W_{ls}, were studied during the hours and days following the operations of the experimental procedures to be described. The importance of the portal circulation and of the size of the organs for the granulopectic activity have been established independently. The direct relationship between the circulation and the regeneration of the liver has been emphasized; it illustrates the direct relationship which exists generally between blood flow and organ size.

METHODS

The granulopectic activity of the R.E.S. has been investigated in rats after each of the following procedures:

(*a*) Ligation of the inferior vena cava below the renal veins.
(*b*) Ligation of a main branch of the portal vein.
(*c*) Partial hepatectomy.
(*d*) Removal of the intestine to reduce portal blood flow.

The experiments were carried out on white male rats weighing 150 ± 50 g. The operative procedures were performed under light ether anaesthesia.

(*a*) The inferior vena cava was ligated below the renal veins in forty animals. All the animals recovered after the operation.

(*b*) The left main branch of the portal vein which irrigates approximately 60–70% of the liver was ligated in fifty animals. There was a moderate percentage of mortality (about 30%) after this procedure. However, the thirty-six animals who survived the first 48 hr after the operation usually survived indefinitely.

(*c*) Partial hepatectomy was carried out on forty animals. All the liver tissue irrigated by the left branch of the portal vein was removed after ligation of this vein. Over 80% of the animals survived this procedure (thirty-three animals).

To investigate the granulopectic activity of the R.E. cells of the liver and spleen, we have used a suspension of carbon (C 11/1431 a) specially prepared by Gunther Wagner, Hanover. This preparation contained about 100 mg of carbon/ml., suspended in a solution of fish glue with a small amount of phenol as preservative. This carbon suspension contained particles homogeneous in size (about 250 Å), it was very stable in the serum and absolutely non-toxic. This preparation was centrifuged at 5000 rev/min for 15 min in order to get rid of the small number of larger particles of carbon present in the original preparation. The supernatant was analysed for carbon by weight after the carbon had been precipitated by concentrated NaOH and the proteins hydrolysed by boiling; it contained generally about 90 mg carbon/ml. This suspension of carbon was then diluted with a solution of gelatin neutralized with ammonia in double-distilled water. A dilution of this ink containing 16 mg of carbon/ml. and 1% gelatin was prepared. The suspension was kept at about 40° C before injection to avoid solidification of the gelatin.

At varying time intervals, ranging from 1 hr to 16 days after operation, groups of animals in each series received injections of the preparation described above (8 mg carbon/100 g body weight). The injections were made in the dorsal vein of the penis in the unanaesthetized rat. The rate of clearance of carbon from the blood was investigated for this dose of carbon according to the techniques described previously (Biozzi *et al.* 1953). Blood samples were obtained at regular intervals by puncturing the retro-orbital venous plexus (Halpern & Pacaud, 1951) with a fine capillary glass pipette previously washed with heparin and calibrated to 0·025 ml. This quantity of blood was then lysed in 2 ml. of 0·1% (w/v) Na_2CO_3 and the amount of carbon in the blood

determined electrophotometrically using a red filter. This procedure was repeated as many times as necessary as the animals are not affected by the bleedings. The mean granulopectic index, K, was calculated in each case from the equation describing the clearance of carbon from the blood, $C = C_0 10^{-Kt}$. The animals were sacrificed after the experiment; the extent and nature of the collateral circulation was investigated at autopsy. The combined weight of the functional liver and of the spleen was recorded (W_{ls}) and the ratio of the body weight to the weight of the organs W/W_{ls} was calculated. The mean corrected granulopectic index was calculated in each case from the following equation: $\alpha = W/W_{ls} \sqrt[3]{K}$. The results of these experiments are presented in Figs. 1–3.

(*d*) The total removal of the intestine was performed on ten rats. All animals withstood the operation well. Two hours later the granulopectic activity of the R.E.S. was investigated in these animals and in ten control animals of the same origin. These animals were also injected with the carbon suspension C 11/1431 a (8 mg carbon/100 g body weight), and the rate of clearance of carbon from the blood studied as described above. The carbon concentrations, at the times indicated in Fig. 4, were recorded as the means of their logarithms.

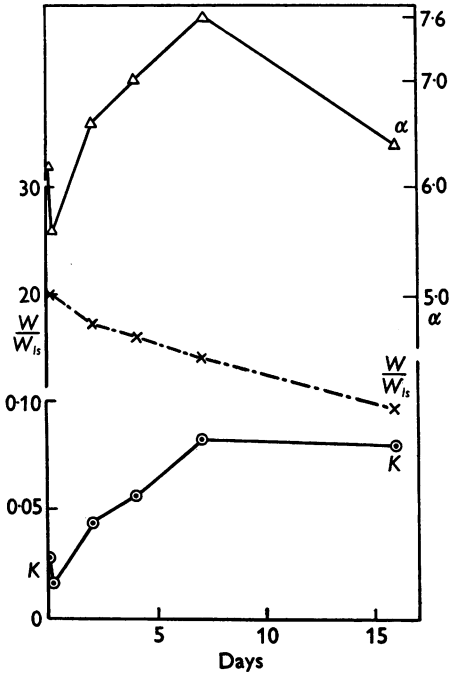

Fig. 1. Variations of the granulopectic index, K, the ratio of body weight to the combined weight of the liver and spleen, W/W_{ls}, and the corrected granulopectic index, α, in the days following the ligation of the inferior vena cava in the rat. (1 hr, 6 rats; 2nd day, 5 rats; 4th day, 8 rats; 7th day, 14 rats; 16th day, 7 rats.)

RESULTS

Effect of ligation of the inferior vena cava upon the granulopectic activity of the reticulo-endothelial system

If the variations of K are examined (Fig. 1), it is seen that shortly after the ligation of the inferior vena cava, this index decreases. Then it increases above normal values up to the 7th day when it reaches its maximum. The relative

weight of the liver and spleen increases considerably and progressively up to the 16th day. α shows an immediate decrease after the operation, then a progressive increase above normal values up to the 7th day and at last it returns to normal around the 16th day.

The values presented are mean values; the increase of granulopectic activity observed on the 7th and 16th day after the ligation of the inferior vena cava vary with each animal. The experimental values of K obtained in fourteen rats on the 7th day were: 0·170, 0·125, 0·110, 0·085, 0·071, 0·140, 0·064, 0·095, 0·044, 0·050, 0·044, 0·036, 0·040, 0·096. The mean value recorded in Fig. 1 was 0·083. These variations were observed also on the 16th day when values of K from 0·050 to 0·125 were recorded.

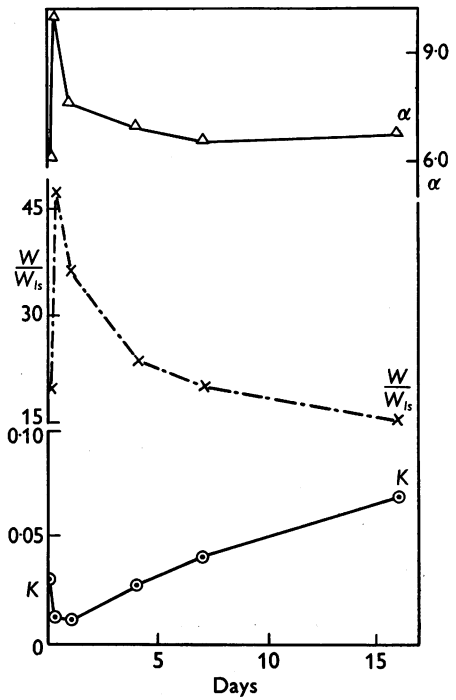

Fig. 2. Variations of the granulopectic index, K, the ratio of body weight to the combined weight of the liver and spleen, W/W_{ls}, and the corrected granulopectic index, α, in the days following the ligation of the left branch of the portal vein in the rat. (4 hr, 7 rats; 16 hr, 7 rats; 4th day, 6 rats; 7th day, 7 rats; 16th day, 9 rats.)

Effect of ligation of the left main branch of the portal vein upon the granulopectic activity of the reticulo-endothelial system

The data presented in Fig. 2 show that immediately after ligation of the left main branch of the portal vein, K decreases considerably. However, the C.G.I. which is the relationship of the granulopectic activity to the combined weights

of the irrigated liver and of the spleen increases markedly in the first few hours. This increase is due to the fact that much more blood passes through the functional liver left than does normally, since all the blood that would normally go through the left branch of the portal vein has to go through the right branch. Within 4 days, however, the situation has nearly returned to normal, the increased blood flow through the remaining functional liver has caused a regeneration of the organ. Seven days after the operation, the animals show a normal granulopectic activity and normal weights of liver and spleen with respect to body weight. The C.G.I. is then within normal limits.

Effect of partial hepatectomy on the granulopectic activity of the reticulo-endothelial system

These experiments are in every way comparable with the previous ones in which the left branch of the portal vein was ligated. Both experimental procedures affected the same amount of liver tissue. The only difference was that in this case the part of the organ remaining without portal circulation was removed and was not left to necrose locally. The small differences observed between the results in Figs. 2 and 3 are attributable to that particular change in technique.

It should be mentioned that these experimental procedures, such as ligation of the left branch of the portal vein or partial hepatectomy, caused some transient portal hypertension and that as a consequence the size of the spleen was generally found to be enlarged.

Effect of decreased portal blood flow upon the granulopectic activity of the reticulo-endothelial system

A total removal of the intestine was carried out. The data in Fig. 4 show that a reduction of portal blood flow through the liver, caused by the removal of the intestine, is accompanied by a considerable diminution in the granulopectic activity of the liver, although the weight of this organ remains unchanged.

DISCUSSION

These series of experiments establish the importance of portal blood flow upon the granulopectic activity of the reticulo-endothelial cells of the liver.

When portal blood flow is decreased, through removal of the intestine without change in liver size, the granulopectic activity is decreased considerably. When portal blood flow is increased, either through the whole liver by ligation of the inferior vena cava and formation of a collateral circulation (inferior vena cava to portal vein), or through part of the liver by partial hepatectomy or by ligation of the left branch of the portal vein, the granulopectic activity is much greater than it should be considering only the size of the organs.

The mechanism by which blood flow affects the phagocytic activity of the cells is not known; however, it would seem probable that the increased blood flow increases the rate of granulopexy by augmenting the number of particles which come into contact with the macrophages per unit of time. In any case

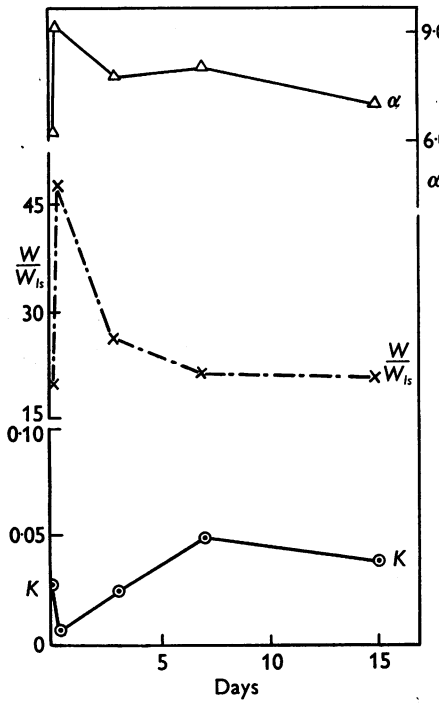

Fig. 3

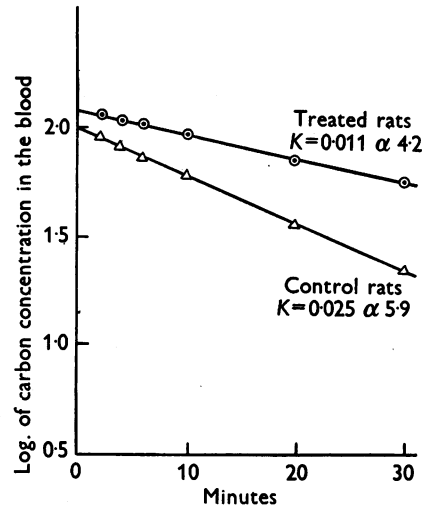

Fig. 4

Fig. 3. Variations of the granulopectic index, K, the ratio of body weight to the combined weight of the liver and spleen, W/W_{ls}, and the corrected granulopectic index, α, in the days following partial hepatectomy in the rat. (4 hr, 6 rats; 3rd day, 7 rats; 7th day, 10 rats; 15th day, 10 rats.)

Fig. 4. Effect of total resection of the intestine upon the granulopectic activity of the R.E. cells of the liver and spleen in the rat.

the blood flow which, as we have seen, is in harmony with the size of the organ is a factor in the rate of phagocytosis of the liver. The relationship between organ size and blood flow can be observed very clearly in these experiments; conditions of equilibrium are obtained when the liver has attained a size corresponding to its new irrigation (Figs. 1–3).

When the inferior vena cava is ligated there is first a decrease of the granulopectic activity attributable to the retention of blood below the ligation, and the poorer mixing of blood in the general circulation. This condition does not last over 24 hr when a collateral circulation, inferior vena cava to portal vein, is

established. This is in agreement with the findings of Reynell (1952), who induced portal hypertension in the rat by stenosing the portal vein and observed that the collateral circulation, portal vein to vena cava, is established within 24 hr. After 24 hr and until the 7th day, there is an increase of the G.I. and of the C.G.I. in particular which can be attributed to the formation of this collateral circulation from the inferior vena cava to the portal vein. This collateral circulation has been verified at autopsy. This increase in blood flow through the liver is associated with an increase in size of the organ as seen in Fig. 1. The approximation of the C.G.I. to normal values in spite of a much elevated value of K, at the end of the 16th day, may mean that the liver has now attained a size related to the increased blood flow determined by the formation of the new collateral circulation. It should be noted that an increase in portal blood flow through the liver is followed first by a considerable increase of granulopectic activity and only later by an increase in the size of the organ.

There are individual variations in the increase of granulopectic activity after ligation of the inferior vena cava. It is thought that these depend upon the relative importance of the new collateral circulation, inferior vena cava to portal vein, or to inferior vena cava at a point above the ligature.

Immediately after hepatectomy the granulopectic activity decreases considerably while α, which reflects the relationship of granulopectic activity to organ size, increases, presumably as a result of an increased blood flow through the remaining liver fraction. This increase in circulation is associated with a proliferation of the remaining liver until an equilibrium is attained around the 15th day, at which time all the values are within normal limits and the livers have regained normal weight. The speed with which the liver is regenerated either after partial hepatectomy or after ligation of the left branch of the portal vein is very remarkable. Within 1 week the liver size is nearly back to normal. This is in agreement with data reported in the literature about liver regeneration (Mann, 1927; Himsworth, 1947; Cameron, 1952). Our experiments give further proof of the importance of the blood flow in determining compensatory hypertrophy of the liver. Mann and his co-worker (1922, 1940, 1943) have observed that regeneration of the liver does not occur in partially hepatectomized dogs if the portal vein is constricted.

The slight differences in the speed of recovery of function and organ size in the animals with the left branch of the portal vein ligated or with partial hepatectomy may be attributable to an added stimulation to regeneration in these animals by substances absorbed from the necrotic tissues.

The data presented show that the functional test of granulopexy can give pertinent information about conditions affecting the portal circulation in the liver as well as about conditions affecting the phagocytic activity of the macrophages themselves. This test, therefore, can be used as a functional test of liver circulation. However, in the interpretation of the results obtained with this

method, in studies dealing with experimental liver pathology, extreme care should be taken to consider the factors which affect the circulation and the macrophages themselves separately.

SUMMARY

1. The data presented indicate that the granulopectic activity of the liver and spleen is conditioned by the amount of blood flowing through these organs.

2. An increase in portal circulation through the liver determined by ligation of the inferior vena cava is followed first by an augmented granulopectic activity and later by an increase in size of the organ.

3. A reduction of portal blood flow brought about by the resection of the intestine is accompanied by a decrease of the granulopectic activity.

4. Ligation of the left branch of the portal vein or partial hepatectomy affects granulopectic activity in the same way. In both cases there is a decrease of the granulopectic activity, but to a lesser degree than might be expected considering the reduced amount of functional liver. The compensatory factor is presumably the increased blood flow through the remaining functional liver caused by portal hypertension.

5. The progressive regeneration of the liver parenchyma after hepatectomy or after ligation of the left branch of the portal vein is reflected in a progressive decrease to normal of the granulopectic activity related to the combined weight of liver and spleen.

6. It follows from the data reported that the size of the liver is essentially determined by the blood that flows through it.

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