THE PHYSIOLOGICAL ACTIVITY OF CERTAIN ALCOHOLS AND ETHERS TO GOLDFISH

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Many alcohols and ethers that were laboratory curiosities a decade or two ago are now produced in industrial quantities and much interest has developed among investigators and others as to their possible utilization. Further, a note by Reid Hunt on the toxicity of ethylene glycol[1] has led the authors to undertake a comparison of the physiological activities of various alcohols and ethers on goldfish. Since goldfish are convenient to handle, and are relatively prompt to respond to their environment, thus giving results that are reliable and comparable, they are frequently chosen as the agencies for such measurements.[2]

EXPERIMENTAL METHODS AND RESULTS

Since ordinary tap water is non-toxic to goldfish and none of the compounds in the series that were tested react with the dissolved salts, aerated tap water was used as the solvent in this study. Water distilled from tin vessels or stored in tin vessels has been found to be toxic to goldfish. In all experiments 250 cc. of solution per fish were used and unless otherwise stated three fish were simultaneously submerged for each test. Three fish were introduced simultaneously into 750 cc. of solution as consecutive introduction may lead to uncertain results. Apparently absorption of the solute takes place so that the concentration of the solution is lowered and longer time intervals would be necessary to produce anesthesia in those fish introduced later. Evidence of this effect is supported by the data in Table I. Small fish were selected for experiment since they respond more promptly than larger fish on account of the larger body area per unit weight, and unit body area is a factor in sensitivity to environment.

The fish were maintained in water at a temperature of 31° C. at all times—a convenient temperature to maintain for the summer season.

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[1]Hunt. Ind. Eng. Chem. 24, 361 (1932).

[2]Adams, Ridla, Burnett and Dreger. J. A. C. S. 48, 1758, (1926).
Leffler and Brill. J. A. C. S. 55, 365 (1933).
Drake and Busbey. J. A. C. S. 54, 2930 (1932).

TABLE I

EFFECT OF FISH ON SOLUTION CONCENTRATION (ISOPROPIL ALCOHOL)

Concentration M/L	Fish No.		Time for Anesthesia	Time Before Removal	Condition At and After Removal
		TRIAL I			
0.35	1	Alcohol Isopropyl	3 min. 15 sec.	15 min, 15 sec.	Recovered extremely slowly.
0.35	2	" "	3 " 35 "	18 "	" " "
0.35	3	" "	3 " 50 "	20 "	Dead when removed.
0.35	4	" "	4 "	17 "	Recovered rather slowly.
		TRIAL II (Solution from Trial I)			
0.35	1	Alcohol Isopropyl	3 min. 50 sec.	18 min.	Recovered slowly.
0.35	2	" "	4 " 10	19 "	" "
0.35	3	" "	4 " 30 "	20 "	" "
0.35	4	" "	4 " 35 "	21 "	Recovered extremely slowly.
		TRIAL III (Solution from Trial II)			
0.35	1	Alcohol Isopropyl	4 min. 30 sec.	22 min.	Recovered slowly.
0.35	2	" "	4 " 40 "	24 "	" "
0.35	3	" "	5 "	26 "	Recovered very slowly.
0.35	4	" "	5 " 05 "	28 "	Dead when removed.

NOTE—Eastman Kodak Chemicals were used in most of the experiments.

In the first trial the average length of time for anesthesia to appear was 3 min. 40 sec., and 20 min. for death to take place; in the second trail the corresponding times are 4 min. 15 sec. and no fish died at 21 min. exposure; in the third trial the average time for anesthesia to take place on the four fish was 4 min. 50 sec. and death did not ensue until 28 min. of exposure. Each trial required a longer time than the preceding—rather conclusive evidence of a loss in concentration due to absorption of the solute as care was taken not to dilute the solution when new fish were introduced.

In the experiments recorded in the tables that follow all solutions were discarded after four fish had been submerged in them and new solutions made when needed.

Thus they were never subjected to abrupt changes in temperature which would make results more uncertain.

To determine the anesthetic state, a thermometer (weight 38.5 gms.) was allowed to rest on the tail of the fish. When no movement of the fish resulted from this pressure, it was concluded that anesthesia had set in.

Proof that aerated doubly distilled water as the solvent has no advantage over aerated tap water in such tests as are described is presented in Table II.

TABLE II

COMPARATIVE EFFECTS OF ETHYL ALCOHOL IN AERATED DOUBLY DISTILLED WATER AND AERATED TAP WATER ON GOLDFISH

Fish No.	Fish Size		Concentration of Sol.	Time for Anesthesia, min.	Time Before Removal, min.	Condition at and After Removal
	Length mm.	Weight gms.				
Doubly Distilled Water						
1	41	2.1	M/L 0.4	65	70	Recovered
2	40	1.9	.....	80	90	Recovered slowly
3	42	2.3	.....	140	165	Remained anesthetized. Died later
Tap Water						
1	42	2.3	0.4	57	65	Recovered slowly
2	39	1.8	.....	75	85	Recovered slowly
3	42	2.3	.....	100	175	Died later

The data in Table II do not indicate any determinable differences in doubly distilled and tap water as solvents for ethyl alcohol, and tap water has been used throughout the rest of the experiments.

Solutions were made up of such strengths that anesthesia took place in less than ten minutes. The length of time for death to result was also determined. These results are listed in Table III and those that follow.

TABLE III

EFFECTS OF MONOHYDROXY ALCOHOLS ON GOLDFISH

Alcohol	Concentration M/L	Fish No.	Fish Size		Time for Anesthesia	Time Before Removal	Condition At and After Removal
			Length mm.	Weight gms.			
Methyl	0.60	1	41	2.1	.....	120 min.	No effect.
"	2.00	1	40	1.9	4 min.	5 " 30 "	Recovered readily.
"	2.00	2	39	1.8	4 " 50 "	7 " 30 "	Died without recovery.
"	2.00	3	39	1.8	4 " 55 "	9 "	Dead when removed.
Ethyl	0.40	1	42	2.3	57 min.	65 min.	Recovered readily.
"	0.40	2	39	1.8	75 "	85 "	Recovered slowly.
"	0.40	3	42	2.3	150 "	175 "	Died without recovery.
"	1.00	1	40	1.9	3 " 10 sec.	4 " 30 sec.	Recovered slowly.
"	1.00	2	43	2.5	4 " 30 "	6 " 35 "	Died without recovery.
"	1.00	3	42	2.3	6 "	9 "	Died without recovery.
n-Propyl	0.30	1	39	1.8	40 sec.	5 min. 30 sec.	Recovered.
" "	0.30	2	38	1.6	45 "	7 "	Recovered slowly.
" "	0.30	3	39	1.8	50 "	8 "	Died when removed.
" "	0.15	1	39	1.8	4 min.	15 " 30 sec.	Recovered.
" "	0.15	2	39	1.8	4 " 10 sec.	18 " 30 "	Recovered very slowly.
" "	0.15	3	39	1.8	5 "	21 " 31 "	Dead when removed.
iso-Propyl	0.20	1	37	1.5	10 min. 30 sec.	45 min.	Recovered slowly.
" "	0.20	2	39	1.8	17 "	48 "	Died without recovery.
" "	0.20	3	37	1.5	18 "	52 "	Dead when removed.
" "	0.35	1	37	1.5	3 " 15 sec.	15 " 15 sec.	Recovered extremely slowly.
" "	0.35	2	39	1.8	3 " 35 "	18 "	Recovered extremely slowly.
" "	0.35	3	38	1.6	3 " 50 "	20 "	Dead when removed.
" "	0.35	4	38	1.6	4 "	14 "	Recovered rather slowly.

TABLE III—(Continued)

Alcohol	Concentration M/L	Fish No.	Fish Size		Time for Anesthesia	Time Before Removal	Condition At and After Removal
			Length mm.	Weight gms.			
n-Butyl	0.40	1	39	1.8	30 sec.	45 sec.	Died without recovery
" "	0.40	2	41	2.1	34 "	45 "	Died without recovery.
" "	0.40	3	41	2.1	40 "	45 "	Died without recovery.
" "	0.05	1	40	1.9	50 "	1 min. 15 "	Recovered slowly.
" "	0.05	2	40	1.9	1 min.	1 " 35 "	Recovered slowly.
" "	0.05	3	41	2.1	1 " 5 "	1 " 50 "	Died without recovery.
" "	0.040	1	38	1.6	1 min. 30 sec.	3 min. 30 sec.	Recovered.
" "	0.040	2	39	1.8	2 "	5 " 30 "	Recovered.
" "	0.040	3	40	1.9	2 " 10 sec.	7 " 30 "	Died without recovery. (Apparent anesthesia as gills stopped movement, but swimming movements continued.)
" "	0.035	1	38	1.6	9 " 35 "	15 "	Recovered.
" "	0.035	2	40	1.9	10 "	19 "	Recovered.
" "	0.035	3	41	2.1	11 "	22 "	Died without recovery.
iso-Butyl	0.01	1	38	1.6	.....	90 min.	No effect.
" "	0.08	1	42	2.3	2 min. 20 sec.	3 " 30 sec.	Recovered promptly.
" "	0.08	2	40	1.9	2 " 20 "	5 " 30 "	Recovered promptly.
" "	0.08	3	43	2.5	2 " 30 "	7 " 30 "	Recovered promptly.
" "	0.08	4	41	2.1	2 " 20 "	9 " 30 "	Recovered very slowly.
" "	0.08	5	42	2.3	2 " 30 "	11 " 30 "	Recovered very slowly.
" "	0.08	6	42	2.3	2 " 30 "	13 " 30 "	Recovered very slowly.
" "	0.08	7	43	2.5	2 " 25 "	15 " 30 "	Recovered very slowly.
" "	0.08	8	42	2.3	2 " 30 "	17 " 30 "	Died without recovery.

TABLE III—(Continued)

Alcohol	Concentration M/L	Fish No.	Fish Size		Time for Anesthesia	Time Before Removal	Condition At and After Removal
			Length mm.	Weight gms.			
sec-Butyl	0.05	1	45	2.7	16 min.	31 min.	Recovered promptly.
" "	0.05	2	37	1.5	18 " 30 sec.	38 "	Recovered slowly.
" "	0.05	3	44	2.6	19 "	45 "	Recovered slowly.
" "	0.10	1	38	1.6	2 " 35 "	6 " 30 sec.	Recovered promptly.
" "	0.10	2	37	1.6	2 " 35 "	7 " 30 "	Recovered promptly.
" "	0.10	3	38	1.6	2 " 45 "	8 " 30 "	Recovered very slowly.
" "	0.10	4	38	1.6	2 "	10 " 30 "	Recovered very slowly.
" "	0.10	5	39	1.8	2 " 50 "	18 "	Recovered very slowly.
" "	0.10	6	38	1.6	2 " 40 "	21 "	Dead when removed.
tert-Butyl	0.10	1	38	1.6	20 min.	40 min.	Recovered very slowly.
" "	0.10	2	38	1.6	23 "	50 "	Recovered very slowly.
" "	0.10	3	39	1.8	27 "	60 "	Dead when removed.
" "	0.20	1	39	1.8	4 "	11 "	Recovered slowly.
" "	0.20	2	38	1.6	4 " 15 sec.	16 "	Recovered slowly.
" "	0.20	3	40	1.9	5 " 15 "	21 "	Recovered very slowly.
" "	0.20	4	39	1.8	4 " 25 "	26 "	Dead when removed.
Benzyl	0.25	1	38	1.6	Less than 20 sec.	30 sec.	Dead when removed.
"	0.05	1	38	1.6	20 sec.	30 "	Recovered slowly.
"	0.005	1	40	1.9	7 min.	19 min.	Recovered promptly.
"	0.0075	1	40	1.9	2 " 10 sec.	10 "	Recovered promptly.
"	0.0075	2	39	1.8	2 " 15 "	20 "	Recovered promptly.
"	0.0075	3	40	1.9	2 " 30 "	45 "	Recovered less promptly.
"	0.0075	4	40	1.9	2 " 20 "	60	Recovered less promptly.
"	0.0075	5	40	1.9	2 " 25 "	75 "	Recovered less promptly.
"	0.0075	6	39	1.8	2 " 20 "	90 "	Recovered less promptly.
"	0.0075	7	39	1.8	2 " 30 "	105 "	Recovered slowly.
"	0.0075	8	39	1.8	2 " 20 "	120 "	Dead when removed.

Not much comment is needed on the results of the experiments that are recorded in Table III.

Picaud is reported by Frankel[3] as finding the following ratios of toxicities on dogs for the normal alcohols when ethyl alcohol is given a value of 1.

TABLE IV

COMPARATIVE TOXICITIES OF ALCOHOLS TO FISH

Alcohols	Picaud	Our Results
Methyl	⅔	½
Ethyl	1	1
n-Propyl	2	3
n-Butyl	3	20
iso-Propyl	.....	1½
iso-Butyl	.....	5
sec-Butyl	.....	5
tert-Butyl	.....	5
Benzyl	.....	20

These results agree quite well except those for n-butyl alcohol.

TABLE V

COMPARATIVE NARCOTIC EFFECTS OF ALCOHOLS

Alcohols	Schneegans and Mering on Dogs[4]	Our Results on Fish
Methyl	Indecisive	½ (?)
Ethyl	1	1
n-Propyl	1	6
iso-Propyl	6	4
n-Butyl	2	25
iso-Butyl	.....	12
sec-Butyl	3	10
tert-Butyl	3	5
Benzyl	.....	125

The narcotic effects for the butyl alcohols are contrary to the statement attributed to Mering by Frankel[5] who is reported as finding that primary alcohols are less active narcotics than secondary alcohols and these less active than tertiary alcohols.

These differences in results may possibly be due to a difference in methods of administration on the experimental animals used or to both.

[3]Frankel, Die Arzneimittel-Synthese, Fourth Ed. (1919). Julius Springer, Berlin p. 132.

[4]Loc. cit. p. 473.

TABLE VI

EFFECTS OF GLYCOLS AND GLYCEROL ON GOLDFISH

Compound	Concentration M/L	Fish No.	Fish Size		Time for Anesthesia	Time Before Removal, min.	Condition At and After Removal
			Lgth mm.	Wgt. gms.			
Ethylene glycol	4.0	1	37	1.5	10 min.	13 min.	Recovered slowly.
" "	4.0	2	38	1.6	11 "	15 "	Died without recovery.
" "	4.0	3	39	1.8	11 " 30 sec.	17 "	Dead when removed. (Anesthesia incomplete in all three cases.)
Propylene glycol	1.0	1	37	1.5	.....	60 min.	No effect in 60 minutes.
" "	3.0	1	38	1.6	12 "	27 "	Died 2 hours later.
" "	3.0	2	38	1.6	13 "	30 "	Died later.
" "	3.0	3	40	1.9	15 "	33 "	Died later.
" "	3.0	4	38	1.6	12 " 30 sec.	24 "	Recovered readily. (Anesthesia incomplete in all cases.)
Trimethylene glycol	1.0	1	38	1.6	6 min.	15 min.	Recovered very slowly.
"	1.0	2	39	1.8	6 "	19 "	Recovered very slowly.
"	1.0	3	38	1.6	6 " 30 sec.	23 "	Recovered very slowly.
"	1.0	4	38	1.6	6 " 40 "	27 "	Died later. (Anesthesia incomplete in all cases.)
Glycerol.....	2.0	1	40	1.9	16 min.	17 min.	Died later.
"	2.0	2	39	1.8	19 "	20 "	Recovered slowly.
"	2.0	3	40	1.9	17 "	18 "	Recovered slowly. (Anesthesia incomplete in all cases.)

A study of the data in Table VI leads to the conclusion that the glycols and glycerol are not anesthetic to goldfish and that glycol is the least toxic to goldfish of the group investigated. These effects are in accord with a statement in Fränkel. (3d) 3d) (Loc. cit. p. 452).

TABLE VII
EFFECTS OF SEVERAL ETHERS ON GOLDFISH

Compound	Concentration M/L	Fish No.	Fish Size		Time for Anesthesia	Time Before Removal	Condition At and After Removal
			Lgth mm.	Wgt. gms.			
Diethyl ether....	0.30	1	38	1.6	20 sec.	1 min.	Recovered promptly.
" "	0.30	2	41	2.1	30 "	1 " 30 sec.	Recovered promptly.
" "	0.30	3	43	2.5	30 "	2 "	Recovered slowly.
" "	0.15	1	37	1.5	40 "	1 " 20 sec.	Recovered promptly.
" "	0.15	2	41	2.1	47 "	1 " 40 "	Recovered promptly.
" "	0.15	3	41	2.1	58 "	2 "	Recovered slowly.
" "	0.05	1	38	1.6	5 "	11 " 30 sec.	Recovered promptly.
" "	0.05	2	42	2.3	5 "	16 "	Recovered promptly.
" "	0.05	3	42	2.8	6 " 15 sec.	36 "	Recovered slowly.
" "	0.05	4	42	2.3	6 " 20 "	105 "	Recovered slowly.
" "	0.05	5	41	2.1	6 " 30 "	120 "	Dead when removed.
B, B'-dihydroxy-diethyl ether	0.6	1	38	1.6	.....	25 min.	No effect.
" "	2.0	1	38	1.6	.....	25 "	No effect.
" "	3.0	1	39	1.8	8 min.	17 "	Recovered very slowly.
" "	3.0	2	41	2.1	8 "	19 "	Dead when removed.
" "	3.0	3	41	2.1	8 " 30 sec.	21 "	Dead when removed. (Anesthesia incomplete in all cases.)
Ethylene glycol monoethyl ether	0.6	1	37	1.5	1 min. 55 sec.	11 min. 30 sec.	Recovered very slowly.
" "	0.6	2	37	1.5	2 " 10 "	13 " 30 "	Dead when removed.
" "	0.6	3	37	1.5	2 " 15 "	15 " 30 "	Dead when removed. (Anesthesia complete.)
Diethyleneglycol monoethyl ether	1.0	1	38	1.6	2 min.	8 min.	Recovered slowly.
" "	1.0	2	39	1.8	2 "	9 "	Recovered slowly. (Decidedly groggy end 5½ hours.)
" "	1.0	3	40	1.9	2 " 30 sec.	10 "	Dead when removed.

The results with the ethers are readily predictable. The presence of free hydroxyl groups lowers the narcotic powers of the ethers.

Mering used dogs and employed hypodermic injection. Our results would be governed by the amount of topical absorption which is dependent on relative solubility of a particular alcohol in water and fish tissue and the activity of the alcohol after absorption. There may be a marked difference in the distribution coefficients of isomeric alcohols. This problem might warrant further study.

A similar statement is found in Percy May's "The Chemistry of Synthetic Drugs."[6]

Benzyl alcohol has an enhanced narcotic effect over ethyl alcohol in comparison with its increase of toxicity, the ratio for ethyl alcohol (narcotic effect/toxicity) being one while that for benzyl alcohol is five.

A comparative measurement of the toxicities and narcotic activities to goldfish of methyl, ethyl, propyl (iso- and normal), butyl (normal-, iso, secondary-, and tertiary-) and benzyl alcohols; of ethylene, propylene, and trimethylene glycols, and of glycerol; and of diethyl, $\beta\,\beta$-dihydroxydiethyl, ethyleneglycol-monoethyl, and diethyleneglycol-monoethyl ethers has been made. The results are not in all cases in agreement with the data recorded in the chemical literature.

CONCLUSIONS

1. The toxicity of normal-butyl alcohol has been found to be higher than that recorded in the literature. The comparative toxicities of the different butyl alcohols is in disagreement with those recorded in the literature. Likewise their narcotic activities are not in accord with the recorded activities. Introduction of hydroxyl groups lowers the narcotic activity and in some cases causes it entirely to disappear.

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[5]Loc. cit. p. 133.

[6]May, Percy and Dyson, G. M. The Chemistry of Synthetic Drugs, Fourth Ed. Rev. (1939). (Longman's Green and Company, London, pp. 23 and 56).