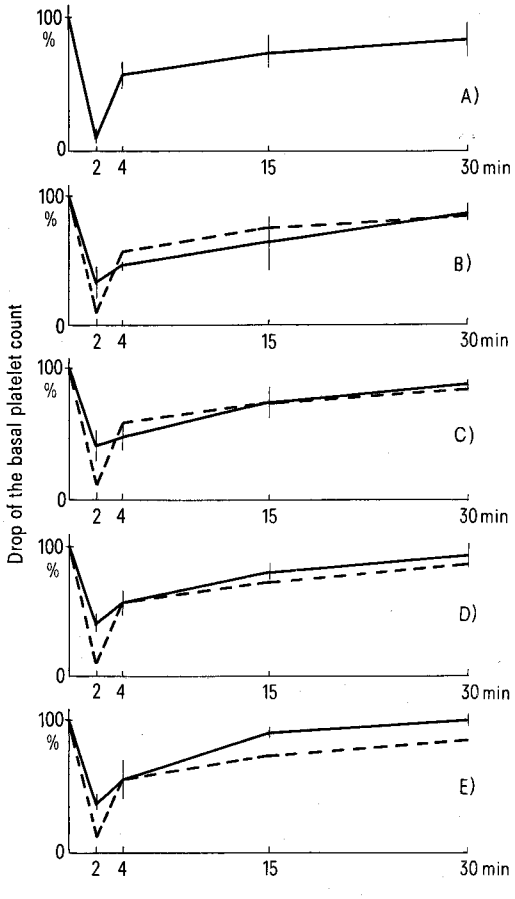
ADP-Induced Platelet Aggregation in vivo After Exclusion of Different Circulatory Districts

It is known that the infusion of ADP in the rat induces a drop in the platelet count[1,2] which has been mainly attributed to the trapping by the lungs of the circulating platelet aggregates[3]. On the other hand, the peculiar role of the lungs in regulating the platelet behaviour was previously pointed out[4,5]. The aim of the present research was to investigate whether other circulatory districts might participate in the trapping of the platelet aggregates besides the pulmonary district.

Methods. Albino Wistar rats (350–450 g) were anaesthetized with NaNembutal-Urethane i.p. 1 femoral vein was catheterized and connected to an infusion pump (Harvard Apparatus Co., Millis, Mass., USA, Model 940), through a polystan tube. Heparin in saline (0.25 mg/ml; Fluka AG, Chemische Fabrik Buchs SG, Switzerland) was infused at the speed of 0.103 ml/min. 1 carotid artery was catheterized and clumped in order to collect blood samples. The exclusion of the splenic district was obtained with a ligature of the splenic hilum; the bilateral exclusion of the kidneys tying up the renal arteries and veins; the exclusion of a limb (the posterior one, whose femoral vein was not catheterized) with a ligature of the femoral artery and vein; the exclusion of the cerebral circulation by means of a ligature of the not catheterized carotid artery followed by the contemporaneous ligature of the jugular veins. In the last case, the respiratory activity was maintained by means of a small-animal respiratory pump (S.R.I., Croydon, Surrey). The blood pressure was measured from the tail artery with a manometer (Type 'BP recorder', W + W Electronic, Basel Switzerland). Both in the control and in the operated animals, 2 control blood samples were collected. Successively 2 ml/kg body wt. of a solution of ADP (0.427 mg/ml Na_3ADP, C. F. Boehringer and Söhne H-Mannheim) were infused in the femoral vein at a speed of 0.786 ml/min; consequently the infusion lasted about 1 min for a rat of 400 g. At intervals of time from the beginning of the infusion, blood samples were collected by means of 'Unopette Disposable Pipetting System' (Becton Dickinson, France). The platelet count was performed in the routine way. The percent drop of the basal platelet count was calculated for each rat, then the mean of these values was estimated. Statistical analysis was performed with the 2 sample *t*-test for the limiting value of 0.05 probability.



Effect of ADP i.v. (0.854 mg/kg body wt.) in the rat: A) control; B) with splenic circulation tied up; C) with renal circulation tied up; D) with circulation of a limb tied up; E) with cerebral circulation tied up. The dotted line in B, C, D, E represents the control.

Results. The i.v. infusion of ADP reduces the number of the circulating platelets. As can be seen in the Figure, a mean drop of 44.7% was observed in the controls (A) 2 min after the beginning of the ADP infusion, then the platelet number promptly rises and reaches 90.58% of the basal concentration at 30 min. A similar drop in the platelet count was observed in the animals previously subjected to the exclusion of one of the following circulatory districts: splenic, renal, cerebral and of one hind-limb. These operations did not induce any change in the basal platelet count. In all cases the deepest reduction of the platelet number was measured 2 min after the beginning of the ADP infusion, but it did not in any case reach the extent observed in the control rats (see B, C, D, E, in the Figure and in the Table where the statistical significance of the results is reported too). The time course of the recovery of the basal platelet number was more rapid than the control in the animals submitted to the exclusion of the cerebral or of the hind-limb circulation. In addition, after the exclusion of the cerebral circulation, we observed a marked increase in the blood pressure.

Discussion. The present results confirm the reduction of the platelet concentration following the i.v. injection of ADP in the intact animal[6,7], moreover they show that a similar drop occurs also in the animals previously submitted to the exclusion of one of the following circulatory districts: splenic, renal, cerebral and of one hind-limb. This drop, obviously due to a trapping of the platelet aggregates by the microvasculature, is less in respect to the control, and of about the same extent after each reduction of the circulatory bed which we performed. It can be suggested that any microvascular endothelium provides a trapping action, depending on the size of the platelet aggregates formed by the i.v. infusion of ADP. Our results seem to be in agreement with the suggestion of LJUNGQVIST[8] and FREDE and BENNER[9] concerning the

[1] R. D. MACKENZIE, J. G. HENDERSON and J. M. STEINBACH, Thromb. Diath. haemorrh. *25*, 30 (1971).
[2] I. KOBAYASHI and P. DIDISHEIM, Thromb. Diath. haemorrh. *30*, 178 (1973).
[3] J. SWEDEMBORG, G. TAYLOR and P. OLSSON, Scand. J. clin. Lab. Invest. *5*, 27 (1971).
[4] M. G. DONI, Experientia *30*, 550 (1974).
[5] D. BOTTECCHIA and M. G. DONI, Experientia *29*, 211 (1973).
[6] G. V. R. BORN and M. J. CROSS, Nature, Lond. *197*, 974 (1963).
[7] A. NORDÖY and A. B. CHANDLER, Scand. J. Haemat. *1*, 16 (1964).
[8] U. LJUNGQVIST, in *Platelet Aggregation* (Ed. J. CAEN; Masson & Cie, Paris 1971), p. 227.
[9] K. E. FREDE and K. U. BENNER, Pflügers Arch. *324*, 319 (1971).

Mean values and standard deviations of the percent drop of the platelet count in respect to the basal number for every group of experiments (5 rats for each case reported, 6 rats for the control).

Group of experiments	Variation of the basal platelet count after ADP			
	2 min	4 min	15 min	30 min
A) Control	-44.70 ± 2.60	-21.95 ± 5.14	-14.17 ± 5.86	-9.42 ± 6.38
B) Exclusion of the splenic circulation	-33.09 ± 5.34 $t = 3.94 > 2.262$	-27.03 ± 1.48	-19.22 ± 10.48	-8.43 ± 3.70
C) Exclusion of the renal circulation	-29.73 ± 6.44 $t = 4.30 > 2.262$	-26.51 ± 5.07	-13.33 ± 6.50	-6.57 ± 2.21
D) Exclusion of a limb circulation	-31.77 ± 3.53 $t = 5.89 > 2.262$	-22.25 ± 5.59	-10.85 ± 3.93	-5.25 ± 5.52
E) Exclusion of the cerebral circulation	-30.35 ± 2.81 $t = 7.85 > 2.262$	-22.17 ± 7.17	-5.66 ± 2.43	-1.85 ± 2.04

The t-values are reported for p 0.05 of the data measured at the maximum of the effect of ADP (2 min) in each group of experiments in respect to the control.

trapping of the platelet aggregates by the renal and the hind-leg microvasculature, besides the well known pulmonary district[1,2]. The degree of the maximum drop of the platelet count observed 2 min after the beginning of the ADP injection, was similar in all the operated animals in spite of the different blood flow, length, rheological and metabolic characteristics of the renal, splenic, cerebral and of hind-limb circulations which we excluded respectively. Consequently the contribution of each circulatory district to the trapping of the platelets is not valuable in our experimental conditions; however, we suggest that the exclusions leave out a part of the total trapping endothelium, while some contemporaneously occurring haemodynamic changes may affect the pool of the platelets trapped elsewere. For example we observed a marked increase in the blood pressure following the exclusion of the cerebral circulation and a less marked increase following the renal exclusion. Moreover, the enhanced sympathetic activity occurring after the exclusion of the cerebral circulation might be responsible also for the increased velocity and extent in the recovery of the platelet count observed in this case. We suggest that the spleen, which is known to sequester platelets[10] and release haematic cells in such circumstances[11,12] was obviously induced to squeeze out and put in circulation the sequestered platelets.

Summary. The effect of the ADP infusion on the basal platelet count was studied in controls and in rats submitted to the exclusion of the following circulatory districts: splenic, renal, cerebral and of a hind-limb. After these exclusions the ADP-induced thrombocytopenia was less marked than the controls.

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[10] R. M. ASTER, J. clin. Invest. *45*, 645 (1966).
[11] J. BARCROFT, H. A. HARRIS, D. ORAHOVATS and R. WEISS, J. Physiol., Lond. *60*, 443 (1925).
[12] K. KRAMER and U. C. LUFT, Am. J. Physiol. *165*, 215 (1951).

Change in Levels of Cholesterol and Free Fatty Acids of Lymphoid Cells During Tumor Growth

It is well known that the structural lipid of mammalian cell membrane consists primarily of phospholipid and cholesterol in fixed proportions specific for species and cell types[1-4]. Studies on the lipids from normal lymphocytes and leukemic cells indicated marked decrease of cholesterol in leukemic cells as compared to normal lymphocytes[5,6]. However, little information is available about the lipid composition of lymphocytes from animals with carcinoma, except leukemia. Previously we reported high levels of cytotoxic free fatty acids in the splenic lymphoid cells from guinea-pigs[7]. The present study demonstrates marked change in levels of cholesterol and free fatty acids of lymphoid cells from different tissues of mice following the growth of Ehrlich's ascites carcinoma.

Adult female mice of *dd*N strain, weighing 24–26 g, were used throughout. The mice were inoculated i.p. with Ehrlich's ascitic tumor cells (5×10^6 cells/mouse). At intervals of 5 and 10 days after tumor implantation, the animals were killed by cervical dislocation. The thymus, spleen and lymph nodes (cervical and mesenteric lymph nodes), all of these tissues were removed and pooled from 100 individual mice, which had been fed with diet and given water ad libitum. Suspensions of lymphoid cells were prepared as follows: the pooled tissues were cut into small pieces, suspended in phosphate-buffered saline (pH 7.2)[8] and filtered through gauze. Then, the cell suspensions were centrifuged for 10 min at

[1] D. B. WEINSTEIN, J. B. MARSH, M. C. GLICK and L. WARREN, J. biol. Chem. *244*, 4103 (1969).
[2] P. SIEKEVITZ, New Engl. J. Med. *283*, 1035 (1972).
[3] S. J. SINGER and G. L. NICOLSON, Science *175*, 720 (1972).
[4] M. S. BRETSHER, Science *181*, 622 (1973).
[5] E. L. GOTTFRED, J. Lipid Res. *8*, 321 (1967); *12*, 531 (1971).
[6] M. INBAR and M. SHINITZKY, Proc. natn. Acad. Sci., USA *71*, 4229 (1974).
[7] S. KIGOSHI and R. ITO, Experientia *29*, 1408 (1973).
[8] A. B. STAVITSKY, J. immun. *72*, 360 (1953).