might be altered in a manner that would favor binding of NADPH more than heparin. This situation would be analogous to that demonstrated by SOLOMON and SCHROGIE[7] in which thyroxin increased the affinity between warfarin and its receptor site and thereby potentiated the anticoagulant response to warfarin[8].

Zusammenfassung. Leberhomogenate aus mit Thyroxin injizierten Ratten reduzieren den Ring A von Kortison schneller als Homogenate von normalen Tieren. Die Reduktion von Kortison mit Kontrollpräparaten konnte mit Heparin leicht verhindert werden, nicht aber durch Einspritzen von Thyroxin.

R. C. TROOP

Division of Clinical Pharmacology, University of Tennessee Medical Units, Memphis (Tennessee 38103, USA), 14 April 1969.

[7] H. M. SOLOMON and J. J. SCHROGIE, Clin. Pharmac. Ther. *8*, 797 (1967).

[8] This study was supported in part by research grant No. AM-09151 from the National Institutes of Health, U.S.P.H.S.

PRO EXPERIMENTIS

A Quantitative Method for Measuring the Uptake of Colloidal Carbon by Mouse Tissues

Out of several methods available for the study of the phagocytic function of the reticuloendothelial system in experimental animals, the one using colloidal carbon, introduced in its present form by HALPERN et al.[1,2], is among the most popular[3]. The main advantage of this method is that colloidal carbon can easily be determined in blood samples when the rate of clearance is studied and can also be readily seen in histological preparations. Difficulties can however arise when quantitative determinations of carbon uptake by organs or tissues are required.

The only method available for measuring carbon uptake by individual organs is the one originally described by HALPERN, BIOZZI et al.[1]. It is based on weighting the dry carbon residue after digestion of organs in strong alkali and repeated extractions and washings. This method is, however, limited by the amount of carbon injected into animals. Thus, with a dose of 16 mg/100 g body weight, which is generally used for measuring the reticuloendothelial phagocytic activity, the amount of carbon likely to be found in organs of individual laboratory animals smaller than rats is below that easily detectable on weight basis.

In the course of studies of the phagocytic activity of the reticuloendothelial system after irradiation it had become necessary to determine the amount of carbon taken up by organs of individual mice. A method, based on spectrophotometric measurement of carbon, has been developed which, being more sensitive, can be applied to small laboratory animals and limited amounts of carbon.

A suspension of colloidal carbon (C11/1431a) was obtained from Günther Wagner, Pelikan-Werke, Hanover (Germany). As the concentration of carbon is not specified for this preparation, amounts of carbon will be given as volumes of the original suspension. A series of dilutions of this suspension was prepared by diluting volumes of 20–100 µl (measured with microcaps-disposable micropipettes, Drummond Sci. Co.) up to 100–1000 ml distilled water. The optical density of these dilutions was measured on a Unicam SP 600 spectrophotometer at 800 nm with a 1 cm path length. From these readings the standard curve shown in Figure 1 was constructed. By making use of this curve and Table I any amount of original carbon suspension from as little as 0.25–400 µl or more can be easily determined.

Male CBA/H mice, aged 5–5½ months and weighing between 28 and 34 g were injected i.v. via a tail vein with 0.3 ml of carbon dilution containing 50 µl original carbon suspension (1 part of original carbon suspension plus 5 parts of 1% BDH gelatin in distilled water, pH 7). When the same volume of carbon dilution was diluted to 500 ml with distilled water it gave an OD of 0.300. This amount of carbon injected per mouse is close to 16 mg/100 g body weight for a 30 g mouse[4]. One group of the injected mice were bled 60 min later under slight ether anaesthesia from the orbital venous plexus and a 10 µl blood sample was lysed in 2 ml of 0.2% solution of Na_2CO_3 and used for estimating the amount of carbon in the blood. These mice were killed by cervical dislocation immediately after the blood sample had been taken

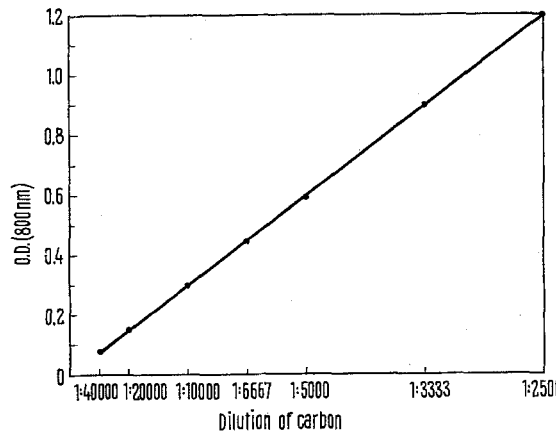

Fig. 1. Standard curve for dilutions of colloidal carbon C11/1431a.

[1] B. N. HALPERN, G. BIOZZI, G. MENÈ and B. BENACERRAF, Ann. Inst. Pasteur *80*, 582 (1951).

[2] B. N. HALPERN, B. BENACERRAF and G. BIOZZI, Br. J. exp. Path. *34*, 426 (1953).

[3] B. BENACERRAF, in *The Liver* (Ed. CH. ROUILLER; Academic Press, New York and London 1964), vol. II, p. 37.

[4] G. BIOZZI, B. BENACERRAF and B. N. HALPERN, Br. J. exp. Path. *34*, 441 (1953).

and their liver, spleen, lungs and one kidney were removed, weighed immediately on a torsion balance and digested. The other group of injected mice were killed 24 h later and the same organs taken. In addition bone marrow from both femurs and tibias was expelled and suspended in 2 ml Tyrode's solution and the cell number counted in a haemocytometer. Organs and bone marrow were obtained in a similar manner from a group of uninjected mice.

All the tissues were individually digested in a mixture of potassium hydroxide, ethanol and gum arabic[5]. For this purpose 25 ml of about 8% KOH in 70% ethanol (specific gravity 0.945–0.950) and 50 ml of 2% solution of gum arabic were added to each liver and 2 ml and 4 ml of each solution respectively to other organs and bone marrow suspensions. After 12–18 h at 37 °C tissues were completely digested and carbon particles remained in a fine suspension with no visible formation of aggregates. If carbon suspension alone was incubated under these conditions in the presence of the digestion mixture no change in OD reading occurred.

Tissue digests were then diluted in such a manner as to obtain concentrations of carbon suitable for spectrophotometric measurements. A filtered solution of a detergent (Haemo-Sol, Meinecke & Company, Inc., Baltimore, Maryland, USA) was used for diluting in order to clarify particularly liver digests (some other detergents have been tried with similar success). The presence of the detergent did not modify the OD of carbon suspension. Liver digests were diluted to 500 ml and spleen digests to 50 or 100 ml with 1% Haemo-Sol and their OD measured. Lungs, kidney and bone marrow digests were diluted to 10 ml with 2% Haemo-Sol. In order to clarify lungs and kidney digests the former were heated to about 55 °C and the latter were left at 4 °C for 2–3 days to sediment. Dilutions of organ digests prepared in the same way from uninjected mice were used as blanks for OD measurements. In order to test the amount of carbon recovered by the above procedure 50 µl of colloidal carbon were added in vitro to each of seven livers that were to be digested. The average amount of carbon recovered from this series was 49.53 µl (S.E. ± 0.16), or 99.1%.

Histological examination of carbon distribution was made on a number of tissues obtained from mice killed 24 h after injection of carbon. Tissues were fixed in Bouin's fluid or formol saline and sections stained with haematoxylin and eosin.

A small amount of carbon particles could be seen in sections of most tissues, mainly in endothelial cells lining capillaries and in occasional reticular cells. This accounts most probably for the failure to recover all the injected carbon in the organs used for quantitative measurement of carbon in this study. Somewhat more carbon was seen in lung sections, but most of the carbon was found in the spleen, liver and bone marrow (Figure 2).

[5] S. Fisher, W. B. Riley and C. D. Shorey, J. Path. Bact. *96*, 463 (1968).

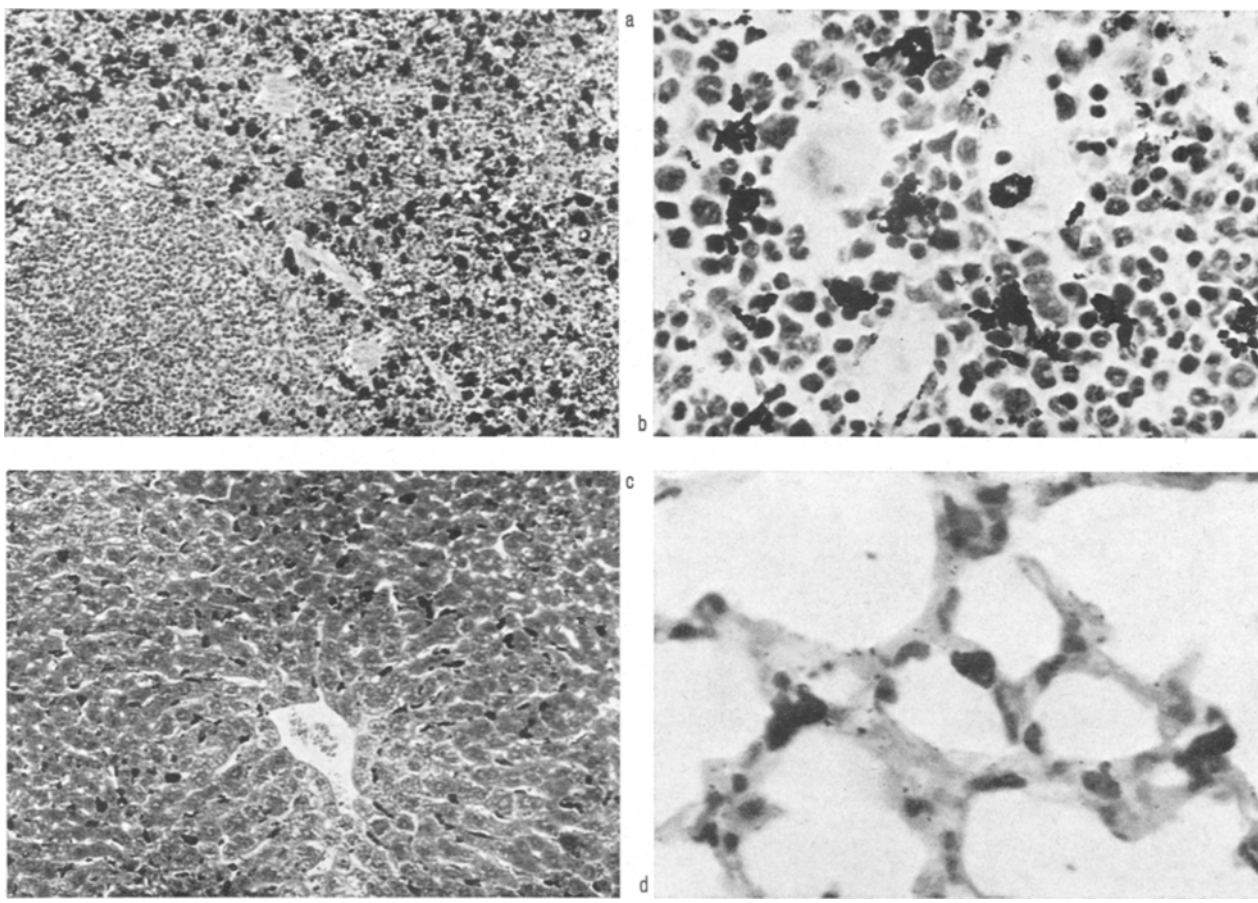

Fig. 2. Carbon particles in sections of (a) spleen (× 180), (b) bone marrow (× 720), (c) liver (× 180), and lung (× 880) 24 h after i.v. injection of 50 µl suspension of colloidal carbon. Note relative amounts of carbon particles in different tissues.

Table I. Standard dilutions of colloidal carbon

Dilution factor	Amount of original carbon suspension (μl)						
1:40,000	25	12.5	6.25	2.5	1.25	0.625	0.25
1:20,000	50	25	12.5	5	2.5	1.25	0.5
1:10,000	100	50	25	10	5	2.5	1
1: 6,667	150	75	37.5	15	7.5	3.75	1.5
1: 5,000	200	100	50	20	10	5	2
1: 3,333	300	150	75	30	15	7.5	3
1: 2,500	400	200	100	40	20	10	4
Total volume of carbon dilution (ml)	1000	500	250	100	50	25	10

Table II. Average weights and carbon content of mouse organs 1 and 24 h after an i.v. injection of 50 μl original carbon suspension[a]

	60 min	24 h
No. of mice	5	6
Body weight (g)	32.2 ± 0.8	31.9 ± 1.1
Organ weights		
Liver (g)	1.46 ± 0.06	1.56 ± 0.04
Spleen (mg)	69 ± 1	95 ± 3
Lungs (mg)	150 ± 8	162 ± 7
Kidney (mg)	228 ± 6	238 ± 8
Nucleated bone marrow cells (millions)[b]	N.D.	58.0 ± 4.5
Carbon content[c]		
Blood[d]	16.30 ± 0.43	N.D.
Liver	24.52 ± 0.69[e]	30.97 ± 0.50
Spleen	4.70 ± 0.04[e]	8.20 ± 0.25
Lungs	0.68 ± 0.17[e]	0.43 ± 0.04
Kidney	0.50 ± 0.02[e]	< 0.25
Bone marrow (4 bones)	N.D.	0.88 ± 0.03
Bone marrow (total)	N.D.	8.37 ± 0.26
Total carbon recovered[f]	47.20 ± 1.19	48.08 ± 0.49

[a] All values are means ± standard errors. [b] Recovered from 2 femurs and 2 tibias. [c] In μl equivalents of the original carbon suspension. [d] Total blood volume 4.94 ml/100 g body wt. [e] Corrected for the blood content of organs (see text). [f] Values for the kidney multiplied by 2. N.D., not determined.

Table III. Uptake of colloidal carbon per unit weight of mouse tissues 24 h after an i.v. injection of 50 μl original suspension

Carbon (μl equivalents) per 1 g wet weight			
Liver	Spleen	Lungs	Bone marrow[a]
19.88 ± 0.34	86.48 ± 3.47	2.64 ± 0.16	33.88 ± 1.87

[a] Calculated from the amount of carbon taken up by a known number of nucleated bone marrow cells (present experiment), specific gravity of rat bone marrow = 1.063 (HULSE, personal communication) and number of nucleated cells per mm³ of bone marrow[7].

Amounts of carbon (in μl equivalents of the original carbon suspension) found in the blood and organs of injected mice are given in Table II. The blood mass was estimated at 4.94 ± 0.04 ml/100 g body weight by means of ^{59}Fe-labelled syngeneic red cells (unpublished observation). Amounts of carbon in organs of mice killed 60 min after the injection were corrected for carbon in the blood taking the following figures for the blood content of organs (in per cents of total blood volume): Liver 7.0, spleen 0.34, lungs 2.2, kidneys 3.0 (unpublished observations). In such a way figures in Table II give net amounts of carbon in organs. Carbon uptake by the whole mouse bone marrow was estimated in the following way. Since no data have been found for the total marrow cellularity in mice it was assumed that marrow cellularity of mice is similar to that of rats and the figure of 17×10^8 nucleated cells per 100 g body wt. reported by DONOHUE et al.[6] was taken. The present experiment provided information on the amount of carbon taken by a known number of nucleated bone marrow cells and by simple arithmetic the figure quoted in Table II was obtained.

The present method for quantitative determination of colloidal carbon uptake by organs after an i.v. injection enables accurate measurements of small amounts of carbon and with satisfactory reproducibility. As reported by other authors[1,2,4] most of the carbon is removed from the blood stream by the liver and the spleen, while negligible amounts are taken up by other tissues with the exception of the bone marrow. The importance of bone marrow cells in the uptake of injected carbon particles appears to have been overlooked or underestimated. It can be readily seen at autopsy and in histological sections, as well as by the present method, that substantial amount of carbon is deposited in the bone marrow. If the amount of carbon taken up by various tissues is given per unit of tissue weight, as a measure of their relative capacity for the uptake of particles the spleen is the most efficient, followed by the bone marrow, liver and lungs (Table III). It remains to be seen whether the bone marrow has the same activity towards other particulate substances.

An additional observation in the present experiment is the stimulatory effect of colloidal carbon injection on spleen and liver enlargement. In mice killed 24 h after carbon injection, as compared to those killed at 1 h, spleen and liver weights (per 100 g body wt.) were 297 ± 9 mg vs 215 ± 5 mg ($P < 0.001$) and 4.90 ± 0.07 g vs 4.54 ± 0.08 g ($P < 0.01$), respectively.

Résumé. On décrit une méthode spectrophotométrique quantitative pour la détermination de la répartition du carbone colloïdal dans les organes d'une seule souris. La sensibilité de la méthode est de 0,25 μl de la suspension de carbone colloïdal C11/1431a. La rate est le tissu qui a présenté la plus grande capacité de fixation du carbone. Elle est suivie par la moelle osseuse, la foie et les poumons.

V. S. ŠLJIVIĆ

Medical Research Council, Radiobiology Unit, Harwell, Didcot (Berkshire, England), 9 May 1969.

[6] D. M. DONOHUE, B. W. GABRIO and C. A. FINCH, J. clin. Invest. *37*, 1564 (1958).
[7] E. V. HULSE, Acta Haemat. *31*, 50 (1964).