TABLE II. Some Intra-specific Differences in the Enzymatic Activities in Mice and Rats.

Wt, g	Kidney Q_{O_2}	Heart Q_{O_2}	Liver Q_{O_2}	Brain Q_{O_2}
	Succinoxidase—Mice			
16	153	188	115	72.4
26	168	203	116	54.2
32	162	208	115	67.5
38	218	234	84.2	57.3
46	160	240	101	55.5
	Malic dehydrogenase—Rat			
230	68.4	127	121	
360	96.0	139	96.9	
	Succinoxidase—Rat			
140	86.5		52.0	40.0
172	95.7		59.6	29.9
210	108		62.5	49.5
265	93.0		54.7	34.1

trolling factors may be different from those effective in basal metabolism(1), cytochrome c(7), and cytochrome oxidase(8). The constant enzymic activity within a single species indicates indirect genetic factors regulating metabolic enzymes rather than hormonal or other direct somatic factors. That the enzymatic variation might be due partly to differences in homogenization, to deleterious effects of freezing (cow), or to dietary differences in the different species appears improbable under the experimental conditions.

Summary. Heart, kidney, liver, and brain of the cow, rat, and mouse were assayed for maximal activity of succinoxidase and malic dehydrogenase. The levels of the two enzymes generally reflect the inverse relationship of metabolism with body size in that the tissues of the cow were lowest, those of the rat intermediate, and those of mouse highest in activity for these enzymes. The enzyme levels did not vary as an exact power function of body weight, nor were there any intraspecific changes in enzyme levels with changes in body size.

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Hemolytic Action of Water Soluble Compounds Related to Mephenesin. (20170)

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The description of the muscle-relaxing and paralyzing action of mephenesin(1) led to extensive trials of the drug as a substitute for curare during anesthesia(2). The use of concentrated solutions of mephenesin by the intravenous route was, however, soon abandoned because of the frequent occurrence of intravascular hemolysis, hematuria and anuria (3). During the past few years a number of compounds structurally related to mephenesin have been discovered that have higher water solubility than mephenesin and because of this appear more suitable for intravenous administration. The present paper describes the hemolytic action of several of these compounds and compares this property with the paralyzing and lethal action of these drugs.

Methods. Determination of water solubility was made by spectrophotometric measurement of saturated solutions of each of the compounds at the wave length corresponding to maximum absorption, using the specific absorbency previously determined for each of the compounds. The partition ratio of the compounds between water and oil ($K_{w/o}$) was likewise measured spectrophotometrically using cottonseed oil, U.S.P. as the oil phase. The partition ratio of the non-aromatic ether No. 13 was obtained by measurement of the refractive index of the aqueous solutions.

TABLE I. Physical and Biological Properties of Glycerol Ethers.

Compound No.	Compound	M.p. or B.p. (mm), °C	Water sol'y 26°C, %	$K_{w/o}$, 26°C	PD_{50}* i.p., mg/kg	LD_{50}† i.p., mg/kg	Time for M/10 sol. to produce 75% hemolysis, min.	% conc. causing 75% hemolysis in 100 min.
1	3-phenoxy-1,2-propanediol	64–66	7.0	6.1	490 ± 26	1400 ±125	26	1.18
2	3-*o*-toloxy-1,2-propanediol	70–71	1.2	1.3	180 ± 18	560 ± 36	0.2	.46
3	3-*o*-methoxyphenoxy-1,2-propanediol	81–82	7.0	13.4	320 ± 23	1200 ± 67	52	1.69
4	3-*o*-ethoxyphenoxy-1,2-propanediol	66–67	>50	4.9	235 ± 15	730 ± 42	3.5	.99
5	3-(2,6-dimethoxyphenoxy) 1,2-propanediol	52–53	>50	10.1	370 ± 24	1200 ± 67	52	1.84
6	3-benzyloxy-1,2-propanediol	109–111 (0.1)	∞	3.6	660 ± 69	1650 ± 70	42	1.31
7	3-*p*-methylbenzyloxy-1,2-propanediol	57–58	10	4.9	360 ± 21	730 ± 40	1.1	.89
8	3-*o*-methoxybenzyloxy-1,2-propanediol	145–148 (0.3)	∞	8.1	340 ± 18	1150 ± 72	25	1.28
9	3-(β-phenylethoxy)-1,2-propanediol	156–157 (2.0)	∞	6.1	380 ± 33	830 ± 55	7	.92
10	3-(α-phenylethoxy) 1,2-propanediol	130–133 (0.5)	∞	3.8	350 ± 38	765 ± 36	11	1.03
11	2-phenoxy-1,3-propanediol	68–69	>50	36.0	760 ± 50	1860 ±123	130	1.82
12	2-*m*-toloxy-1,3-propanediol	66–67	9	4.0	365 ± 19	1050 ± 35	8	.97
13	2-*o*-methoxyphenoxy-1,3-propanediol	64–66	>50	5.3	800 ± 47	1710 ± 83	50	1.69
14	2-*o*-ethoxyphenoxy-1,3-propanediol	157–160 (2.0)	3.8	2.1	340 ± 16	1000 ± 84	1.5	.77
15	2-benzyloxy-1,3-propanediol	39–40	>50	36.0	650 ± 55	2300 ±207	110	1.88
16	1-ethoxy-3-isopropoxy-2-propanol	105–107	∞	2.5	765 ± 43	2480 ±141	1200	4.14

* PD_{50} median paralyzing dose in mice.

The *paralyzing action* was tested in white mice of the CF-1 strain weighing 18-22 g. Groups of 10-20 mice were injected intraperitoneally at dose levels increasing in geometric progression by a factor of approximately 1.5. An animal was considered paralyzed when it lost the righting reflex for at least one minute. The dose producing paralysis in one-half of the animals was taken as a measure of muscle relaxing and paralyzing activity. The mortality occurring 7 days after administration of the compounds was used in calculating the LD_{50} dose. Hemolytic activity was determined according to a modification of Glassman's technic(4). The compounds were dissolved in 0.9% sodium chloride solution containing 0.015 M phosphate buffer adjusted to pH 7.4. Freshly washed rabbit red cells were added to various dilutions of each compound and the time required to achieve 75% hemolysis as determined by visual comparison with the prepared standard was taken as the end point.

Results. Certain physical and biological properties of the compounds are summarized in Table I. The high water solubility of certain compounds was rather unexpected. Thus compound No. 4, the 3-*o*-ethoxyphenoxy-1,2-propanediol, is considerably more soluble than the methoxy analogue, while the corresponding propoxy compound (not included in the table) has a solubility of less than 0.5%. It is of interest to note that the 2-*o*-ethoxyphenoxy-1,3-propanediol (No. 14) does not share the high solubility of its isomer. The 2-*m*-tolyl-1,3-propanediol (No. 12) differs from its isomers in possessing high water solubility.

There is considerable variation in the values of the partition ratios of the various compounds and there appears to be little relationship between this factor and the paralyzing activity of the compounds. Thus mephenesin (No. 2), the most active paralyzing agent of the series had a K value which does not differ too greatly from that of compound No. 16, the least active paralyzing agent. Compound No. 4 which is almost as potent a paralysant as mephenesin has a K value more than 3 times greater than mephenesin. An even more striking instance of the lack of correlation between the value of the partition ratio and central depressant activity is obtained by comparing the two isomeric benzyl ethers (No. 6 and 15). Both these compounds possess similar paralyzing activity although one has a K value 10 times greater than the other.

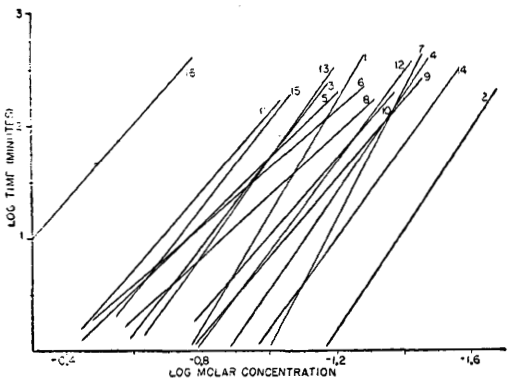

FIG. 1. Hemolytic action of certain glycerol ethers shown by plotting the logarithm of time required for 75% hemolysis against the logarithm of the molar concentration of the compounds. The curve numbers refer to the compound numbers in Table I.

The hemolytic action of the compounds is illustrated in Fig. 1 in which the logarithm of the time required for 75% hemolysis is plotted against the logarithm of the molar concentration of the compounds. The dosage response curves for the various compounds do not differ too greatly in their slopes. This may suggest a similar mechanism of hemolysis. It appears likely that the differences in hemolytic action between these compounds are quantitative rather than qualitative. Table I gives 2 parameters that can be used as a basis of comparison. One is the concentration in grams per 100 ml causing 75% hemolysis in 100 minutes. The other is the time in minutes required for a M/10 solution to produce 75% hemolysis.

The relationship between paralyzing activity and hemolytic action is of interest. Mephenesin, the strongest paralyzing agent, also has the strongest hemolytic action, while compound No. 16 which is the weakest paralysant also possesses least hemolytic action. The other compounds studied fall between these two extremes.

There appears to be a general relationship between hemolytic action and toxicity. Mephenesin exhibits greatest hemolytic action and toxicity while compound No. 16 is least

noxious in both respects. The remaining compounds show fair correspondence between toxicity and hemolytic value.

Discussion. To produce muscular relaxation during anesthesia a centrally acting relaxant such as mephenesin has certain advantages over peripherally acting relaxants because it permits relaxation without depressing respiration(5). Although mephenesin proved an effective drug for the production of muscular relaxation during anesthesia(6) the intravenous use of the drug was almost completely abandoned because of the occurrence of intravascular hemolysis. It was shown that the hemolytic action of mephenesin is independent of the total amount injected but depends on the concentration of the solution used, the presence of other hemolytic agents used as solvents and the rate of injection(7). Several of the water-soluble compounds reported in this paper have been previously described(8) but data concerning their hemolytic power have not been published. Ginzel(9) investigated the hemolytic action and pharmacological properties of *o*-methoxy, *o*-ethoxy and *o*-propoxyphenyl glycerol ethers and recommended the methoxy compound for clinical use because of its low hemolytic action. The present paper confirms the low hemolytic action of this compound. The 3-(2,6-dimethoxyphenoxy)-1,2-propanediol originally prepared by Meltzer and Doczi(10) and considered unsatisfactory by them, appears equally effective and non-hemolytic as the *o*-methoxy compound. Lambooy(11) prepared the very soluble *o*-fluoro analogue of mephenesin but did not evaluate its hemolytic action. Hine, *et al.*(12) found that 1-ethoxy-3-isopropoxy-2-propanol was very water-soluble and recommended the further study of the compound. The present paper shows that the hemolytic action of this substance is very low making the compound in this respect superior to all others tested. The very weak paralyzing action of the compound may make its clinical use impractical.

To administer mephenesin safely by the intravenous route it is desirable to use a solution not stronger than 1%. The average effective dose of mephenesin for relaxation during anesthesia is 1 g, necessitating the infusion of 100 ml of liquid. If it is assumed that the molar activity of the compounds in man is proportional to that observed in mice it would be necessary to inject 1.62 g of compound No. 3 to obtain a similar effect as observed after 1 g of mephenesin. The safe concentration of the solution would be 3.66% so that only about 44 ml of this solution would be required. To obtain equipotent effects the injection of 51.5 ml of a 2.15% solution of compound No. 4 or 52.5 ml of a 9% solution of No. 16 would be necessary. Since the detoxification mechanisms are probably taxed less after administration of the more potent compound the use of compound No. 4 would be preferable to compound No. 16.

Summary. The hemolytic action of 16 water-soluble ethers of glycerol has been examined and compared with their paralyzing and toxic action. Mephenesin was the most potent paralyzing agent and also possessed strongest hemolytic action. The 3-*o*-ethoxy-1,2-propanediol which approached mephenesin in paralyzing action, had only about one-half of its hemolytic activity. Several other water-soluble compounds which have considerably weaker hemolytic activity have been found. These compounds, however, were also much poorer paralyzing agents than mephenesin. No significant correlation between the solubility of the compounds, their water-oil partition ratios, and their paralyzing and hemolytic action was evident.

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