THE PHAGOCYTOSIS OF COLLOIDAL PARTICLES OF DIFFERENT SIZES

G. B. D. SCOTT*, H. S. WILLIAMS AND P. M. MARRIOTT

From the Department of Morbid Anatomy, Royal Free Hospital School of Medicine, and the Department of Medical Physics, Royal Free Hospital, London

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DURING experiments involving the possible effects of Trypan Blue on the ability of the reticuloendothelial system of the rabbit to phagocytose radioactive colloidal gold, marked variations in the rate of clearance of identical doses of the latter from the circulation were encountered.

An investigation of this phenomenon, including electronmicrographs of the various samples of the colloid, not only gave information on the relationship between the numerical distribution of the different sizes of particles in individual colloids and the rate of clearance of their radioactivity from the circulation but suggested possible modes of behaviour of the reticuloendothelial system when confronted with particles of different sizes in differing numbers.

While the experimental data presented is limited and the conclusions reached can be only tentative, the implications are considered to be of some importance and to point the way to future studies.

MATERIALS AND METHODS

Male hybrid Dutch rabbits weighing 1–2 kg. were fed on the S.G.I. diet with cabbage and unrestricted water.

The colloidal gold was prepared by the United Kingdom Atomic Energy Authority for therapeutic purposes. Samples of radioactive colloid were mixed with inactive colloid in the proportion of 1 : 10 by weight, only 2 samples of the latter variety being used throughout the experiments.

The rabbit was under ether anaesthesia throughout and the doses of gold were injected into the marginal vein of the right ear. The level of circulating radioactivity was measured by positioning the left ear under a scintillation detector, heavily shielded by lead from the rest of the animal, thus ensuring that the radiation measured was virtually only that emitted by the blood in the ear. The detector was connected to a ratemeter, the output of which was recorded on a potentiometric chart recorder. The counting rate was recorded continuously until a steady background was reached and after subtraction of background, the curves were plotted on semi-logarithmic graph paper. The slope (K) of the line, plotted in this way, represented the fraction of the material cleared per unit time. The technique of continuous recording was easier than taking repeated blood samples and revealed features of the clearance trace which might otherwise have gone undetected.

Electronmicrographs of the various active and inactive colloids were prepared in the following manner. Serial dilutions of a small quantity of the sample concerned were made until the material was sufficiently dilute to allow adequate separation and visualisation of the particles. A drop of the diluted colloid was placed on a small circle of plastic film (Formar, polyvinyl formal), already mounted on an electron microscope grid, and allowed to dry, a process which did not result in aggregation of particles. Electronmicrographs were taken

* Address until July 1968: Dept of Pathology, The Medical College of Virginia, Richmond, Virginia, U.S.A.

and enlarged to × 100,000. The diameters of 500 separate particles were measured, using a × 6 magnifier with a built-in millimetre scale, and the resultant measurements were converted to Ångström units by means of a nomogram (Kay, 1965).

RESULTS

The clearance traces were similar to those illustrated by Meredith (1961), a rise in circulating radioactivity, consequent on the injection of the colloid, being followed immediately by a rapid fall, the curve of the trace becoming flatter as phagocytosis proceeded and background was approached.

Doses of 60 mg. and 100 mg. of a preparation of colloidal gold with a mean particle diameter of 350 Å produced clearance rates of 0·24 and 0·13 min.$^{-1}$ respectively, but since doses of 100 mg. were often lethal in rabbits, use of this particular preparation in the original experiment was discontinued.

Colloidal gold of smaller particle diameter, stabilised in 3·5 per cent gelatin, was chosen and injected in doses of 0·8 mg. per 100 g. of body weight, thereby involving doses of from 9·0–15·0 mg.

Ten radioactive samples of this variety of colloid were tested and, when mixed individually with one or other of the two batches of the corresponding inactive colloid in the proportions already described, 5 gave clearance slopes of value ranging from 0·08–0·15 min.$^{-1}$. The other 5 were cleared from the circulation faster, giving slope values of from 0·25–0·5 min.$^{-1}$. All samples were tested in 3 or more rabbits and each behaved similarly throughout.

Because of these variations in clearance rates electronmicrographs of three of the 5 samples of radioactive colloid giving clearance rates of from 0·08–0·15 min.$^{-1}$ and of both batches of inactive colloid were prepared. All five of the radioactive samples producing the higher clearance rates were examined in this way.

Measurement of the diameters of 500 particles of those samples of radioactive material resulting in the lower range of clearance rates as well as the two inactive colloids showed the peak of the distribution curves to occur at 40 Å. The curves were asymmetrical, smaller numbers of larger particles being present, the largest diameters varying from 110–190 Å (Fig. 1).

Examination of the 5 radioactive colloids giving the higher clearance rates showed that 2 had particle distribution curves within the range already described (Fig. 1). In 2 others, while the peak of the curve still occurred at 40 Å, small numbers of much larger particles, with diameters up to 270 Å, were present (Fig. 2A). The 5th sample contained virtually 2 separate populations of particles, a larger one, the commonest diameter of which was 50 Å, and a smaller one containing particles of diameters from 170–300 Å (Fig. 2B).

When their clearance traces were plotted on semi-logarithmic graph paper, seven of the 10 samples of radioactive colloid gave slopes which were straight lines over the significant part of the curve except close to the background level of radioactivity, where small variations in the estimation of the latter could have a marked effect on the slope.

Straight line slopes were produced by all radioactive samples giving low slope values (0·08–0·15 min.$^{-1}$) as well as by 2 of the 5 giving the higher ones (Fig. 2A), while those given by the very large doses of the colloid of 350 Å mean particle diameter were of this type. The 3 other samples giving the higher slope values produced traces which were angulated when plotted, a fast first component being followed by a considerably slower one, the alteration in clearance rate occurring when the level of circulating radioactivity was still high enough to exclude the possibility of errors in plotting due to the immediate proximity of the background (Fig. 3A).

Two of these last 3 radioactive colloids had particle diameter distribution curves within the range illustrated in Fig. 1, while the third was the sample containing the two populations of particles (Fig. 2B) and like the rate of clearance, the angulation of the slopes was repeated in successive animals.

Administration of twice the standard dose (1·6 mg./100 g. body weight) of 2 of the radioactive-inactive mixtures giving angulated slopes resulted in straight line

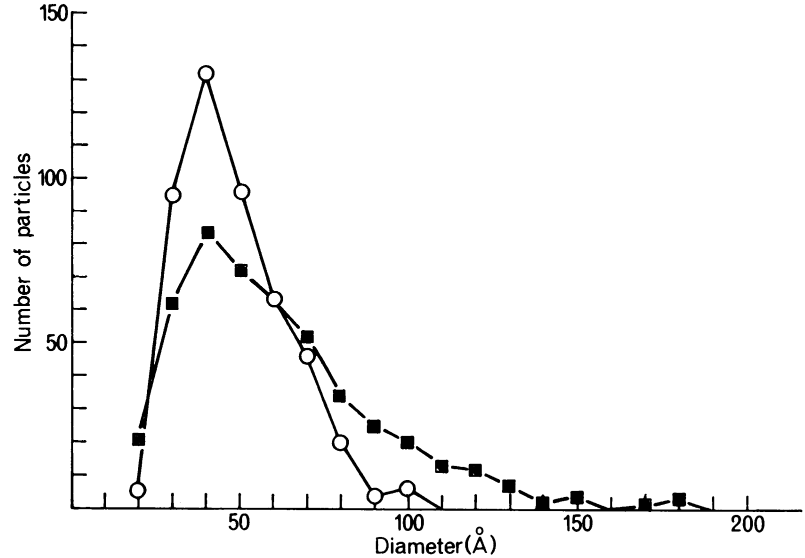

FIG. 1.—Range of particle diameter distribution curves of inactive colloidal gold, 5 radioactive colloids giving lower slope values (0·08–0·15 min.$^{-1}$) and straight line clearance slope and 2 giving higher values (0·20–0·50 min.$^{-1}$) and angulated clearance slopes.

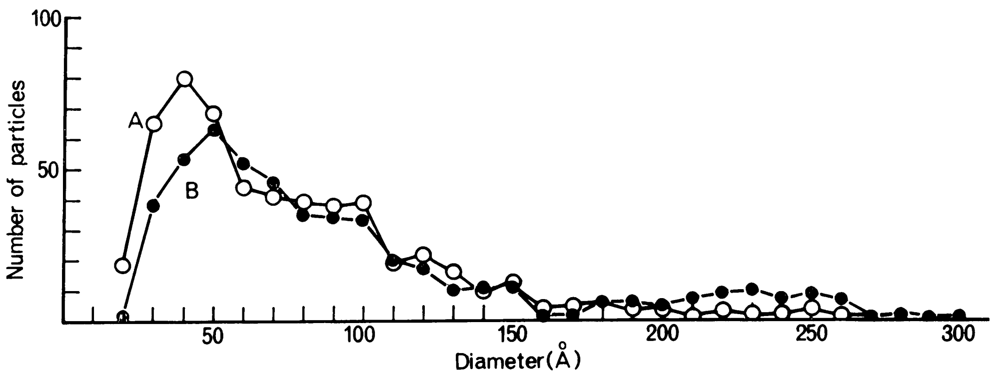

FIG. 2.—Particle diameter distribution curves of 2 radioactive colloids giving higher clearance rates. (A) gave a straight clearance slope, (B) an angulated one.

slopes of value (Fig. 3B) approximately equal to that of the second and slower component of the angulated variety (Fig. 3A).

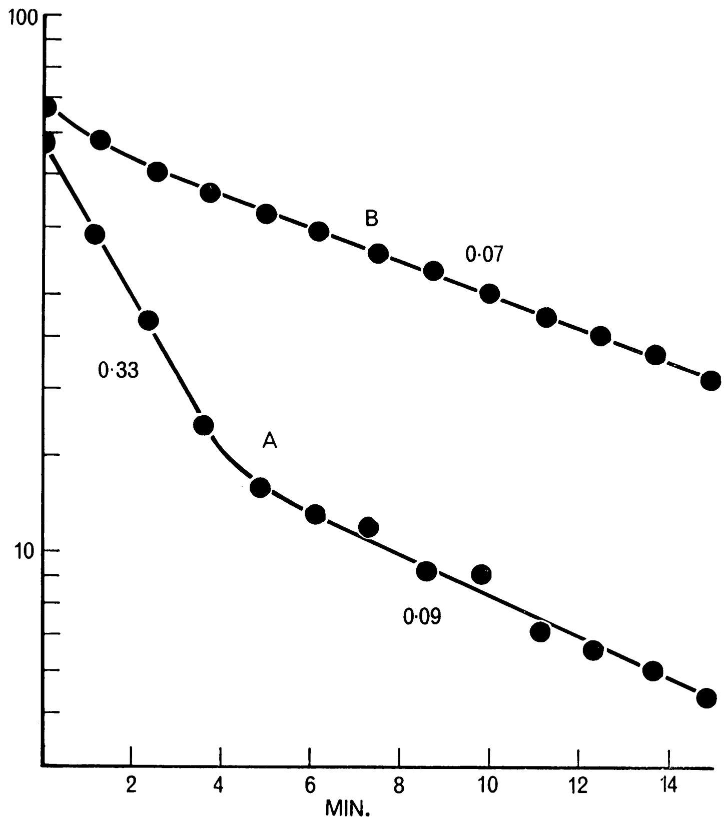

FIG. 3.—(A) An angulated slope produced with dose of 0·8 mg. of radioactive-inactive colloid gold mixture per 100 g. body weight. (B) Straight line slope produced by doubling the dose.

DISCUSSION

The preparation of electronmicrographs of the samples of radioactive and inactive colloidal gold allowed the numerical distribution of the various diameters of particles in the colloid to be determined and enabled the rate of clearance of the colloid from the circulation to be studied in relation to this rather than to the mean or commonest particle diameter only.

Since a standard mixture of radioactive and inactive colloid was used throughout, the rate of clearance was that of the radioactive component as influenced by that of the inactive. Only 2 batches of inactive colloid were used throughout these experiments and the faster clearance rates occurred with both. Thus any difference between the numerical distribution of the diameters of their particles did not have any significant effect on their rate of clearance. Differences in clearance rate were therefore due to differences in the radioactive colloid.

Using radioactive colloids with diameter distribution curves as illustrated in Fig. 1, injection of 0·8 mg. of this radioactive-inactive mixture per 100 g. of body weight usually gave clearance slopes of values varying from 0·08–0·15 $min.^{-1}$. The volumes of the largest individual particles present in these radioactive colloids (Fig. 1) ranged from 21–107 times those with a diameter of only 40 Å. Since a particle of 190 Å in diameter is equivalent in volume to 107 of those with one of 40 Å, a very high proportion of the total mass of the colloid resided in a relatively small number of large particles. Conversely, the removal of one such particle from the circulation would result in a massive reduction in the circulating radioactivity.

This would explain the faster rates of clearance, given by those colloids containing particles of up to 300 Å in diameter (Fig. 2). An even higher proportion of the total mass resides in these very large particles and the removal of one of the largest particles, with a volume 216 times that of one with a diameter of 50 Å, would represent an even greater reduction in the level of circulating radioactivity.

These observations illustrate the intimate relationship which can exist between the numerical distribution of the various sizes of particles in a radioactive colloid and the rate of clearance of its radioactivity from the circulation.

The majority of clearance traces, including some given by colloids containing very large particles, resulted in straight lines when plotted, showing that the rate of clearance was not altering during the period of clearance. This points to the reticuloendothelial system phagocytosing the largest and the smallest particles in constant proportions.

The angulated clearance slopes given by some colloids show that in these instances the rate of clearance was decreasing, a phenomenon which could be explained on the grounds that the reticuloendothelial system was tending, initially, to phagocytose the larger particles in preference to the smaller ones.

Using the colloid with a mean particle diameter of 350 Å, straight line slopes were obtained with doses 5–7 times greater than those given using the colloid of smaller particles. Therefore, progressive saturation is probably not the explanation for the angulation of the clearance slopes, since even these massive doses were not saturating the system during the period of their removal from the circulation.

The reproducibility of angulated slopes in successive rabbits receiving identical doses of the same batch of radioactive-inactive mixture suggests that the factor responsible resides in the colloid itself. It is interesting that, of the radioactive colloids with particle diameter distribution curves of the type illustrated in Fig. 1, the only two to be cleared at the higher rate, gave angulated slopes.

Since angulation of the slope could be eliminated by doubling the weight of gold and therefore the number of particles injected (Fig. 3), selective phagocytosis of particles, according to their size, may depend on the number of particles to which the reticuloendothelial system is exposed. Koenig, Heyssel, Melly and Rogers (1965) also obtained two component clearance slopes but found that the prior injection of increasing quantities of gelatin converted these slopes into single component slopes with lower K values. This suggests that selective phagocytosis may be inhibited also by the presence of factors in the blood.

If selective phagocytosis be the explanation for the angulated clearance slopes, these experiments show that the reticuloendothelial system, particularly the Kupffer cells of the liver, will, under certain circumstances, distinguish between particles whose volumes differ by a factor of only 64 and under other circumstances treat particles, whose volumes differ by a factor of 370 and over, as if they are the same size.

Since presumably particles must come into physical contact with the cells of the reticuloendothelial system, prior to being phagocytosed, it would be more realistic to express this in terms of surface area rather than of volume. Thus the cells of the reticuloendothelial system may be capable of distinguishing between particles whose surface areas differ by a factor of 14 but incapable of distinguishing between those differing by one of 36 according to the prevailing conditions.

SUMMARY

The use of electronmicrographs in an investigation of differences in the rate of clearance of identical doses of radioactive colloidal gold from the circulation of rabbits, enabled the relationship between the distribution of the different sizes of particles in individual colloids and the rate of clearance of the radioactivity to be studied. Data suggested that under certain circumstances the reticuloendothelial system could perform selective phagocytosis according to the size of the particle.

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