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OBSERVATIONS ON THE PASSAGE OF A COLLOID FROM CEREBROSPINAL FLUID TO BLOOD AND TISSUES

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The permeability of the membranes separating blood and cerebrospinal fluid has been the subject of many investigations. In a common type of experiment, introduction of a substance into one of these fluids is followed, after appropriate intervals, by a determination of its concentration in the other. When an injection is made into the subarachnoid space, it is clear that the results of blood analysis will depend not only upon the rate of passage of the test material through the membranes, but also upon the rate of its removal from the blood. Injection of substances into the vascular system also involves this factor.

The behaviour of the material in the blood is of particular importance when foreign colloids are studied, since here there is likely to be a slow passage through the membranes associated with a rapid removal from the blood. This communication is a short survey of such a problem.

Colloidal palladium was used in this work ; it was selected because a stable and reasonably homogeneous suspension could be prepared. Colloidal palladium has seldom been referred to in biological literature, but brief references to it have been made by Rebière (1908), Neuberg, Caspari, and Löhe (1912), Duhamel (1919). Courmont and Dufourt (1913), and Goffin and Goffin (1922). The *Extra Pharmacopoeia* (1932) mentions " Pallamine," a colloidal palladium.

MATERIALS

Preparation of the palladium.—Samples of Pd^{109} were obtained from the Harwell pile by irradiation for one week of a number of 300 mg. samples of palladium sponge. In the maximum neutron flux available, the calculated activity was 420 mc./g. $\pm$ 20 per cent at the end of the irradiation period.

Palladium was separated from the silver impurity by one of two methods. The sponge was dissolved in 5 c.c. of warm aqua-regia containing between 16 and 20 mg. $AgNO_3$ to serve as carrier. In the first method the solution was diluted to 0.5 litre with distilled water and the palladium precipitated with dimethyl glyoxime and filtered off. After being washed, the precipitate was dried, when it separated from the filter paper as a thin wafer which was again dissolved in a small volume of aqua-regia. The solution was heated and concentrated hydrochloric acid added drop by drop until nitrogen peroxide ceased to be evolved. The mixture was next evaporated to near-dryness, then diluted to 50 c.c. with distilled water and centrifuged, after which a sample of the solution was

examined microscopically in order to ensure the absence of gross particulate matter; 0.5 N-NaOH was titrated against a small sample of the solution and the end-point determined either with litmus or by the appearance of a persistent brown precipitate with a clear supernatant.

In the second and more rapid method, advantage was taken of the solubility of AgCl in concentrated HCl. The sponge and carrier were dissolved as before and concentrated HCl added to remove the nitric acid. The solution was evaporated almost to dryness and then diluted to 50 c.c. with distilled water. Much of the AgCl was then precipitated and removed by centrifugation. The evaporation and dilution were repeated with further carrier.

Preparation of the colloid.—The method was essentially that of Paal and Amberger (1904). The colloid was dialysed against running tap-water for ten hours. Occasionally a few brown flakes were seen in the colloid, but these were absent after dialysis.

Concentration of colloid.—The colloid was concentrated under reduced pressure, small plugs of glass wool, separated by drilled baffles of perspex, being arranged in the neck of the flask to reduce the frothing frequently attending this operation. The colloid, now some 20 c.c., was centrifuged for one hour at 1,300 g. There was usually about 0.1 c.c. of sludge at the bottom of the cups and this was discarded. The colloid was dense black in bulk, whereas films appeared dark brown, and the final concentration was usually a little less than 1 per cent Pd. Small samples were set aside for gravimetric assay and electron microscopy. Non-sterilized samples stored for 12 months have shown very little precipitate.

Size of particles.—Electron microscopy of a typical colloid showed a distribution of particle diameters as follows: 54%, 200A; 15%, 300A; 15%, 100A; 11%, 400A; 5%, 500A. In those samples which had not been centrifuged a few particles of about 1 μ were seen. In all samples examined there appeared to be a number of particles of less than 100A, but these were too small for adequate resolution by the electron microscope available.

Stability and dialysable fractions.—Aggregates of particles were not seen microscopically in samples of circulating blood and very few after incubation with whole blood, plasma, or cerebrospinal fluid. The colloid was able to pass a cellophane membrane in amounts which were usually below the limits of accurate assay.

RESULTS

Blood transport.—From naked eye and microscopical studies it appeared probable that the colloid was carried mainly in the plasma during the first hour at least. This was supported by a series of blood analyses after intravenous injection of the colloid; it was found that the concentration of the colloid in whole blood agreed closely with that obtained for plasma after the appropriate corrections for packed cell volume had been made.

Toxicity of colloid.—It was frequently noted that the colloid could be given intravenously without marked change in blood pressure or respiratory rhythm. With large doses a change in respiratory rhythm and a fall in blood pressure were seen, but successive similar doses administered at the same rate produced effects which were usually less than those produced by the initial dose (Fig. 1). The changes indicated are not entirely due to the protective agent.

It was found that reasonably steady records of cerebrospinal fluid pressure could be obtained under pentobarbitone anaesthesia (40 mg./kg. intraperitoneally)

and that, after an appropriate volume of cerebrospinal fluid had been allowed to escape, the colloid could be introduced into the subarachnoid space without any subsequent gross fluctuation of the cerebrospinal fluid pressure. Further, the presence of the colloid did not prevent those changes in the cerebrospinal fluid pressure which are produced by the introduction of hypertonic fluids into the stomach.

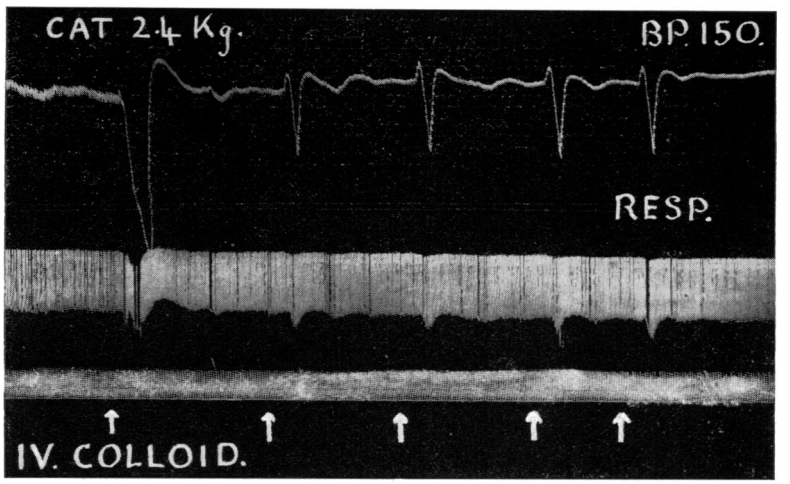

FIG. 1.—The effects upon blood pressure and respiratory rhythm of repeated intravenous injections of 6 c.c. of colloidal palladium containing 6.86 mg. Pd/c.c. The injections were made at the arrows. (Time mark at 5 sec. intervals.)

Assay procedures.—In all estimations of radioactive material a 10 c.c. solution counter was used with an Sc 200A Dynatron scaling unit. The usual corrections were made and also density corrections in the counting rates obtained from those samples which had been digested in sulphuric and nitric acids. Tissues were assayed for Pd after first digesting them in sulphuric and nitric acids in Kjeldahl flasks.

In a typical experiment, designed to investigate the accuracy of the assay procedures, a number of gravimetric assays were performed on a large bulk of radioactive colloid and appropriate dilutions of this digested with liver slices. In ten separate radioactive estimations no value obtained differed from the gravimetric assays by more than 2 per cent, when the concentration of palladium determined by radioactive methods was less than 1 μg./c.c.

Rate of removal of the colloid from the blood.—Cats were used in all animal experiments. They were anaesthetized with pentobarbitone (40 mg./kg. intraperitoneally) and various doses of the colloid were introduced by direct injection into the femoral or saphenous vein. Blood samples were taken from the femoral or carotid artery at intervals during a period of about one hour and assayed for palladium. A total of 10–12 c.c. of blood was taken during each experiment and solid heparin or sodium citrate crystals were used as anticoagulants. The amounts of palladium injected varied between 3.9 μg./kg. and 2.8 mg./kg. The results

(Fig. 2) indicate that over this range the blood level fell, during the first thirty minutes of the experiments, to between 48 per cent and 4 per cent of that observed one minute after the end of the injection.

It was clear that there were considerable differences in the rates of removal of colloidal palladium from the blood, but, on the whole, large doses were cleared more slowly than small doses. The influence of the size of the dose on the rate of its removal from the blood was investigated in two cats by administering a large dose (1.92 mg./kg.) to one of them and a small dose (0.59 mg./kg.) to the other. On the next day, the cat which had received the large dose was given a small one (0.48 mg./kg.), and the cat which had received the small dose was given a large one (2.36 mg./kg.). In each cat the larger dose was removed from the blood more slowly than the smaller dose (Fig. 2), and at the end of the first day the smaller dose

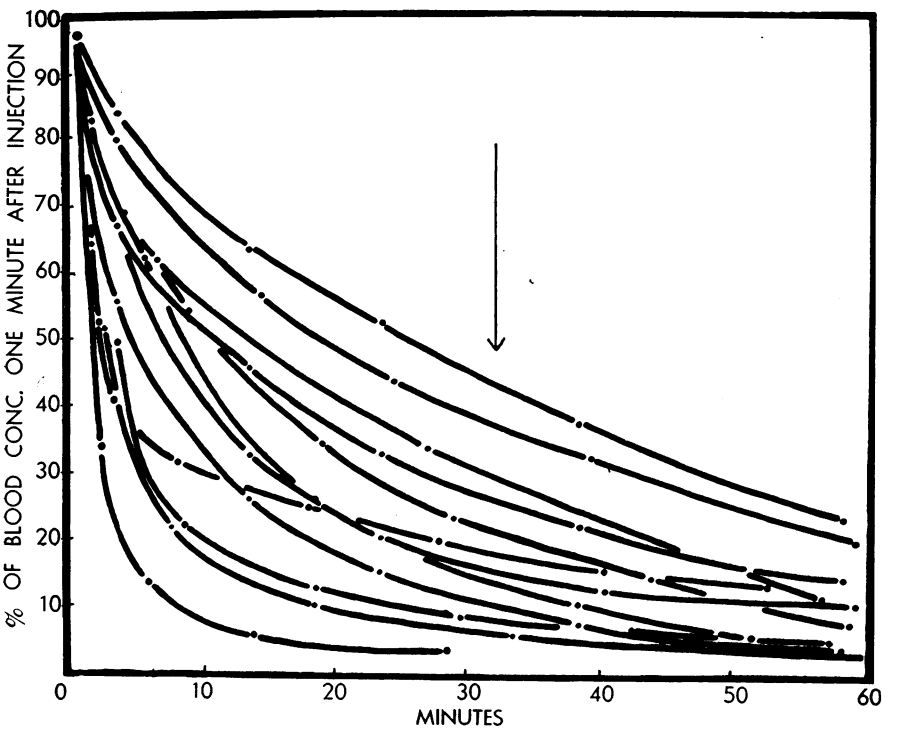

FIG. 2.—The fall in concentration of palladium in the blood of cats after intravenous injection of various doses of colloidal palladium. Following the line of the arrow, the dose (mg./kg.) corresponding to each curve from above downwards is 2.36, 1.92, 2.82, 1.41, 1.34, 2.08, 0.48, 1.03, 0.59, 1.74, 0.22, 0.004.

was practically cleared, while a measurable concentration of the larger dose remained in the blood. The lowest curve (Fig. 2) indicates the limit of assay with the particular technique employed; with the radioactivity of the palladium at its maximum, the lowest blood concentration which could be satisfactorily determined was 0.0013 μg./c.c.

Fate of intravenous colloid.—Twelve experiments were performed. The animals received intravenous doses of colloid containing between 50.2 and 0.01 mg. of palladium. Samples of arterial blood were taken one minute after injection and at the end of the experiment. The animals were killed by opening the chest, and ten minutes later samples of lung, kidney, liver, spleen, and brain were taken for palladium assay. In the earlier experiments lung was not sampled. As far as possible, specimens were removed from similar sites in a particular organ. Table I indicates that on the whole the fall in blood level was more rapid with smaller doses, though here, as in Fig. 2, there are some anomalous results. Samples of cisternal cerebrospinal fluid were usually obtained by cisternal puncture at the end of the experiment, but the concentration of palladium was usually far below the limits of assay.

TABLE I

DISTRIBUTION OF PALLADIUM IN THE TISSUES OF CATS AFTER INTRAVENOUS INJECTIONS OF COLLOIDAL PALLADIUM

Wt. of cat (kg.)	Total dose (mg.)	Duration of exp. (min.)	Fall of concn. in blood %	% of total dose/g. of tissue					% of dose in urine
				Lung	Spleen	Liver	Kidney	Brain	
3.5	50.2	64	70	1.3	0.41	0.44	0.019	0.0012	1.6
3.6	36.6	75	80	0.52	0.38	0.51	0.018	Trace	0.97
3	24.4	69	85	2.04	0.74	0.41	0.015	0.0016	1.07
2.5	15.2	60	74	2.4	0.76	0.58	0.036	0.0017	0.98
2.2	10.0	62	88	1.7	0.63	0.70	0.022	0.0012	0.82
3.8	10.0	61	87	1.5	0.34	0.45	0.013	0.0006	0.88
1.5	7.6	60	85	3.9	1.9	0.79	0.043	0.0023	0.84
1.9	6.1	65	89	No sample	0.66	0.79	0.019	Trace	1.04
2.5	6.1	73	86	No sample	0.70	0.80	0.019	0.0045	Blood-stained 0.12
3	5.0	64	94	1.05	0.34	0.510	0.034	Trace	1.21
2.5	2.5	60	93	1.2	0.72	0.52	0.048	0.0012	1.3
2.6	0.01	60	96	4.2	0.86	0.90	Trace	Trace	0.58

It appears from these results that the lung contained a larger quantity of palladium per unit weight than any of the other tissues examined. The liver or spleen occupied second place and the total excretion of colloid in the urine was about 1 per cent.

Passage from subarachnoid space to blood.—The vertebral laminae at the root of the tail were removed and a polythene cannula of 1 mm. bore was introduced into the subarachnoid space and tied in position with two spaced silk ligatures. Cerebrospinal fluid was allowed to drip from the cannula and the volume replaced with colloid administered by means of a burette. The burette was adjusted so that the level of colloid within it was never more than 16 cm. above the sacral wound. Colloid was allowed to run in for various periods of time and the volumes introduced varied between 0.7 and 1.9 c.c. The animals were prone throughout the experiment. Blood samples were taken at intervals as before, but it was found that large volumes were required in order to detect the small quantities of circulating colloid. A total of 15–20 c.c. of blood was removed from some animals during the

course of an experiment. Fig. 3 shows the results obtained. There was a considerable variation in the rate of rise of blood concentration and this did not appear to depend either on the total dose administered or upon the volume of colloid introduced.

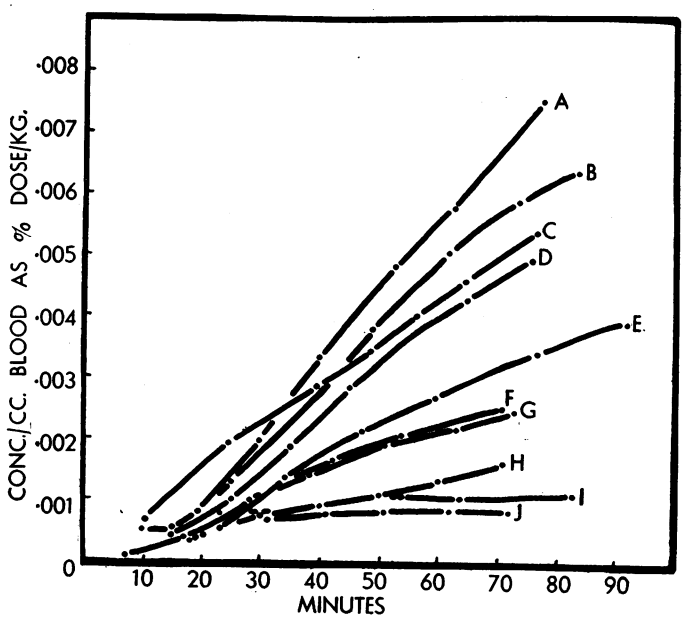

FIG. 3.—The rise in concentration of palladium in the blood after the introduction of various doses of colloidal palladium into the subarachnoid space of cats. Doses in mg./kg. are: A, 1.27; B, 2.82; C, 2.06; D, 1.59; E, 0.74; F, 2.54; G, 3.04; H, 4.36; I, 1.72; J, 1.01.

Tissue distribution after subarachnoid injection.—At the end of the experiments described above, some of the animals were killed and the tissues assayed for palladium. Table II shows that the lungs, as after intravenous injection, contained more palladium per unit weight of tissue than the spleen, liver, or brain. In this series, however, the kidney often contained more than the lung. The brain as a rule

TABLE II

DISTRIBUTION OF PALLADIUM IN THE TISSUES OF CATS AFTER SUBARACHNOID INJECTION OF COLLOIDAL PALLADIUM

Wt. of cat (kg.)	Dose (mg.)	Length of exp. (min.)	Blood concn. at end of exp. μg./c.c.	% of total dose/g. of tissue					% of dose in urine
				Lungs	Spleen	Liver	Kidney	Brain	
3.3	14.4	71	0.074	0.0034	0.0014	0.0014	0.0013	0.0031	0.081
3	7.6	71	0.066	0.0027	0.0014	0.0014	0.0037	Trace	0.093
2.5	7.6	73	0.077	0.0014	No sample	0.00062	0.0042	Trace	0.067
2.5	7.0	84	0.18	0.0036	0.0021	0.0024	0.0074	Trace	0.26
2.6	4.48	84	0.020	0.0091	0.0024	0.0039	0.0013	Trace	0.066
2.8	4.38	78	0.082	0.0023	0.0018	0.0014	0.0050	0.014	0.28
2.7	2.00	92	0.029	0.011	0.0065	0.0042	0.015	Trace	0.056

held such small amounts of palladium that assay could not be satisfactorily performed. In two brain samples, where assay appeared possible, the results obtained were probably due to contamination from the colloid introduced into the spinal subarachnoid space.

Discussion

The present studies indicate that colloidal palladium, with the characteristics described, is rapidly removed from the blood stream of the cat, and that the lungs collect a higher concentration of palladium than any of the other tissues examined, with liver or spleen in the second place. These findings are in accord with those of Drinker and Shaw (1921) and Lund, Shaw, and Drinker (1921), who worked with an acacia-protected colloidal manganese dioxide. These workers also noted that the large initial trapping of colloid by the lungs seemed peculiar to the cat and that even in this animal the lungs subsequently lost much of their colloid to the liver.

The rapid removal of particulate matter from the blood associated with deposition in the liver has been demonstrated for a number of colloids such as yttrium, zirconium, and columbium (Dobson *et al.*, 1949), gold (Sheppard *et al.*, 1951), manganese (Sheppard *et al.*, 1947), chromic phosphate (Jones *et al.*, 1944), and certain brominated dyes (Moore *et al.*, 1943), though with these much dye was also found in the faeces.

Drinker and Shaw (1921) also noted that large doses of colloid usually persisted longer in the blood than small doses, though sometimes a small dose was slowly removed. These anomalies they attributed to some peculiarity in the animal. These observations have been confirmed by the author using colloidal palladium. Dobson *et al.* (1949), from their work with mice, rats, and rabbits, considered that particle size in their colloids governed not only the rate of removal of such material from the blood but also its distribution among the tissues. These conclusions were not supported by Sheppard *et al.* (1951) in their studies with gold sols, though few experiments were reported and the particle size range was apparently small.

In the present research unpredictable variations in the rate of blood clearance were recorded, even with samples of the same preparation of colloid, so that differences in particle size could not be the major factor.

Few of the present results show an " exponential " removal from the blood of the type suggested by Sheppard *et al.* (1947 and 1951) for colloidal gold under " proper " conditions.

It is clear that accurate representation of the rate of removal of particulate matter from the blood presents difficulties. These arise in part from errors made in recording the time of sampling during the period of most rapid removal, and in part from the assumption of a time at which a uniform distribution of the material in the blood occurs.

It is felt that a detailed consideration of the results obtained after subarachnoid injection can serve no useful purpose at the present time, since, of the many variables involved, some have been inadequately controlled or present difficulties in measurement. In this series of experiments it appears that the colloid passed from the subarachnoid space into the blood. The resulting blood concentrations showed

some variation, attaining a maximum of 0.0077 per cent of the injected dose per kg. in 78 minutes.

A comparison of the relative tissue concentrations after intravenous and subarachnoid injections reveals no striking differences except in the kidney. Here after intravenous injection the concentration was 1/100th to 1/50th that in the lung, but after subarachnoid injection both tissues held comparable concentrations.

SUMMARY

1. Details of the preparation of a radioactive palladium colloid are given and some of its properties are described.

2. After intravenous injection of the colloid, the rate of fall of the concentration of palladium in the blood depended partly upon the size of the dose.

3. After subarachnoid injection a small and variable concentration of colloid appeared in the blood.

4. Tissue assays after injection by either route showed that the lungs held a higher concentration than any other tissue examined except the kidney, which, after a subarachnoid dose, showed a concentration comparable with that in the lung.

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