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THE RELATIONSHIP BETWEEN BINDING OF THIOPENTAL TO PLASMA AND ITS DISTRIBUTION INTO ADIPOSE TISSUE IN MAN, AS MEASURED BY A SPECTROPHOTOFLUOROMETRIC METHOD*

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Abstract—Binding *in vitro* of thiopental to human plasma was measured by a spectrophotofluorometric method as well as by radioactive procedures. At relatively high concentrations of thiopental, binding remains fairly constant. However, below 6 mg/l., per cent binding increases inversely with concentration. This phenomenon, occurring also *in vivo* hours after thiopental administration, alters the distribution of the drug between plasma and adipose tissue; eventually the adipose tissue to plasma concentration ratios become lower with diminishing plasma concentration. Such a decrease may be considered the result of a passive mechanism which reduces the effectiveness of certain drugs. Administration of dextran to human subjects was found to lower the concentration of thiopental in plasma mainly by increasing blood volume. A spectrophotofluorometric method for the assay of thiopental has been developed. Specificity has been proven by a quantitative thin-layer chromatographic technique.

INTRODUCTION

THE EXTENT of binding to plasma proteins varies widely among barbiturates[1] and drugs in other series.[2, 3] This binding plays an important role in tissue distribution and in other processes such as glomerular filtration.

Numerous studies have established that binding follows the law of mass-action, being dependent on a number of factors, including protein and drug concentrations.[4] When interpreting tissue distribution data, it is not customary to correct for these two factors because their influence is usually of a lower degree of magnitude than the errors of the analytical method. However, in some cases changes in extent of binding produce measurable alterations in tissue distribution. The present paper indicates that as the binding of thiopental to plasma increases with decreasing total plasma concentrations of the drug, thiopental accumulation in human adipose tissue diminishes.

These studies, requiring measurements at low concentrations, were not feasible until a sensitive spectrophotofluorometric procedure for the assay of thiopental had

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been developed. This method was subsequently adapted for use with thiohexital, (DL-5-(1-methyl-2-pentynyl) 5-allyl-2-thiobarbituric acid.[5]

MATERIAL AND METHODS

Thiopental-2-^{14}C (Tracerlab) was purified by serial recrystallizations with twice the amount of thiopental* from 50% ethanol–water. The melting point of the purified material was 158–159°, which agrees with that reported by Tabern and Volwiler,[6] and its sp. act. was 3·84 μc/mg. Radioactive purity was established by the technic of isotope dilution.

Dextran,* average molecular weight 75,000 (range, 20,000–200,000), was obtained as a 6% solution in normal saline. Diphenylcarbazone and dichloromethane were of superior quality grade, whereas isoamyl alcohol was a spectroquality reagent (Matheson, Coleman and Bell), and all other chemicals, unless otherwise specified, are ACS reagent grade. Crystalline human albumin (lipid content <1 per cent) was purchased from Nutritional Biochemical Corp., Cleveland, Ohio.†

Petroleum ether (City Chemical Corp., New York, N.Y.; b.p. 60–68°) was used initially and, when it became unavailable, *n*-hexane practical (b.p. 66–69°) from the same source was substituted. These solvents were further purified as follows: 3 l. were placed in a clean glass-stoppered bottle and shaken for 5 min with 90 g of anhydrous Na_2SO_4. Then 30 g of activated charcoal (Merck or Penick, U.S.P.) was added and the contents were again shaken for 5 min. The solids were separated by suction filtration followed by gravity filtration. The filter paper employed was from a recently opened lot and the first few ml of solvent filtered was discarded. The solvent was examined in regard to fluorescent impurities as follows: 25 ml *n*-hexane was shaken with 2 ml of 2·5 N NaOH for 5 min and separation of the phases was obtained by centrifugation. The organic solvent was removed and the fluorescence of the NaOH solution measured at the same settings used in the spectrophotofluorometric method. The solvent 'blank' (above NaOH reading) and the readings for NaOH alone were less than the equivalent of 0·1 and 0·02 mg/l. of thiopental sodium respectively. The solvent was stored in glass-stoppered vessels at room temperature in the dark. 'Blank' was low even after several months.

Isoamyl alcohol (Allied Corp., General Chemical Division, New York, N.Y.), purified grade, was washed as previously described.[7] When equal amounts of washed isoamyl alcohol and 2·5 N NaOH were shaken, upon separation of phases the thiopental sodium equivalent measured in the aqueous phase was less than 0·1 mg/l. Spectroquality reagent solvent can be used in the method without purification. The glassware was washed with cleaning solution, water, and finally rinsed with distilled water. Distilled water with low fluorescence (equivalent to less than 0·03 mg/l. of thiopental) was utilized in all dilutions and solutions.

Fluorometric assay of thiopental in biological samples

The spectrophotofluorometric method for thiopental assay is essentially the same as the double extraction spectrophotometric method of Brodie and associates,[7]

* Thiopental and Dextran used for this study were generously donated by Abbott Laboratories, North Chicago, Ill.

† Recently, R. F. Chen (*J. biol. Chem.* **242**, 173, 1967) reported lipid content of human albumin from various commercial sources.

with three modifications. The first, of course, is the use of fluorescence to measure lower concentrations; the second is the need for highly purified solvent; and the third is the elimination of the 'wash' with plasma samples.

The drug is extracted from 0·75 M NaH_2PO_4 solution or homogenate of biological material* into either 1·5% isoamyl alcohol in petroleum ether (v/v) or 1·5% isoamyl alcohol in hexane (v/v) by shaking for 1 hr. Here, if convenient, the method may be interrupted; the thiopental is stable overnight at room temperature under these conditions. After separation of the aqueous phase by centrifugation at 500 *g* for 5 min, an aliquot of the organic solvent is shaken with 2 ml of 2·5 N NaOH. The mixture is then transferred into a 50-ml conical centrifuge tube. Separation of the organic phase is achieved by centrifugation followed by aspiration with a disposable capillary pipet attached to suction. It is important to remove the last traces of solvent by holding the capillary pipet 0·2–0·5 cm above the surface of the aqueous phase for 10–20 sec. When bile, whole blood, or tissue homogenates (20 per cent, w/v, in water) are analyzed, a buffer wash as described by Brodie *et al.*[7] is used prior to the extraction into NaOH. Adipose tissue is homogenized with 0·1 N NaOH (4 ml/g) and the mixture is centrifuged for 5–10 min at 500 *g*. Three distinct layers form;† the upper layer is removed by aspiration and the middle layer, which is the major fraction, is analyzed. All homogenizations are performed at 0–5° in glass homogenizers.

The Aminco–Bowman spectrophotofluorometer equipped with a 150 W, 7·5 Å xenon lamp, which is energized with a Sorensen power supply and a.c. voltage regulator 1000 S, is used with the following settings: activation 305 and fluorescence 505 mμ (Fig. 1). The instrument is adjusted to a thiopental sodium standard of 1 mg/l. in 2·5 N NaOH made from a 100 mg/l. aqueous solution, both prepared freshly each day. To be consistent with the method of Brodie *et al.*,[7] standards are prepared as the sodium salt; concentrations in plasma, etc. are reported as acid.

The slit arrangement used is No. 3 and the rotary slit setting is made 2 mm wide, and a specially selected IP 21 phototube is employed.‡ To facilitate readings, a thin, sharp-cut, yellow, heat-resistant filter may be attached to the shutter mechanism to eliminate scattered light; this causes a slight (2·5 per cent) loss of transmitted energy. Standard fluorescent cuvets are used, after cleaning with dichromate solution, distilled water, and alcohol. Under these conditions a solution of 1 mg/l. of thiopental sodium gives a relative fluorescent intensity (RFI) value of 0·120 above NaOH blank. After correction for blank, readings are converted to concentrations by means of a standard curve. Measurements of concentrations up to 2–3 mg/l. in 2·5 N NaOH can be made directly. Above this concentration, self-quenching occurs and solutions must be diluted to obtain readings in the linear portion of the calibration curve.§ With

* To each sample of biological material an equal volume of 1·5 M NaH_2PO_4 is added.

† When whole homogenates are used, connective tissue interferes with pipetting; the middle phase is free of connective tissues. Control experiments with whole homogenate and with the middle phase, in which thiopental concentrations were compared, gave the same results within experimental error (<10 per cent). Due to the fact that the density of adipose tissue is less than 1, a maximal error of 3 per cent may be introduced in the measurements. To an aliquot of the homogenate an equal amount of 1·5 M NaH_2PO_4 is added for the analysis.

‡ Since the completion of the present investigation, we have found that the EMI 9558 Q photomultiplier tube, when powered with a M-600 photometer (Schoeffel Instrument Co., Westwood, N.J.), gives a 2-fold increase in sensitivity and greater stability than the RCA tube.

§ With a microcuvette higher concentrations can be measured. In this case, to reduce scattering, a narrower slit setting (No. 2) has to be used.

unknown concentrations, it is sometimes helpful to determine by u.v. spectrophotometry what further dilutions should be made.

A final concentration as low as 0·1 mg/l. can be measured by fluorescence. In the present procedure, in contrast to the u.v. spectrophotometric method, an increase from 1 to 3 ml of biological fluid affects fluorescent blank only slightly; blanks must

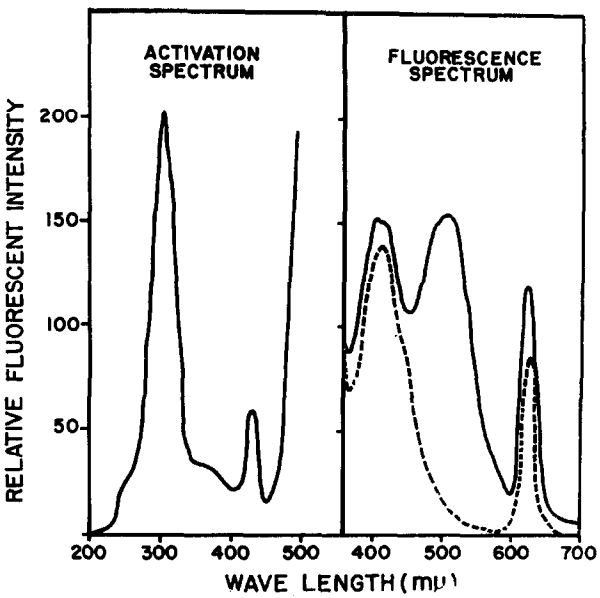

FIG. 1. Left panel, activation spectrum of thiopental (505 mμ). Right panel, solid line, fluorescence spectrum of thiopental; dotted line, fluorescence spectrum for 2·5 N NaOH (activation 305 mμ). Concentration of thiopental, 1 mg/l. Sensitivity setting, maximum.

be diluted in the same manner as unknown samples. Furthermore, for plasma samples the wash with 1·5 M NaH_2PO_4 was obviated because further reduction of already low blanks was negligible, and in control studies the same results were obtained with or without the wash. Recoveries for 1–5 μg of drug range from 85 to 95 per cent. The presence of pentobarbital,* phenobarbital, secobarbital, dextran, meperidine or sulfobromophthalein does not interfere with the method, but that of salicylate does. Hemolysis does not appreciably increase blank readings in the fluorescence procedure as it does in the spectrophotometric method.

Quantitative thin-layer chromatographic procedures

The samples of thiopental are prepared for chromatography as follows. An aliquot of biological material is placed in a 60-ml bottle and the drug is extracted in a manner similar to that used in the spectrophotofluorometric method, except for the use of dichloromethane as the solvent. After shaking and centrifuging, the organic phase is separated and dried with 0·5–1·0 g of anhydrous Na_2SO_4. A measured aliquot of

* Since in 2·5 N NaOH pentobarbital has an activation peak of 265 and fluorescence peak of 404 mμ, pentobarbital and thiopental can be measured independently in the presence of each other. With the above settings, pentobarbital can be measured accurately in concentrations as low as 0·3 mg/l. in 2·5 N NaOH.

the solvent (as large as possible) is evaporated at room temperature, *in vacuo*, in a conical tube. Subsequently, the material is chromatographed on 20 × 20 silica gel G (E. Merck, Darmstadt, Germany) plates, 0·17 mm thickness; the residue is spotted by using 0·1 to 0·2 ml of ethanol followed by two washes of 0·05 ml each. After 15–30 min to allow for equilibration, development is carried out in a tank using benzene–glacial acetic acid (8:1 v/v) as the solvent system. These chromatographic systems have been previously described.[8, 9] When ethanolic extracts obtained from adipose tissue are analyzed by this procedure, the plate is not allowed to equilibrate before immersion, because solvent vapors are taken up in the area of sample application, which causes broadening.

Suitable thiopental internal standards in biological material are concurrently chromatographed and visualized by spraying with mercurous sulfate reagent, followed by 0·1% diphenylcarbazone in chloroform.[10] Areas containing drug (thiopental is stable on the plate for several days) are removed from unsprayed plate zones with a microvacuum collector purchased from Brinkman Instrument Co. The thimble (cleaned by refluxing for 3 hr with acetone) and the inside of the collector itself are rinsed with up to 25 ml dichloromethane, and the drug is extracted from the solvent by shaking with 5 ml of 2·5 N NaOH. After centrifugation and removal of solvent, concentrations in NaOH are determined spectrophotometrically or spectrophotofluorometrically, or by both methods. With this method the recovery is above 90 per cent and the limits of detectability by using the spray are 1–2 μg, whereas by spectrophotofluorometry, the limit is 0·5 μg.

Specificity, accuracy and sensitivity of methods

In order to prove specificity of the spectrophotofluorometric method, thiopental was extracted into 1·5% isoamyl hexane, and then into NaOH. The NaOH extract was acidified and extracted with dichloromethane and the procedure described above was followed.

The fluorometric method can be used with low concentrations of thiopental and in the presence of substances which interfere with the spectrophotometric assay. The latter is usually not sensitive to thiopental concentrations below 1 mg/l. in the final extract. Concentrations as low as 0·1–0·3 mg/l. above reagent blank in NaOH can be measured by fluorometry, although provisions have to be made to reduce electronic instability. The average reagent blank is 0·1 μg and the average plasma blank 0·3 μg for 1–3 ml plasma. Thus, when 3 ml plasma, containing 0·3 μg is analyzed, readings are twice plasma blank. When the amounts to be determined are within the range of the spectrophotometric method, results obtained by both methods are in close agreement (generally within 5 per cent). Provided blank is low, concentrations higher than 2 mg/l. in the final NaOH extract can be measured by either method, and usually then the spectrophotometric method may be more convenient. Blank readings are lower with the spectrophotofluorometric method, which is particularly advantageous at low drug concentrations in biological fluids.*

* A comparison was made between the reagent blanks in the present method and that of the spectrophotofluorometric method of Swagzdis and Flanagan[11] applied to the measurement of thiopental. By using the relatively expensive spectroquality reagent, butyl ether, the reagent blank was 0·2 mg/l. which is equivalent to the present method; however, with washed superior grade butyl ether, it was considerably higher.

Higher fluorescence was obtained with 2·5 N NaOH than with lower concentrations of base. Spectrophotofluorometric measurements indicate that thiopental is relatively stable in 2·5 N NaOH. After 24 hr of standing at room temperature, no measurable change in reading was observed. This contrasts with the instability of other thiobarbiturates.[12]

Comparisons were made between the spectrophotofluorometric and quantitative thin-layer chromatographic procedures as follows. Two subjects received 1·8 g thiopental sodium i.v. in 15 min. Samples of blood, withdrawn during the period from 45–210 min after the dose, were analyzed by both the spectrophotofluorometric and the quantitative thin-layer procedures (see Table 1). About 0·025 mg per spot was

TABLE 1. COMPARISONS AMONG VARIOUS ANALYTIC METHODS FOR THIOPENTAL IN PLASMA*

Spectrophotometric	Fluorometric	Radioassay with ^{14}C-labeled thiopental	Quantitative thin-layer chromatography
34·5	35·5		
25·0	25·0		25·5
23·6	23·6		
22·2	22·0		21·0
15·2	15·2		15·5
14·7	14·8		
11·9	11·7		11·5
10·7	10·7		11·0
9·4	9·4		
5·2	5·3	5·5	
4·2	4·1		
4·1	4·0	4·1	
3·4	3·5	3·5	
2·9	2·7	2·7	
2·0	1·7		
1·2	0·94		
0·97	0·74		
1·1	0·83		
	1·0	1·1	
	0·70	0·69	
	0·51	0·53	
	0·17	0·16	
	0·11	0·12	

* All results (mg/l.) represent average of at least two determinations, with a minimum precision of ±3 per cent.

applied to the thin-layer plate. To determine the specificity of the method for adipose tissue, two subjects, while undergoing elective abdominal surgery, received 0·9 g of thiopental sodium intravenously; tissue samples obtained 2 hr later were treated similarly as the blood samples. The specificity of the spectrophotofluorometric method for plasma as determined by quantitative thin-layer chromatography is at least 95 per cent, whereas with adipose tissue it is 90–100 per cent, based on concurrent analyses of internal standards. The R_f under the conditions used was 0·47 ± 0·05 (S.D.). Comparisons were also made by using low concentrations <1 mg/l. of ^{14}C-labeled thiopental and the fluorescence method. As measured by the fluorescence method, thiopental is stable in plasma when kept at −20° for 48 hr in concentrations as low as 1 mg/l. Under these conditions, at concentrations of 30 mg/l. or higher, the drug is stable for at least one month.

Measurement of binding

Binding to plasma proteins was determined in duplicate for each concentration by equilibrium dialysis, generally for 24–48 hr at 37°, through a Visking membrane against 17 ml of 1/15 M KH_2PO_4–Na_2HPO_4 buffer at pH 7·4, as previously described.[13, 14] One ml of pH 7·4 Sörensen buffer was added to control bags; labeled or unlabeled drug was dissolved in 1 ml of buffer and added to other bags.

For some experiments, equilibrium concentrations as previously determined were placed inside and outside the dialysis bags, which were then incubated for 24 hr. In other experiments the bags were placed in a shaking bath at 115–120 c/min and incubated for only 16 hr.

In order to determine the effect of dilution of the 5 ml of plasma in the bag by 1 ml of buffer, undiluted plasma was placed in the bags and drug was added to the outside phase.

To measure the effect of the addition of dextran, control bags were prepared with 6 ml buffer and 1 ml normal saline. Other bags contained 5 ml plasma, 1 ml dextran solution, and 1 ml buffer. In these experiments the amounts of drug added were such that the equilibrium concentration inside the bag was 5 mg/l. The possible binding of the thiopental to dextran was measured by placing 5 ml dextran solution in a bag with 1 ml buffer containing drug. Experiments were also carried out with 5 ml of 5% (w/v) human albumin in buffer (final albumin concentration is 4·1% w/v). Prior to analysis, absence of leakage of protein was verified by adding 10% (w/v) trichloroacetic acid to an aliquot of the outside phase.

^{14}C-thiopental was combusted to $^{14}CO_2$ and trapped as $Ba^{14}CO_3$, as previously described.[15] The precipitates were assayed for carbon-14 activity with a 3-place gas flow counter (Nuclear-Chicago Corp.). When counts were low, samples were concentrated to dryness *in vacuo* before combustion.

The partition coefficients between Sörensen buffers of different pH's and pre-equilibrated peanut oil were determined at room temperature by a previously described method,[14] simultaneously in duplicate.

Observations in man

The subjects selected for the present study were adult surgical patients. The adipose tissue biopsies were obtained during elective surgery.

Studies of the effect of dextran. Three human subjects each received 500 mg thiopental sodium intravenously in 3 min. At appropriate intervals thereafter, samples of 1–2 drops of saturated, blood were collected in the presence of oxalate, and centrifuged to obtain plasma for analysis. About 3 hr after thiopental administration, 500 ml dextran solution was administered over a period of 5–8 min.

Uptake by adipose tissue. Five subjects received 300–500 mg of thiopental sodium intravenously in 3–5 min, and 5 other subjects received 1·1 to 1·5 g thiopental sodium in 15 min. This was followed by cyclopropane anesthesia for abdominal surgery. A series of simultaneous samplings of blood and adipose tissue was obtained 4–6 hr after thiopental administration. Arterial blood pH was measured by the Astrup technic. Care was taken to avoid hypoventilation and hypotension, which could have resulted in fluctuations in blood pH and perfusion of adipose tissue, by meticulous maintenance of ventilatory and circulatory adequacy.

RESULTS

Comparisons of spectrophotofluorometric, spectrophotometric, ^{14}C, and quantitative thin-layer chromatographic procedures

The results of comparative studies of the various analytic methods for plasma are given (Table 1). Good agreement was obtained by the present fluorescence, radioassay, and quantitative thin-layer chromatographic procedures. Below 3 mg/l., the results with the fluorescence method were lower than with the spectrophotometric procedure.[7] Below 1–2 mg/l. the discrepancies of the latter method are due to the fact that the measurements are hindered by high plasma blank and low absorbance.

Studies of binding

When thiopental concentrations were below 6 mg/l., per cent binding to plasma proteins increased as thiopental concentrations decreased. At drug concentrations of 0·54 to 4·4 mg/l. as much as 90–91 per cent (within ±1 per cent absolute error) was

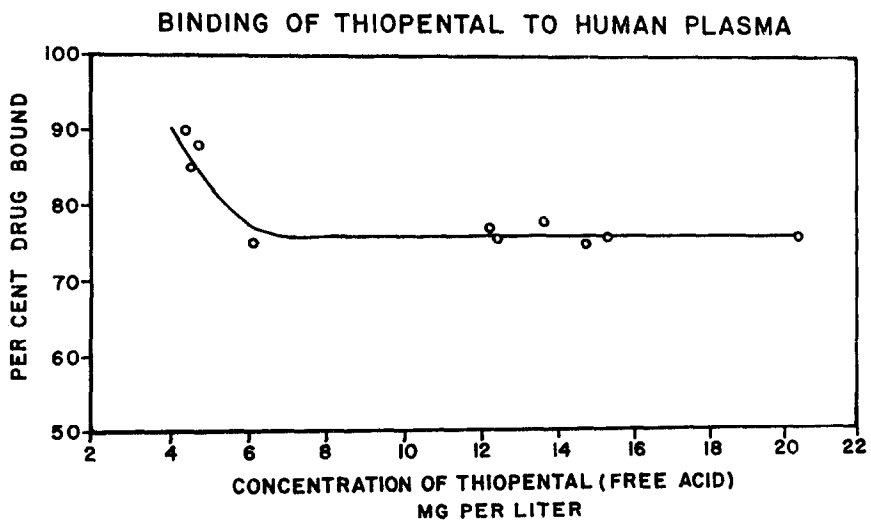

FIG. 2. Binding of thiopental to human plasma. Plasma from a different subject was used for each determination in duplicate. Below and including 6·1 mg/l. the same pooled plasma was used.

bound to human plasma proteins (Fig. 2, Table 2), as compared to 75 per cent reported previously for the range of 10–50 mg/l.[7] Analogous results were obtained with crystalline human albumin (Table 3). The findings noted with spectrophotofluorometry were confirmed with radioactive tracer technics (Table 2). Whether dialysis experiments were performed with shaking or statically for 24–48 hr, the extent of binding was found to be the same, within experimental error. Individual differences in binding were also observed, which apparently become more marked at concentrations below 10 mg/l. (Fig. 2, Table 2) and are presumably due to differences in albumin concentrations. In fact, binding to plasma of a subject with hypoalbuminemia (albumin, 2·1%; total protein, 3·8% w/v) was found to be 66 per cent with a concentration of 5·3 mg/l. inside the bag. The binding of thiopental to plasma of a subject with macroglobulinemia was measured and found to be the same as that for a normal subject.

Because of the poor solubility of thiopental in pH 7·4 Sorensen buffer (0·2 mg/ml at room temperature, which is of the same order of magnitude reported by Bush *et al.* in water),[16] it was frequently convenient to dissolve the drug in 1 ml Sorensen buffer and add it to 5 ml plasma, which results in a 16 per cent decrease in plasma protein concentration. This dilution effect on binding was found to be negligible within experimental error.

TABLE 2. BINDING OF THIOPENTAL-2-^{14}C TO HUMAN PLASMA PROTEINS

Plasma	Concentration inside bag (mg/L*)	Per cent bound	Per cent Albumin (w/v) and A/G ratio
A	3·9	79·6	3·2 (1·8)
	0·50	84·1	3·2 (1·8)
B	5·3	87·6	4·5 (2·1)
	0·69	90·4	4·5 (2·1)
C	4·0	88·5	4·9 (2·1)
	0·53	90·0	4·9 (2·1)
	0·16	90·8	4·9 (2·1)
D	3·9	87·9	4·8 (2·3)
	0·54	91·0	4·8 (2·3)

* As acid, calculated from ^{14}C data. Average results for two bags are within 0·2 per cent.

TABLE 3. BINDING OF THIOPENTAL TO HUMAN ALBUMIN

Concentration inside bag (mg/l*)	Per cent bound
1·5	87
2·2	82
2·3	81
3·3	80
4·3	77
5·7	75
6·0	75
12	76

* Each value is an average of two bags; concentrations as acid. The estimated absolute error is ± 1 per cent.

Goldbaum and Smith[1] showed that phosphate ion did not influence the binding of barbiturates at relatively high drug concentrations. Since our studies are concerned with lower concentrations, an experiment was carried out in which the buffer was 0·03 M phosphate and 0·03 M NaCl; the concentration inside the bag was 7 mg/l. The binding was unchanged as compared to that of a control with 0·06 M Sörensen buffer.

Changes of pH in the range 7·35 to 7·5 with a plasma concentration of 7·0 mg/l· had no significant effect on binding (<1 per cent). On the other hand, the partition coefficients between peanut oil and pH 7·3, 7·4, and 7·5 buffer were 75, 65, and 60 respectively.

Studies with dextran

In 3 subjects, plasma concentrations of thiopental were measured before and after the administration of dextran. After dextran, plasma levels of thiopental fell sharply and thereafter continued to decline at about the same rate (Fig. 3). Dialysis studies *in vitro* indicated that thiopental is not bound to dextran; other studies showed no measurable displacement of thiopental from plasma binding sites by the polymer.

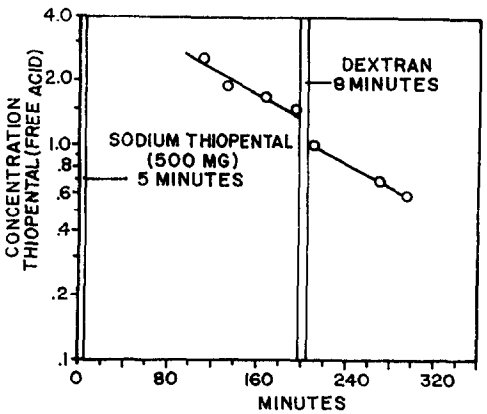

FIG. 3. The effect of dextran on plasma levels of thiopental. In two other subjects the change was from 1·4 and 2·2 to 1·0 and 1·7 mg/l. respectively, before and after dextran.

Uptake by human adipose tissue

In subjects receiving 300- to 500-mg doses of thiopental sodium, low plasma concentrations were determined at chosen sampling times. The subcutaneous fat/plasma partition ratio ranged from 5·7 to 10·2, and the omental fat/plasma ratio from 6·7 to 14; the respective average ratios were 6·7 and 8·5 (Table 4). When a large dose (1·5 g) was administered, the adipose tissue/plasma ratios (R) were about twice those found in the experiments with lower doses (Table 5). Within the sampling period the ratios were fairly constant, R being estimated to be accurate within 15 per cent.

Binding determinations were also performed on plasma of the same subjects studied for uptake of thiopental by adipose tissue, and the results are given in Table 6. The ratio of concentrations in adipose tissue/free drug (R′) ranged from 42 to 70; the arterial pH remained fairly constant (Table 6). The discrepant low value of R, obtained with subject 2 despite the administration of a large dose, is partially due to the relatively high binding. When this factor is taken into account (in R′), the discrepancy is partially resolved. The possibility of surgical interference with blood flow was examined in some experiments by comparing concentrations in duplicate samples of subcutaneous fat taken from the same and different areas. No significant differences (<10 per cent) in concentrations were found. Adipose tissue from other subjects, who received gaseous anesthetics but no barbiturates, exhibited a blank equivalent to plasma blank.

TABLE 4. THE UPTAKE OF THIOPENTAL IN HUMAN ADIPOSE TISSUE AFTER LOW DOSES

Dose (mg)	Time* (min)	Concentration† in Plasma (mg/l.)	Adipose tissue‡ (μg/g)	Adipose tissue/plasma ratio (R)
500	337	1·9		
	345		16·5 (O)	8·7
	346		15·0 (S)	7·8
	372	1·8	11·7 (O)	6·7
	375		14·4 (S)	7·9
350	271		6·7 (S)	5·2
	281	1·3	14 (O)	11
	285		7·0 (S)	5·4
	341	1·1	9·1 (O)	8·3
	344		6·4 (S)	5·8
	362	0·94	8·6 (O)	9·1
	364		6·2 (S)	6·6
300	314	1·1	10·9 (O)	9·9
	315		8·2 (S)	7·5
	343	1·1	8·9 (O)	8·1
	344		11·2 (S)	10
	373	0·92	7·5 (O)	8·1
	374		8·9 (S)	9·7
	411	0·85	6·4 (O)	7·5
	414		5·8 (S)	6·8
	420	0·80	6·1 (O)	7·6
	421		6·6 (S)	8·2

* From beginning of injection.
† As acid.
‡ O = omental fat; S = s.c. fat.

TABLE 5. THE UPTAKE OF THIOPENTAL IN HUMAN ADIPOSE TISSUE AFTER HIGH DOSES

Dose (g)	Time* (min)	Concentration† Plasma (mg/l.)	Adipose tissue‡ (μg/g)	Adipose tissue/plasma ratio (R)
1·5	263	5·5	75 (S)	14
	263		80 (O)	15
	330	5·1	73 (S)	15
1·5	199		112 (S)	
	199		106 (O)	
	270		99 (O)	
	370		89 (S)	15
	385	5·2	82 (O)	16
	385		95 (S)	18
1·5	290	5·6	77 (O)	14
			72 (S)	13

* From beginning of injection. The weights of the subjects ranged from 112–135 lb.
† As acid.
‡ O = omental fat; S = s.c. fat.

DISCUSSION

This study was initiated to extend the work of Shideman *et al.*,[17] who measured thiopental concentrations in human adipose tissue (C_a) but not in plasma (C_p). These workers found that peak C_a was achieved in about 1½ hr and that they remained constant for several hours. In the present study, as well as in that of Mark and Brand,[18]

TABLE 6. DETAILED STUDIES

Subject	Age (yr)	Sex	Weight (lb)	Dose* (g)	Time† (min)	Concentration‡		Per cent binding	Albumin conc. (%) and A/G ratio	pH	R	R′
						Plasma (mg/l.)	Adipose tissue (μg/g)					
1	23	F	154	0·5	410	1·2	9·5 (O)	85	4·2 (2·8)		7·9	53
							9·6 (S)				8·0	53
2	46	M	149	1·1	293	3·7	28·6 (O)	85	3·4 (1·5)	7·385	7·7	52
							23·4 (S)				6·3	43
					315	3·6	22·7 (O)	85		7·385	6·3	42
							22 5 (S)				6·3	42
					347	3·2	21·0 (O)	85		7·380	6·6	44
							22·4 (S)				7·0	47
					365	3·1	20·2 (S)	85		7·400	6·5	43
3	55	F	133	0·5	290					7·512		
					309	1·1	15·6 (O)	80	3·5 (2·5)	7·482	14	70
							15·0 (S)				13	65
					330	1·1	14·6 (O)	80		7·521	13	65
							13·9 (S)				13	65
4	70	M	152		237					7·442		
				1·5	290	4·3	5·9 (O)	78	3·5 (2·2)	7·448	14	62
								R′ calculated at pH 7·4 to 7·5:			48 to	53
									Average found:			53

* As sodium salt.
† From beginning of injection.
‡ O = omental fat; S = subcutaneous fat.

it was also shown that constant R values were attained.* It thus seems reasonable to assume that at 5–6 hr, steady state distribution of thiopental has been achieved. The present data may be interpreted to indicate that the increase in R is mainly attributable to a decrease in C_p. It has been shown that a change of pH within physiologic range alters R[7] and the peanut oil to buffer partition coefficient without causing a significant effect on binding to plasma[1] (see Results). Therefore fluctuations in blood pH were kept at a minimum in the present studies (Table 6).

During our studies we found that with high doses R was larger at 5–6 hr after thiopental administration than when small doses were used. Since distribution of drug is dependent on free drug concentration (C_u), based on the following argument, the ratio of C_a/C_u or R′ could explain the discrepancies in R observed with different doses (see Equations 1–3).

$$\frac{C_u}{C_p} = 1 - b \quad \text{constant} \tag{1}$$

b = fraction bound

$$R = \frac{C_a}{C_u} \tag{2}$$

At steady state, including constant pH, R′ is independent of binding and is constant.

$$R = \frac{C_a}{C_p} = (R')(1 - b) \tag{3}$$

Thus, at steady state, R is a function of binding and is not necessarily constant.

R would then decrease with decreasing C_p if relative binding increases. Furthermore, in the special case of a highly bound drug such as thiopental, seemingly small changes in binding (e.g. 80–85 per cent) will result in a significant change in the fraction of free drug (20–15 per cent) with a marked effect on tissue distribution (R).

A survey of the literature regarding binding of thiopental revealed studies with bovine serum albumin.[1, 21] The studies of Goldbaum and Smith,[1] which are more detailed, indicated an increase in binding with decreasing plasma concentrations and also examined the effects of varying pH and albumin concentrations. However, the lowest concentration of drug studied (about 30 mg/l.) was higher than any in the present investigation. With concentrations of thiopental above 10 mg/l. in human plasma, Brodie *et al.*[7] reported 75 per cent binding.

Kane and Smith[22] found an increase of binding with decreasing concentrations (range, 2–20 mg/l.), but the results of a single experiment with human plasma were given only in a plot from which precise data are difficult to extract. To confirm these latter observations, binding experiments were carried out with plasma of different

* The recent work of Perl *et al.*[19] raises the interesting possibility that steady rate distribution between plasma and adipose tissue is not perfusion-limited, but may also be realized by direct diffusion from neighboring tissues. These factors and the effect of binding are corrections which could eventually be applied to the model proposed by Price *et al.*[20] Modifications involving body composition (in terms of the relative amount of adipose tissue) have already been proposed.[18]

subjects and in a few cases in those subjects in which C_a was analyzed. The results verified that binding is influenced by human plasma albumin and drug concentrations in a specified range. It should be emphasized that some of the concentrations of thiopental used in the present investigation with human plasma and human albumin were much lower than those previously studied.* Since albumin accounts for most of the binding, the differences among various subjects may be due to individual variations of this protein. Within the limits of human experimentation, the present data suggest that the R′ values obtained with low C_p are largely due to a change in binding as postulated in Equation 3. Thus, this observation may be interpreted to indicate a passive mechanism which reduces R and C_a when C_p decreases due to metabolism.†

The partition coefficient for thiopental was reported to be 63 in the system peanut oil and pH 7·4 Sörensen buffer at room temperature,[24] 65 in the present study, and 58 in the system olive oil and buffer at 37°.[22] An estimate of R at steady state may be obtained from these data by making the following assumptions: (1) the partition coefficient between the lipid contained in adipose tissue and plasma water is about the same as that between peanut oil, the composition and polarity of which approximates human adipose tissue, and buffer (about 60); (2) a correction is made for the water content of adipose tissue, which is about 20 per cent in a normal subject;[25] (3) a correction is made for binding to plasma proteins; (4) the small amount of protein and other substances in adipose tissue does not appreciably contribute to the binding of thiopental; and (5) the pH gradient between adipose tissue and plasma water is constant.

R′ is then calculated to be 48. Since R is a function of binding, when *b* is 0·75, 0·875, and 0·9, R would be 12, 6, and 4·8 respectively. Brodie *et al.*,[26] in a study concerned with the dynamics of distribution of thiopental in dogs, observed a decrease of R at late sampling times; no correlation with the extent of binding was then attempted.

The present observations of a change of binding that affects distribution of a drug is in contrast to the phenomenon of quasisaturation of plasma binding sites, transiently achieved, after injection of a high dose of a drug such as phenylbutazone. In such a situation a relatively large proportion of the drug exists in plasma in the unbound form and hence diffuses into tissues and sites of biotransformation.[13]

The time course of thiopental concentrations measured in human adipose tissues in the present experiments confirms previous estimates of the importance of this tissue as a major depot, provided that at least 30 min elapse after drug administration.[18] A rate-limiting factor in the slow biotransformation of thiopental may be the accumulation of large amounts of the drug in adipose tissue, an inert, poorly perfused compartment from which it is gradually released. This mechanism has been suggested not only for thiopental[26] but also for another fat-soluble drug, Dibenamine.[27]

In three preliminary studies, a sudden fall in plasma concentrations of thiopental was observed concurrently with the administration of dextran. Since one drug can displace another from protein binding sites *in vitro*,[1] in animals[28] and in man,[29, 30]

* Plasma containing 4 g% albumin, using mol. wt. = 60,000 for albumin, is 670×10^{-6} M in albumen. Binding was measured with concentrations of 0·16 mg/l ($0{\cdot}66 \times 10^{-6}$ M) and higher of drug.

† In the case of thiopental in adipose tissue this mechanism probably has no major influence on pharmacologic activities. However, Campion and North[23] have shown that change in binding of barbiturates is important in peritoneal dialysis and may also be crucial in hemodialysis and intoxication.

and since it has been postulated that dextran increases the toxicity of barbiturates in animals by a displacement mechanism,[31] this question was further investigated in man. Three possible explanations for our findings were entertained: (1) an increase in blood volume; (2) a change in binding due to dilution of plasma; (3) a displacement of thiopental by dextran.

Our experiments have eliminated the last two, leaving the effect mainly attributable to the increase in blood volume. It is also realized that a change in pH, due to the administration of the slightly acid dextran solution, could also have contributed to the change in plasma level. For both these reasons, dextran administration was avoided in all other experiments.

Some of the experimental data reported above could not have been obtained with the spectrophotometric method.[7] Although the latter method can be improved to a certain extent,[32] the present studies required the development of a more sensitive, specific spectrophotofluorometric method for plasma and adipose tissue. Udenfriend *et al.*[33] had previously determined the fluorescence characteristics of thiopental and based upon this information they had developed a double extraction method of analysis for the drug in water. They suggested that their modification of the u.v. method of Brodie *et al.*[7] could be adapted for the analysis of thiopental in plasma and tissues. However, the modification described in the present study is more sensitive and yields a lower blank than that of Udenfriend *et al.* Flame ionization gas chromatography also has the potential for measurement of small quantities of thiopental.[34] However, the fact that quantitative extraction of drug from biological fluids requires comparatively large volumes of solvents (even after volume reduction through evaporation) renders such a method impractical for the accurate measurement of low concentrations.

In addition to the present study the spectrophotofluorometric method for thiopental has made it possible to investigate the metabolism of this drug by human liver *in vivo* and *in vitro*.[35] It was also used in studies concerned with the placental transfer of the drug in the human neonate,[36] and to estimate blood flow in omental and subcutaneous fat.*

* L. C. Mark *et al.*, unpublished observations.

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Note added in proof. It has now been found (J. M. Perel *et. al.*, unpublished observations) that the fluorescence of thiopental is increased 70 per cent when read in 2·5 N NaOH in D_2O (99:86 %, distilled from EDTA or $KMnO_4$). The reagent blank shows no increase.

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