CN^-, OH^-, $OC_6H_5^-$, and $PO_4\equiv$ are ineffective. The released benzyl radicals arylate the anions. In a microorganism, if such arylation occurs, a free and functionally active mercapto group, essential to the metabolism of the organism, would be converted into a nonactive thioether group.

Conclusions. It may be suggested, tentatively, that the activity of iodonium compounds may, in some cases, be due to interaction with certain mercapto groups essential to the microorganisms. This formulation rests largely on observations of the high reactivity of iodonium compounds toward sulfide, hydrosulfide and similar ions in simple chemical systems. That the detail of the mechanism of inhibition is different for *Cl. histolyticum* and *M. tuberculosis* is apparent from the experiments involving thioglycollate. In the case of *M. tuberculosis* we have not found a mercapto compound which antagonizes the effect of the iodonium compounds. That both sulfonium and iodonium compounds inhibit Monilia, while the iodonium compounds alone inhibit the growth of tubercle bacilli, suggest that a similar mechanism of action may be involved with both compounds for Monilia, but not in the case of *M. tuberculosis*.

15588

Effect of Some Barbiturates on Tissues *In vitro*.

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It is well known to workers with tissue cultures that outgrowths from explants of brain, ganglia and spinal cord can be easily obtained in fluid or semisolid media even without the addition of embryonic extract. The cultivation of spinal ganglia has proved to be a valuable test material in a variety of experimental studies (Burt,[1,2] Meyer and Jablonski,[3] Murnaghan,[4] Weiss,[5-7] Weiss and Wang[8,9]). Relatively little use has been made of nerve cord cultures even though the technic for obtaining such preparations is considerably easier than that required for the dissection of ganglia.

The procedure for cord cultures which has been used in our laboratories is schematized in Fig. 1 to 4. The necks of embryos of suitable ages are cut (Fig. 1) at their base with scissors or knives. The spinal cord is expelled from the neural canal by compressing the tissue in the mid-dorsal line with 2 pairs of forceps moved alternately in the direction of the sectioned vertebral column. Thus a cervical (Fig. 2) and a thoracolumbar segment (Fig. 3) of cord can be obtained. These are washed in Tyrode's solution and then transferred to a ground glass slide and cut with sharp knives. For this purpose we have found fragments of wafer razor blades soldered in the eye of a needle more satisfactory than cataract knives (Fig. 4a). Transverse sections varying from ¼ to ½ mm can be cut easily by moving knife tips, placed at right angles to the cord segment, in opposite directions (Fig. 4 and 4a). After washing in Tyrode's solution hang-

1 Burt, A., *J. Cell. and Comp. Physiol.*, 1943, **22**, 145.

2 Burt, A., *J. Cell. and Comp. Physiol.*, 1943, **22**, 205.

3 Meyer, H., and Jablonski, W., *J. Anat.*, 1937, **72**, 62.

4 Murnaghan, D. P., *Anat. Rec.*, 1941, **81**, 183.

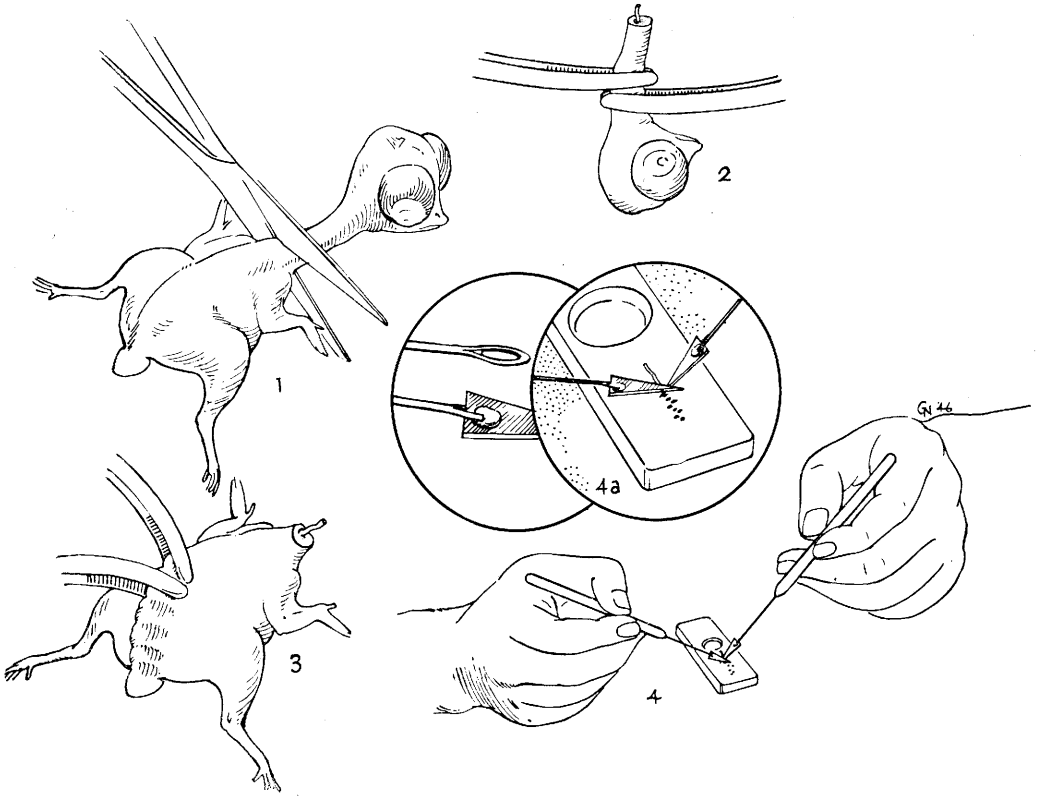
5 Weiss, P., *J. Exp. Zool.*, 1934, **68**, 393.

6 Weiss, P., *Anat. Rec.*, 1944, **88**, 205.

7 Weiss, P., *J. Exp. Zool.*, 1945, **100**, 353.

8 Weiss, P., and Wang, H., *Anat. Rec.*, 1936, **67**, 105.

9 Weiss, P., and Wang, H., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 273.



ing drop preparations were made by placing cord sections in a drop of 25% embryonic extract from chicks incubated for 6-10 days with or without the inclusion of test solutions. A drop of 50% rooster plasma was then mixed thoroughly into the medium.

A detailed description of the morphological aspects of the resulting outgrowth as a function of the age of the cord and other variables will be reported in a separate study. The conditions found most suitable for the purpose of the present report consisted of using cords from chicks incubated for 9 days. Heart fragments from the same individuals and cultivated for 72 hours were used for comparison of growth in nonnervous tissue. The medium was measured out as uniform-sized drops from No. 27 needles mounted onto sterile 1 cc tuberculin syringes. Living cultures were observed after 24 and 48 hours of incubation and were then fixed. Bouin's solution was found to be superior to a variety of other fixatives as a preparation for staining with Bodian's technic.

TABLE I.
Outgrowth from Spinal Cord After 48 Hours and Heart After 72 hours. 9-day Chick Embryos.

	Seconal sodium		Pentobarb. sodium		Sodium amytal	
Conc.	Cord	Heart	Cord	Heart	Cord	Heart
1:800	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
1:1600	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
1:3200	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	½ ½ 1 0
1:6400	0 0 0 0	1 ½ 1 1	1 ½ 1 1	2 2 3 2	2 1 1 2	1 1 2 1
1:8000	1 0 ½ 1	2 2 2 2	2 1 2 2	3 2 4 3	3 3 3 2	2 2 2 1
1:12800	1 2 2 1	3 3 3 3	2 1 2 0	3 3 2 3	—	—

Outgrowth of 1.6 mm = 4+; 1.2 = 3+; 0.8 = 2+; 0.4 =1+; a few short fibers = ±.

Approximately 700 cultures were prepared to test the effect of Seconal Sodium, Pentobarbital Sodium, Sodium Amytal and 14 barbiturate compounds supplied by Dr. K. K. Chen. The authors are deeply indebted to The Lilly Research Laboratories for their kind assistance and support of this study.

Using an ocular micrometer it was possible to measure the amount of outgrowth in living cultures. At the end of 48 hours the maximum length of fibers in control cultures was found to be approximately 1.6 mm. This value served in a scale for grading growth in experimental cultures as follows: outgrowth of 1.6 mm = 4+; 1.2 = 3+; 0.8 = 2+; 0.4 = 1+; a few short fibers = ±. As a practical tool for an extensive survey of a large number of drugs at various concentrations, this system of tabulation was found too cumbersome and effort was directed simply toward finding the minimum concentration of a test solution which would first prevent all outgrowth of fibers and, in the case of heart fragments, of all outwandering cells. After some preliminary study test concentrations for the various barbiturates were prepared at final concentrations of 1:800, 1:1600, 1:3200, 1:6400, 1:8000, 1:12,800 and 1:16,000. Four cultures were set up for each tissue and test solution. All component materials for a given experiment were constant except the barbiturate factor. Each experiment was repeated and the dosage was overlapped several times so that the whole series of results were comparable. Tyrode's solution was used in the preparation of all barbiturate concentrations.

Table I shows that total inhibition of fiber outgrowth was obtained at 1:6400 for Seconal Sodium and 1:3200 for both Pentobarbital Sodium and Sodium Amytal. Outgrowth from heart fragments seemed somewhat more resistant to the deleterious effects of the barbiturates.

The other barbituric acid derivatives fall into 2 groups, as listed in Table II. Compounds 1 to 8 are convulsants and compounds 9 to 14, hypnotics. The median convulsive dose (CD_{50}), median lethal dose (LD_{50}), and median anesthetic dose (AD_{50}) have

TABLE II. Convulsive ("D), Lethal (LD), and Anesthetic Dose (AD) of Barbiturates in Relation to Outgrowths from Chick Spinal Cord and Heart Fragments *in Vitro*.

Compounds	Mol. wt Na salt	Route	CD_{50} ± S.E. (Rats) mg/kg	LD_{50} ± S.E. (Rats) mg/kg	Inhibition Cords, 48°	Inhibition Hearts, 72°
1. 1,3-dimethyl butyl allyl barbituric acid	284	i.v.	—	50.0 ± 3.02	1:6400	1:1600
2. Sodium 3,3-dimethyl allyl ethyl thiobarbiturate	263	,,	12.1 ± 0.95	53.1 ± 2.42	1:1600	1:800
3. 3,3-dimethyl allyl allyl barbituric acid	268	,,	8.89 ± 0.52	16.7 ± 0.45	1:3200	1:800
4. Sodium N-methyl-3,3-dimethyl allyl ethyl barbiturate	261	,,	1.39 ± 0.21	4.29 ± 0.48	1:1600	1:800
5. Sodium isobutyl 1-methyl allyl thiobarbiturate	277	,,	3.9 ± 0.39	21.57 ± 1.66	*1:800	1:800
6. 1,3-dimethyl butyl ethyl barbituric acid	272	i.p.	9.74 ± 0.36	18.13 ± 0.61	1:3200	1:800
7. 3,3-dimethyl allyl allyl thiobarbituric acid	284	i.v.	8.92 ± 0.535	94.08 ± 8.47	1:12800	1:6400
8. 3,3-dimethyl allyl ethyl barbituric acid	256	,,	5.28 ± 0.31	8.84 ± 0.32	*1:800	*1:800
			AD_{50} ± S.E. (Rats) mg/kg			
9. n-propyl-2-methyl allyl barbituric acid	256	i.p.	114.7 ± 3.7	265.1 ± 7.2	*1:800	1:800
10. sec-butyl-2-methyl allyl barbituric acid	270	,,	76.3 ± 3.1	184.8 ± 3.4	1:800	1:800
11. Allyl-1-methyl allyl barbituric acid	254	,,	69.01 ± 2.76	135.6 ± 6.23	1:800	*1:800
12. Ethyl-3-methyl allyl barbituric acid	242	,,	88.43 ± 6.7	229.7 ± 5.10	*1:800	*1:800
13. n-propyl-1-methyl allyl barbituric acid	256	,,	76.52 ± 2.66	227.5 ± 5.5	*1:800	*1:800
14. iso-propyl-3-methyl allyl barbituric acid	256	,,	66.1 ± 3.4	168.3 ± 7.5	*1:800	*1:800

* Subtotal inhibition.

been determined in The Lilly Research Laboratories. It is obvious that out of the whole series the hypnotic barbiturates are less toxic in whole animals than the convulsant barbiturates. Our results indicate that there is some correlation in tissue culture. For example, compounds 9 to 14 which have a lower toxicity *in vivo* were all found to inhibit cellular outgrowth at 1:800 or a little less. Compounds 1 to 8 which are more toxic *in vivo* were found similarly more damaging to tissues *in vitro*. In the latter group, notable exceptions were observed. Thus, although the LD_{50} of compound 8 was only 8.84 $\pm$ 0.32 mg/kg in rats, nerve outgrowth from cord and migration of cells from heart fragments was not completely inhibited at 1:800. Compound 5 was also characterized by a high toxicity *in vivo* but by a low toxicity *in vitro*. Another unpredictable effect was found for 3,3-dimethyl allyl allyl thiobarbituric acid (No. 7), which had an LD_{50} of 94.08 $\pm$ 8.47 mg/kg, but which proved to be the most toxic member of the series in terms of outgrowth of living tissue. No fibers were observed on repeated tests from cords—in a medium containing 1:12,800 of this compound. Heart cells showed no migration at 1:6400. It may be concluded, therefore, that while there is a general correspondence between the toxicity of barbiturates *in vivo* and the presence of some especially vulnerable tissue of the whole organism would modify results obtained in tissue cultures. Cyto-pharmacological work of this type (*e.g.* Jacoby, Medawar and Willmer,[10] Pomerat,[11] Pomerat, Capouya and Greenleaf,[12] Pomerat, Lay and Emerson,[13] Salle[14]) agree in demonstrating that tissue cultures offer a stringent test of toxic effects which may ultimately serve as an aid in exploring possible mechanisms of action.

These studies suggest that it is not enough, in appraising possible drug toxicity, to be content merely with estimations of gross lethal effects. It may also be helpful to survey drug toxicity with respect to particular organs and tissues under appropriate conditions.

Summary. Hanging drop tissue cultures of spinal cord from chicks incubated for 9 days were used to test the inhibitory action of various barbiturates on the outgrowth of nerve fibers. Using concentrations of 1:800, 1:1600, 1:3200, 1:6400, 1:8000 and 1:12,800, total inhibition was obtained at 1:6400 for Seconal Sodium, and at 1:3200 for both Pentobarbital Sodium and Sodium Amytal. The convulsive, lethal and anaesthetic dose of 14 other barbiturate compounds was generally well correlated with *in vitro* estimates of toxicity. However, some notable exceptions were found. For example, the lethal dose of 3,3-dimethyl allyl ethyl barbituric acid was 8.84 $\pm$ 0.32 mg/kg; and the second most damaging compound tested *in vivo,* but belonged to the least toxic group tested *in vitro*. In contrast, 3,3-dimethyl allyl allyl thiobarbituric acid which had a lethal dose of 94.08 $\pm$ 8.47, proved to be the most toxic member of the entire series in terms of outgrowth of living tissue. Corresponding cultures using fibroblast outgrowth from chick heart fragments generally proved somewhat more resistant to barbiturates than fiber outgrowths from cord explants. These results suggest that the survey of drug toxicity should include studies on particular tissues in addition to estimates of gross lethal effects.

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[13] Pomerat, C. M., Lay, M., and Emerson, G. A., *Tex. Rep. on Biol. and Med.*, 1944, **2**, 316.

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