2. Mannono-(1→5)-lactone caused 50 % inhibition of the hydrolysis of 6 mM-*p*-nitrophenyl α -mannoside at 0.080 mM by a rat-epididymal preparation and at 0.098 mM by a limpet preparation. The concentration for 50 % inhibition of the hydrolysis of 2.5 mM-phenyl β -mannoside by the limpet preparation was 0.013 mM.

3. Concentrations of mannono-(1→4)-lactone causing 50 % inhibition under the same conditions were 40 mM for rat-epididymal α -mannosidase, 26 mM for limpet α -mannosidase and 7.1 mM for limpet β -mannosidase.

4. Both mannonolactones acted competitively on rat-epididymal α -mannosidase, with values for K_i of 32 and 0.071 mM for the (1→4)- and the (1→5)-lactone respectively.

5. Glucono-(1→5)-lactone caused 50 % inhibition of the hydrolysis of 6 mM-*p*-nitrophenyl α -mannoside at 8.2 mM by the rat-epididymal preparation, and of 5 mM-phenyl α -glucoside at 0.85 mM by the same preparation. With the limpet preparation the concentrations for 50 % inhibition of the hydrolysis of 5 mM-*o*-nitrophenyl β -glucoside and 5 mM-phenyl α -glucoside were 0.092 and 2.2 mM respectively.

6. Concentrations of glucono-(1→4)-lactone causing 50 % inhibition under the same conditions were 23 mM for rat-epididymal α -mannosidase, 1.3 mM for epididymal α -glucosidase, 0.24 mM for

limpet β -glucosidase and 3.2 mM for limpet α -glucosidase.

7. Neither mannono-(1→4)- nor mannono-(1→5)-lactone caused any inhibition of the limpet β -glucosidase, and they were alike in their very feeble inhibitory power towards rat-epididymal α -glucosidase.

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Transport of Organic Compounds in the Mammal

PARTITION OF DIELDRIN AND TELODRIN BETWEEN THE CELLULAR COMPONENTS AND SOLUBLE PROTEINS OF BLOOD

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The investigations described in this paper are concerned with the problem of transport of two insecticides, dieldrin and Telodrin, in the blood of animals. We have measured particularly the distribution of these two substances between erythrocytes and plasma lipoproteins and proteins *in vivo* and *in vitro*. A knowledge of the transport of physiologically active substances which are not normally present in the body is fundamental to an understanding of the metabolism and the effect of these substances on animals.

MATERIALS AND METHODS

Insecticides. Dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-exo-1,4-endo-5,8-dimethanonaphthalene) and Telodrin (1,3,4,5,6,7,8,8-octachloro-1,3,3a,4,7,7a-hexahydro-4,7-methano-isobenzofuran) were crystallized from acetone-methanol and acetone-hexane to constant melting point (dieldrin, m.p. 177°; Telodrin, m.p. 123°). The purified dieldrin and Telodrin migrated as single substances in reversed-phase paper chromatograms developed with two solvent systems (Mitchell, 1958); phenoxyethanol-AgNO₃ spray

was used for locating the substance on the paper (Mitchell, 1958).

Radioactive chemicals. [^{14}C]Dieldrin (sp. activity $54\ \mu\text{C}/\text{mg.}$) and [^{14}C]Telodrin (sp. activity $14\ \mu\text{C}/\text{mg.}$), synthesized from [^{14}C]hexachlorocyclopentadiene, were obtained from The Radiochemical Centre, Amersham, Bucks., who also supplied 3,3',5-tri-[^{131}I]iodothyronine. The [^{14}C]Telodrin, which contained some impurities, was purified by chromatography on a Florisil column (Hopkin and Williams Ltd., Chadwell Heath, Essex) with *n*-hexane.

Reagents and solvents. All reagents and solvents (supplied by Hopkin and Williams Ltd.) were, except when otherwise stated, of AnalaR grade. Diethyl ether was distilled over Na wire. Dimethylsulphoxide, laboratory-reagent grade, was redistilled; the fraction boiling between 66° and $69^\circ/10\ \text{mm. Hg}$ was collected for use.

Buffers for electrophoresis. Veronal buffer, pH 8.6, $I\ 0.1$, was made by dissolving sodium diethylbarbiturate (10 g., laboratory-reagent grade), sodium acetate trihydrate (6.5 g.) in water with $0.1\ \text{N-HCl}$ (64.4 ml.) added and the whole diluted to 1 l. Phosphate buffer, pH 6.5, $I\ 0.045$, was made by dissolving $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (0.587 g.) and Na_2HPO_4 (1.490 g.) in 1 l. of water. Acetate buffer, pH 4.5, $I\ 0.1$, was prepared by dissolving sodium acetate (82 g.), acetic acid (7.5 g.) and EDTA (1 g., laboratory-reagent grade) in 1 l. of water.

Low-voltage electrophoresis. Serum proteins were separated by electrophoresis either on cellulose acetate strips in veronal buffer, pH 8.6, at $12\ \text{V/cm.}$ for 3 hr., or in agar gel (Bodman, 1960) with the three buffers described above (pH 8.6, 6.5 and 4.5) at $6\ \text{V/cm.}$ for 5 hr. A Kohn bath (Shandon Scientific Co., Cromwell Road, London) was used for the electrophoresis at 25° . A 0.2% solution of Ponceau S (G. T. Gurr Ltd., London) in aq. 3% (w/v) trichloroacetic acid was used for staining serum proteins on the cellulose acetate strips. The staining of proteins in agar gel was done with a 0.002% bromocresol green (G. T. Gurr Ltd.) solution in aq. 3% (w/v) trichloroacetic acid; the protein zones became blue after exposure of the dried gel to NH_3 .

Experiments with blood in vitro. Blood was withdrawn by cardiac puncture from adult male rabbits (New Zealand White strain) and from 350 g. male rats (Carworth Farm strain). Serum which separated immediately after clotting was used in all except one of these experiments. Insecticide was equilibrated with serum for 30 min. at 37° . Either a solution of the insecticide in a few microlitres of ethanol was added direct to the serum, or the ethanolic solution was dried on the bottom of a tube and the resulting film was taken up into serum.

In the remaining experiment, blood (10 ml.) was treated with heparin (200 $\mu\text{l.}$ of a 2.5% solution in 0.9% NaCl), and was equilibrated with insecticide by incubation at 37° for 30 min. Plasma was separated from 3 ml. of the insecticide solution in blood by centrifugation (2000 rev./min. for 10 min.); the cells were washed twice with sodium citrate solution (22 g./l.). After spinning, leucocytes plus platelets were separated layerwise from the denser erythrocytes, which were further purified by washing and resuspension in the sodium citrate solution. Rabbit and rat erythrocytes were haemolysed with 6.7 mm.- NH_3 soln. (Goldblatt, Henson & Somerville, 1960). Erythrocyte contents and stroma were separated by centrifugation, and the stroma were washed repeatedly with the NH_3 soln.; the washings were added to the cell contents. A 4 ml.

portion of the insecticide solution in heparinized blood was mixed with one-ninth of its volume of sodium citrate solution (3.8%), and centrifuged (1500 rev./min. for 5 min.). The platelet-rich plasma was spun (15000 rev./min. for 5 min.) and the resulting button of platelets was purified by repeated resuspension and washes with 3.8% sodium citrate (Biggs & Macfarlane, 1953; Strumia, 1959).

A 3 ml. portion of the insecticide solution in heparinized blood was mixed with 1 ml. of Dextraven (a solution of 6% of dextran in 0.9% NaCl; Bengel Laboratories, Holmes Chapel, Cheshire) and maintained at room temperature until sedimentation of the erythrocytes was complete. The supernatant leucocyte suspension was spun at 50g for 3 min. and the pellet of leucocytes purified by repeated washing and resuspension in 0.9% NaCl (Dennes, Tupper & Wormald, 1961). The purity of erythrocyte, leucocyte, platelet and (erythrocyte) stroma preparations was checked by microscopic examination.

Experiments with animals. ^{14}C -labelled insecticide was injected into the femoral vein of male rats, either in a small volume (microlitres) of ethanol, or in an ethanol-saline suspension, and intraperitoneal injection of other rats was made with a solution of ^{14}C -labelled insecticide in dimethyl sulphoxide. Specimens of blood were withdrawn from the tail vein in both experiments. In the experiment involving intraperitoneal injection, blood was transferred to a tube containing heparin (100 $\mu\text{l.}$ of a 2.5% solution in 0.9% NaCl, for 5 ml. of blood), or a dry film of either ammonium oxalate (10 mg. for 5 ml. of blood) or NaF (5 mg. for 5 ml. of blood), and the erythrocytes and plasma were separated as described above.

Measurement and detection of radioactivity. A γ -scintillation detector (EKCO Electronics Ltd., Southend) was used for the measurement of ^{131}I , contained in annular polythene cups surrounding an encapsulated NaI crystal.

A coincidence detector circuit (Isotopes Development Ltd., Aldermaston, Berks.) was used for the measurement of ^{14}C by liquid scintillation counting. Provided that allowance was made for the different degrees of efficiency of counting, comparable values were obtained for the measurement of radioactivity in preliminary experiments: (1) when serum was blended with NE 213 scintillator [Nuclear Enterprises (G.B.) Ltd., Edinburgh, Scotland], (2) when the protein was denatured, and the activity removed by extraction with *n*-hexane, and (3) when the activity was extracted with ether, the solvent removed and the residue dissolved in NE 213 scintillator. The last method gave the highest efficiency (67% theoretical), and was used for nearly all the work. Where radioactivity could not be extracted with either *n*-hexane or ether, as with the hydrophilic metabolites in urine, a solution of these substances in urine or wet ethyl acetate was mixed with the NE 220 scintillator [Nuclear Enterprises (G.B.) Ltd.]. This method gave an efficiency of 50% theoretical. A 2 ml. portion of each fraction of eluate from the DEAE-cellulose column was shaken with 8 ml. of 0.3% 2,5-diphenyloxazole in toluene, and the resulting mixtures were measured without separation of the phases, and with an efficiency of 18% theoretical, in a Tri-Carb scintillation counter (Packard Instrument Co., Inc., La Grange, Ill., U.S.A.). The possibility of variable quenching of the scintillations by different samples under the various conditions that were used in the counting procedure was frequently checked, and measure-

ments of ^{14}C radioactivity were corrected accordingly. n -[1- ^{14}C]Hexadecane was used as an internal standard.

Radioactivity was detected in agar gel and on cellulose acetate strips by radioautography with Ilfex X-ray film (Ilford Ltd., Ilford, Essex).

Ultracentrifugation. A MSE Superspeed 40 preparative ultracentrifuge was used for all the separations, which were effected in three tubes (5 ml.) in the swing-out head, rotor no. 29314, at an average acceleration of 135 000*g* (40 000 rev./min.). The density steps (1.065, 1.125 and 1.200 g./ml.) which were used were obtained by mixing sera with the high-density electrolyte solutions: *d* 1.1170 (NaCl), *d* 1.2424 (D_2O , NaNO_3), *d* 1.3920 (D_2O , NaNO_3). Centrifuging was continued for 18 hr. and the techniques were those described by de Lalla & Gofman (1954). The contents of each tube were divided somewhat arbitrarily into five fractions, which were withdrawn from the top. Where a visible boundary existed between phases, division between fractions was made there. Thus, at the density step 1.065, the two topmost fractions comprise approx. 1 ml., and they contain the floatable lipoprotein (de Lalla & Gofman, 1954). The bottom fraction is viscous and is separated by a visible boundary from the next fraction, which is coloured, and which in turn is separated by a less sharp boundary from the middle fraction.

Solubility measurements. Insecticide (20 mg., particle size 5μ) was shaken at 120 strokes/min. with 5 ml. of either serum or water for 8 hr. at 20° . The samples were centrifuged at 40 000 rev./min. for 20 min., bottom and top fractions were discarded and the middle fraction was centrifuged again. The middle fraction was again removed, and the dieldrin or Telodrin content was estimated by gas-liquid chromatography. Since a *n*-hexane extract was made for this estimation, it was important to reject top and bottom fractions, which might have contained minute particles of insecticide.

A Perkin-Elmer Fractometer with an electron-capture detector was used for the estimation (Goodwin, Goulden & Reynolds, 1961). Back-flush columns (Pye catalogue no. 12540), of working length 4 ft., were filled with Embacel (May and Baker Ltd., Dagenham, Essex), which had been coated with stationary phase, and preconditioned by heating at 250° for 18 hr. before use. The running conditions were: 2% Apiezon L (Shell Chemical Co. Ltd.) plus Epikote 1001 (Shell Chemical Co. Ltd.) (80–100 mesh size); temp. 185° ; inlet pressure 25 lb./in. 2 ; flow rate for N_2 , 135 ml./min.

DEAE-cellulose-column chromatography. DEAE-cellulose-column chromatography was effected by a straightforward adaptation of the method of discontinuous gradient elution (Sober, Gutter, Wyckoff & Peterson, 1956). Whatman DE 50 powder, which had been washed with acetone, cycled twice with *N*-HCl and *N*-NaOH and equilibrated at pH 7.0 with 5 mM-sodium phosphate, was packed in a chromatography tube (36 cm. $\times$ 1 cm.), under a pressure of 10 lb./in. 2 . The column was loaded with 1 ml. of test serum and percolated (20 ml./hr.) with buffer in the cold room at 5° .

A stepwise elution was employed: fractions 1–66, 5 mM-sodium phosphate, pH 7.0; 67–103, 0.02 M-sodium phosphate, pH 6.3; 104–177, 0.03 M- NaH_2PO_4 , pH 4.6; 178–243, 0.05 M- NaH_2PO_4 , pH 4.6; 244–264, 0.05 M- NaH_2PO_4 , pH 4.6, in 0.02 M-NaCl; 265–307, 0.05 M- NaH_2PO_4 , pH 4.6, in 0.05 M-NaCl; 308 onwards, 0.5 N-NaOH in M-NaCl.

Protein was estimated in all the fractions (5 ml.) at 270 $m\mu$ with a Unicam spectrophotometer (SP. 500), and radioactivity was determined on 2 ml. portions as previously described. Fractions which formed a peak of radioactivity were pooled and concentrated by ultrafiltration in a MD 35–15 apparatus (Membranfilter Gesellschaft A.-G., Göttingen, Germany), fitted with membrane LSG60 (pore size $< 5\mu$). Individual proteins in the concentrates were separated on cellulose acetate strips by electrophoresis at pH 8.6.

RESULTS

Binding and solubility properties of dieldrin and Telodrin. 'Chromatographic' methods were devised to investigate the chemical affinity of macromolecules and other organic compounds for the two insecticides. With water as solvent Telodrin remains stationary on all the supporting media. In contrast with the other mobile phases, an aqueous solution of either bovine albumin or sodium taurocholate moves the insecticides on paper and paper treated with gelatin (Table 1). The fact that Telodrin moves on paper treated with either petroleum jelly or silicone oil only when dilute sodium taurocholate is used as solvent, indicates that it combines with bile salt more strongly than with albumin, and such a mechanism may account for the presence of a small quantity of Telodrin in

Table 1. *Effect of the stationary phase on the chromatographic mobility of Telodrin in aqueous solvents*

Stationary phases were prepared by treatment of Whatman no. 1 filter papers with a solution of gelatin, petroleum jelly or silicone, as appropriate. The figures are R_F values for Telodrin.

Treatment of supporting material	Superimposition of bile salt on the Telodrin spot	Mobile phases				
		Water	5% Sodium taurocholate	5% Albumin	0.5% Gelatin	5% Lecithin
Untreated	—	0	0.6–1.0	1.0	0	0–0.1
	Present	Streaking	—	0	0.4–0.7	0–0.1
Aq. 0.5% gelatin	—	0	0.08–1.0	1.0	—	0
	Present	—	—	0–0.25	—	—
3% Petroleum jelly in ether	—	0	0.1–0.4	0	0	0
3% Silicone in ether	—	0	0.4–0.6	0	0	0

bile after treatment of the animal with this insecticide.

The difference between the solubilities of dieldrin and Telodrin in serum and water shows that serum constituents exert a powerful solubilizing effect (Table 2). A value of 195 $\mu\text{g./ml.}$ has previously been reported for the solubility of dieldrin in rabbit serum (Weikel, Laug & Tomchick, 1958). Ultra-filtration of serum that had previously been equilibrated with dieldrin and Telodrin showed that these compounds were absent from the filtrate, unless the initial concentration exceeded the solubility in serum, when the concentration of free compound in solution did not exceed 0.05 $\mu\text{g./ml.}$

Partition of radioactivity between the constituents of rabbit and rat blood after admixture in vitro. The distribution of [^{14}C]dieldrin and [^{14}C]Telodrin between the cellular components and the plasma of rabbit and rat blood was investigated *in vitro*, and results for Telodrin show that only a small proportion of radioactivity is found in the erythrocyte stroma, leucocytes and platelets, and the remainder is bound to the plasma constituents and erythrocyte contents (Table 3). The proportion of insecticide distributed between the plasma and the cells is 37:19 for Telodrin (in the present work) and 38:20 for dieldrin (Weikel *et al.* 1958).

After electrophoresis, the radioactivity in the erythrocyte contents is distributed into three peaks (Fig. 1), one of which remains at the start line, possibly in association with denatured protein. An intense peak of radioactivity migrates towards the

cathode in combination with haemoglobin, and a third peak of radioactivity, which may be associated with lipoprotein, travels towards the anode behind two bands of protein. The partition of [^{14}C]dieldrin and [^{14}C]Telodrin between rabbit and rat serum protein after separation at pH 8.6 and pH 4.5 by electrophoresis (Fig. 2) shows that the pattern of peaks of radioactivity is extensively altered by this change in pH. At pH 8.6, there are two sharp peaks of radioactivity, which in rabbit serum move with the mobility of albumin and the α -globulins respectively, and which in rat serum move with the mobility of pre- and post-albumin. A third peak has been observed, in some rabbit and rat sera, between these peaks. Four incompletely resolved peaks of radioactivity are seen in rabbit and rat serum after electrophoresis at pH 4.5, where the proteins are also incompletely resolved (Fig. 2).

In experiments where blood was removed from rats 3 hr. after intubation with 2 ml. of an aqueous 50 % emulsion of arachnis oil, and from rats which had been starved for 24 hr., the partition of [^{14}C]dieldrin and [^{14}C]Telodrin between the serum proteins gave two peaks of radioactivity after separation by electrophoresis at pH 8.6.

After separation at pH 8.6 by electrophoresis the distribution of radioactivity amongst rabbit serum

Table 2. *Solubility of dieldrin and Telodrin in rabbit serum and water*

Figures are concentrations ($\mu\text{g./ml.}$) at 20°.

	Dieldrin	Telodrin
Serum 1	—	56
	—	65
Serum 2	210	158, 162
	144	141
Water	0.05	0.03

Table 3. *Distribution of ^{14}C -labelled Telodrin between plasma and cellular components of rabbit and rat blood after admixture in vitro*

Results are expressed as counts/100 sec. in the amount of blood constituent contained in 1 ml. of whole blood.

	Rabbit	Rat
Plasma	371 200	350 200
Erythrocytes	—	110 700
Erythrocyte contents	156 900	—
Erythrocyte membranes	20 500	—
Leucocytes	5 700	6 700
Platelets	3 300	—
Total	557 600 (97.8 %)	467 600 (98.2 %)

Fig. 1. Partition of [^{14}C]dieldrin (—) and [^{14}C]Telodrin (----) between the erythrocyte contents after electrophoretic separation at pH 6.5 in phosphate buffer (I 0.025). Open bars represent the relative positions of erythrocyte proteins. [^{14}C]Telodrin (5×10^5 counts/100 sec.) and 19×10^5 counts/100 sec. of [^{14}C]dieldrin were separately incubated with 1 ml. of whole blood. In the Figures arrows indicate the origin.

proteins remained unaltered at seven concentrations of [^{14}C]Telodrin (Fig. 3); the smallest and greatest concentrations ($\mu\text{g./ml.}$) were 1.5 and 60.

Crossing electrophoresis for study of interaction between serum constituents and dieldrin and Telodrin.

Electrophoretically separated serum proteins were moved across a stationary line of either dieldrin or Telodrin by chromatography at right angles to the initial electrophoresis. The formation of two wedge-shaped loops (Cairns-Smith, 1961), which coincided in location with albumin and the α -globulins, confirmed the interaction between the insecticides and serum constituents.

Since dieldrin and Telodrin are water-insoluble, and since purified protein and lipoprotein fractions of plasma were not employed in these experiments, the areas of the loops cannot be used for calculation of the number of protein-binding sites (Cairns-Smith, 1961).

Distribution of [^{14}C]Telodrin, proteins and lipoproteins in fractions of rabbit serum after ultracentrifugation. The method of ultracentrifugal flotation is especially useful for the separation of lipoproteins. Unlike the serum proteins, the lipoproteins have densities from 0.93 to 1.16 (g./ml.), and the three density steps chosen (Table 4) cause flotation in the preparative ultracentrifuge of, respectively, the low-density lipoproteins, the low-density plus high-density lipoprotein-2, and the low-density plus total high-density lipoproteins (Lindgren & Nichols, 1960).

The concentration of radioactivity in the various fractions, withdrawn from top to bottom of the tube at the first density step (1.063), follows the protein concentration in these fractions (Table 4). Since the floatable lipoprotein is contained in fractions 1 and 2, the absence of radioactivity suggests that Telodrin is not associated with the

Fig. 2. Partition of [^{14}C]dieldrin (----) and [^{14}C]Telodrin (—) between rabbit and rat serum proteins after electrophoretic separation at pH 8.6 and pH 4.5. Open bars represent the relative positions of the major serum proteins. (a) and (b) represent recorder traces for the radioactive insecticides in rabbit serum at pH 8.6 (veronal buffer) and pH 4.5 (acetate buffer) respectively, and (c) and (d) represent similar traces in rat serum at these pH values. [^{14}C]Telodrin (6×10^5 counts/100 sec.) and 24×10^5 counts/100 sec. of [^{14}C]dieldrin were separately incubated with 1 ml. of serum in all cases. In (b) and (d), α_1 - and α_2 -globulin have been located by the method of Burgi & Schmid (1961).

chylomicra and lipomicros. The first and second fractions at the second density step (1·125) contain the floatable lipoprotein, and since fraction 2 has a high concentration of radioactivity and an elevated cholesterol value, which reflects a high lipoprotein content (Table 4), the radioactivity in this fraction may be associated with high-density lipoprotein-2. At the third density step (1·200), the concentration of radioactivity in the fractions follows the cholesterol values, and high concentrations of Telodrin in fractions 1 and 2 may thus be associated with high-density lipoprotein-2 and -3. Serum proteins begin to appear in lipoprotein-containing fractions at the third density step (Fig. 4). In an electric field, the high-density lipoprotein fraction moves with the mobility of α_1 -globulin. After removal of all the lipoproteins, the lipid in the

serum exists in the form of specific complexes with albumin. High concentrations of albumin and of radioactivity occur in fraction 5 at the second density step, and in fractions 4 and 5 at the first density step (Fig. 4).

Distribution of [14 C]dieldrin and [14 C]Telodrin amongst the constituents of rabbit serum after passage through a DEAE-cellulose column. Five successive bands, containing γ -globulin, β -globulin, albumin, the α -globulins and a protein with the same electrophoretic mobility as albumin respectively, were obtained from this column by a stepwise elution method, adapted from the discontinuous gradient method of Sober *et al.* (1956). Radioactivity was mainly associated with the third, fourth and fifth bands (Fig. 5); the fifth band had the highest ratio of radioactivity to protein of any fraction of eluate. The distribution of radioactivity in respect of the serum proteins is qualitatively similar for rabbit serum containing dieldrin and Telodrin. Some protein remained on the column but this protein was not associated with high radioactivity, and could be eluted with alkali.

Experiments with rats in vivo. After intraperitoneal administration of [14 C]dieldrin and [14 C]Telodrin the proportionation of radioactivity between plasma and erythrocytes remained roughly constant, in spite of differences in time and in concentration of radioactivity (Table 5), but the actual concentration of radioactivity after 2 hr. was higher than after 4 days.

When the radioactivity in the blood of rats was followed after intravenous injection of [14 C]Telodrin, rapid unloading of the blood was found (Table 6). Extrapolation of a logarithmic plot of the time-course for the concentration of radioactivity in the first specimens of withdrawn blood gave an apparent value of less than 2% of the dose at zero time.

Nature of the radioactivity in the experiments in vivo. After treatment of animals with [14 C]dieldrin or [14 C]Telodrin, the distribution of 14 C between the soluble proteins of blood *in vivo* is identical with the distribution *in vitro*. Thus the radioactivity was due to unchanged starting materials and not to metabolites. This was confirmed for Telodrin by gas chromatography, when all the label was accounted for as Telodrin.

In another experiment the urine of a rat was collected for 8 days, after injection with [14 C]Telodrin (24×10^6 counts/100 sec.). The metabolites in the urine (2×10^6 counts/100 sec.) were extracted with ethyl acetate, which was then washed with 0·1N-sodium hydroxide, and one-half of the concentrate (8×10^5 counts/100 sec.) was taken up into rabbit serum. After electrophoresis of a suitable portion, a strong peak of radioactivity remained near the origin, in the position occupied

Fig. 3. Partition of different concentrations of [14 C]-Telodrin between rabbit serum proteins after electrophoretic separation at pH 8·6. (a), (b), (c), (d), (e), (f) and (g) represent the partition of radioactivity of these concentrations (μ g./ml. of rabbit serum) of [14 C]Telodrin: 60, 48, 24, 12, 6, 3, 1·5.

Table 4. *Distribution of [¹⁴C]Telodrin, proteins and lipoproteins in fractions of rabbit serum after ultracentrifugation*

[¹⁴C]Telodrin (6.3×10^5 counts/100 sec., 20 μ g.) was equilibrated with serum for 30 min.; the mixture was then spun in different salt solutions at 135 000g and separated into fractions (for details, see the Materials and Methods section).

Density steps (g./ml.)	Fractions	Concn. of [¹⁴ C]Telodrin (counts/100 sec./0.1 ml. of tube contents)	Concn. of protein (mg. of protein N/0.1 ml. of tube contents)	Concn. of cholesterol (mg./0.1 ml. of tube contents)
1.063	1	7340	0.07	1.53
	(top)			
	2	2320	0.03	1.25
	3	13040	0.39	3.38
	4	119230	2.32	3.8
1.125	5	34180	1.51	1.7
	(bottom)			
1.200	1	2270	0.08	—
	2	27230	0.05	6.13
	3	18770	0.25	2.30
	4	10400	0.39	0.55
	5	39980	1.97	2.8
1.200	1	61280	0.40	7.11
	2	38080	0.35	2.50
	3	10260	0.33	1.92
	4	8320	0.43	0.75
	5	41350	—	3.64

Fig. 4. Relative concentrations of the soluble proteins in fractions of rabbit serum after ultracentrifugation (see Table 3). ▨, γ -Globulins; ▩, β -globulins; ▤, α -globulins; □, albumins.

by β -globulin (Fig. 6). These results and others demonstrate that the distribution of metabolites of Telodrin, from urine and faeces, amongst the serum constituents (Fig. 6) is entirely different from that found for the initial substance, and the proportion of radioactive metabolites in circulating blood is therefore too small to affect the experimental results, in which the radioactivity can accordingly be ascribed to unchanged dieldrin or Telodrin.

Effect of dieldrin and Telodrin on the binding of 3,3',5-tri-[¹³¹I]iodothyronine to rabbit serum proteins. After electrophoresis at pH 8.6, the radioactivity in the serum proteins was distributed into three peaks (Fig. 7). An intense peak of radioactivity migrates with serum albumin and two incompletely resolved peaks move with the mobility of

Fig. 5. Separation of the soluble proteins of rabbit serum by DEAE-cellulose-column chromatography, and their association with [¹⁴C]Telodrin. [¹⁴C]Telodrin (5×10^5 counts/100 sec.) was incubated with 1 ml. of test serum, which was loaded on to the column (for details, see the Materials and Methods section). Major constituents recorded for the bands of protein were separated on cellulose acetate by electrophoresis at pH 8.6. —, Concentration of protein, as measured by $E_{1\text{cm}}$. at 270 m μ ; ---, radioactivity.

β -globulin and post-albumin. This distribution of radioactivity remained unchanged when Telodrin-saturated serum was substituted for ordinary serum in this experiment. Measurement of free and bound 3,3',5-tri-iodothyronine, following the method of Shapiro & Rabinowitz (1962), confirmed the foregoing result.

DISCUSSION

There is strong evidence for the binding of dieldrin and Telodrin with the soluble proteins of the circulating blood. This interaction involves

Table 5. *Distribution of radioactivity between blood plasma and erythrocytes in rats after intraperitoneal injection of [¹⁴C]dieldrin and [¹⁴C]Telodrin respectively*

Blood of rats 1 and 4 was taken 2 hr., and of rats 2, 3, 5 and 6 4 days, after injection. Dose of each rat: 1.5×10^7 counts/100 sec. (0.15 mg.) of [¹⁴C]dieldrin or 2.4×10^7 counts/100 sec. (0.45 mg.) of [¹⁴C]Telodrin. Results are expressed as counts/100 sec. in the amount of blood constituent contained in 1 ml. of whole blood. Animals weighed 500 g.

Rat no.	...	...	Dieldrin-treated animals			Telodrin-treated animals		
			1	2	3	4	5	6
Plasma			4320	2054	2314	2995	707	556
Erythrocytes			2071	1518	1302	1744	391	361

Table 6. *Concentration of radioactivity in rat blood after intravenous administration of [¹⁴C]Telodrin*

Dose: 76×10^5 counts/100 sec. for rat 1 (320 g. wt.) and 70×10^5 counts/100 sec. for rat 2 (200 g. wt.).

Time after injection (min.)	Counts/100 sec./ml. of blood		% Dose in the circulating blood	
	Rat 1	Rat 2	Rat 1	Rat 2
4	1500	—	0.32	—
8	950	—	0.20	—
14	750	—	0.16	—
15	—	4240	—	0.61
30	—	1920	—	0.27
35	620	—	0.12	—
60	—	2370	—	0.34
69	550	—	0.12	—
95	650	—	0.14	—
120	—	2450	—	0.35
18 hr.	650	—	0.14	—

Fig. 6. Partition of the ¹⁴C-labelled metabolites of Telodrin in rat urine (—) and faeces (----) between rabbit serum proteins after electrophoretic separation at pH 8.6. Open bars represent the relative positions of the major serum proteins. [¹⁴C]Telodrin (24×10^6 counts/100 sec.) was injected intraperitoneally into the animal; 2×10^6 counts/100 sec. were recovered in the urine, of which 8×10^5 counts/100 sec. were incorporated into rabbit serum; 12×10^6 counts/100 sec. were recovered in the faeces, of which 8×10^5 counts/100 sec. were incorporated into rabbit serum.

albumin, a constituent of the α-globulin fraction which may be lipoprotein in nature, and a third constituent which is eluted after the α-globulins from DEAE-cellulose, and which contains protein

Fig. 7. Distribution of 3,3',5-tri-[¹³¹I]iodothyronine amongst the proteins of rabbit serum after electrophoretic separation at pH 8.6 in veronal buffer. Open bars represent the relative positions of the major serum proteins. Less than $0.1 \mu\text{m-mole}$ of 3,3',5-tri-[¹³¹I]iodothyronine was added to 1 ml. of serum.

with the same electrophoretic mobility as albumin. Unlike the binding of thyroxine with serum proteins (Tata, Widnell & Gratzner, 1961), the binding of dieldrin and Telodrin to constituent

proteins is not decreased with increasing concentration of insecticide, and the increasing concentrations are not accompanied by spill-over from one protein to another.

The carrier function of serum proteins for organic compounds has long been established (Bennhold, 1931, 1932) for serum albumin, and Schmid (1956) found that bilirubin and bilirubin glucuronide combined with serum albumin and α -globulin. Haemin is also bound to albumin and to high-density lipoprotein in human serum (Nishida & Kummerow, 1962). Thyroid hormones have a more complicated relationship with serum proteins, and 3,3',5-tri-iodothyronine is bound to albumin, post-albumin and β -globulin in rat serum; dieldrin and Telodrin do not interfere with the binding of this substance to serum proteins. Thyroxine is transported principally by thyroxine-binding prealbumin, albumin and thyroxine-binding globulin in human serum (Tata, 1961; Tata *et al.* 1961), of which the thyroxine-binding proteins possess highly specific binding sites for the hormone. Cortisol and corticosterone are transported in blood by serum albumin and a corticosteroid-binding globulin (Bush, 1957; Daughaday, 1956). This corticosteroid-binding globulin has an electrophoretic mobility at pH 5.2 between that of acidic α_1 -glycoprotein and the slower prealbumin which contains thyroxine-binding protein (Daughaday, 1958), and is eluted from DEAE-cellulose in the region of α_1 -globulin (Sober *et al.* 1956). The combination of dieldrin and Telodrin with the soluble proteins of blood somewhat resembles the complex binding of corticosteroids and thyroid hormones, but the actual pattern for the distribution of dieldrin and Telodrin amongst the serum proteins appears to be specific for these substances.

Haemoglobin is largely but not entirely responsible for the binding of dieldrin and Telodrin in erythrocytes. An unknown constituent of the erythrocyte contents combines with a small proportion of the insecticides, and it migrates in an electric field in the reverse direction to haemoglobin. In experiments with whole blood, the binding by erythrocytes is strongest for oestrone and oestradiol, and least intense for cortisol and corticosterone (Sandberg, Slaunwhite & Antoniadis, 1957); work with oestradiol has shown that haemoglobin was largely responsible for this binding (Bischoff & Bryson, 1960).

Erythrocyte stroma are freely permeable to oestradiol (Bischoff & Katherman, 1948; Bischoff & Bryson, 1960), and present evidence indicates that these membranes are permeable to dieldrin and Telodrin. Thus in erythrocytes these insecticides are not bound to the stroma, but to the erythrocyte contents, and the fact that the proportiona-

tion of dieldrin and Telodrin *in vivo* between blood plasma and erythrocytes remains approximately constant at different times and at widely different concentrations demonstrates the permeability of stroma to these insecticides.

The initial rate of removal of dieldrin and Telodrin from blood is very rapid, followed by a slow roughly logarithmic rate of removal. In these respects dieldrin and Telodrin resemble thalidomide (Faigle, Keberle, Riess & Schmid, 1962), 2-naphthylamine (Goldblatt *et al.* 1960) and reserpine (Sheppard, Lucas & Tsien, 1955).

Repeated extraction with ether removes all of the radioactively labelled insecticides from whole blood, and bile salts displace bound dieldrin and Telodrin from bovine albumin. In general, unloading in the kidney and liver is caused by breakdown of the carrier proteins, by changes in pH after penetration into the tissues and by phagocytosis. When [131 I]ovalbumin was injected intravenously into animals, radioactivity was found within a few minutes in the cytoplasmic granules of liver and other reticuloendothelial organs. Radioautography demonstrated that ovalbumin was concentrated in the macrophages of lymph nodes and in liver Kupffer cells, from which Haurowitz & Crampton (1952) claimed that this substance penetrated the cellular membrane, probably by phagocytosis. Since all of the plasma proteins enter phagocytic cells at the same rate (McFarlane, 1957), a mechanism for the rapid unloading of bound dieldrin and Telodrin from blood involving pinocytosis seems likely, especially since many water-soluble and water-insoluble substances are rapidly removed from circulating blood. The withdrawal of these substances into the sinusoid spaces of the liver would provide an alternative explanation of their rapid removal from blood.

SUMMARY

1. In 'chromatographic' experiments, Telodrin interacts with sodium taurocholate and with bovine albumin, not with gelatin.
2. Solubilities of dieldrin and Telodrin are 4000 times as great in rabbit serum as in water.
3. Dieldrin and Telodrin are located mainly in the erythrocyte contents and plasma of rabbit and rat blood and not in the leucocytes, platelets or stroma.
4. On electrophoretic evidence at pH 8.6, dieldrin and Telodrin are associated with haemoglobin and an unknown constituent in the erythrocyte contents, with albumin and α -globulin in rabbit serum, and with pre- and post-albumin in rat serum. In rabbit and rat serum at pH 4.5, these substances are partially separated into four zones by electrophoresis.

5. Ultracentrifugation of rabbit serum causes flotation of a proportion of Telodrin with high-density lipoprotein which has similar electrophoretic properties to those of α_1 -globulin.

6. DEAE-cellulose-column chromatography separates from rabbit serum three bands of radioactive dieldrin or Telodrin, associated with albumin, the α -globulins and a protein with the same electrophoretic mobility as albumin respectively.

7. After intraperitoneal injection of single doses of [^{14}C]dieldrin or [^{14}C]Telodrin into the rat, the radioactivity in blood is shared between plasma and cells, and the ratio remains roughly constant for 4 days.

8. The very rapid initial rate of removal of dieldrin or Telodrin from blood is followed by a roughly logarithmic rate of removal.

9. Dieldrin and Telodrin do not interfere with the binding of 3,3',5-tri-[^{131}I]iodothyronine to serum proteins.

10. Free permeability of the erythrocyte surface to the insecticides is suggested by the proportionation of dieldrin and Telodrin between blood plasma and cells *in vivo* at different times and different concentrations.

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