DETERMINATION OF BLOOD AND OTHER TISSUE CONCENTRATIONS OF PARACETAMOL IN DOG AND MAN

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The even distribution of non-conjugated paracetamol in the tissue waters of the dog has been confirmed. The mean tissue water: plasma water concentration is 1·1. A technique for the determination of non-conjugated paracetamol in man has been devised from existing methods. It has increased sensitivity and is applicable to the routine determination of a large number of samples. This method determines only non-conjugated paracetamol, the sulphated and glucuronated conjugates do not interfere with its accuracy, and it gives results comparable with previous methods. In man, 45 min. after oral administration of paracetamol, the whole blood: plasma concentration ratio is virtually constant at 1·1, indicating that the determination of non-conjugated paracetamol in whole blood should give a satisfactory indication of tissue level.

THE only detailed study which appears to have been made on the distribution of paracetamol (*N*-acetyl-*p*-aminophenol) in tissues (Brodie and Axelrod, 1949) was done using one dog given phenacetin by mouth. Paracetamol levels were determined because these workers had previously shown it to be the major metabolite of phenacetin. Subsequently, Clark (1951) demonstrated that the metabolic pathways of paracetamol in man and the dog were similar. Because of the limited evidence available we have repeated the work of Brodie and Axelrod on the tissue distribution of paracetamol in the dog.

The original analytical method of Brodie and Axelrod was used. A modification of this was subsequently used for the estimation of blood concentrations in man.

EXPERIMENTAL METHODS

Tissue Studies of Paracetamol in Dogs

Five dogs were given 300 mg./kg. paracetamol by mouth. After 2 hr. they were killed by intravenous pentobarbitone. The dose and time were chosen so that the tissue concentrations of the drug could be compared directly with those of Brodie and Axelrod (1949) for non-conjugated paracetamol values after administration of an equivalent dose of phenacetin.

Determination of tissue levels was made by the method of Brodie and Axelrod (1948, 1949), except that the pH for extraction was changed because it gave unsatisfactory results for liver homogenates. Tissues were homogenised in 0·1N hydrochloric acid, and the homogenate neutralised and buffered at pH 6·6, before the extraction of non-conjugated paracetamol. By adding known amounts of the drug to tissues from treated animals, recovery was found to be 86 $\pm$ 3 per cent from liver

homogenate to which 6–96 mg./kg. had been added and 96 ± 4·5 per cent for muscle homogenate to which 6–96 mg./kg. had been added.

Results from the tissues of the treated animals are in Table I. The figures have also been calculated to give the proportion of paracetamol in tissue water related to its concentration in plasma water—assuming that water represents 92 per cent of the total plasma, and that the tissue water contents per cent are: liver 72, kidney 77, heart 76, spleen 75, lung 76 and muscle 73. These figures were also used by Brodie and Axelrod (1949).

Analytical Techniques in Man

Because the method of Brodie and Axelrod (1949) was found to be unsuitable for large numbers of samples, a method was devised which was a combination of the procedures of Lester and Greenberg (1947) and of Brodie and Axelrod (1948). The detailed method is described below. Essentially, whole blood is triturated with sodium sulphate to give a dry friable mass from which free paracetamol can be extracted with ether.

TABLE I

TISSUE LEVELS OF PARACETAMOL IN DOGS 2 HR. AFTER AN ORAL DOSE OF 300 MG./KG.
(Values given as mg./kg. net weight of tissues)

Tissue	Dog, sex and wt. (kg.) A F, 8·5	B M, 11·2	C M, 11·7	D M, 10·1	E M, 10·7	Mean ± s.e.	Brodie and Axelrod‡
Plasma	169	156	103	123	147	140 ± 12	96
Liver*	210	130	120	112	149	144 ± 18	99
Kidney	228	159	108	116	136	149 ± 22	104
Heart	204	132	92	113	133	135 ± 19	79
Spleen	178	109	86	96	115	117 ± 16	80
Lung	182	114	99	101	127	125 ± 15	88
Brain§	176	135	83	87	140	124 ± 17	82
Muscle	179	139	95	99	146	132 ± 16	69
Fat§	24	21	13	14	18	18 ± 2	—
	Tissue water : plasma water concentration ratios						
Liver	1·59	1·06	1·51	1·15	1·29	1·32 ± 0·10	1·32
Kidney	1·62	1·21	1·25	1·12	1·10	1·26 ± 0·09	1·29
Heart	1·47	1·02	1·08	1·11	1·09	1·15 ± 0·08	1·00
Spleen	1·29	0·85	1·02	0·95	0·96	1·01 ± 0·07	1·00
Lung	1·30	0·88	1·16	0·99	1·05	1·07 ± 0·07	1·11
Brain	1·23	1·01	0·95	0·83	1·12	1·03 ± 0·04	1·00
Muscle	1·34	1·12	1·16	1·01	1·25	1·18 ± 0·06	0·90
Fat	0·26	0·25	0·23	0·23	0·23	0·24 ± 0·01	—
Mean†	1·43	1·02	1·16	1·02	1·12		1·09
± s.e.	0·06±	0·05±	0·07±	0·04±	0·04±		0·06±

* Value recorded corrected for 86 per cent recovery; all other tissues assumed to give 100 per cent recovery.
† Excluding fat.
‡ Only one animal, therefore no deviations can be calculated. The dose was 2·7 g. phenacetin; weight of dog not stated.
§ The final coloured solutions obtained from brain and fat were almost invariably opalescent. This was compensated by reading their optical density at 600 mμ and subtracting the value so obtained from the reading at 515 mμ. The reading of the diazo compound *per se* at 660 mμ is negligible, but the standards are routinely read the same way for this comparison.

Paracetamol is mainly excreted as the sulphate ether (about 67 per cent) and as the glucuronate (about 33 per cent), (Lester and Greenberg, 1947), and it seemed desirable to ensure that these metabolites would not interfere with the determination of non-conjugated drug. Addition, at

the level of 50 μg./ml., of either of these substances to whole blood, and to whole blood containing a standard amount of paracetamol was found not to increase the level of non-conjugated drug determined.

A number of samples were analysed by the methods of Brodie and Axelrod (1949) and also by the modification finally adopted. The results are shown in Table II. By analysing whole blood samples containing known amounts of drug over an extended period, the percentage standard deviation of the method, at the levels of 10, 20 and 40 μg./ml. whole blood was established as ±5–7 per cent.

TABLE II

COMPARISON BETWEEN METHODS OF ASSAY FOR NON-CONJUGATED PARACETAMOL

Subjects	Modified method μg./ml. whole blood	Brodie and Axelrod method μg./ml. whole blood
A	24·6	25·4
B	14·0	15·8
C	5·5	5·8
D	16·9	16·8
E	17·4	18·0
F	12·4	11·3
G	24·3	22·6

Reagents. Sodium sulphate (anhydrous granular), ether (Analar), 0·1N sodium hydroxide, 40 per cent sodium hydroxide, α-naphthol reagent, hydrochloric acid, *n*-butanol, isopentanol, and potassium chloride (Analar).

The ether and isopentanol were purified by shaking successively with 40 per cent sodium hydroxide, water, 10 per cent hydrochloric acid and water; they were finally dried with anhydrous sodium sulphate.

The α-naphthol reagent was prepared by reacting 1 ml. of 5 per cent ethanolic solution of α-naphthol with 10 mg. of potassium dichromate and 1 ml. of 2N hydrochloric acid for 3–5 min. 19 ml. of 5 per cent ethanolic solution of α-naphthol were then added.

Procedure. Anhydrous granular sodium sulphate was added slowly to a sample of whole blood (2 ml.) with mixing until a dry friable mass was obtained. After standing for 5 min. the mass was extracted in a Soxhlet thimble for 1 hr. with purified ether (100–150 ml.) containing 1·5 per cent of purified isopentanol. The extract was reduced to 50–70 ml. by evaporation on a steam-bath and then extracted with portions of aqueous 0·1N sodium hydroxide (5 ml., 2 ml.). The combined aqueous extracts were treated with concentrated hydrochloric acid (1·5 ml.) and heated in ½ in. diameter test tubes, covered with glass marbles, for 45 min. in a boiling water-bath. The solution was cooled to room temperature (25°), α-naphthol reagent (5 drops) and 40 per cent sodium hydroxide (2·5 ml.) were added and the mixture allowed to stand for 2–3 min. The solution was then saturated with solid potassium chloride and extracted with butanol (5 ml.). The extract was dried with anhydrous sodium sulphate to give a clear solution and the developed colour read on a spectrophotometer at 635 mμ. The paracetamol content of the original blood was obtained from a standard graph prepared by the addition of

known amounts of the drug to whole blood, and treatment by the foregoing method.

Relationship of Whole Blood to Plasma Levels in Man

In the original blood level studies reported by Brodie and Axelrod (1948, 1949), Lester and Greenberg (1947), Weikel (1958) and Carlo, Cambosos, Feeney and Smith (1955), whole blood or plasma levels were used, although none of these workers made any specific reference to the ratio between the two. It was therefore of interest to determine this ratio for man. Assays for paracetamol in blood and plasma from 9 subjects 45 min. after administration of 1 g. of the drug, are recorded in Table III, together with the ratios. This ratio remains virtually constant at about 1·1, whatever the amount of drug in the blood and plasma.

TABLE III

SINGLE ASSAY OF PARACETAMOL FROM BLOOD AND PLASMA 45 MIN. AFTER THE ADMINISTRATION OF 1 G. PARACETAMOL

Subject	Whole blood μg./ml.	Plasma μg./ml.	Ratio
1	4·5	4·5	1·0
2	26·6	25·5	1·04
3	12·8	13·5	0·95
4	17·3	15·8	1·09
5	18·0	17·3	1·04
6	25·2	21·5	1·17
7	20·2	18·3	1·11
8	24·8	22·1	1·11
9	23·2	22·1	1·05
		Mean	1·062

Discussion

In dogs, paracetamol is distributed evenly in all the tissues with the exception of fat. The mean values were higher than those reported by Brodie and Axelrod (1949); this may, however, be explained by the fact that these authors administered the drug as phenacetin and only part of this would have been converted to paracetamol at the time the dog was killed. Nor did they state the weight of the animal.

A more important finding was the confirmation that the mean tissue to plasma concentration ratio (again excluding fat) in the dog is slightly above unity throughout. In other words, the compound showed no special preference for any one tissue but occupied all of the available water space.

In man, we have demonstrated that the ratio between whole blood and plasma concentration is also close to 1·1. It follows that an estimate of tissue levels should be obtained by a simple determination of whole blood or plasma concentration—especially as Clark (1951) has shown that the drug is dealt with in similar fashion in both species.

The analytical method we now recommend for the examination of large numbers of samples has been shown to give results comparable to those of Lester and Greenberg (1947), and Brodie and Axelrod (1949), but with a spectrophotometric sensitivity about $2\frac{1}{2}$ times greater. Previous work has assumed that only non-conjugated paracetamol is

estimated. We have demonstrated that the two major conjugated forms of paracetamol do not interfere with the estimation of non-conjugated drug.

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