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THE TRANSPORT OF BERYLLIUM IN RAT BLOOD

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Abstract—$BeSO_4$ injected i.v. into rats is transported in the plasma in two forms. These have been studied, using ^{7}Be as a tracer, by ultracentrifugation, gel filtration and ion exchange chromatography techniques. Some of the rats were also given ^{32}P. It was found that, while a small part of the dose remained of small molecular size, most of the $BeSO_4$ was converted to aggregates of beryllium phosphate. These aggregates were bound to plasma globulin, most probably α-globulin. The molecular proportions of these aggregates could not be determined.

THE TOXIC effects of beryllium are varied and complex and, to a large extent depend on the route of entry.[5, 13, 23, 24] When given by mouth $BeSO_4$ can lead to phosphate-deficiency rickets whereas when given i.v., it can produce 'mid-zonal' necrosis of the liver.[2, 19] Different chemical forms of beryllium can produce different effects even when given by the same route.[8] The fate of soluble beryllium salts also depends on the dose. The smaller the quantity injected the greater the fraction excreted or taken up by bone so that the injection of carrier-free ^{7}Be does not reproduce the distribution of toxic doses of beryllium.[20] Although it is not yet known how beryllium produces its toxic effects within cells[1] much of what happens after the intravenous injection of different doses of $BeSO_4$ must depend on the way beryllium is transported in the circulation.

It is generally agreed that after injection the beryllium in the blood stream is in two forms—diffusible and non-diffusible.[14] The diffusible fraction is thought to be determined by the small amounts of organic acids, such as citrate, in the plasma.[10] The particulate matter formed after the i.v. injection of $BeSO_4$ was considered by Cheng[8] to be beryllium phosphate and the outstanding question is whether or not this, or some other fraction of the dose, is bound to the plasma proteins.[2, 4, 10, 18] Our experiments were designed to answer this question and evidence for the presence of circulating protein-bound beryllium will be presented.

MATERIALS AND METHODS

Female albino rats of the Porton strain weighing between 190 and 220 g and fed on M.R.C. diet 4 lb[6] were used. Doses of $BeSO_4$ (A.R., British Drug Houses, Poole, Dorset) are expressed in terms of Be. They were injected into a tail vein or into a jugular vein via a polythene cannula inserted under ether anaesthesia. The i.v. LD_{50} was determined with groups of 4 rats.[25] The $BeSO_4$ solutions were made radioactive

by the addition of carrier-free $^{7}BeCl_2$ (Radiochemical Centre, Amersham, Bucks.). Samples of blood were collected from the abdominal aorta under ether anaesthesia 1·5–16 min after i.v. injection of $BeSO_4$. Serum or plasma (anticoagulant—heparin) was prepared by centrifuging the blood at 1800 *g* for 25 min. In some experiments the rats were injected i.p. with 25 μc ^{32}P/100 g body wt. 20 min before the injection of the $BeSO_4$. The ^{32}P was given as sodium phosphate (^{32}P) injection B.P. (Radiochemical Centre, Amersham, Bucks).

The effect of $BeSO_4$ on the disappearance of injected ^{32}P from the circulation was studied in rats in which the left jugular vein had been cannulated with polythene tubing (i.d. 0·4 mm) under ether anaesthesia 2–5 days before the experiments.[12] On the day of the experiment the rats were given 75 i.u. heparin/100 g body wt. via the cannula 10 min–2 hr before the start. Radioactive phosphate (approx. 25 μc/100 g body wt.) was injected into a tail vein. Blood samples were taken in one of two ways. In some experiments 0·1 ml samples were withdrawn from the cannula as described by Ashby, Heath and Stoner,[3] clearing the dead space before each sample. In other experiments the dead space was cleared with a syringe before the first sample and before the sample immediately following the injection of $BeSO_4$. The capillary pipette of Ashby *et al.*[3] was again used and columnar flow in it was assumed. About 0·07 ml blood was sucked into the tube and two drops of blood from the needle were discharged into a tared tube containing the protein precipitant. In this way a sample of about 0·02 g was obtained from the blood which had entered the pipette at the end of the withdrawal. These blood samples were added to 0·9 ml of either 5 or 3% trichloroacetic acid and the protein precipitate separated by centrifugation. The radioactivity of the samples was extracted by the trichloroacetic acid and measured in the supernatant as described below. The water content of the blood was assumed to be 82 per cent.

Ultracentrifugation. Serum or plasma samples were subjected to 138,000 *g* (calculated for base of tube) for 1 hr (Spinco; Rotor 40). Immediately after ultracentrifugation the tubes were carefully removed from the rotor, frozen in liquid nitrogen and cut into 5–8 approximately equal sections. Each sample was weighed and its ^{7}Be and protein contents estimated.

Gel filtration. Sephadex G-100 (140–400 mesh) (Pharmacia, Uppsala, Sweden) was used in columns of 21–26 cm $\times$ 1·33 cm^2 with flow rates of 11·5–25 g buffer/hr. The columns were equilibrated with either 0·066 M Sørensen's phosphate buffer (pH 7·0) or 0·01 M Tris (hydroxymethyl) aminomethane (L. Light & Co. Ltd., Colnbrook, Bucks) adjusted to pH 7·5 with HCl. In further series of experiments the Sephadex was equilibrated with Tris-HCl buffer (pH 7·5) containing beryllium lactate (0·0116 per cent) (Murex Ltd, Rainham, Essex) and ^{7}Be as tracer. Samples (1·0–1·43 ml) of the solutions to be studied were layered on to the top of the column and developed by the passage of buffer. For ease of working the effluent was collected in weighed fractions (1·1–1·2 g) and their radioactivity and protein contents estimated.

Human γ-globulin (Fraction II), β-globulin (Fraction III) and α-globulin (Fraction IV–I) were obtained from the Nutritional Biochemical Corporation, Cleveland, Ohio, U.S.A. Crystalline bovine plasma albumin was obtained from the Armour Pharmaceutical Laboratories, Eastbourne, England. The solutions of these proteins (20 mg/ml) were made in 0·01 M Tris-HCl buffer containing Be lactate and ^{7}Be, except in the case of the γ- and α-globulins when the solutions were prepared as

follows. An excess of protein was mixed with buffer and shaken for 2 hr. The suspensions were centrifuged at 1800 *g* for 15 min and the supernatant removed. For the α-globulin the protein concentration of this supernatant was 15·6 mg/ml and for the γ-globulin 4·9 mg/ml. The latter solution was concentrated to 14 mg/ml by dialysis for 2 hr against carbowax. These concentrations were derived from a comparison of the u.v. extinction of the solutions at 280 mμ with that of a standard albumin solution.

For some experiments solutions of β-globulin (Fraction III) and α-globulin (Fraction IV–I) in 0·01 M Tris-HCl buffer (pH 7·5) were purified on a Sephadex G-100 column. The fractions of the effluent containing the protein peak were pooled and passed directly down a Sephadex G-100 column equilibrated with Tris-HCl buffer (pH 7·5) containing beryllium lactate and ^{7}Be as above. Fractions of effluent were collected as before for the estimation of radioactivity and protein content.

Ion-exchange chromatography. Plasma was first de-salted on a column of Sephadex G-25 (Pharmacia, Uppsala, Sweden) equilibrated with 0·01 M Tris-HCl buffer (pH 7·5). Samples of the effluent containing the plasma proteins were applied to columns (12–15 cm × 0·79 cm^2) of DEAE-cellulose (Whatman, DE 23, New Fibrous) equilibrated with 0·01 M Tris-HCl buffer (pH 7·5) and the protein fractions eluted with Tris-HCl buffer containing increasing concentrations of NaCl. Radioactivity and protein were estimated as below.

Radioactivity measurements. Radioactivity due to ^{7}Be was measured with a Packard Auto-gamma scintillation counter. Radioactivity due to ^{32}P was measured with a Packard Tri-carb scintillation spectrometer. For ^{32}P, 1·0 ml samples of the column effluent or 0·6–0·7 ml samples of the trichloroacetic acid extract of blood diluted to 1·0 ml with water were used, with 15 ml XDC scintillation medium I[7] and in the double labelling experiments a correction was made for the counts due to ^{7}Be. Sixteen per cent of the radioactivity of ^{7}Be recorded in the gamma-counting system was also counted in the ^{32}P counting system. No correction of the ^{7}Be counts for those due to ^{32}P was required as only 0·9 per cent of the radioactivity of ^{32}P recorded in the gamma-counting system.

Analytical methods. Plasma inorganic phosphate was determined by the method of Fiske and Subbarow.[11] The protein content of the effluent from the columns was estimated spectrophotometrically by u.v. extinction at 280 mμ in cells of 5 mm light path. Identification of the proteins in the peaks was attempted by electrophoresis on cellulose acetate strips.[15]

RESULTS

The i.v. LD_{50} of $BeSO_4$ in these female rats was 0·51 mg Be/kg body wt. (95 per cent fiducial limits, 0·481–0·537 mg). When blood was removed after the i.v. injection of doses of labelled $BeSO_4$ between 0·03 and 0·51 mg Be/kg, almost all the circulating radioactivity was in the plasma (mean, 85·6 per cent; range, 72–98 per cent). Experiments on the distribution of beryllium within the plasma gave the following results.

Ultracentrifugation

After ultracentrifugation the beryllium remained evenly distributed in the plasma samples from rats given 0·03 mg Be/kg body wt. (Fig. 1A). As the dose of labelled $BeSO_4$ was increased the percentage of the radioactivity recovered in the bottom 10 per cent of the ultracentrifuged sample rose steadily until, after a dose of 0·1 mg

Be/kg body wt., the profile shown in Fig. 1B was obtained. The u.v. extinction profile was the same for all doses and not different from that seen after the ultracentrifugation of normal plasma. Neither the speed of the injection (10–90 sec.) nor the time (1·5–15 min) when the blood was taken from the rat influenced this distribution.

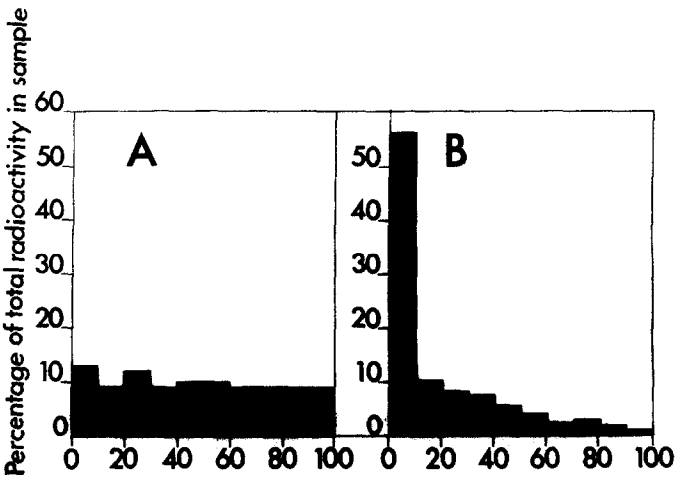

FIG. 1. The histograms show the effect of ultracentrifugation on the distribution of radioactivity in plasma, (A) from a rat given 0·03 mg Be/kg body wt. i.v. and (B) from one given 0·51 mg Be/kg body wt. i.v. as labelled $BeSO_4$. The radioactivity in each fraction is expressed as percentage of the total radioactivity of the plasma sample and the length of the sample has been divided into 100 arbitary units (bottom = 0).

Gel filtration

Three series of experiments were performed with plasma from rats injected with $BeSO_4$.

In the first series the Sephadex columns were equilibrated with 0·066 M phosphate buffer pH 7·0. Plasma (1 ml) from an animal given 0·51 mg Be/kg body wt., as labelled $BeSO_4$, 15 min before, was applied to the top of the column. Experiments were also performed with ultracentrifugation fractions of the same plasma, one with the bottom fraction i.e. the first 10 per cent of the plasma sample containing 56 per cent of its total radioactivity, and the second with the combined middle and top fractions. Two protein peaks with similar characteristics were found in all cases, as shown for the ultracentrifuged fractions in Fig. 2. The radioactivity profile also showed two peaks, the major one having an elution volume very different from that of the protein peaks. The smaller peak had the same elution volume as the first protein peak.

In the second series of experiments, the columns were equilibrated with 0·01 M Tris-HCl buffer pH 7·5. The samples of plasma (1 ml) applied to the top of the column were obtained from rats which had received either 0·51 or 0·03 mg Be/kg body wt. (as labelled $BeSO_4$, i.v. 15 and 5 min respectively before the blood was removed and centrifuged.

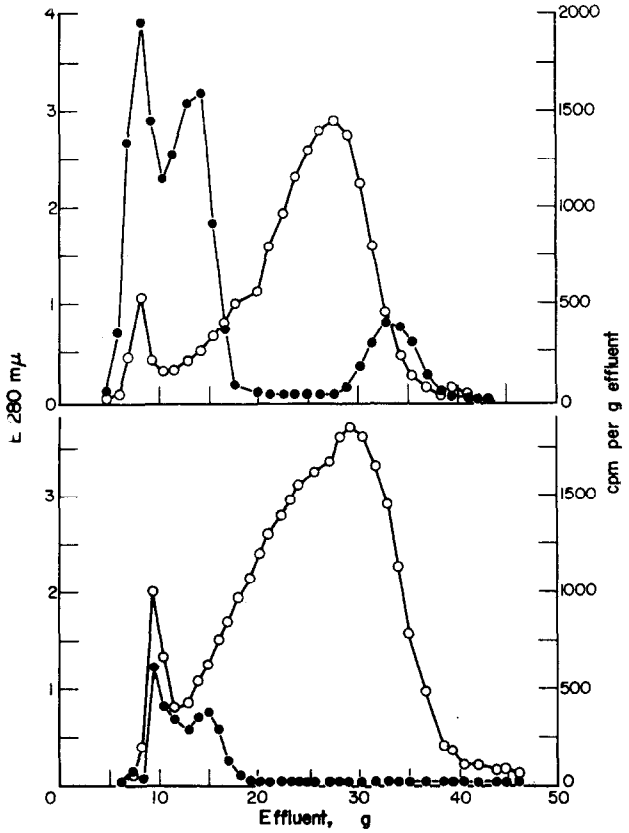

FIG. 2. Radioactivity (○—○) and u.v. extinction ($E_{280m\mu}$; ●—●) profiles of the effluent from Sephadex columns developed with 0·066M phosphate buffer (pH 7·0) after the application of ultracentrifuged plasma from a rat 15 min after an i.v. injection of labelled $BeSO_4$ (0·51 mg Be/kg body wt.).

Upper diagram. Results with upper 90 per cent of plasma sample. Lower diagram. Results with bottom 10 per cent of plasma sample. In both case, gel bed volume, 34 ml, flow rate, 13·0 g buffer/hr.

Two protein peaks were again found but a much larger percentage of the total radioactivity accompanied the first protein peak (Figs. 3 and 4). After 0·51 mg Be/kg body wt. practically all the radioactivity was associated with this peak. After 0·03 mg, while much of the radioactivity was still associated with the first protein peak, a second, broad radioactive peak was found, not clearly associated with protein and having a much larger elution volume. Electrophoresis experiments on cellulose acetate strips confirmed the presence of globulins in the first protein peak and of albumin in the second.

The third series of experiments was carried out with plasma from rats injected i.p. with ^{32}P. When plasma was tested 30 min later a single peak of ^{32}P radioactivity was found in the effluent from the Sephadex column developed with Tris-HCl buffer (Fig. 5A). When $BeSO_4$ (0·51 mg Be/kg body wt.) was injected i.v. 10 min before the removal of the blood, the effluent from the column to which the plasma had been applied showed two radioactive peaks, the first appearing with the first protein peak and the second corresponding to the peak of the control plasma (Fig. 5B).

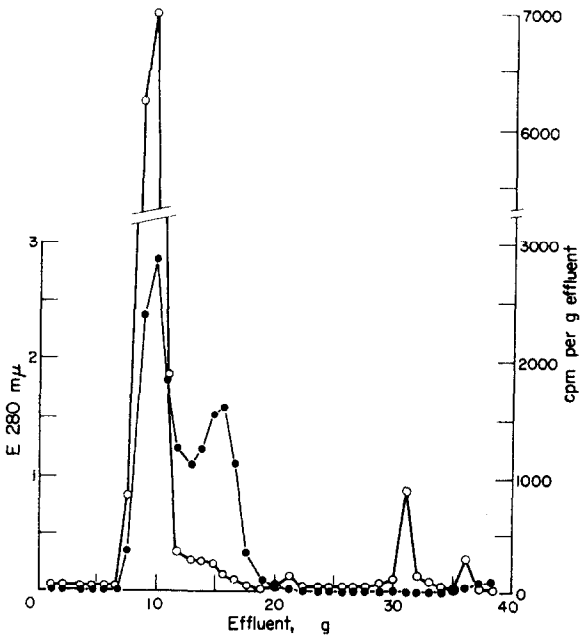

FIG. 3. Radioactivity (○—○) and u.v. extinction ($E_{280m\mu}$; ●—●) profiles of the effluent from a Sephadex column developed with 0·01M Tris buffer (pH 7·5) after the application of 1·0 ml plasma from a rat 15 min after an i.v. injection of labelled $BeSO_4$ (0·51 mg Be/kg body wt.). Gel bed volume, 34 ml. Flow rate, 13·3 g buffer/hr.

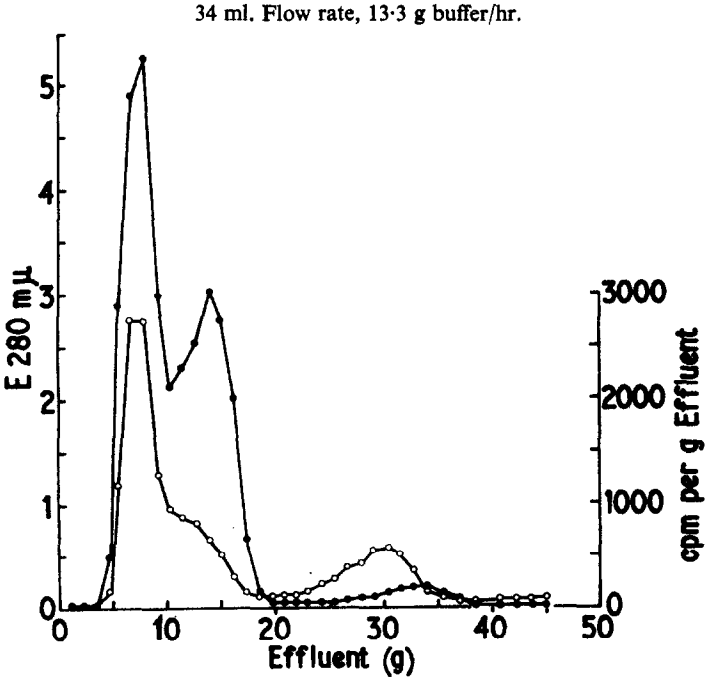

FIG. 4. Radioactivity (○—○) and u.v. extinction ($E_{280m\mu}$; ●—●) profiles of the effluent from a Sephadex column developed with 0·01 M Tris-HCl buffer (pH 7·5) after application of 1·0 ml plasma from a rat 5 min after an i.v. injection of labelled $BeSO_4$ (0·03 mg Be/kg body wt.) Gel bed volume, 34 ml. Flow rate, 13·3 g buffer/hr.

When the dose of $BeSO_4$ was also labelled with ^{7}Be the radioactivity in the effluent due to ^{7}Be ran with the first protein peak, superimposed on the ^{32}P activity of this peak (Fig. 5C).

Further experiments were performed with normal plasma or protein solutions.

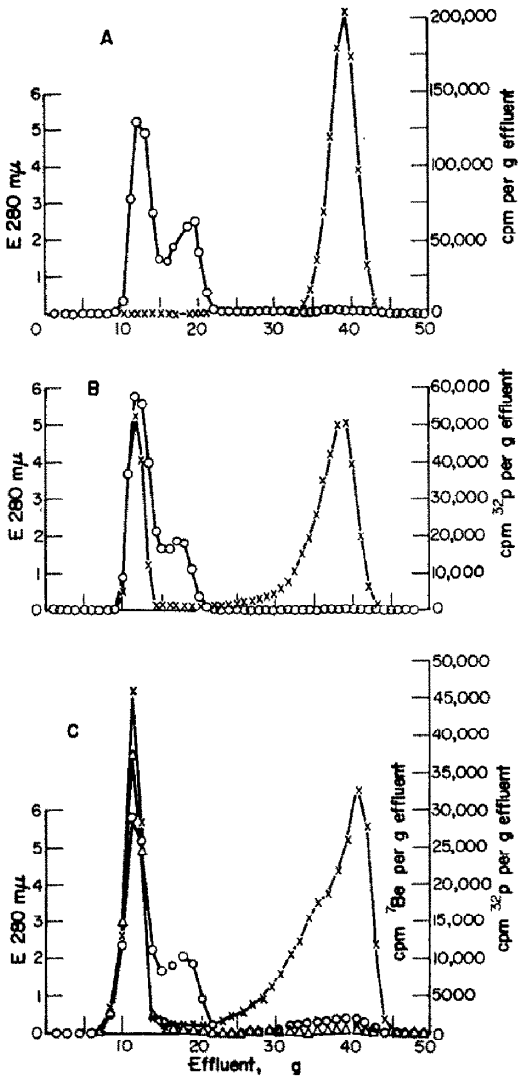

FIG. 5. Radioactivity and u.v. extinction ($E_{280m\mu}$; ○—○) profiles of the effluent from Sephadex columns developed with 0·01 M Tris-HCl buffer (pH 7·5) after the applications of plasma samples. Radioactivity due to ^{32}P, X—X; due to ^{7}Be, △—△.

A. 1·0 ml plasma from rat 30 min after i.p. dose of ^{32}P.

Gel bed volume 33·3 ml. Flow rate, 11·9 g buffer/hr.

B. 1·0 ml plasma from rat 30 min after i.p. dose of ^{32}P and 10 min after i.v. injection of 0·51 mg Be/kg body wt.

Gel bed volume, 33·3 ml. Flow rate, 11·5 g buffer/hr.

C. 1·0 ml plasma from rat 30 min after i.p. dose of ^{32}P and 10 min after i.v. injection of 0·51 mg Be/kg body wt. labelled with ^{7}Be.

Gel bed volume, 33·3 ml. Flow rate, 15·4 g buffer/hr.

For some the columns were equilibrated with 0·01 M Tris-HCl buffer (pH 7·5) containing Be lactate (0·0116%) and ^{7}Be as tracer. When normal plasma was passed down these columns the appearance of the first protein peak was associated with a marked increase in the radioactivity of the effluent (Fig. 6). The basal radioactivity of the effluent was subsequently depressed for a time.

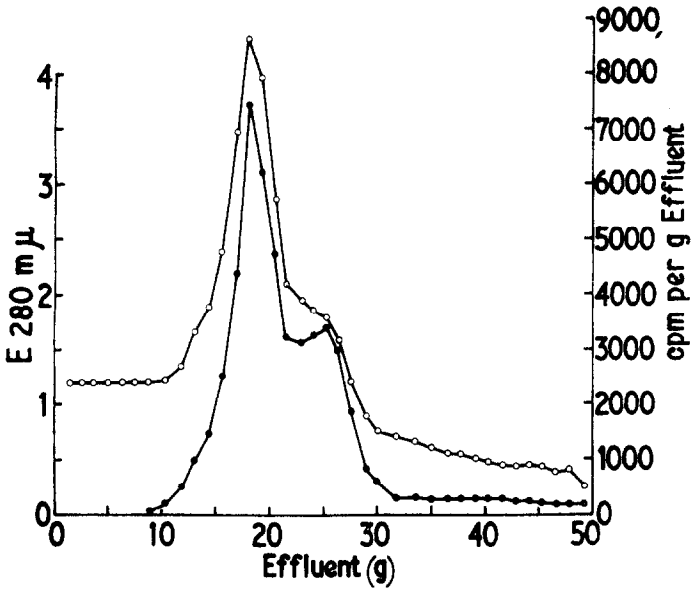

FIG. 6. Radioactivity (○—○) and u.v. extinction ($E_{280m\mu}$; ●—●) profiles of the effluent from a Sephadex column continuously washed with 0·01 M Tris-HCl buffer (pH 7·5) containing 0·0116% beryllium lactate labelled with ^{7}Be. Normal Plasma (1·0 ml) was applied to the top of the column at the origin. Gel bed volume, 34·5 ml. Flow rate, 25·0 g buffer/hr.

The results obtained when plasma protein fractions were passed down these 'radioactive' columns are summarized in Table 1. All appeared in the effluent as single protein peaks but only in the case of the α-globulin fraction was this protein peak accompanied by any radioactivity. However, with all the globulin (but not the albumin) preparations a broad peak of radioactivity appeared in the effluent long after the protein had been discharged from the column. The elution volume of this peak was greater than for any of the radioactive peaks previously found in these experiments and suggested that the ^{7}Be was associated with small molecular weight impurities. The α- and β-globulin fractions were, therefore, further purified on Sephadex G-100 columns. When this purified α-globulin was passed down the 'radioactive' column a single peak of radioactivity was found in the effluent corresponding to the protein peak (Fig. 7). The passage of the purified β-globulin had no significant effect on the radioactivity of the effluent.

The gel filtration of mixtures of ^{7}Be labelled $BeSO_4$ solutions and α-globulin or albumin, with or without Na_2HPO_4 on Sephadex G-100 columns developed with 0·01 M Tris-HCl buffer (pH 7·5) did not show any association between beryllium and protein (J. Vacher; unpublished results). The pH of these mixtures was very low (1·5) and the position of the ^{7}Be peak in the effluent was the same as when the ^{7}Be was added to the column in a solution of $BeSO_4$.

A slightly different experiment showed that it was possible to form aggregates of beryllium phosphate *in vitro*. Sufficient labelled $BeSO_4$ solution to react with 20 per cent of the phosphate in 5·0 ml Krebs–Ringer's solution (141·3 m-mole NaCl, 5·65 m-mole KCl, 3·03 m-mole $CaCl_2$, 1·41 m-mole KH_2PO_4, 1·41 m-mole $MgSO_4$ in 1·0 l.) containg 3·2% albumin (pH 7·4) was rapidly injected into it while it was being

TABLE 1. EFFECT OF PLASMA PROTEIN FRACTIONS

Applying to a Sephadex G-100 column (25 cm × 1·33 cm^2; gel-bed volume 33 ml) equilibrated with 0·01 M Tris buffer pH 7·5 containing Be lactate (0·116%) and ^7Be. The Table shows the amount of protein applied, the flow rate of the buffer and the elution volumes of the protein and radioactivity peaks.

Plasma protein fraction applied to column	Buffer flow rate (g./hr)	Elution volume of peaks (g)	
		Protein	Radioactivity
Albumin (20 mg : 1·0 ml)	16·7	21	—
α-globulin (20 mg : 1·28 ml)	15·7	15·2	15·2 and 42·7
β-globulin (20 mg : 1·0 ml)	17·2	15·6	41·7
γ-globulin (20 mg : 1·43 ml)	15·7	18·1	42·7

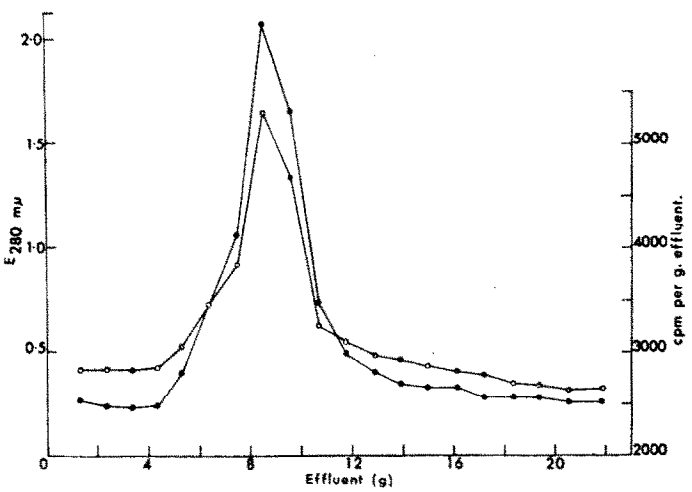

FIG. 7. Radioactivity (○—○) and u.v. extinction ($E_{280m\mu}$; ●—●) profiles of the effluent from a Sephadex column continuously washed with 0·01 M Tris-HCl buffer (pH 7·5) containing 0·0116% beryllium lactate labelled with ^7Be. Purified human α-globulin (see text) added to the top of the column at the origin. Gel bed volume, 34 ml. Flow rate, 14·0 g buffer/hr.

rapidly agitated in a plastic tube on a Vortex Jr. Mixer (Scientific Industries Inc., Queens Village, New York). The mixture was then centrifuged at 1800 *g* for 10–15 min. This spun down the larger particles which had been formed so that the concentration of radioactivity at the bottom of the tube was nearly twice that at the top. A sample (1·0 ml) of the top fraction was passed down a Sephadex G-100 column and two

radioactive peaks were found in the effluent, the main one preceding the albumin peak. (Fig. 8.) The position of the main radioactive peak indicated that aggregates of beryllium phosphate could be formed with similar molecular size to plasma globulins. Additional tests for the protein binding of beryllium were therefore necessary using dissimilar methods of protein separation.

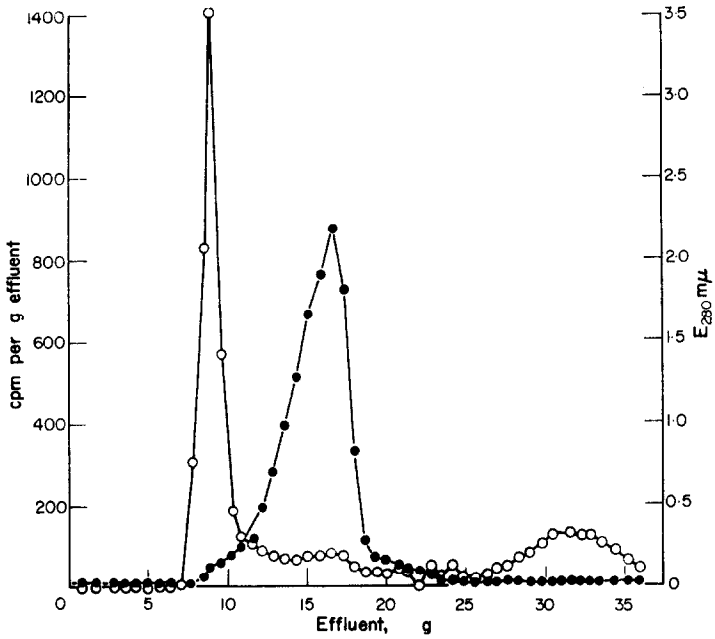

FIG. 8. Radioactivity (○—○) and u.v. extinction ($E_{280m\mu}$; ●—●) profiles of the effluent from a Sephadex column developed with 0·01 M Tris-HCl buffer (pH 7·5) after application of 1·0 ml supernatant of a mixture of $BeSO_4$ in Krebs–Ringer's solution containing 3·2% albumin (pH 7·4). For details of preparation see text. Sufficient $BeSO_4$ added to complex 20% phosphate in the Krebs–Ringer's solution. Recovery of radioactivity in effluent 84 per cent.
Gel bed volume, 34 ml. Flow rate, 5·5 g/hr.

Ion-exchange chromatography

It was confirmed that rat plasma does not separate into pure fractions when chromatographed on DEAE-cellulose.[17] Although this method does not give good separation of the α-globulins and albumin[22] it provides a method of protein separation quite distinct from that used above, distinguishing by charge rather than size. The nature of the buffer used and the initial pH were dictated by a desire to maintain Be in the plasma as in *in vivo* and were probably not ideal for this type of protein separation.

Plasma from 3 rats 5–7 min after the i.v. injection of 0·51 mg Be/kg body wt. was examined in this way. The protein profile (Fig. 9) was very similar in each case, the most variable feature being the amount which was not adsorbed and passed through before the addition of NaCl. This was very large in the case of the rat bled 5 min after the injection of $BeSO_4$ and this protein was accompanied by a large amount of radioactivity. Otherwise most of the radioactivity was eluted between 100 and 300 mM NaCl and the relative heights of the peaks were similar in each case. Not all the protein

peaks were associated with radioactivity but the radioactive peaks coincided with protein ones with one exception, which is shown in Fig. 9. The comparatively slow elution of radioactivity by 100 mM NaCl was a constant finding.

The radioactive beryllium phosphate aggregates prepared in Krebs–Ringer's solution containing 3·2% albumin (see above) were also tested in this system. The pH of this solution was 4·7 and the $BeSO_4$ was added either at that pH or after neutralization to pH 7·4. Like the plasma, the solutions were desalted on a Sephadex G-25 column. In the effluent from this column the peak of the radioactivity followed that of the albumin. The two peaks were combined for chromatography on DEAE-cellulose (Fig. 10). The complex profile of the crystalline bovine serum was clearly shown. ^{7}Be was present in many of the fractions but the majority was eluted from the column by 100 mM NaCl. At this NaCl concentration peak radioactivity was only attained after a number of fractions had been collected and the fall in the radioactivity

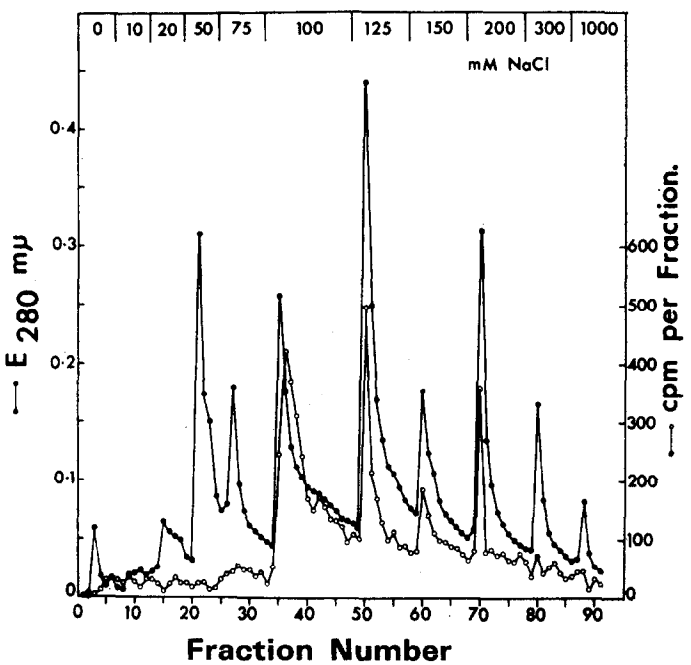

FIG. 9. Radioactivity (○—○) and u.v. extinction ($E_{280m\mu}$; ●—●) profiles of the effluent from a DEAE-cellulose column developed with 0·01 M Tris-HCl buffer (pH 7·5) containing increasing concentrations of NaCl (shown on scale at top of the figure) after application of desalted plasma obtained from a rat 7 min after an i.v. injection of labelled $BeSO_4$ (0·51 mg Be/kg body wt.).
Column volume, 10 ml. Fraction volume, 5·0 ml.

of the subsequent fractions was rather slow. This bore a similarity to the elution pattern at this NaCl concentration in the plasma experiments.

Effect of beryllium on the rate of disappearance of ^{32}P from the circulation

From the preceding results an i.v. dose of $BeSO_4$ would be expected to alter the rate of disappearance from the circulation of injected ^{32}P. This possibility was tested in 31 rats. Typical results, at short and longer intervals after the administration of

^{32}P, are shown in Figs. 11 and 12. The smallest dose of $BeSO_4$ used, 0·03 mg Be/kg body wt., had about the same effect as an equivalent volume of 0·9% NaCl adjusted with H_2SO_4 to a similar pH. However, a striking effect was seen after the injection of 0·51 mg Be/kg body wt. Immediately after the injection of $BeSO_4$ there was a large increase in the radioactivity of the acid-soluble fraction of the blood. (^{32}P radioactivity was not precipitated by trichloroacetic acid in the beryllium treated rat.)

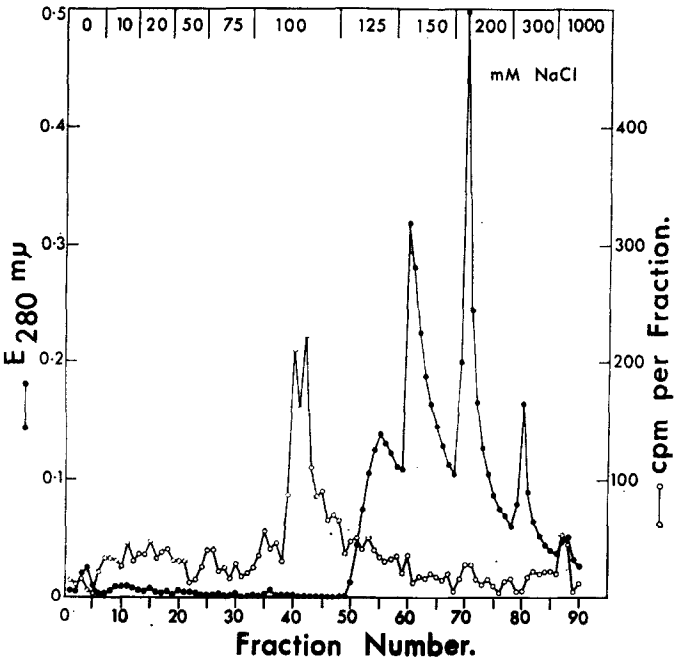

FIG. 10. Radioactivity (○—○) and u.v. extinction ($E_{280m\mu}$; ●—●) profiles of the effluent from a DEAE-cellulose column developed with 0·01 M Tris-HCl buffer (pH 7·5) containing increasing concentrations of NaCl (shown on scale at top of the figure) after application of the desalted supernatant of a mixture of $BeSO_4$ in Krebs–Ringer's solution containing 3·2% albumin (pH 4·7). For details of preparation see text. Sufficient $BeSO_4$ added to complex 20% phosphate in the Krebs–Ringer's solution. Recovery of radioactivity in effluent 65 per cent. Column volume 11 ml. Fraction volume 5·0 ml.

Intermediate doses reproduced this effect in lesser degree. Because of the effect of the acidified NaCl solution it was difficult to decide whether the $BeSO_4$ had any significant effect on the subsequent rate of removal of ^{32}P.

The mean (±S.D.) inorganic phosphate concentration in the plasma of these rats was 1·85 ± 0·18 μg atoms P/ml (n = 6). Assuming a plasma volume of 4·2 ml/100 g body wt.[3] this would give a plasma phosphate pool of 7·8 μg atoms P/100 g body wt. An injection of 0·51 mg (56·7 μg atoms) Be/kg body wt. could therefore complex up to 50 per cent of the plasma phosphate pool, assuming the formation of $Be_3(PO_4)_2$. In the homeostatic reactions which follow, the plasma phosphate pool will be replenished by radioactive phosphate from the extravascular space to give the effect shown in Figs. 11 and 12.

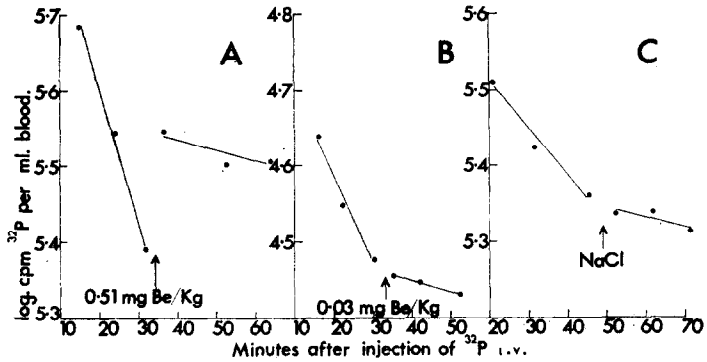

FIG. 11. Effect of different intravenous doses of $BeSO_4$ (A and B) and of an equivalent volume of 0·9% NaCl acidified to pH 3·7 with H_2SO_4 (C) on the rate of disappearance of ^{32}P from the acid soluble fraction of the blood. Each curve refers to a separate rat. Curves fitted by eye.

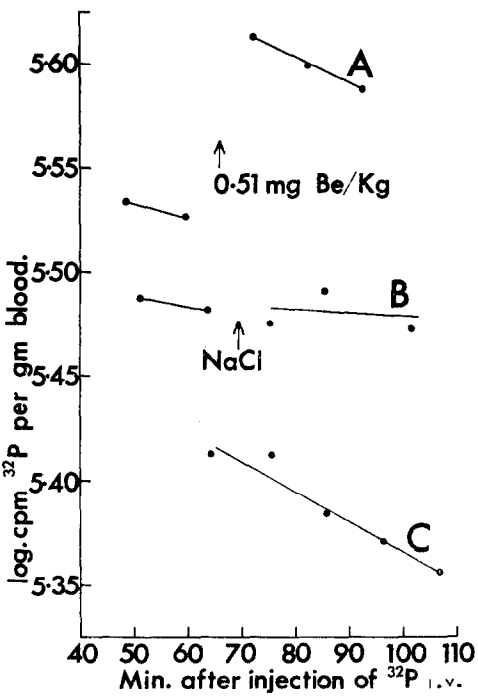

FIG. 12. The rate of disappearance of ^{32}P from the acid-soluble fraction of the blood. Each curve refers to a separate rat: (A) shows the effect of an i.v. dose of $BeSO_4$, (B) the effect of an equivalent volume of 0·9% NaCl acidified to pH 3·7 with H_2SO_4 and (C) the rate when no injection was given. Curves fitted by eye.

DISCUSSION

In agreement with previous workers[10, 14] we found that after the i.v. injection of $BeSO_4$ the circulating Be was almost entirely in the plasma where it existed in two main forms. A small fraction remained of small molecular size (Figs. 3 and 4) and

presumably represented the diffusible form associated with the plasma organic acids.[10] The majority was in aggregates of large molecular size. The main problem was the nature of these aggregates.

The evidence illustrated in Figs. 5, 11 and 12 established that, after the injection of $BeSO_4$, beryllium phosphate was produced, as envisaged by others,[8, 10] to form one ingredient of the aggregates. There are several beryllium phosphates. Two which are likely to be formed under these conditions are $Be_3(PO_4)_2$ and $Be_3(PO_4)_2.Be(OH)_2$ but it is not possible to say in what proportions. From the ultracentrifuge data it would appear that the aggregates increased in size as the dose was raised.

There was no evidence in any of these experiments that these aggregates were bound to native plasma albumin. This confirmed *in vivo* what others had found *in vitro*.[4, 10] Gel-filtration on Sephadex G-100 with Tris-HCl buffer suggested that the beryllium phosphate was associated with the plasma globulins in the first protein peak. The results with Sephadex G-100 columns labelled with ^7Be would be difficult to interpret in detail but the findings strongly suggested that the association was specifically with α-globulin. An abnormal α_1-globulin has been described in rat serum 24 hr after the administration of beryllium[16] but it is not known if this is related to these changes immediately following the i.v. injection of $BeSO_4$. Globulin binding has also been reported in the case of radiogold.[21]

Although these results were persuasive they did not establish the binding of beryllium by plasma globulin. Beryllium phosphate aggregates, not bound to protein, could be prepared *in vitro* and the majority of those not sedimented by the initial centrifugation were of comparable molecular size to the plasma globulins, according to gel-filtration. Further evidence for the protein binding of beryllium was therefore required.

This was obtained using DEAE-cellulose. In the effluent from these columns ^7Be was associated with some, but not all, of the protein peaks. The fact that ^7Be followed protein in a definite pattern when the plasma proteins from rats injected with labelled $BeSO_4$ were separated both by size and by charge is strong evidence of attachment to plasma protein. Although DEAE-cellulose does not separate the plasma proteins into pure fractions the ^7Be profile in the effluent was consistent with attachment to α-globulin.[9] However, not all the beryllium phosphate aggregates formed *in vivo* may be protein bound. There was a slight difference in behaviour of the protein and radioactivity during elution by 100 mM NaCl which could be explained by the presence of unbound aggregates. When beryllium phosphate aggregates were produced *in vitro* and absorbed on DEAE-cellulose the majority were eluted by 100 mM NaCl but more slowly than those formed *in vivo*.

Although we can now speak with fair confidence of the binding of circulating beryllium phosphate to plasma protein, most probably α-globulin, the molecular proportions of the complex are not known; nor is the binding 'strong'. The beryllium was removed from the protein by trichloroacetic acid and by phosphate buffer (Fig. 2) and the complex was not formed when the components were mixed *in vitro* at a low pH. The influence of the form of the circulating beryllium on its removal from the plasma will be considered in a further communication.

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