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Authors

Dobson, Ernest L
Kelly, Lola S
Finney, Caroline R
et al.

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The Association of Blood Platelets and Colloidal Carbon Removal

Ernest L. Dobson, Lola S. Kelly, Caroline R. Finney
and Justine J. Lynch

DISCLAIMER

ACKNOWLEDGEMENT

SUMMARY

When mice were given three successive hourly injections of colloidal
carbon, platelets disappeared almost completely from the circulation.
Antiplatelet serum injected during the course of carbon clearance caused
an increase in the rate of carbon removal, whereas mice pretreated with
antiplatelet serum had normal rates of carbon removal. In normal mice,
successive carbon injections showed progressively more rapid clearance
and this effect was greatly diminished by pretreatment of the mice with
antiplatelet serum. It is suggested that platelet-carbon aggregates
lodge or are formed in the sinusoids of the RE organs and serve as sticky
clumps which subsequently trap carbon injected at later times. The rate
of recovery of platelet levels toward normal yields an estimate of 2.5
days for the half life of mouse platelets.

INTRODUCTION

Tait and Elvidge (1926) observed that the injection of colloids produced a thrombocytopenia. In the course of experiments on the kinetics of colloidal carbon removal from the blood of mice, we observed an association of the carbon with platelets in blood smears. It seemed appropriate therefore to study the kinetics of platelets in mice during the course of carbon clearance.

METHODS

Swiss Webster mice six to eight weeks old were used in all experiments.

Phagocytic function was determined essentially by the carbon clearance method discussed by Benacerraf et al. (1957). Twelve mg per mouse of colloidal carbon [1] were injected intravenously. Blood was collected from the tip of the tail at appropriate time intervals. The concentration of the carbon was measured spectrophotometrically and plotted on semilog paper as a function of time. The fractional disappearance rate constant, k, defined by

$$C = C_o e^{-kt}$$

has been used as a quantitative measure of phagocytic activity of the RE system [2].

Blood for platelet counts was obtained by cardiac puncture. Platelet counts were done according to the method of Brecher and Cronkite

(1950).

Rabbit anti-mouse platelet serum was produced by the injection into rabbits of isolated mouse platelets mixed with Freunds adjuvant. The serum was adsorbed with mouse red cells before use. 0.05 cc of serum was injected intravenously into mice.

RESULTS

When mice were given repeated injections of 12 mg of colloidal carbon at hourly intervals, a progressive increase in the carbon clearance rate was observed confirming our earlier observations (Dobson et al., 1967). The data are shown in Table 1.

Aggregation of carbon particles in blood can readily be seen by examining a blood smear. Gabrieli et al. (1967) have studied the aggregation of carbon suspensions in rat plasma. However, our observations that platelets and carbon particles were often found associated in smears of whole blood suggested that platelet count determinations might prove interesting following multiple injections of colloidal carbon.

Fig. 1 shows platelet concentrations as a function of time in mice given multiple injections of carbon. The first injection of 12 mg carbon resulted in no apparent decrease in platelet levels. The second injection given one hour later resulted in an immediate and dramatic decrease in circulating platelet levels. These levels had returned to normal by 20 minutes. The third injection of colloidal carbon resulted in the same dramatic drop in platelet levels which, however, remained low.

A drop in platelet count following the injection of particles was described by Tait and Elvidge in 1926, by numerous authors since, and most recently by Wiedmeier et al. (1969). In vitro aggregation of platelets by latex particles has also been described (Glynn et al., 1965).

To investigate the effect of platelet removal on carbon disappearance, rabbit anti-mouse platelet serum was injected intravenously during the course of carbon clearance. The rate of carbon clearance was always promptly increased by this injection. The half times for carbon removal before and after the antiplatelet serum injection are shown in Table 2. In contrast, no effect was seen when the antiplatelet serum was injected ten minutes or more before the carbon.

When mice were depleted of platelets by the injection of antiplatelet serum prior to multiple injections of carbon, the increase in carbon removal rate normally seen with multiple injections (Table 1) was greatly diminished. This may be seen in Table 3. It is interesting to note that while the increase in the removal rate of carbon after the third injection is much less marked in these platelet depleted mice, blockade certainly did not result from the two previous injections of carbon.

Mice, whose platelet levels had been depleted by multiple carbon injections, were measured for their blood platelet levels on a daily basis for six days. A gradual return toward normal was observed as illustrated in Fig. 2. Data points are shown with standard errors of the mean. The solid line represents the theoretical curve whose equation is shown.

DISCUSSION

When repeated injections of colloidal carbon were given at hourly intervals, a progressive increase in the rate of carbon removal was observed (Dobson et al., 1967). It was pointed out in this paper that this observation is inconsistent with the usually accepted theories on the induction of blockade. Specifically it does not fit either the satiation theory or the opsonin depletion theory. Thus it appears that there are other factors responsible for the phenomenon. Fig. 1 shows a very close association between removal of repeated injections of carbon and platelet disappearance. However, it is clear that circulating platelets are not necessary for the removal of carbon since mice which had been pretreated with antiplatelet serum had a normal half time for carbon clearance. On the other hand, animals pretreated with antiplatelet serum no longer demonstrated the fourfold acceleration of carbon removal following the multiple carbon injections.

One possible explanation for these observations could be that platelet-carbon aggregates may lodge or be formed in the sinusoids of the RE organs and serve as sticky clumps which subsequently trap carbon injected at later times. When these sticky clumps are finally engulfed by the phagocytic cells and are no longer in direct contact with circulating carbon, blockade ensues. Some confirmation of this hypothesis can be found in the work of Salvidido and Crosby (1960) who observed clumps of agglutinated India ink particles and platelets in the RE organs. This followed a temporary depletion of platelets from the blood.

This theory would explain the very rapid removal of a fourth carbon injection at a time when circulating platelet numbers are low. It would also explain the delay in the induction of blockade observed by Parker and Finney (1960).

Dineen et al (1968) have reported a gradual production of thrombocytopenia following the injection of zymosan which they ascribe to the hyperphagocytosis produced by this agent. This is presumably a different phenomenon from the rapid platelet depletion observed in the current studies.

The observations on the kinetics of circulating thrombocyte populations permit the estimation of some parameters concerning platelet life. The rapid restoration to normal levels following the second carbon injection suggests a large pool of reserve thrombocytes which can be mobilized readily. While there is some disagreement in the literature concerning platelet reserve pools, Zucker et al (1961) and Craddock et al (1960) interpreted their data to indicate the existence of platelet pools that can be mobilized.

Following the near total depletion by 3 carbon injections, platelet restoration occurred slowly. The rising platelet level could be fitted almost precisely by the following equation: $C_t = 1.5 \times 10^6 (1-e^{-0.28t})$ which corresponds to a possibly over-simplified hypothetical model in which platelets are produced at a constant rate of 8.4×10^9 platelets per day if no reserves are assumed and 2 or 3 times this value if reserve pools equal to or double the circulating number exist. The model also assumes random destruction or removal with a half time of $2\frac{1}{2}$ days. This

value is in fair agreement with the varying values for the half life

measured by Ebbe et al (1966) and Hjort and Paputchis (1960) for rat

platelets.

Footnotes

1. Gunther Wagner, Hanover, Germany, Suspension no. C11/1431a, prepared
    as described by Parker and Finney (1960).

2. The approximation of the disappearance curve to an exponential
    function and the relationship of the disappearance constant to the
    "phagocytic index" is discussed by Dobson et al. (1967).

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Table 1

Half time (min.) of removal of colloidal carbon
(12 mgm) injected at hourly intervals.

1st injection (15 mice)	2nd injection (10 mice)	3rd injection (15 mice)	4th injection (4 mice)
28.8 ± 1.9	11.6 ± 2.4	5.9 ± 1	5.2 ± 0.4

Table 2

The acceleration of carbon clearance by the
injection of antiplatelet serum during
course of carbon clearance.

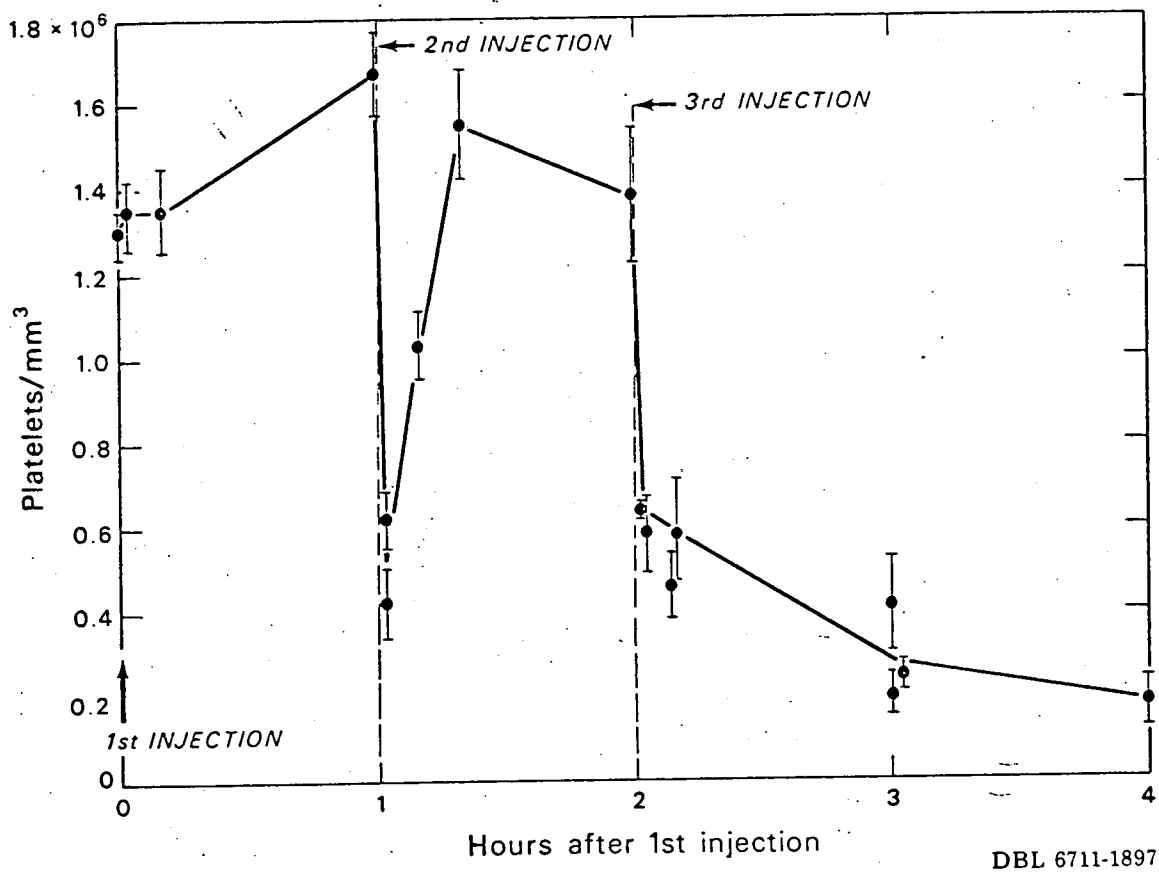| $T\frac{1}{2}$ of carbon disappearance before anti-platelet serum	Time of anti-platelet serum injection following carbon injection	New $T\frac{1}{2}$ of carbon disappearance after antiplatelet serum	Increase in removal rate constant (k_2/k_1)
37.9 min.	15.9 min.	11.2 min.	3.4
33.0	16.3	19.0	1.7
24.9	25	8.5	2.9
25.5	25	9.6	2.7
Av. 30.3 $\pm$ 3.1		Av. 12.1 $\pm$ 2.4	Av. 2.7

Table 3

Half time (min) of removal of the third hourly colloidal
carbon injection (12 mgm) in mice treated with antiplatelet
serum one day previously and appropriate controls.

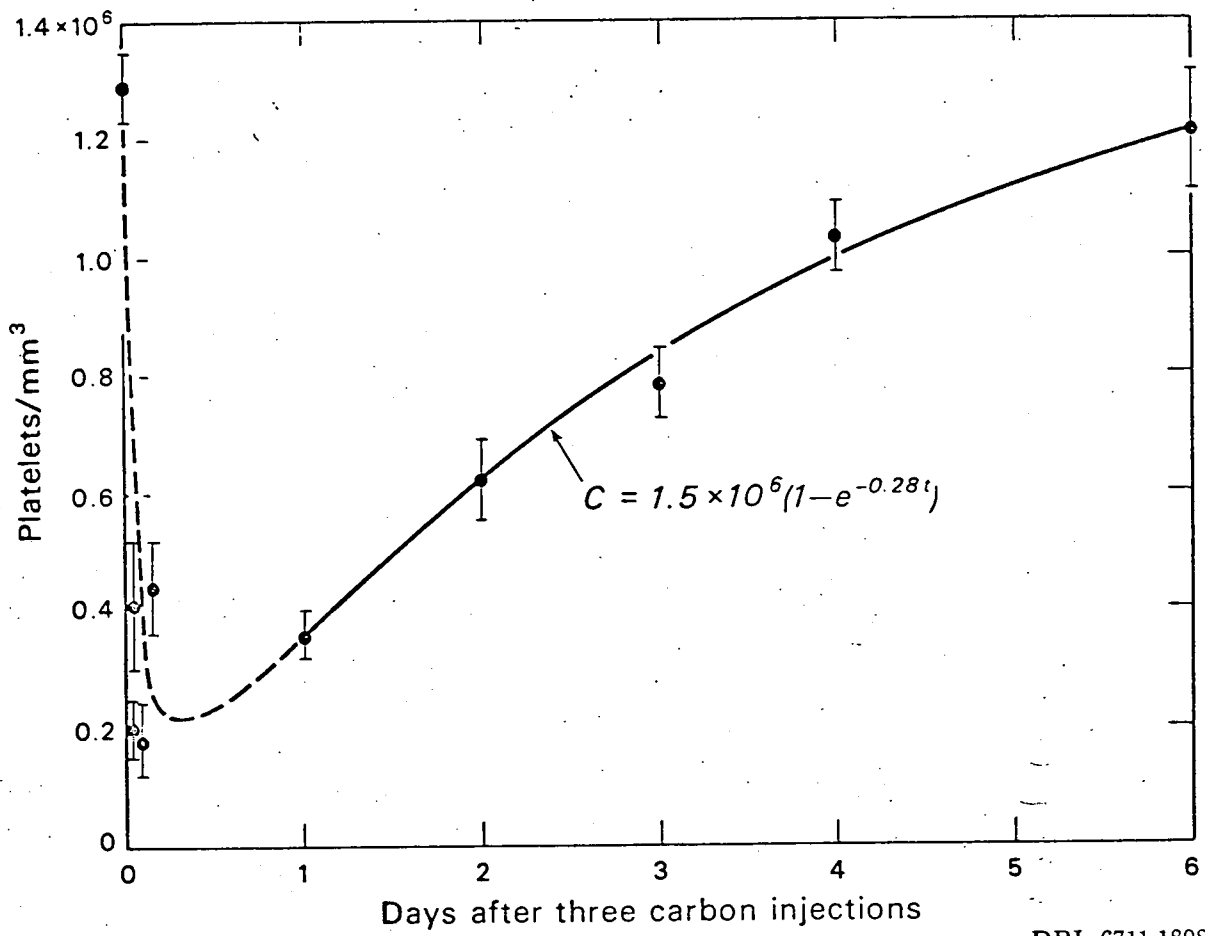| Single carbon injection controls (12 mice)	Multiple carbon injections	
	No antiplatelet serum (12 mice)	Antiplatelet serum (10 mice)
32.0 ± 2.0	8.0 ± 0.9	17.9 ± 2.2

FIGURE LEGENDS

Fig. 1 The effect of multiple carbon injections on blood platelet levels.
12 mg of colloidal carbon was injected at zero time and at 1 and
2 hours as indicated by the arrows. Errors shown are standard
errors of the mean.

Fig. 2 The recovery of blood platelet levels as a function of time
following depletion by multiple carbon injections. Errors shown
are standard errors of the mean.

DBL 6711-1897

$$C = 1.5 \times 10^6 (1 - e^{-0.28t})$$

Platelets/mm³ vs. Days after three carbon injections

DBL 6711-1898