Summary. Rats exposed to 6 p.p.m. ozone by volume for 4 hours developed severe pulmonary edema and hemorrhage with significant increases in lung weight, lung protein content, and lung 5-HT when related to body weight. Ozone exposure was found to decrease MAO activity; this was an effect of dilution by edema rather than destruction of the enzyme. Ozone exposure did not alter blood 5-HT content. Possible mechanisms involved in the lung 5-HT increase after exposure to ozone were discussed briefly.

The authors appreciate 5-HT made available by Vismara Terapeutici, Casatenova Brianza, Italy, through California Corp. for Biochemical Research.

1. Mittler, S., Hedrick, D., King, M., Gaynor, A., *Indust. Med. & Surg.*, 1956, v25, 301.

2. Mittler, S., King, M., Burkhardt, B., *A.M.A. Arch. Indust. Health*, 1957, v15, 191.

3. Stokinger, H. E., *A.M.A. Arch. Indust. Hyg. & Occup. Med.*, 1954, v9, 366.

4. Stokinger, H. E., Wagner, W. D., Wright, P. G., *A.M.A. Arch. Indust. Health*, 1956, v14, 158.

5. Stokinger, H. E., *ibid.*, 1957, v15, 181.

6. Kabins, S. A., Molina, C., Katz, L. N., *Am. J. Physiol.*, 1959, v197, 955.

7. Parratt, J. R., West, G. B., *J. Physiol.*, 1957, v137, 179.

8. Weissbach, H., Waalkes, T. P., Udenfriend, S., *Science*, 1957, v125, 235.

9. Quilligan, J. J., Boche, R. D., Falk, H. L., Kotin, P., *A.M.A. Arch. Indust. Health*, 1958, v18, 16.

10. Byers, D. H., Saltzman, B. E., *Am. Indust. Hyg. Assn. J.*, 1958, v19, 251.

11. Bogdanski, D. F., Pletscher, A., Brodie, B. B., Udenfriend, S., *J. Pharmacol. Exp. Therap.*, 1956, v117, 82.

12. Sjoerdsma, A., Smith, T. E., Stevenson, T. D., Udenfriend, S., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 36.

13. Udenfriend, S., Weissbach, H., Clark, C. T., *J. Biol. Chem.*, 1955, v215, 337.

14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *ibid.*, 1951, v193, 265.

15. Udenfriend, S., Weissbach, H., Brodie, B. B., 1958, in *Methods of Biochemical Analysis*, vVI (ed. D. Glick), New York: *Interscience*, pp. 95-130.

16. Udenfriend, S., Weissbach, H., *Fed. Proc.*, 1954, v13, 412.

Received February 14, 1961. P.S.E.B.M., 1961, v107.

Passage of Thiopental and Barbital Into Ocular and Cerebrospinal Fluids of Dog.* (26573)

PETER G. DAYTON, LEONARD BRAND, DOLORES TALLER AND LESTER C. MARK

New York Univ. Research Service, Goldwater Memorial Hospital and Dept. of Anesthesiology, Columbia Presbyterian Medical Center, N. Y. City

The passage of barbital and thiopental into ocular and cerebrospinal fluids was investigated in order to extend previous studies of the passage of barbiturates into various compartments such as brain, cerebrospinal fluid, fat and muscle(1-3). The comparison between ocular and spinal fluid was made because embryological and physiological similarities exist between the 2 fluids(4).

Methods and materials. Barbital was determined by the method of Giotti and Maynert(5). Thiopental was measured by the method of Brodie *et al.*(1). When concentrations were low, after extraction of drug by the organic solvent, the drug was extracted into 1 or 2 ml of aqueous phase and measured either in micro or semi-micro cuvettes. The methods gave negligible blank values and recoveries averaged 90-100%.

Mongrel, adult female dogs weighing 7-20 kg were used for this study. Barbital sodium (100 mg/kg) as a 10% neutral solution or thiopental sodium (50 mg/kg) as an alkaline (pH 10.5) 2.5% solution was injected intravenously within 5 seconds. Artificial respiration was given to the thiopental-treated dogs

* This work supported by contract from Office of Surgeon General, Dept. of Army, and by grants from Nat. Inst. of Neurol. Dis. and Blindness, P.H.S. and Abbott Lab., North Chicago, Ill.

TABLE I. Passage of Barbital into Cerebrospinal and Ocular Fluids of Dogs.
Barbital sodium, 100 mg/kg i.v.

Time, min.	Conc. of barbital sodium: Plasma, mg/l	Cerebrospinal fluid, mg/l	Ocular fluid, mg/l	Concentration ratios: CSF P	OF P
9	200	31	47	.16	.24
9	220	48	64	.22	.29
30	150	95	81	.62	.54
30	130	52	64	.40	.49
60	100	97	110	.98	1.1
60	120	110	110	.92	.92
180	94	87	110	.95	1.2
180	130	120	140	.92	1.1

by rhythmic inflation of the chest with room air through an endotracheal tube fitted with a non-rebreathing valve. Adequate thoracic excursions were maintained at a rate of 16-20 per minute until satisfactory spontaneous respiration was resumed. At various time intervals the animals were sacrificed by rapid intravenous injection of 4 to 6 ml of a saturated solution of potassium chloride which produced cardiac standstill within 5 seconds. Heart blood was immediately obtained by cardiac puncture and cerebrospinal fluid was withdrawn from the cisterna magna. Ocular fluid was sampled from anterior chamber of the eye by means of 26 gauge hypodermic needle. The needle was inserted through the cornea near its lateral margin and advanced between the cornea and the iris until the tip was lying free just in front of the lens. Gentle aspiration permitted removal of 0.5 to 1.0 ml of clear ocular fluid. Each eye was used only once as a sample source. The ocular fluid value reported in Tables I and II is the average value; concentrations in the 2 eyes differed within experimental error(4-5%).

Results. Barbital concentrations in plasma were lower the longer the time interval after drug administration (Table I). Ocular fluid/plasma concentration ratios were low at first and rose to maximal values in about 60 minutes. Concentrations half that of plasma were reached in about 30 minutes. Cerebrospinal/fluid plasma concentration ratios were generally slightly lower than ocular fluid/plasma concentration ratios. Results of similar experiments performed with thiopental are shown in Table II. For thiopental, concentration ratios were corrected for the extensive binding to plasma proteins. After making this correction, concentration ratios for thiopental were found much higher than for barbital at 9 minutes. Cerebrospinal fluid/plasma water concentration ratios were consistently (in contrast to barbital) higher at 3 and 9 minutes than ocular fluid/plasma water concentration ratios. The data in Tables I and II indicate that thiopental enters ocular and cerebrospinal fluid more rapidly than barbital and that cerebrospinal fluid/plasma and ocular fluid/plasma ratios of about one eventually are achieved for both drugs.

Discussion. Some of the pertinent physical properties of the 2 barbiturates chosen for this study had been determined previously. The pKa is 7.8 for barbital and 7.6 for thiopental. The partition coefficient between peanut oil and pH 7.4 phosphate buffer was

TABLE II. Passage of Thiopental into Cerebrospinal and Ocular Fluids of Dogs.
Thiopental sodium, 50 mg/kg i.v.

Time, min.	Concentrations of thiopental sodium: Plasma,* mg/l	Cerebrospinal fluid, mg/l	Ocular fluid, mg/l	Concentration ratios: CSF / Plasma water	OF / Plasma water
3	72 (18)	13	6.3	.72	.35
3	94 (24)	8.2	6.1	.35	.25
9	67 (17)	11	6.5	.65	.38
9	62 (16)	14	8.7	.88	.54
30	33 (8.3)	11	9.2	1.3	1.1
30	—	20	12	—	—
30	50 (13)	13	11	1.0	.85
30	23 (5.8)	8.3	6.3	1.4	1.2

* Values in parentheses are calculated values of drug concentrations in plasma water based on 75% binding to plasma protein.

0.23 for barbital as compared with 63 for thiopental(2). Based upon the Henderson-Hasselbalch equation, at pH 7.4, 41% of barbital is present in the unionized form while at the same pH 61% of the thiopental present is unionized. Barbital is not appreciably bound to plasma protein whereas thiopental is extensively bound (65-75%)(9-11). It is evident that the 2 barbiturates differ most strikingly in lipid solubility and in the extent of their binding onto plasma protein; at pH 7.4 both are unionized to about the same extent.

Based upon studies in the rabbit, Mayer, Maickel and Brodie(12) calculated relative permeability constants for penetration into cerebrospinal fluid by various drugs. For thiopental and barbital, they found average constants of 0.69 and 0.029 min^{-1} respectively. These authors suggested that the blood-brain and blood-spinal fluid barriers behave as a lipid boundary which drugs cross by simple diffusion, according to the extent of their dissociation and the lipid solubility of the unionized molecules. These authors (13) have also shown that thiopental leaves the cerebrospinal fluid more rapidly than barbital. Recently, Brodie and co-workers extended their investigations, using the dog (14); permeability constants (cerebrospinal fluid) were similar to those found for rabbits —0.026 for barbital and 0.50 min^{-1} for thiopental, respectively. The present results with cerebrospinal and ocular fluids are consistent with these findings, in that barbital was found to enter both fluids more slowly than thiopental.

Just as there is a blood-brain barrier and a blood-spinal fluid barrier, there is also a blood-ocular fluid barrier to the free passage of certain substances from the general circulation into the intraocular fluid. Ross(6) has suggested that lipid solubility is of greater importance than molecular size in the passage of non-electrolytes into ocular fluid. Other authors(7,8) are in agreement with this concept though factors such as pKa(14,15), diffusion coefficient and state of hydration may also be involved. According to Davson (7), there are two processes for penetration of substances from the blood into ocular fluid: (a) the secretory flow mechanism and (b) the iris diffusion mechanism. The secretory mechanism is associated with the ciliary process and the relatively small posterior chamber; the diffusion mechanism is associated with the iris and the relatively large anterior chamber. These mechanisms involve passage of substances through one or more membranes. Davson(7) has suggested that, for a series of compounds of comparable molecular size, rate of transfer by both mechanisms increases with increase in lipid solubility. However, rate of transfer by the secretory flow mechanism soon reaches a maximum which is probably determined by rate of formation of ocular fluid itself by this process. Beyond this point, a further increase in total transfer rate can still result from further increase in lipid solubility, due to increased transfer by the iris diffusion mechanism. Under the experimental conditions used, thiopental enters both ocular and cerebrospinal fluids more rapidly than barbital. From these data and the works cited above it seems reasonable to implicate high lipid solubility as the key factor in rapid distribution of thiopental into these body compartments. Simple diffusion across lipoid boundaries may be the major mechanism involved.

Summary. Experiments with dogs have shown that thiopental enters ocular fluid and spinal fluid more rapidly than barbital does. This is probably due to the higher lipid solubility of the thio barbiturate.

The authors thank Drs. Bernard B. Brodie and J. J. Burns for helpful criticism.

1. Brodie, B. B., Mark, L. C., Lief, P. A., Bernstein, E., Rovenstine, E. A., *J. Pharm. & Exp. Therap.*, 1950, v98, 85.

2. Mark, L. C., Burns, J. J., Brand, L., Campomanes, C. I., Trousof, N., Papper, E. M., Brodie, B. B., *ibid.*, 1958, v123, 70.

3. Mark, L. C., Brand, L., Dayton, P. G., Taller, D., Papper, E. M., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 606.

4. Wegefarth, P., Weed, H. L., *J. Med. Res.*, 1914, v31, 167.

5. Giotti, A., Maynert, E. W., *J. Pharm. & Exp. Therap.*, 1953, v101, 26.

6. Ross, E. J., *J. Physiol.*, 1951, v112, 229.
7. Davson, H., Physiology of the Ocular and Cerebrospinal Fluids, Little, Brown and Co., Boston, 1959.
8. Langham, M. E., *Physiol. Rev.*, 1958, v38, 215.
9. Goldbaum, L. R., Smith, P. K., *J. Pharm. & Exp. Therap.*, 1954, v111, 197.
10. Taylor, J. D., Richards, R. K., Davin, J. C., Asher, J., *ibid.*, 1954, v112, 40.
11. Schanker, L. S., Shore, P. A., Brodie, B. B., Hogben, A. M., *ibid.*, 1957, v120, 528.
12. Mayer, S. E., Maickel, R. P., Brodie, B. B., *ibid.*, 1959, v127, 205.
13.———, *ibid.*, 1960, v128, 41.
14. Brodie, B. B., Kurz, H., Schanker, L. S., *ibid.*, 1960, v130, 20.
15. Zubrod, C. G., Rall, D. P., *ibid.*, 1959, v125, 194.

Received February 15, 1961. P.S.E.B.M., 1961, v107.

Studies of Alcaptonuria: Collagenase Degradation of Homogentisic-Tanned Hide Powder Collagen.* (26574)

ROBERT AUSTIN MILCH (With the assistance of Richard A. Murray)
Division of Orthopaedic Surgery, The Johns Hopkins University School of Medicine and The Johns Hopkins Hospital, Baltimore, Md.

Studies in this laboratory on the pathogenesis of alcaptonuric arthritis, the third of the 3 temporally related, characteristic components of the alcaptonuria syndrome (homogentisic aciduria, or alcaptonuria; ochronosis; alcaptonuric, or ochronotic arthritis) have been concerned primarily with the interactions of various homogentisic acid solutions with collagen. Since it had been shown previously(1) that homogentisic acid does not interact with unbound chondroitin sulfate, it followed logically that interactions with collagen, the other and major component of cartilage, as well as the other connective tissues, necessarily was implicated.

Indeed, it was later shown(2,3) that homogentisic acid autoxidation polymers (HGA-OP), in diametric opposition to unoxidized, monomeric homogentisic acid solutions (HGA), become more or less irreversibly bound to collagen by a pH-independent reaction. For steric reasons, this results in significant cross-linking of adjacent collagen polypeptide chains and stabilization of the collagen structural lattice. As such, the resulting tanning reaction would be expected to result in a product which would not only resist water solubilization and heat degradation, as has been previously demonstrated, but would also be expected to result in increased resistance to the action of a number of proteolytic enzyme systems. The present report notes the result of one such study.

Material and methods. Twelve gram batches of limed and neutralized (pH 5.4) bovine hide powder collagen (American Standard Hide Powder, purchased from Frank F. Marshall, Ridgeway, Pa.) were passed through No. 20 ASTM sieves, placed in covered Ehrlenmeyer flasks and reacted for approximately 75 hours at room temperature (21°-28°C) with 500 ml aliquots of water or of aqueous, autoxidized, polymeric homogentisic acid (HGA-OP) solutions (3 mg/ml), prepared as outlined previously(4). The liquid phase was removed by suction filtration after the period of interaction and the solid (collagen) phase was freeze-dried. (A considerable quantity of the previously untreated hide powder collagen was lost by solubilization during water treatment, whereas significantly smaller amounts were solubilized during interaction with HGA-OP. No attempt was made here, however, to provide quantitative data on the precise degree of solubilization in either instance.)

Two hundred milligram samples of each collagen preparation were placed into dry, chemically clean screw-top 15 ml graduated

* Aided by a grant from Nat. Inst. of Arthritis and Metab. Dis., Nat. Inst. Health, U. S. Dept. of HEW.