4,4'-Diaminodiphenyl sulphone: Solubility and Distribution in Blood

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4,4'-Diaminodiphenyl sulphone is used in the treatment of leprosy. Comparatively little study has been made of its mode of action, but there is evidence that it resembles the sulphonamides in being a *p*-aminobenzoic acid antagonist, although there are indications of other modes of action. Both in the sulphone and in sulphanilamide transfer of electrons from the amino group to the sulphonyl group imparts a negative charge to the oxygen atoms, and both compounds follow the classification scheme developed by Bell & Roblin (1942), according to which the bacteriostatic activity is a function of this transfer of negative charge [see also Youmans & Doub (1946)]. The metabolic fate of 4,4'-diaminodiphenyl sulphone and sulphanilamide are very similar and Francis & Spinks (1950) found that the toxic effects of the sulphone resemble those of sulphanilamide in respect of nervous symptoms (in the goat) as well as haemolytic effects and other changes in the blood of man.

The parallel extends to the distribution of the drugs in the tissues, where sulphanilamide is so rapidly and evenly distributed that it has been used to measure the water content of the organism (Painter, 1938), and it has been found in animal experiments by Francis (1953) that the concentration of the sulphone in most tissues is of the same order of magnitude as that in the blood, though there was some variation from one species to another.

Both sulphanilamide and 4,4'-diaminodiphenyl sulphone may be in simple free solution in intracellular as well as intercellular phases. Detailed investigations of less-complicated systems, especially blood, have shown, however, that the distribution of sulphanilamide and other sulphonamides is not so simple a matter (Davis, 1942); for instance, for various sulphonamides equal distribution between blood plasma and cells is uncommon; thus the concentration of sulphanilamide in blood cells (calculated on the basis of 'available water') is double the plasma concentration (Anderson & Thomson, 1948; Kael & Wagner, 1954). Binding to plasma protein is also very common among these substances (Langecker & Loppnov, 1955; Davis & Wood, 1942); thus in a series of

experiments on seven sulphonamides, sulphanilamide showed weakest binding, yet the percentage bound was as high as 20 %.

The distribution of 4,4'-diaminodiphenyl sulphone itself in blood between cells and plasma does not seem to have been studied previously; but Kono (1953) concluded from experiments with two other sulphones, promine and promizole, that they were adsorbed on red blood cells in accordance with Freundlich's adsorption isotherm, that this adsorption was inhibited by the presence of blood plasma and that both drugs combined with serum albumin. It was to be expected that 4,4'-diaminodiphenyl sulphone might also show a complex behaviour, especially as other results point in the same direction; Chatterjee & Poddar (1956, 1957) and Poddar & Chatterjee (1957) found its concentration in lepromatous tissue of patients to be ten times that in normal skin and radioautographs showed high concentration of the sulphone in affected blood vessels and nerves. It was not clear, however, whether this high drug concentration was associated with the leprosy bacteria (which were present in large numbers) or with the body tissue. It would obviously be desirable to know to what extent 4,4'-diaminodiphenyl sulphone associates with normal tissue either by adsorption or by virtue of some metabolic activity, and an attempt has been made in these studies to throw further light on this problem.

EXPERIMENTAL

Materials

Blood was obtained from one volunteer only to avoid variation between samples; however, the period covered by the experiments was several months. 4,4'-Diaminodiphenyl sulphone was a recrystallized (from ethanol) laboratory-grade product (m.p. 176–179°).

Methods

Solubility. Solid diaminodiphenyl sulphone and the solvent were shaken in a thermostat for at least 4 hr. The supernatant liquid was isolated by suction through cotton wool and concentration of the sulphone was determined.

Distribution of diaminodiphenyl sulphone in blood. The equilibration was in most cases carried out in tubes 10 cm. long, 0.7 cm. diam., with a capacity of 2–3 ml. Each thin equilibration tube was calibrated so that any volume of liquid in it could be measured.

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The sample temperature was maintained at the equilibration temperature (37°) until the end of the centrifuging by placing a half-solidified eutectic mixture in the extra volume between the thin equilibration tube and the wall of the larger centrifuge tube. A mixture of benzoic acid and acetamide (4:3, w/w) was found to be most convenient (though the hygroscopic nature of the mixture necessitated frequent drying over phosphorus pentoxide). The centrifuge was heated to approx. 37° during centrifuging.

The blood was drawn not more than 1 hr. before the start of the equilibrations. Heparin was used as anticoagulant. The blood was transferred to the equilibration tubes. A known quantity of standard solution of diaminodiphenyl sulphone in iso-osmotic sodium chloride was added to each and the tubes were placed at 37°. During the equilibration the blood cells were kept suspended by gentle stirring.

Afterwards the tubes were centrifuged at approx. 2000 rev./min. (max. radius about 15 cm.) for 15 min. Then as much as possible of the plasma was drained. In some cases iso-osmotic sodium chloride solution was added to the cells, followed by another incubation period, centrifuging and draining. In other experiments, part or all of the plasma was withdrawn or washed away before any of the sulphone was added. Throughout the experiments the volumes of liquid entering and leaving the tubes were measured.

Determination of diaminodiphenyl sulphone. This was done by the method of Bratton & Marshall (1939) for sulphanilamide, as modified by Simpson (1949), in which the sulphone is diazotized and coupled in acid solution with *N*-1-naphthylethylenediamine dihydrochloride. The blood was haemolysed with a saponin solution and acidified with hydrochloric acid. Protein was precipitated with trichloroacetic acid. Sodium nitrite, ammonium sulphamate and coupling reagent were then added and the extinction was measured at 550 m μ . For all determinations use was made of standards and blanks.

RESULTS

Solubility

The solubility of 4,4'-diaminodiphenyl sulphone in water at 37° is 38 mg./100 g. Additional determinations (two to eight at each temperature) showed that the logarithm of the solubility varies linearly with the reciprocal of the temperature between 25° and 40°: the solubility increases by a factor of 2.0 with a 12° increase in temperature. The solubility of the sulphone in blood plasma at 37° (average of four 68 mg./100 g.; s.d. 4%) is much larger than that in water (or in iso-osmotic sodium chloride). This indicates that some kind of compound formation takes place in the plasma.

Experiments with blood

The total number of experiments was 58; the average recovery was found to be 94.5% (s.d. 4.5%). No correlation was found between recovery and the plasma concentration/cell concentration ratio for the sulphone or between recovery and concentration of the sulphone.

The relatively low recovery may indicate that part of the sulphone is bound irreversibly to protein. Simple adsorption on precipitated protein is less likely in view of the above results.

The penetration of diaminodiphenyl sulphone into, as well as passage out of, the cells was too rapid to be estimated with the present technique, since equilibrium was established within about 5 min.

The possibility, mentioned above, of compound-formation in the plasma is supported by the finding that the ratio of total concentration of the sulphone in plasma (D_p) to that in cells (D_c) at equilibrium depended on the degree of dilution of the plasma. The results of experiments with varying amounts of diaminodiphenyl sulphone and plasma are shown in Fig. 1.

Fig. 1. Ratio of plasma concentration to cell concentration (all mM; 1 mg./100 g. $\approx$ 0.04 mM) as a function of plasma dilution: $\circ$, $0.06 < D_c < 0.08$; $\bullet$, $0.12 < D_c < 0.16$; $\square$, $0.24 < D_c < 0.32$; $\blacksquare$, $D_c = 0.80$. Ordinate: ratio of total concentration of diaminodiphenyl sulphone in (diluted) plasma to that in cells (D_p/D_c) after equilibration. Abscissa: x_p = vol. of undiluted plasma/vol. of undiluted plasma plus 0.9% NaCl. The points marked 14 and 17 are average values of 14 and 17 experiments respectively; the vertical lines represent standard errors. Diaminodiphenyl sulphone, dissolved in 0.9% NaCl solution, was added to heparinized blood and equilibrated at 37° for not less than $\frac{1}{2}$ hr. Cells and (diluted) plasma were separated by centrifuging and the concentration of the sulphone in them was determined.

Thus a quantitative discussion may be based on the following two hypotheses: (1) That the cell concentration (i.e. total amount of the sulphone/ml. of cell fraction) at constant chemical potential of the sulphone is independent of variations in x_p (volume of plasma/volume of plasma plus 0.9% sodium chloride); (2) that the sulphone forms complexes with some component of the plasma, probably albumin (see next paper: Linderstrøm-Lang, 1962), and that the formation is reversible.

A probable reaction scheme is

with corresponding equilibrium constants

$$K_1 = \frac{AD}{A \cdot D}, \quad K_2 = \frac{AD_2}{AD \cdot D}, \text{ etc.} \quad (1)$$

where D is free concentration of diaminodiphenyl sulphone in (diluted) plasma and A is free albumin concentration etc.

It may be deduced that

$$\frac{D_p}{D} = 1 + A_p K_1 \frac{1 + 2DK_2 + 3D^2K_2K_3 + \dots}{1 + DK_1 + D^2K_1K_2 + D^3K_1K_2K_3 + \dots} \quad (2)$$

where D_p is the total concentration of the sulphone and A_p the total albumin concentration in (diluted) plasma.

Introducing $D/D_c \equiv 1/K'$, where D_c is the total concentration of the sulphone in cells,

$$\frac{D_p}{D_c} = \frac{1}{K'} + A_p \frac{K_1}{K'} \frac{1 + 2DK_2 + 3D^2K_2K_3 + \dots}{1 + DK_1 + D^2K_1K_2 + D^3K_1K_2K_3 + \dots} \quad (3)$$

If free diaminodiphenyl sulphone is considered to be in ideal solution in the plasma, and if hypothesis (1) above is valid, then K' is independent of A_p at constant D , and D_p/D_c becomes linearly dependent on A_p at constant D , as indicated by the straight line in Fig. 1.

$1/K'$ is D_p/D_c for $A_p = 0$ (i.e. $x_p = 0$); thus the line representing the data for which $D_c \approx 0.14$ mm yields $1/K' = 0.38$. The corresponding $D (= D_c/K')$ is $0.38 \times 0.14 \approx 0.05$ mm.

In a concentrated protein solution the conditions are not ideal. Strictly speaking, the concentration (D') of free diaminodiphenyl sulphone in solution inside the cells in equilibrium with free sulphone (D) outside is not obtainable. However, for salts dissolved in protein solution it is normally found that (1) $(\partial C_s / \partial C_p)_{\mu_s}$ is small, and (2) it is constant and negative (e.g. Güntelberg & Linderstrøm-Lang, 1949), where C_s is salt concentration, and C_p is protein concentration (both g./1000 g. of water)

and μ_s denotes constant chemical potential of the salt. If this can be applied to diaminodiphenyl sulphone the value of $(D - D')$ in g./1000 g. of water will be small, positive and proportional to the haemoglobin concentration. The effect may be expressed as hydration of the protein in solution. Dick (1959) cites values from different authors, and they are in the range 0.1–0.3 g. of water/g. of protein. For haemoglobin Drabkin (1950) gives the hydration as 0.34 g. of water/g. of haemoglobin.

In blood cells approximately one-third of the weight is haemoglobin and two-thirds water; of this water, an amount corresponding to one-third of the amount of haemoglobin is considered unavailable to solutes according to the above-mentioned statement. So the amount of water available (per 100 g. of cells) is

$$100 [1 - \frac{1}{3} (1 + \frac{1}{3})] \approx 60 \text{ g.},$$

and the free concentration (mg./100 g. of cells) to be expected is therefore $0.60D$. The specific gravity of a cell is 1.08, so, per 100 ml. of cells, $D' \approx 0.65D$, and therefore $D_c/D' = (1/0.65) \times (1/0.38) = 4.1$.

DISCUSSION

Less than 5 min. after the addition of diaminodiphenyl sulphone for the attainment of equilibrium between blood cells and their surroundings is not an unreasonable result if the transport mechanism is facilitated diffusion, i.e. including some kind of carrier system, but it may be explained equally well by an adsorption rather than an absorption process. A determination of the temperature coefficient of the equilibrium ratio or work with blood-cell 'ghosts' might yield an answer to the problem, as might a radioautographic study (cf. Poddar & Chatterjee, 1957).

The ratio D_c/D' is about 4, and this suggests that the sulphone is present in much higher concentration inside the cells than outside (in the absence of plasma); any of the following may offer an explanation: (1) active transport; (2) compound formation e.g. with haemoglobin; (3) adsorption on the cell surface.

With an increase in D , $1/K'$ decreases slightly, whereas if saturation phenomena were at work the opposite should be expected. The system may have been far from saturation at the experimental conditions and the binding of one diaminodiphenyl sulphone molecule may have been facilitated by the presence of other molecules of the sulphone already bound.

The absence of saturation phenomena in the concentration range employed, and the rapid establishment of equilibrium, agree with the findings of Wagner & Kacl (1958) for sulphanilamide. They reach the conclusion that the binding

is not an adsorption phenomenon but probably involves diffusion into the blood cells.

The relationship between D_p/D_c and x_p is possibly non-linear (see Fig. 1); this could be caused by an adsorption of diaminodiphenyl sulphone on to the cell surface which was inhibited by the presence of blood plasma (cf. Kono, 1953). Hypothesis (1) above may therefore be valid only to a first approximation; the deviation is not, however, necessarily significant.

In the plasma the solubility data alone show that in this part of the system compound formation between the sulphone and a component does take place. A numerical calculation of the equilibrium constants has been attempted by Linderstrøm-Lang (1962).

SUMMARY

1. The solubility of 4,4'-diaminodiphenyl sulphone in water is 38 mg./100 g. at 37° and decreases by a factor of two with a 12° decrease in temperature.

2. Equilibrium between human blood cells and plasma with respect to 4,4'-diaminodiphenyl sulphone is established within 5 min.

3. The amount of the sulphone contained in, or in other ways attached to, the blood cells is four times an estimated free concentration inside the cells.

4. 4,4'-Diaminodiphenyl sulphone forms complexes with a component in human blood plasma.

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4,4'-Diaminodiphenyl sulphone: Binding to Serum Albumin

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It was shown in the preceding paper (Linderstrøm-Lang & Naylor, 1962) that 4,4'-diaminodiphenyl sulphone, dissolved in blood plasma, forms one or more complexes with a component of the plasma. The present paper describes an attempt to verify that the component is plasma albumin.

EXPERIMENTAL

Materials. 4,4'-Diaminodiphenylsulphone was a recrystallized (from ethanol) laboratory-grade product (m.p. 176–

179°). The serum albumin used was crystallized bovine plasma albumin from Armour Pharmaceutical Co.

Solubility determinations. Solid diaminodiphenyl sulphone and the solvent were shaken in a thermostat overnight. The supernatant liquid was isolated by suction through a sintered-glass disk (G4) and concentration of the sulphone was determined.

Dialysis experiments. The cellophan bags employed were, at the upper end, provided with a short piece of conical glass tubing fitted with a small rubber bung. Thus each bag could be used several times and an effective check for impurities in the cellophan wall was made possible.

Usually, 0.5 ml. of albumin solution in iso-osmotic (0.9%) sodium chloride was added to the weighed bags as the inner phase. These were then immersed in 2 ml. of

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