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IS PHAGOCYTOSIS OF CARBON AN EXPONENTIAL PROCESS?

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ABSTRACT

When the disappearance of colloidal carbon from the blood of rats was measured over a period of more than one half-life, it became evident that the process is not adequately represented by a negative exponential function. During the second and subsequent half-lives removal of carbon accelerated, if the process is regarded as exponential. The form of these curves is the result of two opposite tendencies: the tendency for an equal amount of carbon to be removed per minute, and the tendency for the system to become saturated by the load already removed.

Biozzi, Benacerraf and Halpern (1) found that the concentration of colloidal carbon remaining in the blood of rats following intravenous injection was a negative exponential function of time from injection. However, the halflife of carbon in the blood was directly proportional to the dose of carbon. Despite this anomaly, these curves have usually been regarded as exponentials, and the half-life of colloidal carbon has been used extensively as a measure of phagocytic activity. Parker and Finney (2) obtained data in mice indicating an increasing rate of disappearance rather than a constant exponential rate. Fred and Shore (3) have confirmed this departure from the exponential mode and have analyzed the curves by computer techniques with the aid of a mathematical model.

We have found that the disapperance of colloidal carbon in young Sprague-Dawley rats is usually not exponential, if the process is followed more than one half-life, although it is approximately exponential during the first half-life, and occasionaly remarkably close to exponential for two half-lives.

We have analyzed curves from 105 male Sprague-Dawley rats (mean age, 54 days; mean weight, 217 grams) given various doses of carbon (4) from 4 mg to 32 mg per 100 grams of body weight. The rats were anesthetized with diethyl-ether, and a vein was exposed by an incision on the inner aspect of the thigh. Colloidal carbon suspension, diluted 1:1 with 0.9% NaCl solution (5), was injected with a tuberculin syringe equipped with a 27 gauge needle. A series of 0.02 or 0.04 ml blood samples was obtained from the tail at half-minute or longer intervals and mixed in 4.00 ml of 0.1% sodium carbonate solution. Optical density was determined at 675mμ with a Beckman Model B spectrophotometer. An example of a disappearance curve showing obvious bending is found in Fig. 1. The bending was not always as evident as this, but in a considerable fraction of the curves a definite bending point was found at some point in the second half-life.

The analysis of the curves was made by the following steps: the optical densities were converted to logarithms which were plotted against time; a series of lines was fitted beginning at 2 minutes after injection; the first line was extended until a definite bending point was found;

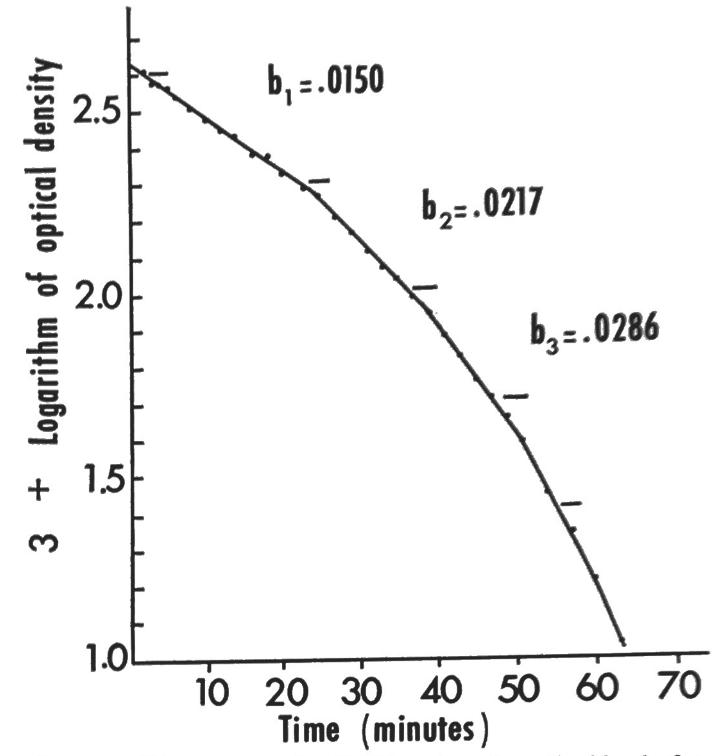

Figure 1. Disappearance of colloidal carbon from the blood of a rat that received 24 mg of colloidal carbon per 100 grams of body weight. The slopes during the first, second and third half-lives are designated b_1, b_2 and b_3 respectively.

a new line was started and extended until a second bending point was found, and so on. This made it possible to divide the points into sets belonging to the first, second, third and in some instances, fourth and fifth half-lives; the slopes were calculated by the method of least squares (6). An objective measure of bending was obtained as the ratio of the second to the first slope, the third to the second slope, and so on. The slope-ratios of different rats receiving the same dose of carbon were averaged logarithmically and the standard errors were calculated.

The results are found in Table 1. The mean slope-ratios and their standard errors are shown as well as the probabilities for comparisons to a slope-ratio of 1.000. In all but the lowest carbon dose the slope-ratio differs from one at the P 0.001 level 7), and in all instances the mean bending is in the same direction, i.e. acceleration of the rate of disappearance when the process is considered as an exponential. The curves continued to bend when slope-ratios were considered for the third, fourth, and fifth half-lives.

An additional analysis for bending within the first half-life was made by dividing the points into two sub-

TABLE 1. Half-lives and slope-ratios obtained from disappearance curves of 105 young, male, Sprague-Dawley rats given various doses of colloidal carbon intravenously.

Dose of carbon (mg/100g)	No. of rats	Half-life of carbon (min.)	S.E. (%)	Slope-ratio b_2/b_1	S.E. (%)	Prob. diff. from 1.000	Slope-ratio b_{1B}/b_{1A}	S.E. (%)	Prob. diff. from 1.000	Slope-ratio b_3/b_2	b_4/b_3	b_5/b_4
4	11	4.3[1]	10.1[2]	1.073[1]	13.4[2]	0.2						
6	9	5.9	6.9	1.108	4.8	<0.001						
8	40	8.2	7.3	1.278	2.3	<0.001						
12	15	12.0	8.5	1.274	3.6	<0.001	1.037[1]	2.8[2]	0.2	1.544[1]	1.063[1]	1.167[1]
16	14	14.6	5.5	1.222	2.4	<0.001	1.082	5.0	<0.1	1.342	1.334	
24	10	22.0	7.8	1.332	2.2	<0.001	0.956	3.4	0.2	1.240	1.473	1.542
32	6	31.0	4.4	1.218	3.7	<0.001	0.953	9.9	>0.3	1.310	1.250	1.332

[1] The means were obtained by logarithmic averaging.

[2] Standard errors are in percentage form and confidence intervals may be obtained by multiplying and dividing the means by 1 plus (the standard errors expressed as decimal fractions) or 1 plus (multiples of the standard errors expressed as decimal fractions). The meaning of the term slope-ratio is made clear in Fig. 1 and in the text.

sets and fitting lines to each sub-set. The mean slope-ratios varied from 0.953 to 1.082. The mean slope-ratios did not differ from 1.000 at the P 0.05 level. The degree of bending seen within the first half-life was not sufficient to be apparent without calculation and a single exponential line appeared to fit the points.

It is easy to answer the question: "Why do the curves bend?". They bend because the reticuloendothelial system tends to remove the same amount of carbon per minute, regardless of the concentration. The evidence for this is found in Fig. 2 which confirms the observation of Biozzi *et al.* (1) that the half-life is directly proportional to the dose of carbon. A more difficult question is: "Why are the curves so close to being exponential?" This has been considered by Biozzi *et al.* (1) who deduced that saturation of the phagocytic cells slows removal of carbon from the blood just enough to make an exponential. It seemed unlikely to us that such a fine balance could be maintained throughout the whole process, and indeed we have shown that it was not, under the conditions we used. As the process continued through the second and subsequent half-lives, a larger and larger fraction of the amount present was removed per minute, but in our experience, the mean slope-ratio never reached 2.000, as it would if no inhibition occurred.

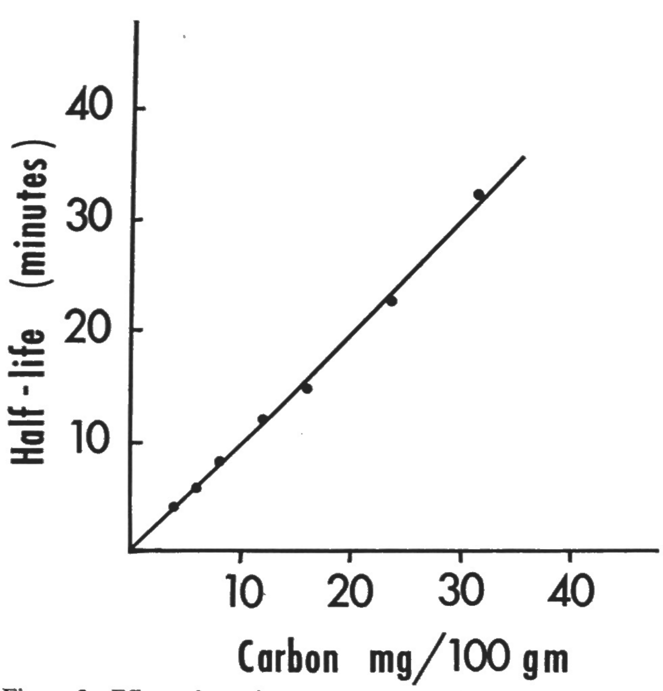

Figure 2. Effect of varying the dose of colloidal carbon on its (first) half-life in the blood of rats. Each point is the geometric mean of determinations from 6 to 40 rats.

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4. C/11/1431a, Pelikan Special Biological Ink; John Henschel and Co., 195 Marine Street, Farmingdale Long Island, New York.
5. Our methods differ from those of Biozzi *et al.* in two respects. We have not added gelatin to the diluent and the disapperance curves were obtained in rats anesthetized with diethyl-ether. Kampschmidt *et al.* (8) and Norman and Benditt (9) have shown that gelatin slows the rate of removal of carbon in normal rats and Kampschmidt *et al.* (8) have shown that addition of NaCl does not affect the rate of removal in normal rats.
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