Turnover of thiourea in the aqueous humors of the rabbit eye

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The rate of accumulation of ^{14}C-labeled thiourea in the aqueous humor of the anterior and posterior chambers of rabbits has been studied. The coefficients of transport by flow and diffusion and the concentration in the "secreted fluid" have been evaluated and compared with corresponding values for urea. The results demonstrate that thiourea penetrates both the iris and ciliary processes more rapidly than urea, an effect ascribed to its greater lipoid solubility.

The penetration of various compounds into the eye has been studied by measuring their rate of accumulation in the aqueous humors following systemic administration. Before data concerning concentration in the posterior chamber became available, conclusions about mode of penetration into the anterior aqueous were based on mathematical formulations designed to evaluate parameters of transport into the anterior chamber alone. Such formulations, however, do not take into account the quantity of a substance which enters the anterior chamber from the posterior chamber, and thus do not permit any conclusions regarding penetration across the anterior surface of the iris, or the nature of either this barrier or that located in the ciliary processes. With the development of techniques for sampling aqueous from the posterior chamber, data became available which made it possible to evaluate separately the kinetics of penetration across each of these barriers.

The effect of lipoid solubility on penetration of substances into the aqueous humor has been studied previously by methods involving only the anterior chamber. The results of these investigations suggested that penetration of the so-called "blood aqueous barrier" may be dependent on lipoid solubility.[1-4] The purpose of the present study is to evaluate the role of lipoid solubility on transport across both of the barriers which separate the blood and aqueous humors. For this purpose, the rates of penetration of thiourea (ether: water partition coefficient 1:140) across iris and ciliary body were determined and compared with corresponding data previously reported[5] for the similar but less lipoid soluble compound urea (ether:water partition coefficient 1:2000).

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Methods

All experiments were performed on young, adult albino rabbits weighing between 1.7 and 2.3 Kg. ^{14}C-labeled thiourea (10 to 20 μc), with or without nonlabeled thiourea (20 mg.), was given parenterally, 40 per cent intravenously and 60 per cent intraperitoneally. For experiments involving time periods in excess of 50 minutes, 10 per cent of the initial dose was given intraperitoneally every 45 minutes. Samples of blood and of posterior and anterior aqueous humors were collected at various times after injection by methods described earlier.[6] In all instances, except those involving time periods of less than 30 minutes, three or more samples of blood were obtained from each rabbit. Vitreous humor was withdrawn with a syringe and 18 gauge needle immediately after the death of the animals.

Radioactivity in intraocular fluids and plasma was determined with a flow gas counter, appropriate corrections being made for self absorption.

To determine whether meaurement of radioactivity reflected accurately the concentration of free thiourea present in different samples, this compound was isolated by chromatographic separation. A 1 per cent solution of picric acid was added in the ratio of 4 parts to 1 of plasma, dialysate of plasma, or anterior aqueous and the supernatant fluids were studied by chromatography on an ion exchange column after removal of excess picric acid with Dowex resin.[7]

Radioactivity was monitored with a continuous flow detector unit attached to an automatic amino acid analyzer. In all instances, a single peak of radioactivity was observed in the location known to correspond to thiourea.

Mathematical considerations. The mathematical considerations used in this study were described previously in detail.[5, 8-10] The mathematical formulation employed considers that substances enter the posterior chamber from the plasma in a unidirectional manner (secretion) and by diffusion from plasma. Net losses from the posterior chamber are presumed to occur by flow to the anterior chamber, by diffusion back to the plasma, and by diffusion to the vitreous and lens (taken together). Diffusion within the unstirred vitreous is calculated on the assumption that the vitreous body is a cylinder with a cross-sectional area equal approximately to that of the interface between the posterior chamber and vitreous humor.[10] Discrete stations are chosen at points equally spaced throughout the vitreous, and, by means of an analog computer, the concentration at each of these stations is calculated by solving a system of simultaneous partial differential equations which relate time, concentration, and distance in the vitreous from the posterior chamber–vitreous interface.

Substances are considered to enter the anterior chamber by flow from the posterior chamber and by diffusion across the anterior surface of the iris and to leave by diffusion to the iris vessels and by flow out at the chamber angle.

Coefficients for diffusion across ciliary processes and iris, and concentration of thiourea in the "secreted" fluid, are determined by selecting values which when used to draw theoretical curves based upon the above considerations produced good fits to the experimental data.

The equations describing these processes are as follows:

Anterior chamber:

$$\frac{dC_a}{dt} = k_{fa}\,(C_h - C_a) + k_{dpa}\,(C_p - C_a) \quad (1)$$

Posterior chamber:

$$\frac{dC_h}{dt} = k_{fh}\,(C_s - C_h) + k_{dph}\,(C_p - C_h) - \left[\frac{DA}{xV_h}(C_h - C_v)\right] x = 0 \quad (2)$$

The meaning of the symbols used in the equations is given in Table 1.

The only parameter of unknown numerical value in equation (1) is k_{dpa}, the diffusion coefficient across the iris. This is determined by selecting values and solving the equation until a fit to the data is obtained.

There are two unknown parameters in equation (2): C_s, concentration in the secreted fluid, and k_{dph}, the diffusion coefficient. While only one equation is available to evaluate these terms, they bear a fixed relationship to each other. This relationship can be determined from a knowledge of the concentration of thiourea in the posterior aqueous to that in the plasma at steady state when $dC_h/dt = 0$. Various combinations of values of C_s and k_{dph} can be used to generate curves permitting selection of a unique combination of C_s and k_{dph} which produces a curve that most closely approximates the data.[7] In previous papers, data for sodium and chloride were analyzed in this manner. The relationship between C_s and k_{dph} was calculated on the assumption that at steady state the concentrations are equal in the posterior chamber and in the compartment of the vitreous nearest to it. Thus, under these conditions, the last term in equation (2) would be zero, since there would be no net gain or loss of sodium or chloride to the posterior chamber.

In the case of urea, however, chemical analyses showed that the concentrations in the posterior aqueous and vitreous were not the same under steady state conditions, there being about 11 per cent excess in the vitreous, so that urea is con-

Table I. Meaning of symbols and units

Symbol	*Designation*	*Units*
A	Area of posterior chamber–vitreous interface	cm.^{2}
C_a	Concentration in aqueous humor of anterior chamber	Relative units* or mmoles per kg. of H_2O
C_h	Concentration in aqueous humor of posterior chamber	Relative units or mmoles per Kg. of H_2O
C_p	Concentration in plasma	Relative units or mmoles per Kg. of H_2O
C_s	Concentration of secreted fluid	Relative units or mmoles per Kg. of H_2O
C_v	Concentration in vitreous humor	Relative units or mmoles per kg. of H_2O
D	Diffusion constant	cm.^{2} per min.
k_{dpa}	Transfer coefficient by diffusion plasma to anterior chamber	min.$^{-1}$
k_{dph}	Transfer coefficient by diffusion plasma to posterior chamber	min.$^{-1}$
k_{fa}	Transfer coefficient by flow into and out of anterior chamber	min.$^{-1}$
k_{fh}	Transfer coefficient by flow into and out of posterior chamber	min.$^{-1}$
V_h	Volume of posterior chamber	cm.^{3}
x	Space variable	cm.

*A relative unit is defined as a percentage of the concentration in the plasma once the concentration reached in the plasma is essentially constant.

stantly diffusing into the posterior chamber from this body. To evaluate the magnitude of the last term in equation (2), information concerning rate of transport of urea from vitreous to posterior chamber was required. This was obtained by injecting labeled urea into the center of the vitreous and measuring the ratios of concentrations in anterior and posterior aqueous to those in the vitreous as a function of time. Transfer coefficients between posterior chamber and vitreous were then calculated from the data and the last term in equation (2) was shown to be 0.36.[5] Similar experiments were performed with thiourea, which substance was also observed to exist in lesser concentration in the posterior aqueous than in the vitreous. However, it was not possible to determine the rate of diffusion into the posterior aqueous when thiourea was injected into the vitreous because of the rapidity with which it diffused out of the vitreous. Thus for thiourea the difference in concentration between vitreous and aqueous humors could not be taken into account when evaluating the relationship between C_s and k_{dph}, as shown in Fig. 3. Failure to do so, however, could result in an overestimate of k_{dph} and C_s of less than 10 per cent.

Results

Fig. 1 shows the concentration of thiourea in plasma and aqueous humors of the posterior and anterior chambers of animals given only the ^{14}C-labeled compound. The concentration in the posterior chamber increased to about 30 per cent of that in the plasma 15 minutes after injection. Thereafter the concentration in the posterior aqueous gradually declined to 15 and 7 per cent after 3 and 6 hours, respectively. Within 24 hours the concentration had fallen to less than 3 per cent of that in the plasma, while in the latter fluid the concentration had decreased only 20 per cent from its initial value.

These results suggested that thiourea became less and less available for transport into the aqueous humor, despite an essentially constant concentration in plasma. Dialyses against physiologic saline performed on plasma withdrawn from rabbits 5 hours after injection showed that almost 90 per cent of the thiourea was bound to plasma proteins. In contrast, when thiourea was added to plasma in vitro, or incubated with plasma or whole blood in vitro for five hours, concentrations in plasma and dialysate were essentially equal, indicating that no binding occurred.

In an attempt to saturate any binding sites in the plasma, nonlabeled thiourea was added to the labeled compound before injection. Dialyses performed on plasma from animals injected with this mixture revealed minimal binding in the first 5 hours. However, when longer time periods were employed significant binding (25 to 30 per cent) was found to exist. A curve showing the degree of binding as a function of time was constructed (Fig. 2).

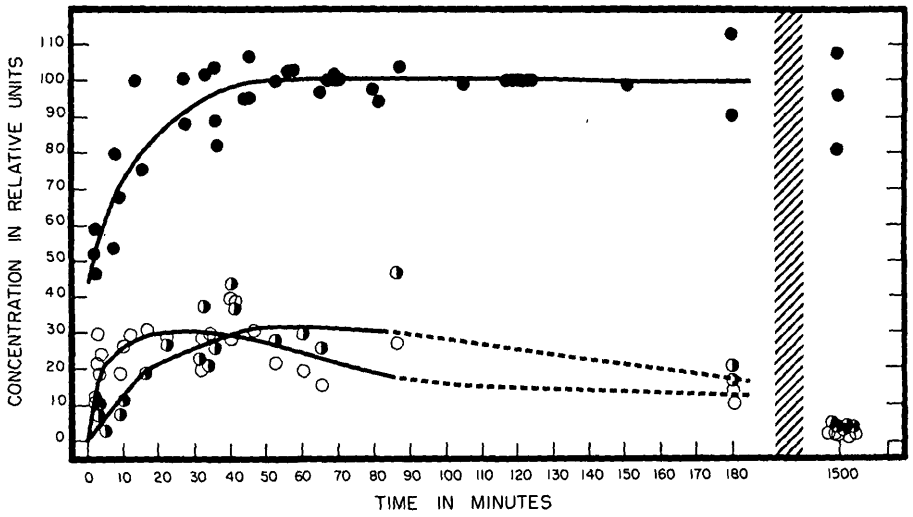

Fig. 1. Concentration of ^{14}C-labeled thiourea in plasma, posterior and anterior aqueous following parenteral administration of tracer material.

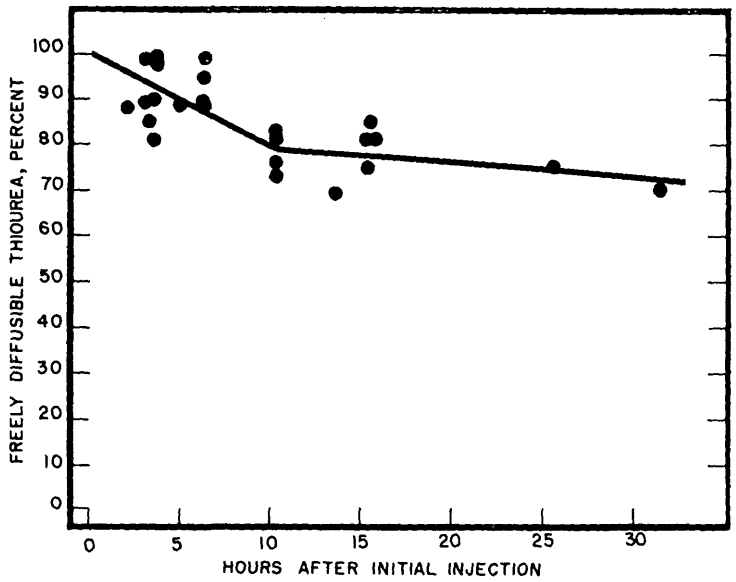

Fig. 2. Percentage of freely diffusible ^{14}C-labeled thiourea in plasma determined by dialysis at various times after initial injection of labeled and nonlabeled material. Points at 25 and 30 hours each represent pooled plasmas from 6 rabbits.

The relative concentration of freely diffusible ^{14}C-labeled thiourea in the plasma and posterior and anterior aqueous of rabbits at various times following parenteral administration is shown in Fig. 4. The data presented in Fig. 2 were employed to determine the percentage of the total thiourea in the plasma which was freely diffusible. The correction applied for the first three hours following injection is only approximately 10 per cent, but at steady state it amounts to about 30 per cent. All values are adjusted relative to the concentration of diffusible thiourea in the plasma at 1500 minutes; the latter value is arbitrarily set at 100 relative units. The

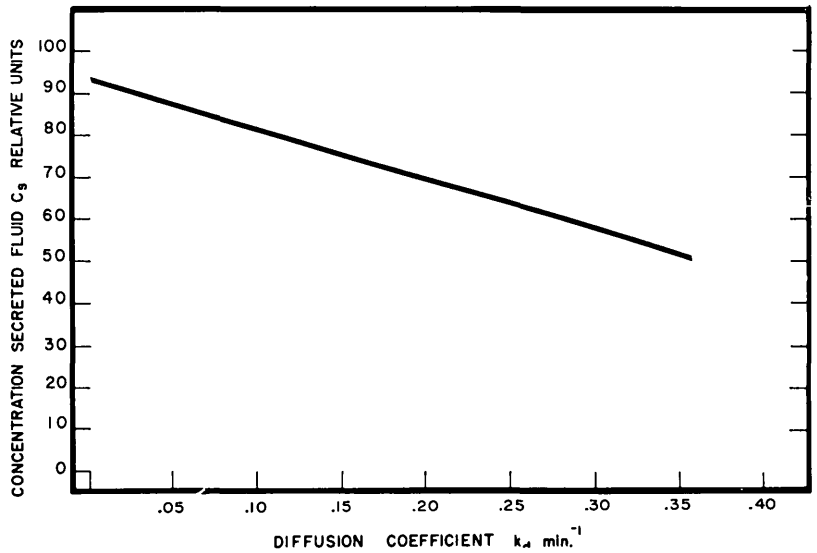

Fig. 3. Relation between concentration of thiourea in secreted fluid and the coefficient of diffusion between plasma and posterior chamber at steady state ($^dC_h/dt = 0$).

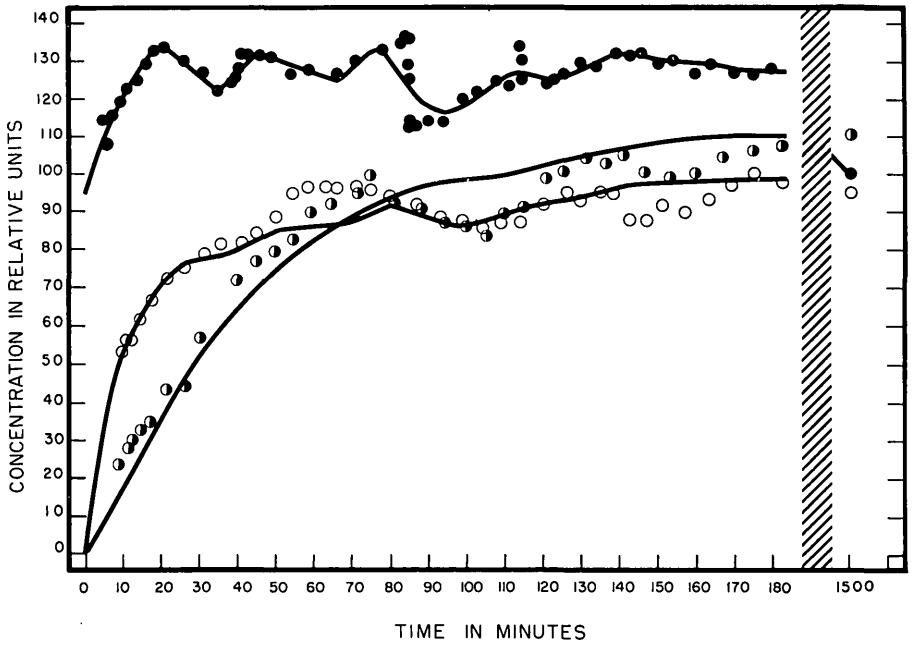

Fig. 4. Concentration of freely diffusible ^{14}C-labeled thiourea in plasma, posterior and anterior aqueous humor of rabbits after parenteral administration of labeled and nonlabeled compound (filled circles, plasma; half-filled circles, anterior aqueous; open circles, posterior aqueous).

values for 1500 minutes are averages of samples of aqueous from the posterior and anterior chamber from 20 eyes. Sliding averages, by sixes, were used to smooth the data.

The line through the data indicating concentration in plasma is drawn as a visual fit, those through the data representing concentration in aqueous humors were generated by solving equations (1) and (2) with an analog computer using values for flow rates, diffusion coefficients, and concentrations in the secreted fluid C_s as indicated in Table II. The table also shows the corresponding values for urea. The value for D was estimated as 0.57×10^{-3} cm.^{2} per minute; A = 1.1 cm.^{2}; V_h = 55 mm.^{3}.

The data shown in Table II indicate that the rate of diffusion of thiourea into the posterior chamber as well as the concentration in the secreted fluid is almost twice that for urea. Further, the diffusion rate across the anterior surface of the iris is likewise much higher for thiourea than urea.

Discussion

The observation that both the rates of diffusion and secretion of thiourea into the posterior chamber are higher than those of urea tends to confirm and amplify the role of lipoid solubility on penetration into the aqueous humors. Cell membranes in general have been shown to be penetrated more rapidly by compounds of high lipoid solubility. This is particularly true of barriers, such as the ciliary body, in which epithelial cells are tightly packed and the passage of materials is presumed to be through cells, rather than between them. The present studies also demonstrate, however, that thiourea enters the anterior chamber by diffusion considerably more rapidly than does urea. The difference in the diffusion coefficient is similar in magnitude to that observed for the posterior chamber. Since the iris contains only a rudimentary endothelium, it may be presumed that the greater diffusion coefficient of thiourea is a consequence of the effect of lipoid solubility on the iris vessels themselves. Pappenheimer[11] demonstrated that capillary permeability is at least in part dependent on lipoid solubility. The structure of the iris vessels also differs from the structure of vessels of similar size elsewhere in the body, in that the capillaries of the iris are remarkably thick-walled. It is possible that lipoid solubility may significantly affect diffusion across such vessels.

The experiments involving the isolation of radioactive substance in the plasma, dialysate of plasma, or aqueous humor demonstrate that the diffusible radioactive substance in plasma is thiourea and that measurement of radioactivity in the aqueous humor accurately reflects the concentration of this substance.

The observation that thiourea binds to plasma only in the living animal suggests that the process requires participation of some organ system. It is also possible that thiourea may bind to the ciliary process. If it does, administration of nonlabeled thiourea (employed to saturate the mechanism of binding to plasma) may also satu-

Table II. Coefficients of transfer by diffusion and flow for thiourea and urea in the posterior and anterior chambers and concentrations in the secreted fluid entering the posterior chamber

	Posterior chamber			*Anterior chamber*	
	Flow k_{fa} *(min.$^{-1}$)*	*Diffusion* k_{dph} *(min.$^{-1}$)*	*Conc. sec. fluid* C_s *(rel. units)*	*Flow* k_{fa} *(min.$^{-1}$)*	*Diffusion* k_{dpa} *(min.$^{-1}$)*
Thiourea	0.06	0.060	86	0.013	0.015
Urea	0.06	0.036	50	0.013	0.010

rate the site of transport into the ocular fluids. This possibility seems unlikely, however, since at early time periods (10 to 30 minutes) when there is no significant binding to plasma proteins, the concentration of thiourea in the posterior aqueous is higher in animals given nonlabeled thiourea (Fig. 4) than in animals given labeled compound alone (Fig. 1). These findings are additional evidence that thiourea is not transported actively by mechanisms involving metabolic processes such as mediate the transport of ascorbic acid or amino acids.

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