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# ROLE OF CHOLESTEROL IN THE DISTRIBUTION OF HALOPERIDOL AND TRIFLUOPERAZINE IN BLOOD AND PLASMA

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**Abstract**—The distribution of labeled haloperidol, trifluoperazine, and tolmetin (control) in human blood and plasma was investigated *in vitro* by the use of immuno- and physico-chemical techniques. Cholesterol was discovered to have a significant binding affinity for haloperidol and trifluoperazine; somewhat lesser affinity was noted for cholesterol oleate ester. Haloperidol had a much higher affinity for cholesterol and the high density lipoproteins than did trifluoperazine, which had a binding affinity also for albumin. Haloperidol demonstrated little, if any, binding to albumin. As anticipated, tolmetin was bound almost completely to albumin. The significance of the erythrocyte membrane, in terms of its binding affinity for anti-psychotic drugs, storage function, service as a vehicle for drug transport, establishment of a distribution equilibrium between plasma and erythrocytes, and maintenance of a drug blood-level, was confirmed by our studies. Binding to erythrocyte membranes, however, was weaker than binding to plasma. Explanation, implication, and clinical application of these findings are offered.

The binding of drugs to plasma proteins has in recent years been subject to intensive study [1]. Plasma proteins and cellular elements have been shown to significantly affect the intra- and extravascular distribution of a drug in the body and the transport to receptor sites of action, as well as metabolism and excretion [2–4]. It has not been determined definitely if the therapeutic value of a drug is reflected by the free drug concentration in the blood [5] or by the ability of drug–protein or drug–cell membrane complex to be delivered and utilized at the site of action [3]. In addition, equilibrium between free and bound forms of the drug will depend on the constant of binding by the drug to a carrier or receptor [4].

Though a number of plasma proteins and formed elements participate as drug binders, albumin is regarded as the most significant binding agent. Albumin has a net negative charge at the normal pH of serum and can interact with anions as well as cations. The majority of drugs can interact with albumin, but the degree of binding may range from small fractions to almost the entire drug concentration. Both acidic drugs, e.g. phenylbutazone and tolmetin, as well as lipid soluble basic drugs, e.g. tricyclic antidepressants and phenothiazines, are believed to have high binding affinities for albumin [6]. Studies involving thioridazine and its metabolites indicate binding affinity to albumin and other, unidentified, plasma proteins [7, 8].

Another pattern of drug partition in blood occurs in the distribution of bound drug between plasma proteins and cellular components, thus forming a three-component system [9]. The importance of the erythrocyte membrane and the lipoproteins as drug-binders for chlorpromazine and imipramine has recently been recognized [10]. Manian *et al.* [3] reinjected washed erythrocytes, after having interacted them with chlorpromazine, into rats and marked tranquilization was induced, indicating that the erythrocytes may transport the drug to brain receptor sites. Hughes *et al.* [4] demonstrated approximately 90 per cent binding *in vitro* of haloperidol in human plasma, with a rapid distribution of free drug between plasma and erythrocytes. *In vivo* studies with haloperidol indicate a free fraction concentration of haloperidol of approximately 8 per cent [11]. This suggests that both the plasma and cellular components may act as sinks or drug reservoirs.

Previously, we observed a prolonged drug-induced Parkinsonian reaction to haloperidol in a patient with benign monoclonal gammopathy (IgM), and the possibility of binding of the drug to the monoclonal IgM as a pathogenetic factor was considered [12]. This hypothesis was not proven, but the unexpected distribution of haloperidol in the patient's serum prompted a series of experiments to determine the partition of haloperidol and other drugs in human blood.

## MATERIALS AND METHODS

*Serum.* Normal human serum and sera from patients with hypercholesterolemia were obtained from random clotted specimens submitted for analysis to the Clinical Chemistry Laboratory of The Mount Sinai Hospital, New York City.

*Albumin.* A 4 per cent solution in saline was prepared from 25% human albumin (Albumisol, Merck, Sharp & Dohme, Philadelphia, PA).

*Drugs.* [$^3$H]Haloperidol (97.4 mCi/mmole) and [$^{14}$C]tolmetin (13.9 mCi/mmole) were provided by McNeil Laboratories, Inc., Fort Washington, PA.

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[$^3$H]Trifluoperazine (33.8 mCi/mmole) was provided by Smith, Kline & French, Philadelphia, PA.

*Binding of drugs to serum.* Unless stated otherwise, 0.1 ml of radiolabeled drug in 0.15 M NaCl (2.2 μg/ml of haloperidol, 2 μg/ml of trifluoperazine, and 12.5 μg/ml of tolmetin) was added to 0.1 ml of serum and incubated for 2 hr at 37°. Of this mixture 0.1 ml was layered on top of a 40–10% sucrose (in 0.15 M NaCl, pH 7.0) gradient (4 ml) prepared in an Auto-Densi-Flow (Buchler Instruments, Fort Lee, NJ), and centrifuged in a Spinco Preparative Ultracentrifuge (Beckman Instruments, Palo Alto, CA, model L75B, rotor SW56) at 56,000 rpm (408,000 *g*) for 8 hr at 4°. Fractions of 0.2 ml were collected. Aliquots of 50 μl of each fraction were mixed with 1 ml hyamine solution and counted in 15 ml of standard scintillation mixture in a Beckman model LS355 Counter.

Every fraction from the sucrose gradient was also tested by double gel diffusion [13] with anti-sera to human albumin, IgG, α-lipoproteins (HDL), and β-lipoproteins (LDL, VLDL). Distribution of these proteins in the gradient was correlated with radioactivity present. A total of twenty tubes was collected, and under these conditions albumin was found in tubes 13–17 with the maximum concentration in tubes 15 and 16, HDL was in tubes 16–18, and LDL and VLDL were in tubes 17–19; free drug was in tubes 19 and 20.

Due to the design of the experiments and the limitation of the numbers of samples that can be run simultaneously in an ultracentrifuge, single tests were used for comparison of different conditions. The reliability of the separation method was determined on a simultaneous separation of four samples of one serum and haloperidol that were treated as if they were individual specimens. The coefficient of variation for corresponding separated fractions was between 4.3 and 5.7 per cent, and one-way analysis of variance of all values obtained showed a nonsignificant difference between samples.

*Binding of drug to serum in the presence of cholesterol and cholesterol ester.* Human serum, with or without tested drug added, was saturated with cholesterol (Van Gelder Fanto Corp., Lot No. S-934/1, Physicians Drug & Supply Co., Philadelphia, PA) or cholesterol oleate ester (U.S. Biochemical Corp., Cleveland, OH) by addition of 2.5 mg/0.2 ml serum (10 μl of 250 mg/ml solution in ether) and incubation for 1 hr at 37°. The insoluble portion was removed by centrifugation at 15,000 rpm (27,000 *g*) for 15 min in a Sorvall RC 5 refrigerated centrifuge (Ivan Sorvall, Norwalk, CT) either before or after addition of radiolabeled drug and incubation for 1 hr at 37°. The distribution of the labeled drug in the supernatant fraction was tested as described above.

*Presentation of data.* Total net counts of different fractions were added and the percentage of the total drug content was calculated. Values above 5 per cent of total were plotted on the background of protein distribution of albumin and lipoproteins.

*Distribution of drug between erythrocytes and serum or solvent.* Either 0.6 ml of packed red blood cells or 1.2 ml of whole blood was diluted to 3 ml in normal saline and incubated for 2 hr at 37° with one of the radioactively labeled drugs (1.3 μg haloperidol, 3.6 μg trifluoperazine, and 3.0 μg tolmetin). Following incubation, the contents were mixed and an aliquot was withdrawn from each tube for counting; the mixtures were then divided equally into three tubes for centrifugation. The erythrocytes and supernatant fraction of one sample were counted, and the sedimented red blood cells of the other two samples were resuspended in 0.8 ml of either normal saline solution or homologous plasma, incubated for 1 hr at 37°, and centrifuged, with subsequent counting of erythrocytes and supernatant fractions.*

*Autoradiography.* Precipitin lines, produced by double gel diffusion [13] and by immunoelectrophoresis [14], of serum with and without drug (control), and of a variety of antibodies (Maloy Co. Springfield, VA) were tested for their radioactivity contents. After the lines were fully developed, the slides were covered with filter paper, dried at 37°, contacted with Kodak XRP film for 2–4 weeks, and developed.

## RESULTS

*Distribution of radiolabeled drug in 4% albumin.* Incubation of a mixture of saline and drug (control) revealed peak concentrations of all three drugs in tubes 19 and 20 (Fig. 1A). Between 60 per cent (haloperidol) and 85 per cent (tolmetin) of the drugs were located, in concentrations above 5 per cent, in tubes 16–20.

High binding affinity for 4 per cent albumin by both tolmetin and trifluoperazine was observed in gradient ultracentrifugation. Peak concentrations of radioactivity coordinated with the distribution of albumin, as determined by immunodiffusion (Fig. 1B). The highest concentrations of trifluoperazine were found in tubes 15 and 16, with a partial decrease in tube 17 and about 20 per cent of the drug present in tubes 18–20. Peak concentrations for tolmetin, higher than those for trifluoperazine, were initiated in tube 14, with a greater decrement in tube 17. Nearly all of the haloperidol was concentrated in the upper portion of the gradient (tubes 18–20), outside the range for albumin, in a distribution pattern similar to the drug–saline mixture, thus indicating little, if any, binding to this protein. [$^{14}$C]Tolmetin was the only drug that could be detected by autoradiography. It was found by electrophoresis on cellulose acetate to be located in the albumin fraction, and by immunoelectrophoresis and double gel diffusion, against specific sera, to be confined to the specific precipitate of albumin-anti-albumin.

*Distribution of radiolabeled drug in normal serum (ns).* Peak concentrations of all three drugs were found in those areas of the sucrose gradient that contained the albumin and HDL fractions of the plasma (tubes 14–18) (Fig. 1C). In the upper areas of the gradient where proteins of lower molecular weight (LDL, VLDL and chylomicrons) were located, decreased concentrations of haloperidol and

* In these experiments the blood was drawn into vacutainer tubes. Preliminary experiments indicated that the presence of Tris (2-butoxyethyl) phosphate (TBEF) [D. Fremstad and K. Bergerud, *Acta pharmac. tox.* **39**, 570 (1976)] did not significantly alter the results of this study.

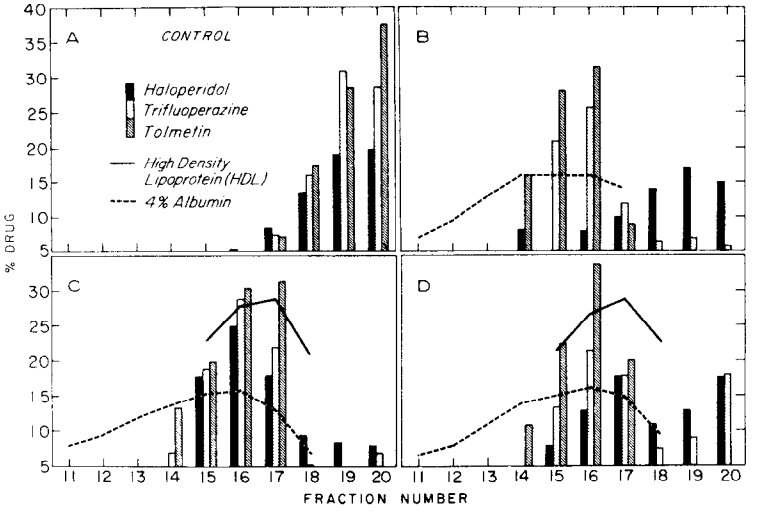


Fig. 1. Binding profiles of haloperidol (■), trifluoperazine (▨) and tolmetin (▧) to saline (A), 4% albumin (B), normal human serum (C), and hypercholesterolemic human serum (D). The height of the bar represents the percentage of drug in each fraction. The dotted and solid lines represent the positions of albumin and HDL respectively. Values below 5 per cent are not shown.

trifluoperazine were noted (tubes 18 and 19). Some radioactivity was found on top of the gradient (tubes 19 and 20), presumably representing binding by VLDL and unbound drug. The absence of tolmetin in tubes 18–20 suggested almost complete binding by albumin. Haloperidol appeared to be bound by the lipoproteins, probably by the HDL, and was present also in fractions 19 and 20. The distribution of trifluoperazine showed a slight shift to the upper part of the gradient. It was decreased in fraction 14 and present in fractions 18 and 20. This indicated that this drug was bound primarily to both albumin and HDL as well as, though to a lesser degree, to LDL and VLDL.

*Distribution of radiolabeled drug in hypercholesterolemic serum (HCS).* The presence of an increased concentration of cholesterol in serum resulted in a marked effect on the distribution of the lipid-soluble drugs haloperidol and trifluoperazine but not of the acidic drug tolmetin (Fig. 1D).

In NS, 70.5 per cent of the haloperidol was found in tubes 14–18, whereas in HCS only 52.9 per cent was found in this region. Of the resulting decrease of 17.6 per cent, a fraction of 13.9 per cent (79 per cent) was actually transferred to tubes 19 and 20 in HCS.

In NS, 82.5 per cent of the trifluoperazine was in tubes 14–18; in HCS, 64.8 per cent was. Of the resulting decrease of 17.7 per cent, a fraction of 15.7 per cent (or 89 per cent) was found in tubes 19 and 20 in HCS.

The distribution of tolmetin was unaffected by a high cholesterol content. It thus appears that the increased concentration of cholesterol altered the binding characteristics of haloperidol and trifluoperazine.

*Binding affinities of radiolabeled drug to cholesterol and cholesterol oleate.* When an excess of cholesterol was added to serum, followed by haloperidol, the distribution of haloperidol between serum and cholesterol that did not dissolve was such that over 50 per cent of the label could be removed by centrifugation of the mixture. In addition, when an excess of cholesterol was added to haloperidol-saturated normal serum, 75 per cent of the haloperidol bound to the undissolved excess of cholesterol and could be removed by centrifugation. As seen in Fig. 2, only about 50 per cent of haloperidol was found in the supernatant fraction when incubated simultaneously with NS and cholesterol, and the addition of cholesterol removed 75 per cent of the haloperidol from NS preincubated with haloperidol.

The finding that the distribution of haloperidol in HCS differed from NS led to the investigation of cholesterol and cholesterol oleate in terms of their

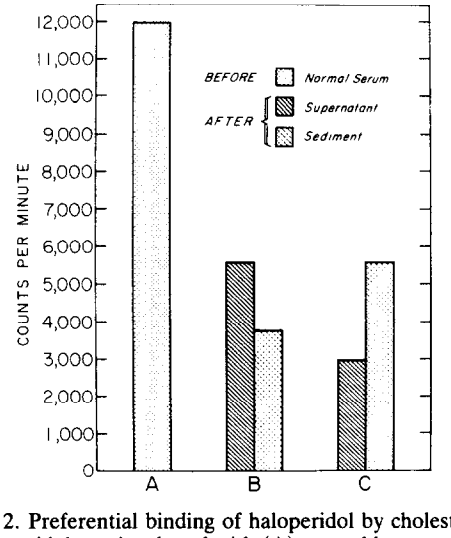


Fig. 2. Preferential binding of haloperidol by cholesterol. Haloperidol was incubated with (A) normal human serum (▨), (B) normal human serum saturated with cholesterol, and (C) with cholesterol after saturation with haloperidol. Distributions in supernatant fraction ▧ and sediment ■ are expressed as counts per minute.

affinities for drugs. The problem was investigated in two ways by determining: (1) the effects of cholesterol and cholesterol oleate on drugs bound in serum [Fig. 3 (B and C)], and (2) the effects of saturation of NS with cholesterol and cholesterol oleate on the distribution of bound drugs [Fig. 3 (D and E)].

The addition of 2.5 mg of cholesterol or cholesterol oleate, in 10 $\mu$l of ether, to 0.2 ml of NS resulted in partial binding of cholesterol and cholesterol oleate to plasma proteins. Some of the added material was removed by centrifugation [Fig. 3 (D and E)].

There was little difference in the distribution of the three drugs in NS (Fig. 1C) when ether was added as a control for these experiments (Fig. 3A). When cholesterol was added to NS that contained the three drugs and the insoluble portion was subsequently removed, it was found that almost all (80.2 per cent) of the haloperidol had been removed with the insoluble portion (Fig. 3B); in addition, a considerable reduction in the concentration of trifluoperazine (37 per cent), and the absence of an effect on tolmetin were noted. When cholesterol oleate was substituted for cholesterol (Fig. 3C), only partial losses of haloperidol (54.3 per cent) and trifluoperazine (23.7 per cent) were observed.

Saturation of NS with cholesterol (Fig. 3D) and cholesterol oleate (Fig. 3E) and subsequent removal of the excess by centrifugation before addition of drugs [Fig. 3 (D and E, respectively)] resulted in a shift of haloperidol and trifluoperazine to tubes 18–20 of the gradient, thus mimicking the distribution of drugs in HCS (Fig. 1D). In all of the above experiments, tolmetin was not affected by either cholesterol or its ester.

*Binding of drug to red blood cells (RBC).* After incubation of blood with tolmetin, no drug was bound to RBC (Table 1). Both haloperidol and trifluoperazine distributed evenly between plasma and RBC (51.6 and 56.3 per cent, respectively, bound to RBC). Subsequent incubation with plasma removed two-thirds of the haloperidol and trifluoperazine associated with the RBC (61.2 and 61.9 per cent, respectively); only one-third of the haloperidol (31.1 per cent), and a fraction (5 per cent) of the trifluoperazine was removed by saline.

When washed red blood cells, suspended in 0.9% saline, were substituted for whole blood (Table 2), 68.1 per cent of the haloperidol and 89.7 per cent of the trifluoperazine were associated with the RBC, which, after a second incubation with homologous plasma or 0.9% saline, gave results comparable to those with RBC obtained from whole blood.

## DISCUSSION

The importance of drug–protein binding as a pharmacokinetic variable was first demonstrated by Davis [15] in his work with sulfonamides. The finding of identical levels of free drug in both plasma and cerebrospinal fluid (CSF) and the absence of bound drug in the latter indicated that a portion of the drug was temporarily retained in the plasma, presumably in bound form (to albumin), and that it was the free drug which was therapeutically effective.

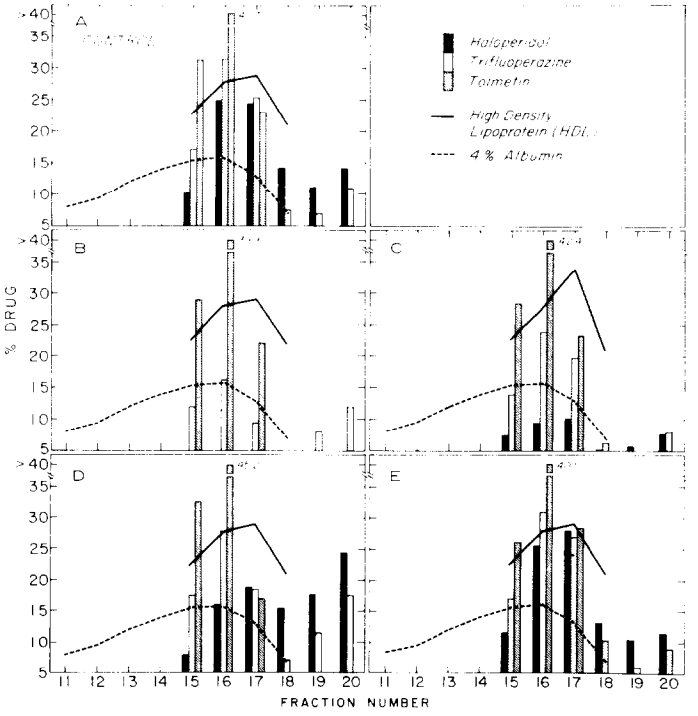


Fig. 3. Binding profiles of haloperidol, trifluoperazine, and tolmetin where ether (control) (A), cholesterol (B), and cholesterol oleate (C) were added to a preincubated mixture of normal human serum and drug with subsequent centrifugation of the entire mixture. Panels D and E are the binding profiles of the three drugs when each drug was added after centrifugation of preincubated mixtures of normal human serum with cholesterol (D) and with cholesterol oleate (E). For further explanation see Fig. 1.

Table 1. Profiles of binding of haloperidol, trifluoperazine, and tolmetin to red blood cells in whole blood*

| Procedure | Percentage of drug bound to cells and supernatant fraction | | | | | |
|---|---|---|---|---|---|---|
| | [$^3$H]Haloperidol | | [$^3$H]Trifluoperazine | | [$^{14}$C]Tolmetin | |
| | Cells | Supernatant fraction | Cells | Supernatant fraction | Cells | Supernatant fraction |
| I. Incubation of radiolabeled drug with dilute whole blood | 51.6 | 48.4 | 56.3 | 43.7 | 0 | 100 |
| II. Second incubation of red blood cells (from first incubation) with homologous plasma | 38.8 | 61.2 | 38.1 | 61.9 | NA† | NA |
| III. Second incubation of red blood cells (from the first incubation) with saline | 68.9 | 31.1 | 95.0 | 5.0 | NA | NA |

* Incubation I was for 2 hr; incubations II and III were each for 1 hr. See Materials and Methods for details. For the calculations of the percentages of drug after the two second incubations, the percentages of drug in cells in the first incubation (51.6 and 56.3 per cent) were used as 100 per cent.

† Not applicable.

Haloperidol, a butyrophenone, and trifluoperazine, a phenothiazine, are both very widely used psychiatric drugs. Tolmetin, a relatively new anti-inflammatory agent, which was available labeled with $^{14}$C, was chosen as a control because it belongs to a group of drugs known to have a very high binding affinity to albumin [6]. As with all pharmacologic agents that act on the central nervous system (CNS), haloperidol and trifluoperazine are lipid-soluble drugs [16]. The blood–brain barrier is a physicochemical membrane of essentially lipid nature [17]. The ability of the substance to penetrate the blood–brain barrier is, therefore, primarily a function of its lipid solubility [18]. It is thought that it is the unbound drug molecule that diffuses through the barrier, entering the CSF, and is taken up at the brain receptor site [6, 18].

Albumin, of all plasma protein, has been considered to be the prime binder of both acidic and basic drugs. Our findings demonstrate that lipid-soluble drugs vary in their binding affinities. Tolmetin, appeared to be almost completely bound to albumin; in contrast, haloperidol appeared to be bound almost completely to proteins that sedimented (in gradient ultracentrifugation) where $\alpha$ (i.e. high density) lipoprotein (HDL) and, to a much lesser extent, $\beta$ (i.e. low density) lipoproteins (LDL) and very low density lipoproteins (VLDL) float. The binding of trifluoperazine reflected, in part, the same distribution as haloperidol, but the distribution relative to tolmetin and haloperidol indicated an affinity for both albumin and lipoproteins.

In contrast to our findings, Bickel [10] demonstrated equal distributions of chlorpromazine and imipramine to albumin, HDL, LDL, VLDL, and chylomicrons. Chlorpromazine and trifluoperazine, both phenothiazines, would be expected to have similar binding affinities. The quantitative difference between our results and those of Bickel, however, may be due to a difference in method. Bickel prepared the lipoprotein fractions from plasma by the flotation method in density media, in contrast to our

Table 2. Profiles of binding of haloperidol, trifluoperazine, and tolmetin to washed packed red blood cells*

| Procedure | Percentage of drug bound to cells and supernatant fraction | | | | | |
|---|---|---|---|---|---|---|
| | [$^3$H]Haloperidol | | [$^3$H]Trifluoperazine | | [$^{14}$C]Tolmetin | |
| | Cells | Supernatant fraction | Cells | Supernatant fraction | Cells | Supernatant fraction |
| I. Incubation of radiolabeled drug with saline suspended washed packed cells | 68.1 | 31.9 | 89.7 | 10.3 | 0 | 100 |
| II. Second incubation of red blood cells (from first incubation) with homologous plasma | 37.2 | 62.8 | 36.6 | 63.4 | NA† | NA |
| III. Second incubation of red blood cells (from first incubation) with saline | 69.0 | 31.0 | 93.2 | 6.8 | NA | NA |

* Incubation I was for 2 hr; incubations II and III were each for 1 hr. See Materials and Methods for details. For the calculations of the percentages of drug after the two second incubations, the percentages of drug in cells in the first incubation (68.1 and 89.7 per cent) were used as 100 per cent.

† Not applicable.

use of a sucrose gradient in a preparative ultracentrifuge. Bickel incubated the drugs with purified albumin and lipoprotein fractions after their separation, whereas we incubated drug with serum prior to separation of these fractions. Our results thus included the possible effects of competitive binding of the drugs to the plasma proteins. His control drug, salicylic acid, revealed binding properties identical to those of tolmetin.

The discovery that free cholesterol and, to a lesser degree, its ester possess drug binding capabilities is of considerable significance. (Brinkschulte and Breyer-Pfaff [19] have recently shown a significant negative correlation between plasma total cholesterol and the free fractions of tricyclic antidepressants.) This supports the hypothesis that HDL significantly binds both haloperidol and trifluoperazine. Since the HDL have a total cholesterol and cholesterol ester content of about 20 per cent versus 45 per cent for the LDL, the preference of haloperidol for HDL requires explanation. It is well established that HDL is released into the blood stream in an incomplete form, i.e. essentially free of cholesterol esters, and Glomset [20] has demonstrated that the enzyme lecithin:cholesterol acyltransferase (LCAT) permits the transfer of fatty acid residues from plasma lecithin to free cholesterol with the formation of cholesterol esters. Free cholesterol reaches the intravascular compartment through the gastrointestinal tract, as a product of catalytic degradation from tissues, and as a result of exchange processes within the bloodstream. The incomplete HDL molecule then acts as a scavenger for both free cholesterol and cholesterol esters, thereby effecting its completion. As a result, the cholesterol is present on the surface of HDL and can react with haloperidol. In contrast, it has been shown by proton resonance studies of LDL that cholesterol is in the middle layer of the molecule covered on the surface with phospholipid [21].

Cholesterol may serve as a vehicle for transport to, as well as through, the blood–brain barrier. An example of a similar phenomenon is the transport of transferrin which, after delivery of iron to the erythropoetic cell, becomes available for repeated transport. We have shown that cholesterol will remove haloperidol, and trifluoperazine to a lesser extent, from whatever they may be bound to in the plasma. Whether cholesterol assists in the diffusion of drug through the barrier or facilitates transport to, incorporation by, and storage at, the brain receptor site are questions that merit further consideration and study. It is free cholesterol, rather than cholesterol ester, that is present in CSF (0.14 mg/100 ml; plasma level average is 160 mg/100 ml) and in brain tissue. The high affinity of haloperidol for free cholesterol is, therefore, of great significance. Albumin in CSF (23 mg/100 ml; plasma level average is 4430 mg/100 ml) may provide additional binding for trifluoperazine. It would thus appear that after the loss of drug from plasma to CSF, re-equilibration with red blood cells takes place and additional drug is then made available for diffusion through the blood–brain barrier.

The significance of the erythrocyte membrane in terms of its binding affinity for lipid-soluble drugs, storage function, service as a vehicle for drug transport, the establishment of a distribution equilibrium between plasma and erythrocytes, and the maintenance of a drug blood-level is confirmed by our studies. Hughes *et al.* [4] added human red blood cells to plasma that contained radiolabeled haloperidol. They did not study the effects of incubation of drug with washed packed cells or with whole human blood. Manian *et al.* [3], using radiolabeled chlorpromazine and its metabolites, studied the characteristics of binding of these compounds to rat red blood cell membranes and rat brain synaptosomes. They did not use whole blood or plasma and did not measure the distribution of drug within and between these systems. Distribution was monitored as a function of time in plasma, red blood cells, and brain after injection of radiolabeled drug-treated erythrocytes into the rat. Bickel [10], hemolyzing erythrocytes with subsequent separation into membranes and cell contents, found binding of chlorpromazine and imipramine almost exclusively to membranes. We have demonstrated that binding to red blood cells is weaker than binding to plasma. Fresh plasma removed twice as much haloperidol from red blood cells as saline. The same amount of trifluoperazine was removed by fresh plasma, but saline removed only a fraction. Binding of tolmetin to red blood cells was not demonstrated. It thus appears that identical results are obtained irrespective of whether washed packed cells or whole blood is utilized at the start of the experiment. Whether or not it is only lipid-soluble drugs that bind to erythrocyte membrane requires further study.

Consideration should be given to the possible *in vitro* conversion of trifluoperazine to its ring sulfoxide, a pharmacologically inactive metabolite, by RBC hemoglobin. The ring sulfoxide, being more polar, might have different binding characteristics and would, therefore, compete with the parent compound for binding sites [22].

Attempts to visualize the binding of these drugs to serum proteins by immunoelectrophoresis and autoradiography were successful only for the $^{14}C$-containing drug tolmetin, which was shown to bind exclusively to albumin.

There are two limitations to this study which should be considered. One is that the separation techniques that were used do not clearly separate the lipoproteins from each other and from albumin [10]. Second, incubating serum with free cholesterol may have created artifactual lipid protein structures that bear little relationship to lipoproteins as they exist in circulating plasma. Therefore, though the red blood cell/plasma distribution studies and the normal and hypercholesterolemic serum binding results all seem to point to a role for cholesterol in drug binding, the presence of a specific carrier protein for lipid-soluble drugs cannot be excluded from consideration. This will be the subject of further studies.

A number of clinical applications are suggested by our studies. Drugs that currently do not have access to the CNS because of impenetrability of the blood–brain barrier, e.g. antibiotics and cytotoxics, could be made lipid soluble by attachment of cholesterol and/or its esters. In contrast to situations involving overdose with barbiturates, hemodialysis

and peritoneal dialysis have not proven useful as life-saving measures in cases of overdose with psychiatric drugs. Removal of these substances might be enhanced by dialysis against a solution containing both albumin and cholesterol. This technique has been successful with drugs that are highly bound to albumin, through dialysis against an albumin-containing solution [6].

It is tempting to speculate that the high affinity of haloperidol to cholesterol may result in absorption of the drug along the entire length of the neuron. In this way it may, in addition to blocking dopamine receptors [23], also serve an altering or stabilizing function within the neuron, thereby increasing the effectiveness of the drug.

Finally, the relationships between the dosage of lipid-soluble drugs, obtained blood levels, and serum cholesterol values also merit further study.

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