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Heroin and Morphine Binding with Human Serum Proteins and Red Blood Cells[1] (38411)

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The specific binding of heroin and morphine to human serum proteins and other constituents of blood has not been completely defined. Previous studies by Olsen (1, 2), indicate that l-methadone, a basic drug, is bound extensively (83–87%) to plasma proteins. Many acidic drugs are similarly bound. Morphine is an amphoteric compound with a basic amine group and an acidic phenolic group. Morphine and heroin (3,6-diacetylmorphine) are extracted into organic solvents at pH 8.5 in the nonpolar, unionized form at this isoelectric point (11).

Blaney and Woods (4), using an ultrafiltration procedure, demonstrated 9–17% morphine binding with serum from tolerant and nontolerant dogs. Nadeau and Sobolewski (5) reported that, using an ultracentrifugation technique, morphine paralleled the distribution of proteins in dog serum, but with increased "binding properties" from morphine-tolerant dogs. Morphine binding to human proteins was suggested by the immunity theory of Berkowitz and Spector (6) and others (7, 8). Chryssanthou (9) hypothesized that morphine forms inactive, reversible complexes while bound to blood and/or tissue.

The data in the present study delineate the *in vitro* binding properties of heroin and morphine to human plasma proteins and red blood cells.

Materials, Methods and Results. Morphine-*N*-methyl-^{14}C, with a specific activity of 57 mCi/mmole was purchased from New England Nuclear Corp. Heroin is synthesized according to the methods of Wright (10) and Small and Lutz (11). Purification is detailed in a previous publication (12).

Human plasma proteins were purchased from the following sources: Sigma Chemical Co., St. Louis, MO: albumin, fraction V; gamma globulin, fraction II. Nutritional Biochemical Co., Cleveland, OH: alpha globulin, fraction IV-1; beta globulin, fraction III; beta lipoprotein, fraction III-0. Schwarz/Mann, Orangeburg, NY: glycoprotein, fraction VI.

Human serum was obtained from five healthy volunteers who were taking no medications. The serum was pooled and stored frozen in 20-ml aliquots. Dialysis tubing (0.25-in. diameter, Union Carbide) was boiled in 0.2 *M* phosphate buffer, pH 7.4, for 30 min before use. Proteins were mixed with 0.2 *M* phosphate buffer, pH 7.4, with gentle agitation for 30 min before use. A 6-in. strip of tubing was filled with 1 ml protein-buffer solution and tied in a U shape with one knot at top using waxed dental floss. The bag was secured in a glass test tube containing 15 ml phosphate buffer and approximately 9000 dpm of morphine-^{14}C or heroin-^{14}C. The tubes were incubated at 37° for 20 hr in a shaking water bath (50 oscillations/min).

Two aliquots of 2 ml of dialysate were taken from each tube for counting in a Nuclear Chicago Isocap 300 liquid scintillation system in 15 ml of Aquasol (New England Nuclear). The ^{14}C efficiency is 90% when counted to a minimum 5% error (min 2000 cpm) with sample channels ratio and chemical quench correction curves.

Experiments were performed to confirm that equilibration between bag and tube contents had been reached in 18 hr (dpm retentate = dpm diffusate). Mechanical binding of radioactive material to cellulose bag and glassware was corrected in each set of experiments by blank tubes without protein, *i.e.*, buffer in bag and tube. The mean dpm in control experiments represents 100% of radioactivity or 0% binding.

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TABLE I. Heroin-^{14}C and Morphine-^{14}C Binding with Serum Proteins.

Protein	mg/100 ml	% bound heroin-^{14}C	% bound morphine-^{14}C
Serum		2.9	1.6
Albumin	50	0.5	2.4
Albumin (high)	200	—	3.0
Alpha globulin	10	0	0.9
Beta globulin	10	5.3	2.4
Gamma globulin	10	0	0.5
Glycoprotein	1	8.0	1.0
Lipoprotein	10	0	6.4

$$\%\text{bound} = 100\% - \frac{A-B}{A} \times 100,$$

where A = control (dpm/ml) and B = experimental (dpm/ml).

Equilibrium dialysis systems were prepared at approximate normal human blood concentrations for each protein. Table I lists the results of studies with heroin-^{14}C and morphine-^{14}C binding to serum and individual proteins. Hemoglobin-free red cell ghosts were prepared by the method of Dodge *et al.* (13) from heparinized human blood and diluted 1:4. Human red cells were washed three times in buffer and diluted 1:8 for use. These data are presented in Table II.

In a separate set of experiments in order to determine the effect of cold heroin on protein binding, concentrations of cold heroin ranging from 0.008 to 0.4 mg/ml were added to buffer (control) and to human serum (experimental). These results are listed in Table III.

Discussion. Neither heroin-^{14}C nor morphine-^{14}C exhibits significant binding with physiological levels of protein in an *in vitro* system (Table I). However, when cold heroin is added to human serum, binding of heroin-^{14}C is in the range 20–39% (Table III). Washed human erythrocytes and red cell ghosts also exhibit significant binding (22–28%) but only in the presence of cold heroin (Table II). However, there is minimal binding (2–3%) of heroin-^{14}C when cold heroin is not present in the system. These data confirm the work of Blaney and Woods (4), of Nadeau and Sobolewski (5), in which cold morphine was added to serum *in vitro*, and the Chryssanthou (9) study where morphine was administered directly into human subjects.

It appears that chemically nondetectable quantities of heroin or morphine present in the radioactive doses used *in vitro* (4.8×10^{-3} μCi$=8 \times 10^{-7}$ mg in 40 μl) do not interfere with the equilibrium dialysis system. Olsen made the same observation (1, 2) using methadone-^{14}C. The addition of microgram quantities of cold heroin or morphine yield data showing binding of the radioactive compound. A possible explanation for this phenomenon is the mechanical affinity of the radioactive-labeled molecules to the nonlabeled moiety, thereby presenting a binding of alkaloid:alkaloid instead of alkaloid:protein. The data in Table III are corrected for this factor by using control dialysis sacs (without protein) containing varying quantities of cold heroin plus heroin-^{14}C. These experiments demonstrated pronounced differences from blanks (without protein, without cold heroin) thereby exhibiting no binding of the ^{14}C compound.

Since protein–heroin-^{14}C binding was low, it was considered that structural difference of heroin compared to morphine may reduce binding site availability, *viz.*, 3,6-acetyl groups for heroin instead of 3,6-hydroxy groups for morphine. However, neither narcotic alkaloid exhibits significant binding, with six serum proteins studies (Table I) indicating that the structural hypothesis is invalid.

In vivo studies (6, 9) demonstrating morphine binding are probably complicated by the presence of conjugates, as suggested by Blaney and

TABLE II. Heroin-^{14}C Binding with RBC Membranes Alone and with Addition of Cold Heroin.

Heroin (mg/ml)	RBC	Ghosts
	(% bound-heroin-^{14}C)	
0	3.2	2.2
0.1	22.5	28.2

TABLE III. Heroin-^{14}C Binding with Serum + Cold Heroin.

Heroin (mg/ml)	Serum (% bound-heroin-^{14}C)[a]
0.008	20.4
0.04	26.7
0.08	30.7
0.4	39.4

[a] Percent bound versus controls: Controls contained cold heroin in buffer; experimental contained cold heroin in serum (2ml/bag).

Woods (4). Morphine glucuronide, since it is ionizable, would have different binding properties from the "free" nonconjugated moieties.

Our data suggest that injected heroin "floats" unbound in the bloodstream until it is deacetylated to 6-monoacetylmorphine (MAM) and then to morphine. The free morphine would also "float" as lipid soluble, un-ionized molecules before it is metabolized to the glucuronide and then excreted by the kidneys. This theory is supported by our previously reported data (12), indicating the presence of free heroin, MAM, and morphine in plasma, perhaps passively en route to receptor tissues for deacetylation, conjugation, or out of these tissues for excretion.

Summary. Equilibrium dialysis experiments were performed to determine the binding properties of heroin and morphine to major blood constituents. The serum proteins studied showed little binding with heroin-^{14}C or morphine-^{14}C. However, the addition of cold heroin to the system increased the observed uptake of the radioactive substance with serum, RBC, ghosts, and washed RBC. These changes in binding may be due to mechanical affinity of the molecules: ^{14}C-labeled plus nonradioactive *in vitro*. Conjugation may alter this pattern *in vivo*. Free heroin and morphine probably float unbound in the blood before further metabolism in other tissues.

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