Methadone Binding to Human Plasma Albumin

Abstract. *The interaction of* L-[*1-*^{3}*H]methadone with human plasma albumin was studied by equilibrium dialysis. The percent of methadone bound to albumin was relatively independent of the concentration of methadone, but was dependent on the concentration of albumin, ranging from 8.0 to 43.8 percent bound as the albumin concentration increased from 0.400 gram to 5.00 grams per 100 milliliters of solution.*

The extent to which a drug is bound to plasma proteins may greatly influence its distribution, rate of metabolism and excretion, and interactions with other drugs. Binding data on drugs therefore can contribute to more rational use of them. The plasma protein binding of many acidic drugs has been studied (*1*). However, there are only four detailed studies on the binding of drugs that are bases (*2*) and no adequate data on the binding of narcotic analgesics. We report here on an in vitro study of the binding of tritium-labeled methadone to human plasma albumin. Albumin represents approximately 50 percent of the total protein in normal human plasma, and could therefore contribute significantly to the total plasma protein binding of methadone (*3*).

Methadone concentrations were chosen to approximate possible amounts in human plasma after a therapeutic dose. For a 70-kg man receiving a single intravenous dose of 10 mg of methadone hydrochloride, the plasma concentration would be about 0.24 μg/ml ($7 \times 10^{-7}M$). This calculation is based on the assumptions that methadone is rapidly distributed in the total body water (41 liters) before excretion or metabolism can occur, and that there is no appreciable tissue or plasma protein binding. Excretion, metabolism, tissue binding, or oral administration of the dose would result in lower plasma concentrations (*4*).

Table 1. Binding of L-[1-^{3}H]methadone to human plasma albumin at different concentrations of albumin in solution. A constant amount of methadone was added to the buffer outside the dialysis bag; at equilibrium the free (unbound) concentration of methadone was $3 \times 10^{-7}M$. The percent of methadone bound is expressed as a mean ± standard deviation. The parentheses indicate the number of experiments performed.

Albumin (g/100 ml)	Methadone bound* (%)
0.400	8.0 ± 1.3 (8)
1.00	19.7 ± 2.1 (8)
2.00	31.0 ± 1.0 (8)
3.00	36.2 ± 1.3 (8)
4.00	38.1 ± 0.8 (7)
5.00	43.8 ± 1.5 (9)

* Corrected for volume occupied by albumin.

Crystalline albumin from human plasma (Calbiochem) was dissolved in 0.067*M* phosphate buffer, *p*H 7.4. Dialysis bags were boiled three times in distilled water and soaked overnight at 2°C in phosphate buffer. The solution of albumin and buffer (2 ml) was placed in the dialysis bag (diameter, 6 mm) which was then tied and put into a glass tube containing 10 ml of a solution of the phosphate buffer and L-[1-^{3}H]-methadone hydrobromide (New England Nuclear, 92.3 mc/mmole). We determined, by thin layer chromatography, that the methadone was greater than 98 percent radiochemically pure. The tubes were rotated (Labquake®, Labindustries) through 240°, 25 times per minute at 37°C. In initial studies measurements were taken every hour for 10 hours, and equilibrium was reached within 6 hours at 37°C for all concentrations of methadone and albumin. The binding of methadone to the bag was constant—4.5 percent ± 0.9 percent—over the concentration range $2.3 \times 10^{-8}M$ to $5.7 \times 10^{-5}M$. In control experiments without albumin (buffer inside and outside the bag), concentration of methadone inside the bag was equal to the concentration outside the bag at equilibrium and therefore no correction for binding of methadone to the bag was necessary in the experiments with albumin.

The *p*H of the buffer outside the bag and that of the solution of albumin and buffer inside the bag was 7.4 after 6 hours of dialysis. The volume of the buffer outside of the bag did not change significantly during the dialysis. Each tube was checked for leakage of protein outside the bag by precipitation tests of the solution with cold trichloroacetic acid. When at equilibrium, duplicate 100-μl samples from the fluid inside and outside of the bag were pipetted into liquid scintillation vials, 1 ml of Nuclear-Chicago solubilizer (Amersham/Searle), was added and the vials were incubated at 37°C for 1 hour. Toluene scintillation solution was then added and the samples counted. Comparison of samples from inside and outside the bag showed that the albumin did not affect the counting efficiency.

The total methadone concentration was calculated from the specific activity (corrected for decay), the efficiency, and the counts per minute per unit volume. The average of counts from duplicate samples was used to calculate the percent of methadone bound to albumin according to the formula:

$$B = \frac{C_I - C_O \text{ (corrected)}}{C_I} \times 100$$

where B is the percentage of bound methadone, C_I is the number of counts from the solution inside the bag, and C_o is the number of counts from the solution outside of the bag.

The number of counts per unit volume of liquid outside of the bag was assumed to equal the number of counts of unbound methadone per unit volume of liquid inside of the bag. However, because the solid albumin occupied a significant amount of the volume of the sample on the inside, a correction was made that reduced the observed counts per minute in the outside sample so that it corresponded to the counts per minute of the unbound methadone in the liquid portion of the inside sample. The correction was based on the partial specific volume of albumin, 0.736 (*5*) and the formula, $W = 100 - (\alpha P)$, where W is the amount (ml) of water in 100 ml of protein solution, α is the partial specific volume of albumin, and P is the amount (g) of albumin per 100 ml of protein solution (*6*). The correction factor is equal to $W/100$.

The percentage of methadone bound to albumin was dependent on the concentration of albumin (Table 1). The free (unbound) concentration of methadone at equilibrium was maintained at $3 \times 10^{-7}M$ and the albumin concen-

Table 2. Binding of L-[1-^{3}H]methadone to human plasma albumin at different concentrations of methadone. The total methadone concentration equals the concentration of methadone, both free and bound to protein, in the dialysis bag at equilibrium. The percent of bound methadone is expressed as a mean ± standard deviation. The parentheses indicate the number of experiments performed.

Total methadone concentration (*M*)	Methadone bound* (%)
Albumin, 0.400 g/100 ml	
2.0×10^{-8}	8.6 ± 0.9 (10)
3.8×10^{-8}	8.7 ± 2.3 (8)
3.7×10^{-7}	8.0 ± 1.3 (8)
3.7×10^{-6}	8.1 ± 0.6 (11)
Albumin, 5.00 g/100 ml	
4.9×10^{-8}	40.8 ± 1.4 (6)
5.3×10^{-7}	43.8 ± 1.5 (9)
4.7×10^{-6}	40.7 ± 0.6 (6)
5.4×10^{-5}	35.6 ± 1.0 (5)

* Corrected for volume occupied by albumin.

tration was varied. The percent of bound methadone increased as the concentration of albumin increased, and this relationship was linear on a semilogarithmic plot of percent of bound methadone versus the log of the albumin concentration.

The percentage of methadone bound to albumin was, however, relatively independent of the methadone concentration when examined at two albumin concentrations, 0.400 g per 100 ml of solution ($6.13 \times 10^{-5}M$) and 5.00 g per 100 ml of solution ($7.67 \times 10^{-4}M$), except that slightly less methadone was bound at the higher concentrations of methadone (Table 2). Because the molar concentrations of drug do not exceed the molar concentrations of albumin studied, saturation of protein binding sites is not likely a factor in the results obtained.

Borgå *et al.* (*2*), using dilutions of human plasma, found that, for desmethylimipramine and nortriptyline, the plot of the ratio of unbound to bound drug against the reciprocal of the plasma concentration was a straight line through the origin. A similar plot of the methadone data from Table 1 is a straight line, but it does not go through the origin. The regression equation for this line is $R = (6.79 \times 10^{-4})A + 0.213$ (*7*), where R is the ratio of unbound to bound L-[1-^{3}H]methadone, and A is the reciprocal of the molar concentration of albumin.

A Scatchard plot r/D versus r, where r is the number of moles of drug bound per mole of albumin, and D is the concentration of unbound drug, results in a curved line (with the binding data at an albumin concentration of 0.400 g/100 ml from Table 2), an indication that there may be several binding sites on the albumin molecule with different affinities for methadone.

To my knowledge, this is the first report on the binding of methadone to human plasma albumin, and the first quantitative study on the protein binding of any of the narcotic analgesics. The results presented here and the two studies by Borgå *et al.*, and Franksson and Änggård (*2*) on other basic drugs, reveal that the binding to protein of some basic drugs differs from that of some acidic drugs as warfarin (*8*), insofar as the percent of the basic drug that is bound seems to be relatively independent of drug concentration.

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References and Notes

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3. Initial studies suggest that methadone binds to plasma proteins other than albumin; the percent of methadone bound in solutions of crystalline albumin is less than the percent bound in human plasma and serum with equal concentrations of albumin.
4. A report of postmortem studies of concentrations of methadone in human blood indicated a range of 0.22 to 3.04 μg/ml. Several of the subjects had taken overdoses of methadone, so the upper value may be atypical for therapeutic situations [A. E. Robinson and F. M. Williams, *J. Pharm. Pharmacol.* **23**, 353 (1971)].
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Polyadenylic Acid Sequences in the Virion RNA of Poliovirus and Eastern Equine Encephalitis Virus

Abstract. *Poliovirus virion RNA contains a single covalently bound sequence of polyadenylic acid which is approximately 49 nucleotides long. A single, slightly longer polyadenylic acid sequence is contained in Eastern Equine Encephalitis virus RNA. Since these viruses are otherwise dissimilar these sequences may play a common role in viral replication, possibly in translation of the viral RNA.*

The virion RNA of two small, unrelated animal viruses, poliovirus, a picornavirus, and Eastern Equine Encephalitis virus (EEE), a group A arbovirus, has been found by us to contain polyadenylic acid [poly(A)]sequences. We now report the quantity, size, and base composition of poly(A) sequences released from each viral RNA by ribonucleases. The data is discussed in light of the known infectious nature of purified RNA from these two viruses and the discovery of poly(A) sequences in the messenger RNA's (mRNA) from mammalian cells (*1–4*) and from several animal viruses (*5–7*).

The EEE virus (New Jersey strain) was prepared in chick embryo cell cultures infected at a multiplicity of 0.1 plaque-forming unit per cell. After an attachment period of 1 hour at 37°C, 240 μc of carrier-free $^{32}PO_4$ was added to each 5-cm dish in phosphate-free Hanks salt solution containing 3 percent calf serum, freed of γ-globulin. Virus was harvested 18 hours later from the supernatant. The virus was concentrated and purified by elution from aluminum phosphate gel (*8*) and zone sedimentation on sucrose gradients (5 to 20 percent), and subsequent sedimentation to a pellet at 100,000*g*.

Poliovirus (type 1, Mahoney) was grown in suspension culture HeLa cells with an input multiplicity of 100 plaque-forming units per cell. The cells were at a concentration of 4×10^6 per milliliter in phosphate-free Eagle's minimal essential medium supplemented with 4 mM glutamine and actinomycin D (4 μg/ml). After 30 minutes, dialyzed calf serum (5 percent) and 18 mc of carrier-free $^{32}PO_4$ was added. The cells were harvested 6.5 hours later by centrifugation and were then subjected to four cycles of freezing and thawing to liberate the virus. The virus was purified by banding at sedimentation equilibrium in a cesium chloride solution (*9*).

RNA was purified from both viral preparations by three successive extractions with hot phenol of virus suspended in buffered sodium dodecylsulfate solution, as described (*10*). The size of the poliovirus RNA was analyzed by sucrose gradient sedimentation with HeLa cell ribosomal RNA's as sedimentation markers. A portion of poliovirus RNA was also sedimented on a sucrose gradient after being dissolved in a dimethyl sulfoxide (DMSO) solution together with an excess of small ($< 10S$), unlabeled, synthetic poly(A). In this latter analysis the DMSO would have denatured any double-stranded RNA present (*11*) while the added unlabeled poly(A) would prevent any contaminating small radioactive poly(A) molecules from readhering to the viral RNA before sedimentation. These sedimentation analyses both indicated that