STUDIES ON LIVER BLOOD FLOW AND PHAGOCYTIC ACTIVITY OF RETICULOENDOTHELIAL SYSTEM IN DOGS TREATED WITH ETHYL PALMITATE†

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INTRODUCTION

It has been reported that an intravenous injection of a colloidal preparation of ethyl palmitate caused a suppression of the phagocytic function of the reticuloendothelial system (RES) and a widespread necrosis of the spleen in mice (Stuart, 1960). These phenomena were also found to occur in rats, guinea-pigs and rabbits (Buchanan and McGregor, 1964; Prosnitz *et al.*, 1969). It has been shown in a previous report that ethyl palmitate administered intravenously to dogs, induced a depressed erythrophagocytosis and a degeneration and focal necrosis of the liver without causing any pathological change of the spleen (Areekul *et al.*, 1973a). The objective of the present experiment was to investigate the effect of 10% ethyl palmitate on the liver blood flow and the phagocytic function of RES in dogs.

MATERIALS AND METHODS

The experiments were performed on 12 dogs of both sexes, weighing between 5.0 and 11.2 kg. Different doses of ^{131}I-AA, 0.03 mg/kg and 5.0 mg/kg, were administered intravenously to study the liver blood flow and the phagocytic activity of the RES respectively, in these dogs. The dogs after being served as the control group for these studies, were given 1.5 mg/kg body weight of 10% ethyl palmitate intravenously for 3 consecutive days and left for 48 hours before the next experiment with these 2 dosages of ^{131}I-AA (ethpalm dogs).

The methods of preparing 10% ethyl palmitate and the microaggregated human serum albumin and labelling with ^{131}I were the same as described in previous reports (Areekul *et al.*, 1973 a, b).

A dose of 0.03 mg/kg of ^{131}I-AA was injected intravenously into a dog and blood samples (2.0 ml) were withdrawn at 3, 5, 7 and 9 minutes. Thirty minutes later, blood samples were taken as the background and a second dose of ^{131}I-AA (5.0 mg/kg) was administered. Blood samples were then taken at 5, 9, 13 and 17 minutes after injection.

Blood was centrifuged and plasma was passed through a resin column (containing 3 ml of Amberlite IRA-400, chloride form) to remove free iodide that had been liberated by metabolic activity of the RES. The plasma was washed out of the resin bed with distilled water to yield a final volume of 10 ml. Plasma proteins were then precipitated with 10% sodium tungstate and 2/3 N sulphuric acid. After centrifugation, the sample was counted in a well-type scintillation counter.

$T\frac{1}{2}$ values were obtained from a semilog-graph of the radioactivity in the plasma plotted against time in dogs receiving 0.03 mg/kg of ^{131}I-AA, and K_e values were estimated from the formula :-

$$K_e = \frac{0.693}{T\frac{1}{2}} \quad \ldots\ldots\ldots\ldots (1)$$

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Blood volume was calculated from a value of red cell volume which was estimated from the relationship between red cell volume (ml/kg) and the haematocrit value (Areekul *et al.*, unpublished data). The liver blood flow was estimated from the equation :-

$$\text{Liver blood flow} = K_e \times \text{blood volume} \quad \ldots\ldots (2)$$

The values of the logarithm (base 10) of the plasma radioactivity in dogs receiving 5.0 mg/kg of ^{131}I-AA was plotted as a function of time on a linear graphic paper and the phagocytic index (K) was estimated from:-

$$K = \frac{\log C_1 - \log C_2}{t_2 - t_1} \quad \ldots\ldots (3)$$

where C_1 and C_2 were the radioactivity in the plasma at time t_1 and t_2 respectively.

RESULTS

The body weight, haemoglobin and haematocrit values, $T\frac{1}{2}$ and the liver blood flow in the normal and the ethpalm dogs are shown in Fig. 1 and Table 1. There was a significant difference ($P < 0.05$) in the mean value of the $T\frac{1}{2}$ of 10 ethpalm dogs (4.4 min) and of 10 normal dogs (2.3 min) whereas the mean value of K_e was lower in the ethpalm dogs than in the normal group, i.e. 0.201 min^{-1} compared with 0.312 min^{-1}. The mean value of the liver blood flow was also lower in the ethpalm group (128 ml/min or 0.46 ml/min/gm liver weight) as compared with the normal group (174 ml/min or 0.71 ml/min/gm liver weight).

The values of the $T\frac{1}{2}$ and the phagocytic index of the RES calculated from eq. 3 in these 2 groups of dogs are shown in Table 2. There was a difference ($P > 0.05$) in the $T\frac{1}{2}$ of the normal dogs (3.8 min) and of the ethpalm group (4.3 min). The mean phagocytic index (K) of the ethpalm group (0.071 min^{-1}) was also found to be lower ($P > 0.05$) than that of

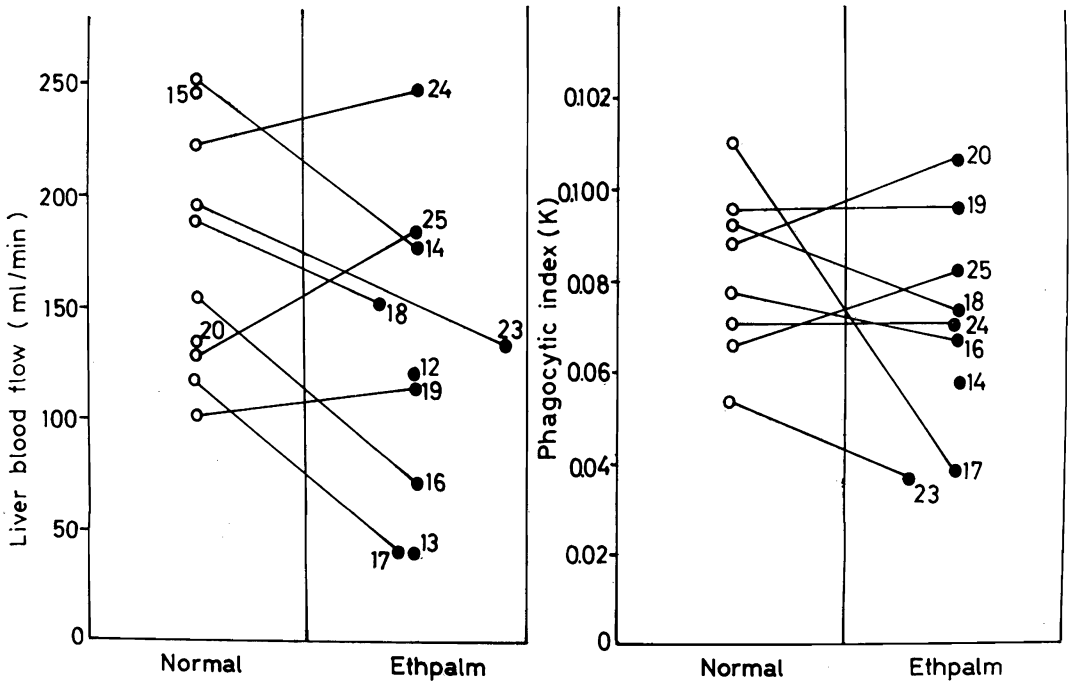

Fig. 1—The individual value of the liver blood flow (ml/min) and the phagocytic index (min^{-1}) in the normal dogs and the ethpalm dogs.

Table 1

Liver blood flow in normal and ethpalm-dogs, measured with ^{131}I-AA 0.03 mg/kg body weight.

No.	B.W. (kg)	Hb. (gm%)	Hct. (%)	$T_{\frac{1}{2}}$ (min)	K_e (min^{-1})	Blood volume (ml)	Liver blood flow (ml/min)	Liver blood flow (ml/min/gm)
Ethpalm-dogs								
D - 12	6.0	7.8	25	3.3	0.210	570	120	0.75
D - 13	8.0	9.4	28	10.6	0.065	617	40	0.16
D - 14	9.0	10.9	33	2.6	0.267	656	175	0.58
D - 16	5.3	6.4	26	4.1	0.169	421	71	0.32
D - 17	5.1	4.9	18	8.3	0.084	477	40	0.18
D - 18	9.8	7.1	23	3.8	0.182	822	150	0.43
D - 19	6.3	12.5	33	2.7	0.256	461	118	0.67
D - 23	10.0	9.4	28	4.0	0.173	776	134	0.38
D - 24	11.2	10.3	29	2.5	0.277	885	245	0.50
D - 25	7.4	10.0	28	2.1	0.329	552	182	0.66
Mean	7.8	8.9	27	4.4	0.201	624	128	0.46
Normal dogs								
D - 14	9.2	12.5	36	1.8	0.385	652	251	0.80
D - 15	10.0	12.5	36	2.0	0.347	706	245	0.69
D - 16	5.0	10.0	35	1.6	0.433	358	155	1.10
D - 17	5.6	9.7	29	2.5	0.277	427	118	0.82
D - 18	9.0	15.2	45	2.2	0.315	595	188	0.61
D - 19	6.3	16.9	45	2.8	0.248	417	103	0.58
D - 20	6.4	10.3	33	2.4	0.286	457	134	0.74
D - 23	10.9	11.2	35	2.8	0.248	780	193	0.50
D - 24	10.2	12.2	40	2.2	0.315	700	221	0.61
D - 25	7.3	13.8	43	2.6	0.267	490	131	0.60
Mean	8.0	12.4	38	2.3	0.312	558	174	0.71

Table 2

The half-disappearance time, and the phagocytic index in normal and ethpalm dogs, measured with ^{131}I-AA 5.0 mg/kg body weight.

No.	B.W. (kg)	Hb. (gm%)	Hct. (%)	$T\frac{1}{2}$ (min)	K (min^{-1})
Ethpalm-dogs					
D - 14	9.0	10.9	33	5.2	0.058
D - 16	5.3	6.4	26	4.4	0.069
D - 17	5.1	4.9	18	7.5	0.039
D - 18	9.8	7.1	23	4.2	0.074
D - 19	6.3	12.5	33	2.6	0.096
D - 20	7.2	9.1	27	3.0	0.106
D - 23	10.0	9.4	28	7.6	0.037
D - 24	11.2	10.3	29	2.5	0.074
D - 25	7.4	10.0	28	2.1	0.083
Mean	7.9	9.0	27	4.3	0.071
Normal dogs					
D - 16	5.0	10.0	35	3.9	0.078
D - 17	5.6	9.7	29	3.0	0.101
D - 18	9.0	15.2	45	3.4	0.093
D - 19	6.3	16.9	45	2.6	0.096
D - 20	6.4	10.3	33	3.5	0.089
D - 23	10.9	11.2	35	5.4	0.054
D - 24	10.2	12.2	40	4.2	0.071
D - 25	7.3	13.8	43	4.6	0.066
Mean	7.6	12.4	38	3.8	0.081

the normal group (0.081 min^{-1}). The individual values of the liver blood flow and the phagocytic index in these 2 groups of dogs are illustrated in Fig. 1.

DISCUSSION

It has been shown in a previous report that an intravenous injection of 10% ethyl palmitate induced a depression of erythrophagocytic activity in dogs (Areekul *et al.*, 1973a). The results in the present studies also showed decreased values of the phagocytic index (K) in dogs receiving ethyl palmitate. This could have been due to the suppression of the phagocytic activity in all the reticuloendothelial cells which were in contact with the blood stream. Since the liver has a higher phagocytic activity than the other organs of the RES, it seemed likely that the depressing effect was exerted mostly on the liver. This impression was confirmed by the finding of decreased liver blood flow in the present studies and by histological sections which showed degeneration and focal necrosis of liver cells induced by 10% ethyl palmitate in these dogs (Areekul *et al.*, 1973a).

The distribution and the fate of ethyl plamitate in the systems of various animal species are not well understood. The physicochemical properties of this compound prevent its passage through the capillary barrier and therefore inhibit entry to lymph glands and thymus. Ethyl palmitate caused selective splenic destruction in mice, rats, guinea-pigs and rabbits (Stuart, 1960; Buchanan and McGregor, 1964; Prosnitz *et al.*, 1969). There was also evidence that the liver phagocytes were affected because of the low values of the phagocytic index in mice, but no significant pathological lesion was observed, presumably due to efficient detoxification by the parenchyma (Stuart, 1960). It has *been* suggested also that the mechanism of action is probably related to a critical concentration of ethyl palmitate within the phagocytes which is followed by cell damage and release of cytotoxic material into the susceptible lymphoid tissue of the spleen in mice (Stuart, 1960). The selective organ of destruction in dogs was found to be the liver which showed dose dependence while there were no pathological changes in the spleen even when the dose of ethyl palmitate was increased to 30%. The mechanism of action of ethyl palmitate in dogs was therefore quite different from that in mice, rats, guinea-pigs and rabbits. Studies on the distribution and the fate of ethyl palmitate in dogs are in progress.

SUMMARY

The effects of 10% ethyl palmitate on the liver blood flow and the phagocytic function of the RES were studied in 12 dogs using 0.03 mg/kg and 5.0 mg/kg of ^{131}I-AA. The results showed a lower liver blood flow in the ethpalm group (128 ml/min or 0.46 ml/min/gm liver weight) than in the normal group (174 ml/min or 0.71 ml/min/gm liver weight). The mean values of the phagocytic index (K) of the ethpalm group (0.071 min^{-1}) was also found to be lower than that of the normal group (0.081min^{-1}). These findings indicated that ethyl palmitate depressed the phagocytic function of the RES and the liver blood flow in these dogs. These results confirmed a previous report that the action of ethyl palmitate in depressing erythrophaogcytosis and causing degeneration and focal necrosis of the liver was dose dependent while there were no significant pathological changes in the spleen.

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