## Antibiotic Activity of Deoxy-Herqueinone (Atrovenetin Monomethyl Ether)

Atrovenetin was first isolated from cultures of *Penicillium atrovenetum* (G. Smith)[1], and subsequently from cultures of the closely related species *Penicillium herquei* (Bainer and Sartory)[2], along with the closely related compounds herqueinone and norherqueinone. The antibiotic activity of cultures of *P. herquei* was shown to be mainly due to the single component atrovenetin[2]. The conversion of herqueinone and norherqueinone to deoxyherqueinone and deoxy-norherqueinone, and the identity of the latter with atrovenetin, was reported by BARTON et al.[3]. Accordingly deoxy-herqueinone should be a monomethyl ether of atrovenetin. In view of the antibiotic activity of atrovenetin, the activity of deoxyherqueinone has now been investigated; it is found to be active against a number of organisms, though to a lesser degree than atrovenetin[4]. Both herqueinone and norherqueinone are found to be completely inactive, while the deoxy derivatives prepared from them are active. The relative activities of atrovenetin and deoxy-herqueinone are given in the Table.

| Organism tested | Min. inhibitory concentrations mcg/ml Atrovenetin 4 | Deoxyherqueinone |
|---|---|---|
| *B. mycoides* | 0.4 | 20.0 |
| *B. subtilis* | 0.5 | 18.0 |
| *S. lutea* | 0.6 | 35.0 |
| *S. aureus* | 0.7 | 20.0 |
| Streptomycin resistant strain gram + ve rods of sublitis group | 0.9 | 18.0 |
| Penicillin resistant *Staphylococcus aureus* | 0.7 | 20.0 |
| *K. pneumoniae* | 0.3 | No activity |

*Zusammenfassung.* Aus Herqueinon erhält man Desoxyherqueinon (Atrovenetin-monomethyl-äther) mit antibakteriellen Eigenschaften. Spektrum ähnlich wie Atrovenetin, obwohl die minimale Hemmungskonzentration höher liegt.

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## Thiopental Binding to Serum Albumin

Most drugs interact with one or more plasma proteins which may influence pharmacological activity. Albumin is the protein component of plasma most often involved in the binding of various substances[1]. The prolongation of sleep with thiopental compared with hexobarbital is caused by the former being more strongly bound to plasma protein[2]. The percentage of binding of barbiturates with protein has been reported elsewhere[2,3]. In this study the number of binding sites of thiopental on bovine serum albumin has been determined. In addition the effect EDTA (disodium ethylenediamiotetraacetate) has on thiopental binding has been studied.

*Method.* The equilibrium dialysis technique described by KLOTZ[4] using bovine serum albumin (Biochemical Research, Cohn Fraction Grade C) was used for this experiment. Dialysis was carried out in 22 · 150 mm test tubes with $^5/_8$ inch dialysis membranes first soaked in 0.1 *M* nitric acid to remove impurities[5] and then suspended against solutions of various concentrations of thiopental in 0.1 *M* tris hydrochloride-tris buffer (pH 7.42) at 7°C for 12 h. Concentrations of thiopental ranged from $7.0 \cdot 10^{-4} M$ to $5 \cdot 10^{-5} M$ and bovine serum albumin was used at a concentration of 0.05 Gm/100 ml ($7.25 \cdot 10^{-6} M$).

The experimental data were analyzed by means of the Scatchard equation[6], $\bar{v}/A = kn - k\bar{v}$; where $\bar{v}$ is the molar ratio of bound thiopental molecules to albumin, A is the free thiopental concentration in *M*, k is the average apparent association constant for binding, and n is the average maximal number of binding sites on albumin. The ratio of $\bar{v}$ to A was determined from the values obtained for bound thiopental at given concentrations of thiopental. If all binding sites are equivalent and independent, plots of $\bar{v}/A$ as a function of $\bar{v}$ will produce a straight line. The intercept on the $\bar{v}/A$ axis is kn, when $\bar{v}$ approaches 0 and the intercept on the $\bar{v}$ axis is n, when $\bar{v}/A$ approaches 0 (Figure).

*Results.* The results shown in the Figure demonstrate a linear relationship between $\bar{v}/A$ and $\bar{v}$ and that the binding sites are equivalent and independent. As shown in the Figure the average maximal bound thiopental with bovine serum albumin was determined roughly by extrapolating the line to $\bar{v}/A$ axis. The value of kn was 50,000, n was 5 and k was 12,000. Calculated $-\Delta F°$ was approximately 5300 cal/mole.

The effect of EDTA on the binding of thiopental to serum albumin is also shown in the Figure. There is a decreased capacity for thiopental binding on albumin in the presence of EDTA in the system. Values determined from our data were: n = 4, kn = 40,000, and k = 10,000. Free energy was 5100 cal/mole.

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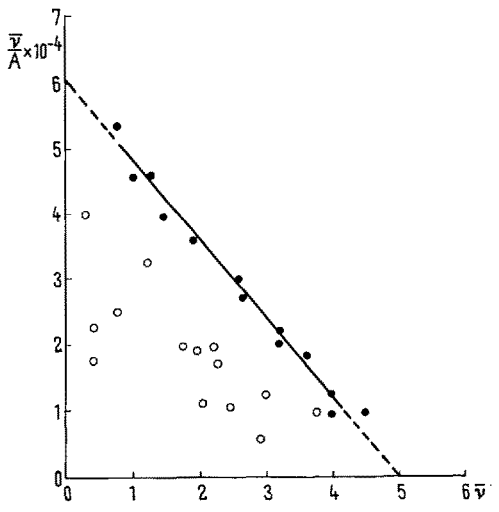
*Discussion.* The pharmacological significance of drug-protein interactions has been reviewed[1,7]. Diminution of drug activity from interaction of drug with protein has been reported. The data of LASSER et al.[8] suggest that potentiation of pentobarbital activity by Urokon is caused by binding of albumin allowing a high level of free or unbound pentobarbital available for central nervous activity. It has been reported that increasing the albumin concentration from 0.5% to 5% increased the amount of bound thiopental from 30% to 85%[2]. BRODIE et al.[3] demonstrated that at plasma levels of 10–50 μg, 75% of thiopental was bound to the non-diffusible component of plasma. The effect of EDTA on the binding capacity of thiopental with albumin was determined. The number of binding sites decreased to 4 and the association constant of bovine serum albumin for thiopental in the presence of EDTA decreased to 10,000. Thiopental in the presence of EDTA is less firmly bound with albumin. ONKST et al.[9] demonstrated that in the presence of calcium binding substances such as EDTA and citrate the induction time for pentobarbital narcosis was shortened[10].




Binding of thiopental by bovine serum albumin at 8°C in tris buffer, pH 7.42. (Open circles indicate the presence of EDTA in the media.)

*Zusammenfassung.* Die Bindungskapazität und die Anlagerungskonstante von Thiopental an Rinderalbumin wurde bei pH 7,42 in Trispuffer bei einer Temperatur von 7°C nach der Methode der Gleichgewichtsdialyse gemessen. Die Bindungskapazität betrug 5 und die Vergleichskonstante war 12000. Durch Zugabe von EDTA wurde die Bindungskapazität auf 4 reduziert; gleichzeitig verringerte sich die Anlagerungskonstante auf 10000.

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## Effects of Serotonin, Anti-Serotonin and Anti-Histamine Drugs on Uracil-Mustard Intoxication

The experimental syndrome produced by acute whole body radiation, as well as the acute intoxication pattern induced by alkylating agents, can be modified by the administration of biogene amines or their antagonists. Studies with serotonin, antiserotonins and antihistaminics have yielded conflicting results[1–3].

Recently, a new alkylating agent, derived from the nitrogen mustard, uracil mustard (UM), has been introduced in the therapy of leukemias and lymphomas[4,5]. Experimental acute UM intoxication produces a pathologic pattern entirely comparable to acute intoxication by radiation or radiomimetic agents[6].

We have studied the effects of the administration of serotonin, anti-serotonin and anti-histaminic drugs on the survival time of rats treated with sublethal doses of UM.

*Methods.* Five groups of adult male albino rats (Wistar strain), having an average weight of 225 g, were given intraperitoneally 1.5 mg/kg 5-bis-(2-chloroethyl)-amino-uracil (UM)[7] diluted in physiological saline + 5% dimethylacetamide (as suggested by PETERING et al.[8]) to a concentration of 0.3 mg/ml.

The first group served as a control. The second group was i.p. injected, 30 min before the administration of UM, with 2.5 mg/kg 1-methyl-lysergic acid butanolamide (methysergide, UML 491)[9] at a concentration of 0.5 mg/ml. The third group was i.p. injected, 24 h after the administration of UM, with the same dose of methysergide as the previous group. The fourth group was i.p. injected, 30 min before UM, with 12.5 mg/kg 5-hydroxytryptamine creatinine sulphate (5-OHT)[10] at a concentration of 2.5 mg/ml. The fifth group was i.p. injected,

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