PHAGOCYTOSIS OF HEAT-DENATURED HUMAN SERUM ALBUMIN LABELLED WITH ^{131}I AND ITS USE AS A MEANS OF INVESTIGATING LIVER BLOOD FLOW

B. BENACERRAF, G. BIOZZI, B. N. HALPERN, C. STIFFEL AND D. MOUTON

From the Laboratory of Experimental Medicine of the Clinique Médicale Propédeutique de l'Hopital Broussais, C.N.R.S. and Centre de Recherches Allergiques de l'Association Claude Bernard, Paris

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WHEN protein solutions are heated colloidal aggregates are formed (Neurath, Cooper and Erickson, 1941 ; Cooper and Neurath, 1943). The degree of aggregation and the size of the aggregates depend, for a given protein, on the concentration, the temperature, the pH and the salt concentration (Joly and Barbu, 1949). In previous experiments concerning the protein aggregates of heated whole serum (Benacerraf, Halpern, Stiffel, Cruchaud and Biozzi, 1955 ; Biozzi, Halpern, Benacerraf, Stiffel and Mouton, 1956) we observed that the cells of the reticulo-endothelial system (R.E.S.) phagocytize the complexes of globulin and albumin (C.A.G.) which can be isolated from heated serum. The kinetics of blood clearance of C.A.G. tagged with ^{131}I follows the laws established for other colloids phagocytized by the R.E.S. (Biozzi, Benacerraf and Halpern, 1953 ; Benacerraf, Stiffel, Biozzi and Halpern, 1954 ; Halpern, Benacerraf, Biozzi and Stiffel, 1954). The blood concentration decreases exponentially with respect to time and the rate of clearance is inversely proportional to the dose of C.A.G. when enough colloids are injected.

In this study we have verified that the R.E. cells phagocytize the aggregates of human serum albumin (C.A.). The sensitivity of the radioactive techniques allows the study of the blood clearance of very small doses of C.A.^{131}I, while the lack of toxicity and the stability in the blood of the protein permit the injection of high doses for the investigation of R.E. phagocytic activity. We have observed that there is in every animal species a critical dose of this protein below which the rate of clearance is constant and independent of the dose of colloid injected. In this range, C.A.^{131}I clearance can be used to measure liver blood flow as efficiently as the chromium phosphate technique (Dobson and Jones, 1952). A study has been made also of the efficiency of portal clearance of C.A.^{131}I in this dose range.

The breakdown of heat-denatured human serum albumin by the cells of the R.E.S. after it has been phagocytized was also investigated.

METHODS

Preparation and Iodination of Human Albumin Complex (C.A.)

The human serum albumin was supplied by the Centre National de la Transfusion Sanguine, available in a 25 per cent solution for clinical use. This solution contained mandelic acid 0·05 M as a stabiliser. A dilution containing 1 per cent albumin was prepared with

normal saline and the pH adjusted to 7. The optical density was then measured in the 550 mμ wave band in a 1 cm. square cell. Heating was carried out as described below until the optical density measured on aliquot samples was increased by 0·085 unit ± 10 per cent. The solution was heated at 70° in a water bath with constant shaking for 20 min.; the optical density was measured, and the temperature was brought up to 75°–80° and the optical density measured again every 5 min. until the required value was reached. This usually required 15–20 min. The solution was then immediately cooled below 50°. The times of heating, however, are not absolutely standardized and are controlled through optical density measurements because small changes of pH determine great changes in the effects of heating. This method was found to be more satisfactory than the use of buffered solutions for heating.

The albumin complex was then isolated by precipitation at its isoelectric point. After cooling to room temperature, the heated solution was acidified with N- or 0·1 N-HCl, until precipitation occurred. The supernatant was discarded after centrifugation and the precipitate washed several times with normal saline at pH 5·4 until the washings no longer contained material precipitable by 20 per cent trichloracetic acid. The albumin complex was then dissolved in normal saline by adding N- or 0·1 N-NaOH until pH 7·6. In this way solutions containing up to 30 mg. of protein per ml. can be prepared. The solution was left overnight in the refrigerator to ensure solubilization. It was then centrifuged and the insoluble fraction discarded. Merthiolate 1/5000 was added. It can be kept in the refrigerator at 4° for several months. Care must be taken not to let it freeze because the C.A. will then precipitate and become insoluble.

Iodination of C.A.

Iodination of C.A. with iodine containing ^{131}I was carried out by the method described for C.A.G. (Biozzi *et al.*, 1956). With this technique, the amount of iodine which was bound to the C.A. varied from 0·42 mg. to 0·72 mg. iodine/100 mg. protein. In order to obtain samples of C.A.^{131}I radioactive enough to allow the measurement of very small doses of the protein injected, several samples of C.A. were iodinated with considerably more ^{131}I; up to 2 millicuries of ^{131}I were used for 3 mg. of protein.

Methods of estimation of C.A.^{131}I

C.A.^{131}I was injected intravenously in a wide range of doses into mice, rats, guinea-pigs and rabbits. Measured blood samples, 0·025 or 0·05 ml., were drawn with a calibrated glass pipette from the retro-orbital venous plexus in mice, rats and guinea-pigs (Halpern and Pacaud, 1951) and from the ear vein in rabbits at standard times after the intravenous injections. The blood samples were spread on round filter paper 2·5 cm. diameter on glass slides and β-radioactivity measured with a Geiger Müller counter. After the animal had been killed and the liver, spleen, lung and kidney had been weighed, the γ-radioactivity of weighed samples of tissues was measured with a scintillating counter keeping the geometry constant. Samples of the injected material were measured in the same conditions.

Methods of estimation of chromium phosphate tagged with ^{32}P

Chromium phosphate containing ^{32}P was prepared by the usual technique by Dr. Noller. The blood clearance of small doses of this colloid was investigated in mice. Chromium phosphate tagged with ^{32}P was measured in blood samples through its β-radiations by the method used for ^{131}I. Chromium phosphate tagged with ^{32}P was measured in the tissues, after hydrolysis of the organs in concentrated NaOH: 98 per cent of the injected radioactivity was found in the liver.

Methods of estimation of small doses of carbon

Blood clearance of a very small dose of carbon was investigated in rats. Carbon suspension C11/1431 a (Gunther Wagner) in doses of 0·5 mg./100 g. was injected into rats intravenously. Estimation in the blood was carried out as follows: blood samples 0·10 ml. each, drawn from the retro-orbital venous plexus with a calibrated pipette washed with heparin were added each to 1·4 ml. of normal saline. The samples were centrifuged to get rid of the cells and the carbon concentration measured in the supernatant electrophotometrically.

RESULTS

Distribution of Injected C.A.^{131}I among the Various Organs

Table I shows the distribution of the injected radioactivity in mice, rats, guinea-pigs and rabbits for various doses of C.A.^{131}I. When very small doses are injected, the rate of clearance is very rapid and the animal can be killed a few min. after the injection. In this case 90 to 100 per cent of the injected radioactivity is detected in the liver and spleen. The liver contains nearly all the injected radioactivity, showing that for very small doses of colloids the phagocytic efficiency of the liver is greater than that of the spleen. When higher doses of C.A.^{131}I are injected the percentage found in the spleen increases as expected from our experience with carbon particles. However, only a fraction of the injected radioactivity can be recovered in the organs analysed; this can be as little as 43 per cent when 5 mg./100 g. of C.A.^{131}I are injected. In the latter experiments, the animals were killed 20–70 min. after injection and these differences are probably due to the rapid metabolism, release into the blood and mainly renal excretion of the C.A.^{131}I. A residual blood radioactivity of 7 per cent of the injected dose can be found.

To substantiate these facts we injected a group of 40 mice with 0·25 mg./100 g. of C.A.^{131}I, a dose nearly completely cleared by the liver in a few minutes. The animals were killed at various times after injection and the radioactivity of the various organs measured as described (Fig. 1). C.A.^{131}I is attacked very rapidly by the liver. From 6 to 30 min. over 50 per cent of the ^{131}I is released from the liver.

Blood clearance of C.A.^{131}I in mice

A group of 85 mice were injected intravenously with various doses of C.A.^{131}I ranging from 0·025 to 10 mg./100 g. Several different preparations were used with similar results. The rate of blood clearance was measured and at the end of the experiments the animals were killed, and the liver and spleen weighed in the wet state. The ratio, W/Wls, of the weights of the liver and spleen to body wt. was calculated.

In Fig. 2 we present typical experiments. For a very small dose of C.A.^{131}I, 0·025 mg./100 g., the blood clearance follows an exponential function with respect to time. The phagocytic index K which is the rate of clearance can therefore be calculated from equation 1 :

$$\frac{\log C_1 - \log C_2}{T_2 - T_1} = K \text{ (decimal logarithm).}$$

When much larger doses of C.A.^{131}I are injected, there remains at the end of the experiment a residual blood radioactivity (Table II), due to the escape of ^{131}I into the blood when it is released from the R.E. cells. When the values of each point of the clearance curve are corrected by subtracting the residual blood radioactivity, the rate of clearance follows also an exponential equation and the phagocytic index K can be calculated (equation 1). This procedure has been followed in the calculation of K when necessary.

TABLE I.—*Distribution of C.A.*^{131}*I after Intravenous Injection in Mouse, Rat, Guinea-pig and Rabbit*

	Dose of C.A.^{131}I.	Interval after injection (minutes).		Liver.	Spleen.	Kidney.	Lung.	Blood.	Total
Mouse	0·25 mg./100 g.	6	Per cent of injected dose	98·0	2·0	0·75	0	—	100·75
			Per cent/g.	77·0	10·0	2·3	—	—	—
	2·5 mg./100 g.	22	Per cent of injected dose	62·0	2·7	2·0	1·2	7·5	75·4
			Per cent/g.	51·5	13·5	7·2	5·3	—	—
Rat	0·25 mg./100 g.	7	Per cent of injected dose	93·0	1·9	1·2	0·1	2·0	98·2
			Per cent/g.	19·5	4·2	1·1	0·1	—	—
	5 mg./100 g.	60	Per cent of injected dose	34·0	1·0	1·1	0·8	7·0	43·9
			Per cent/g.	6·7	2·5	1·0	0·6	—	—
Guinea-pig	0·1 mg./100 g.	6	Per cent of injected dose	91·0	1·0	0·25	1·0	—	93·25
			Per cent/g.	7·9	1·7	0·08	0·3	—	—
	2 mg./100 g.	30	Per cent of injected dose	61·4	1·2	1·5	0·4	5·0	69·5
			Per cent/g.	4·6	3·2	0·34	0·16	—	—
	5 mg./100 g.	70	Per cent of injected dose	36·0	0·8	1·5	0·5	6·0	44·8
			Per cent/g.	2·7	1·6	0·4	0·19	—	—
Rabbit	0·1 mg./ 100 g.	.	Per cent of injected dose	88·0	1·7	0·4	0·3	2·0	92·4
			Per cent/g.	1·14	1·94	0·03	—	—	—

The figures are average values in 3 or 4 animals per dose.

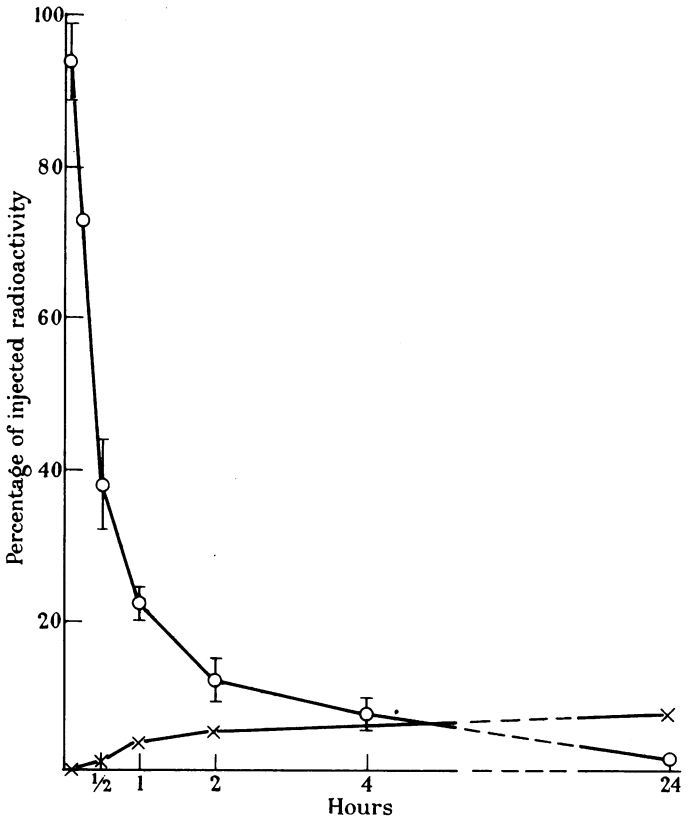

FIG. 1.—Measure of radioactivity of the liver and the thyroid in mice injected with 0·25 mg. per 100 g. of C.A.^{131}I at various times following the injection ○———○ = liver. ×———× = thyroid.

In Table II we present for each dose of C.A.^{131}I the mean phagocytic index K, the residual blood radioactivity, the mean value of W/Wls and the corrected phagocytic index α calculated from equation 2 :

$$\alpha = \frac{W}{Wls}\sqrt[3]{K}$$

TABLE II.—*Blood Clearance of Various Doses of C.A.^{131}I in Mice*

C.A.^{131}I (mg./100 g.).	Number of animals.	Phagocytic index K.	Average residual blood radioactivity per cent injected dose.	$\frac{W}{Wls}$.	Corrected index α.
0·025	5	0·400±0·076	2·5	19·4	—
0·125	5	0·388±0·082	3	16·2	—
0·250	5	0·386±0·110	3	15·9	—
0·500	4	0·285	3	19·8	13·0
1·25	4	0·150	4	18·0	9·5
2·5	39	0·084±0·031	4	18·1	7·7±0·65
5·0	16	0·042±0·016	6·5	18·4	6·5±0·4
10·0	6	0·027±0·019	9	17·3	4·9±0·5

In a previous study of the phagocytic activity of the R.E.S. with carbon particles (Biozzi *et al.*, 1953) we observed that in rats and mice there is a third power relationship between the phagocytic index K and the relative weight of the liver and spleen in the range where the rate of colloid clearance measured by K is inversely proportional to the injected dose. The corrected index α which

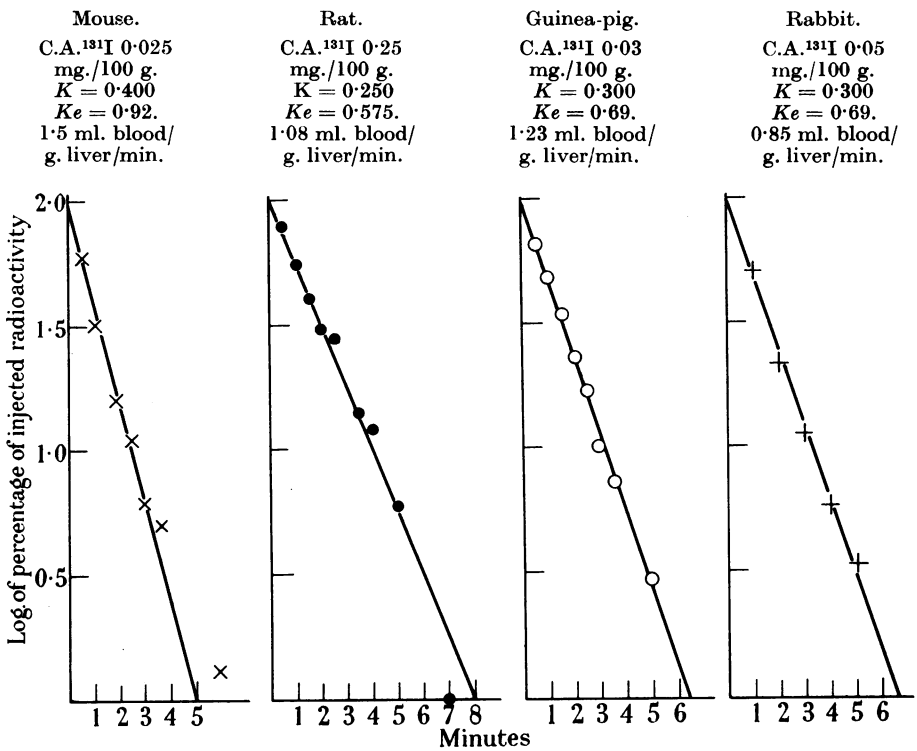

FIG. 2.—Rate of clearance from the blood of small doses of C.A.^{131}I in mouse, rat, guinea-pig and rabbit in the range of dose where the rate is maximum. Measure of " minimum liver blood flow ".

is a measure of tissue phagocytic activity presents much smaller standard deviations than K.

The standard deviations of K and α for doses of C.A^{131}I where $K \times D = ct$ are similar to those reported for carbon particles in mice (Biozzi, Benacerraf, Stiffel and Halpern, 1954). The standard deviations of α are also smaller than those of K. The C.A.^{131}I clearance measures therefore the same phagocytic activity of the R.E.S. as carbon clearance, and the calculation of α from equation 2 effects a real correction. For these reasons we have corrected the observed mean values of K with the help of equation 2, by choosing a standard average value of $W/Wls = 18{\cdot}3$ calculated from all our mice. In Fig. 3 we have plotted the variations of the corrected values of K with respect to the injected dose of

C.A.^{131}I. The data presented show that the rate of clearance of C.A.^{131}I is inversely proportional to the injected dose : $K \times D = ct.$

However there is a critical dose of C.A.^{131}I below which the rate of clearance is no longer a function of the dose and tends to become constant as it reaches its maximum value. In this range the only limiting factor is the blood flow through

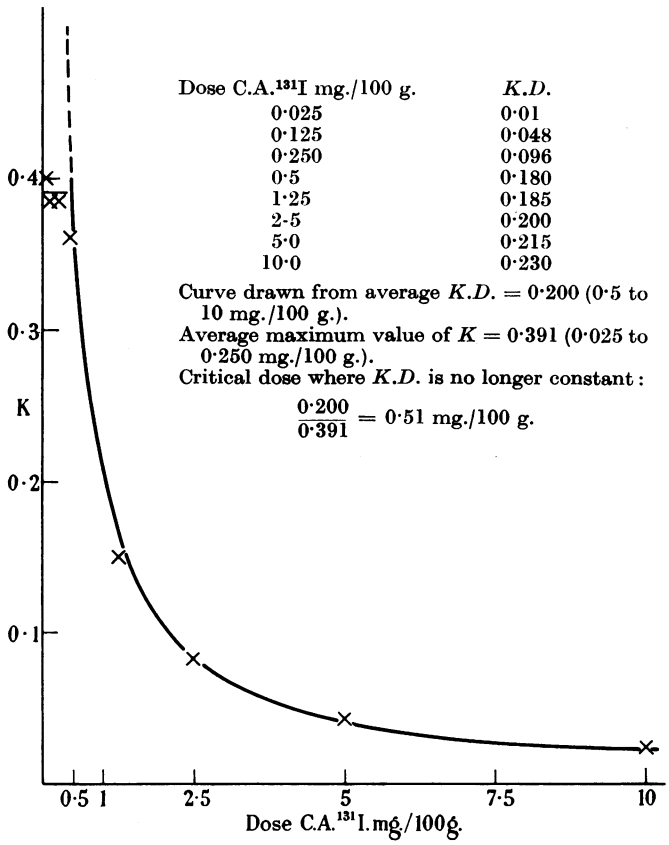

FIG. 3.—Variation of the phagocytic index K in relation with the dose of C. A.^{131}I in mice.

the R.E.S. and principally through the liver. The efficiency of colloid clearance in these organs reaches then a maximum value independent of the dose of colloid injected. The efficiency factor can be measured experimentally as we shall see later. It should be stressed therefore that the colloid clearance test measures the phagocytic activity of the R.E.S., only when a sufficient dose is injected, where $K \times D = ct.$ The limits of this relationship should be established experimentally in each case. The critical R.E. dose can be found in any animal for a given colloid by measuring the maximum rate of clearance, with a very small dose and the rate of clearance in the range where $K \times D = ct$ (Fig. 3).

The critical R.E. dose is then calculated from equation 3 :

$$\text{critical R.E. dose} = \frac{K \times D}{\text{maximum value of } K}$$

Blood clearance of C.A.^{131}I in rats, guinea-pigs and rabbits

The blood clearance of C.A.^{131}I in rats, guinea-pigs and rabbits follows the same laws as in mice (Table III). The rate of clearance is exponential (Fig. 2), the value of K decreases directly with the dose so that $K \times D = ct$. There is a critical R.E. dose also below which the value of K reaches a maximum and no longer changes. The critical R.E. dose of C.A.^{131}I calculated from equation 3 is 0·43 mg./100 g. for the rat, 0·36 mg./100 g. for the guinea-pig and 0·19 mg./100 g. for the rabbit. The value of the critical R.E. dose will vary with the size of the liver and spleen as expected.

TABLE III.—*Blood Clearance of Various Doses of C.A.^{131}I in Rats, Guinea-pigs and Rabbits*

	C.A.^{131}I (mg./100 g.).	Number of animals.	Phagocytic index K.	Average residual radioactivity per cent injected dose.	$K \times D$.
Rats	0·012	3	0·325	1·5	0·0039
	0·05	6	0·341	2·0	0·017
	0·25	6	0·245	2·0	0·061
	0·5	3	0·268	2·0	0·134
	1·0	3	0·107	4·4	0·107
	2·5	5	0·062	5·2	0·155
	5·0	4	0·025	7·0	0·125
Guinea-pigs	0·03	5	0·299	0·0	0·0089
	2·0	4	0·053	5·0	0·106
	5·0	4	0·023	6·3	0·115
Rabbits	0·05	5	0·293	1·5	0·014
	0·10	5	0·280	1·1	0·028
	1·0	4	0·064	—	0·064
	2·5	4	0·018	—	0·045

The Measure of Liver Blood Flow with C.A.^{131}I

Below a critical R.E. dose of C.A.^{131}I, the rate of blood clearance attains a maximum value and the efficiency of clearance in the liver reaches a maximum. This technique can be applied to the measure of blood flow through the liver which for these doses absorbs nearly all the colloid injected.

The application of the colloid clearance test to the measure of liver blood flow has been introduced by Dobson and Jones (1952) with chromium phosphate containing ^{32}P. The principle of the method is as follows. If the liver completely clears the blood of the injected colloid, the specific rate constant Ke calculated from Naperian logarithms and equation 1 measures the fraction of the blood volume passing through the liver per unit time. The volume of blood flowing through the organ will be $Ke \times$ blood volume.

This method measures the true blood flow through the liver only if the efficiency of colloid clearance by the organ is 100 per cent, otherwise it gives a lower value which we call "minimum liver blood flow". This value has to be corrected

for the efficiency factor to measure true liver blood flow. It is essential to know the maximum efficiency of liver clearance of the colloid injected to measure the corrected liver blood flow. Dobson and Jones found an efficiency factor of 79 per cent in mice and 88 per cent in rabbits.

We have used the method of C.A.^{131}I clearance in the range of dosage where the rate of clearance is maximum to measure "minimum liver blood flow" in mice, rats, guinea-pigs and rabbits and we have compared the values observed with those obtained with other colloids. Table IV states the individual values of minimum liver blood flow in mice, measured with C.A^{131}I in the dose range where K is constant, from 0·025 to 0·25 mg./100 g. The values of blood flow are expressed in ml./g. of liver/min. The minimum liver blood flow measured in 15 mice with C.A.^{131}I is 1·3 ± 0·29 ml./g./min. with considerable individual variation.

TABLE IV.—*Blood Clearance of C.A.^{131}I for Doses under 0·5 mg./100 g., where Rate is Maximum. Measure of Minimum Liver Blood Flow in Mice*

Dose C.A.^{131}I (mg./100 g.).	Body wt. (g.).	*Ke* Naperian log.	Liver wt. (g.).	Minimum liver circulation. (ml./g./min.).
0·025	24	1·06	1·150	1·65
	29	0·78	1·400	1·21
	24·5	0·71	1·350	0·97
	24	0·920	1·100	1·5
	26·5	1·12	1·200	1·86
0·125	24·5	1·04	1·400	1·37
	19·5	0·92	1·200	1·12
	24·4	0·76	1·100	1·27
	24·5	0·65	1·150	1·04
	21·0	1·10	1·250	1·39
0·250	24·4	1·15	1·300	1·62
	22·5	1·1	1·100	1·69
	19·0	0·94	1·200	1·09
	24·5	0·65	1·300	0·93
	20·5	0·6	1·150	0·80
Mean values	23·5	0·90	1·22	1·3±0·29

Blood volume value of 1·5 ml./20 g. was assumed in the calculations. Mean value computed from 35 mice.

In Table V the values of minimum liver blood flow in mice calculated from chromium phosphate clearance are presented. The same blood volume 1·5 ml./20 g. was considered. The results are almost identical to those obtained with C.A.^{131}I. The "minimum liver blood flow" measured in 10 mice was 1·2 ml./g./min. Minimum liver blood flow in rats is stated in Table VI, in guinea-pigs and in rabbits in Table VII. The value of minimum liver blood flow was 1·13 ± 0·2 ml./g./min. in rats, in rabbits it was 1·15 ± 0·25 and in guinea-pigs 1·3 ml./g./min. "Minimum liver blood flow" thus differs little in these 4 animal species, and varies more between animals within a single species.

In rats the minimum liver blood flow was also calculated from the values of carbon clearance for the dose of 0·5 mg./100 g. (below the critical R.E. dose for this colloid). The results are identical to those obtained with C.A.^{131}I.

In order to calculate true liver blood flow in mice, rats, guinea-pigs and rabbits, the efficiency of C.A.^{131}I liver clearance has to be measured. Since the values of

Ke in mice for C.A.^{131}I are the same as those for chromium phosphate (Tables IV and V), the efficiency of clearance by the liver must be identical for these two substances in this animal species.

We have considered in mice an efficiency of clearance of C.A.^{131}I of 79 per cent reported by Dobson for chromium phosphate. In the other animal species the efficiency factor was measured as follows: Very small amounts of C.A.^{131}I, 5 to 10 μg. in 0·1 ml. were injected slowly (10 to 20 sec.) into the superior mesenteric vein, so that it would go through the liver before reaching the general circulation. This was done under local anaesthesia. The rate of clearance of C.A.^{131}I was measured. In this case the blood γ-radioactivity was measured in the well

TABLE V.—*Blood Clearance of Small Doses of Chromium Phosphate and Measure of Minimum Liver Blood Flow in Mice*

Dose chromium phosphate (ml./20 g.).	Body wt. (g.).	*Ke* Naperian log.	Liver wt. (g.).	Minimum liver circulation (ml./g./min.).
0·10	23·0	1·72	1·300	2·27
	21·5	0·50	1·100	0·73
	20·0	0·71	1·00	1·06
	19·0	0·62	0·850	1·04
	19·0	0·85	0·900	1·34
	22·0	0·69	1·050	1·08
0·25	24·0	0·76	1·300	1·05
	22·5	0·62	1·300	0·81
	27·5	1·15	1·450	1·63
	21·0	0·71	1·050	1·06
Mean values	21·8	0·833	1·130	1·2

Chromium phosphate 0·5 mg./ml.

TABLE VI.—*Blood Clearance of C.A.^{131}I for Doses under 0·5 mg./100 g. where Rate is Maximum. Measure of Minimum Liver Blood Flow in Rats*

Dose C.A.^{131}I (mg./100 g.).	Body wt. (g.).	*Ke* Naperian log.	Liver wt. (g.).	Minimum liver circulation (ml./g./min.).
0·012	104	0·828	4·500	1·38
	114	0·621	5·000	1·01
	103	0·805	5·000	1·19
0·050	131	0·943	6·200	1·43
	166	0·655	7·900	0·99
	132	0·855	6·300	1·28
	154	0·690	7·100	1·08
	161	1·04	8·300	1·45
	171	0·545	6·500	1·03
0·250	130	0·632	5·800	1·02
	144	0·565	7·100	0·83
	165	0·575	7·300	0·94
	135	0·552	4·750	1·13
	135	0·437	5·500	0·77
	135	0·621	5·200	1·16
Mean values	139	0·691	6·163	1·13±0·2

A blood volume value of 7·2 ml./100 g. was assumed in the calculation of liver blood flow. Mean value computed from 15 rats.

TABLE VII.—*Maximum Blood Clearance of C.A.*^{131}I *and Measure of Minimum Blood Flow in Guinea-pig and Rabbit*

Dose C.A.^{131}I (mg./100 g.).	Body wt. (g.).	*Ke* Naperian logarithm.	Liver wt. (g.).	Blood volume (ml./100 g.).	Minimum liver circulation (ml./g./min.).
Guinea-pig					
0·03	325	0·690	11·15	10·4	2·1
	325	0·747	11·35	5·6	1·2
	332	0·690	15·05	6·4	0·97
	330	0·805	12·40	6	1·28
	310	0·506	10·15	6·75	1·04
Mean values	324	0·687	12·02	7·0	1·3
Rabbit					
0·05	2·500	0·805	101·5	6·1	1·22
	2·100	0·414	65·0	6·7	0·89
	2·320	0·747	81·0	6·1	1·30
	2·920	0·713	96·5	5·6	1·20
	2·350	0·690	113·0	5·9	0·85
0·1	2·500	0·621	85·0	6·0	1·1
	3·250	0·736	116·0	8·3	1·7
	2·500	0·621	81·0	6·1	1·17
	2·300	0·437	65·0	6·7	1·04
	2·350	0·805	113·0	5·9	0·99
Mean values	2·500	0·659	91·7	6·3	1·15±0·25

Individual values of blood volume were considered in the calculation of liver blood flow.

scintillating crystal in order to increase the sensitivity of measurement. The curves were then extrapolated to 0 time. The initial blood concentrations obtained after injection in the mesenteric vein were compared with the concentrations at 0 time had the same dose of C.A.^{131}I been injected in the usual way in the systemic circulation. The ratio of the two concentrations at 0 time gives the efficiency factor of clearance of the colloid by the liver. Care was taken to inject amounts of C.A.^{131}I reaching the liver in a blood concentration where the rate of clearance is maximum.

The efficiency factor varies very little in the 3 animal species studied. The values observed are practically identical with those reported in the literature for chromium phosphate in the live animal by Dobson and Jones (1952) and in the rat liver perfused under physiological conditions by Brauer, Leong, McElroy and Holloway (1956).

TABLE VIII.—*Measure of Corrected Liver Blood Flow in Mice, Rats, Guinea-pigs and Rabbits*

	Minimum liver blood flow (ml./g./min.).	Efficiency factor (per cent).	Corrected liver blood flow (ml./g./min.).
Mouse	1·3	79 (Dobson & Jones)	1·64
Rat	1·13	84	1·35
Guinea-pig	1·3	77	1·68
Rabbit	1·15	82	1·4

In Table VIII the values of corrected liver blood flow for the 4 species studied are presented. Our results show that the amount of blood flowing through the liver per unit wt. varies little in these different species, although the values are somewhat larger in the mouse and the guinea-pig than in the rat and the rabbit.

DISCUSSION

The data reported establish that heat-treated human serum albumin aggregates are phagocytized by the cells of the R.E.S. with a high degree of efficiency, similar to what is observed with unstable preparations of chromium phosphate. Heat denaturation and aggregation of proteins render them readily absorbed by the R.E.S. in spite of the fact that they are soluble at a pH other than their isoelectric point.

The method of preparation and isolation of C.A.^{131}I described yields a product which is phagocytized homogeneously by the cells of the R.E.S. The CA.^{131}I follows all the laws of clearance of homogeneous colloids phagocytized by the cells of the R.E.S which makes it a valuable means of investigating phagocytic activity.

It should be emphasized again that to explore the phagocytic activity of the R.E.S. by the method of colloid clearance, a sufficient dose of the colloid has to be injected in the range where $K \times D = ct$. Below that dose the method is only blood flow-dependent, as only part of the R.E. cells are exposed to the colloid injected.

The use of chromium phosphate (Gabrielli, 1951 ; Heller, 1955) or colloidal gold (Barrow, Tullis and Chambers, 1951) to explore the phagocytic activity of the R.E.S. should therefore be criticized. The results obtained with these methods cannot be considered as a measure of R.E.S. phagocytic activity because doses of these colloids were injected which are cleared from the blood at a rate independent of the dose injected. Chromium phosphate or colloidal gold cannot be injected in the required dosage for R.E.S. exploration because of their instability in the blood and the toxicity of gold. Their particle size moreover cannot be well standardized. There are very few colloids which due to their lack of toxicity, particle size homogeneity and stability can be injected in the required dosage to explore the phagocytic function of the R.E.S. Carbon and C.A.^{131}I are two such colloids. We have used them to explore this function in the same animal species and we have obtained identical results with the two methods (Halpern *et al.*, 1954).

The comparative phagocytic activity of the R.E.S. of the mouse, rat, guinea-pig and rabbit measured by the constant $K \times D$ for each substance is also the same with both substances ; the mouse has the most active R.E.S. and the rabbit the least active.

In this study we offer a new method of measuring the specific cellular phagocytic capacity of the R.E.S. independent of blood flow. We have defined the " critical R.E. dose " as the dose of a colloid phagocytized by the R.E.S. above which $K \times D = ct$ and below which K is maximum. It is a specific measure of cellular activity. The " critical R.E. dose " can be calculated for any animal by measuring both the maximum value of K and a value of K in the range where $K \times D = ct$. When these measures are made on the same animal, the small dose should be injected first, because its saturating effect is negligible on the R.E.S. with respect to the large dose, while the reverse is not true.

The critical R.E. dose varies as expected with the size of the liver and spleen. The critical R.E. dose of C.A.^{131}I in mg./100 g. is 0·51 in the mouse for a $W/Wls = 18{\cdot}3$; 0·43 in the rat for a $W/Wls = 23{\cdot}5$; 0·36 in the guinea-pig for a $W/Wls = 24{\cdot}5$ and 0·19 in the rabbit for a W/Wls = 29. It confirms that the mouse has the most actively phagocytic system, and the rabbit the least, due mainly to differences in the size of the liver and spleen.

Our experience has shown that C.A.^{131}I can be used to measure liver blood flow as efficiently as chromium phosphate and colloidal gold (Vetter, Falkner and Neumayr, 1954). The measure of liver blood flow with C.A.^{131}I has shown that the amount of blood that flows through the liver per unit wt. varies very little in the different animal species studied, and varies more between individuals in a single species. Dobson and Jones, with the chromium phosphate technique, found smaller values of liver blood flow in the rabbit, and slightly higher values in the mouse. The efficiency factors of clearance are however identical for C.A.^{131}I and chromium phosphate in mice, rats and rabbits.

The speed with which C.A.^{131}I is broken down by the R.E.S. is also remarkable. This protein or other protein treated in the same way by heat and properly labelled, lend themselves to the exploration of the metabolic function of the R.E. cells both in the normal subject and under the effect of drugs or disease, due to the efficiency with which they are absorbed by the R.E.S. and metabolized.

SUMMARY

A method is described to prepare, isolate and standardize a complex of heat-denatured human serum albumin (C.A.) which, when injected intravenously, is phagocytized efficiently by the R.E.S. This protein has been labelled with ^{131}I and used to investigate the phagocytic activity of the R.E.S. in various animal species. The R.E. cells phagocytize this substance according to the same laws as apply to other colloidal suspensions. The blood clearance follows an exponential function; the rate of clearance is inversely proportional to the dose of colloid, when sufficient quantity of substance is injected. There is in every animal species a critical dose below which the rate of clearance is maximum and independent of the dose of colloid injected.

C.A.^{131}I can be used to investigate the phagocytic activity of the R.E.S. provided a dose of this protein is injected in the range where $K \times D = ct.$

Below this dose range, the study of C.A.^{131}I blood clearance can be applied to the measure of portal blood flow. A study of portal clearance for these small doses of C.A.^{131}I has been made to calculate the corrected liver blood flow. The values obtained indicate that the liver blood flow per unit weight is very similar in the various animal species studied.

A study was also made of the breakdown of this protein after it has been phagocytized, showing that ^{131}I is very rapidly released from the cells of the R.E.S.

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