THE CHANGES IN THE REACTION OF GROWING ORGANISMS TO NARCOTICS. By H. M. VERNON.

(From the Physiological Laboratory, Oxford.)

It was shown by Führer[1] that the activity of the members of the methyl alcohol series in paralysing the development of sea-urchin ova increased threefold for each increment of molecular weight, or that propyl alcohol was three times more active than ethyl alcohol, butyl alcohol nine times more active, and so on. Traube[2] pointed out that the concentrations of various alcohols, esters and ketones observed by Overton[3] to produce narcosis in tadpoles likewise showed this threefold increase of activity on passing successively from lower to higher members of homologous series, and he claimed these results as a proof that the narcotics acted by virtue of their capillary activity, and not by dissolving in the cell lipoids in accordance with the Meyer-Overton hypothesis of partition coefficients. Thus he showed that aqueous solutions containing equivalents of methyl, ethyl, propyl, isobutyl and iso-amyl acetates in the proportion of 1, $\frac{1}{3}$, $\frac{1}{9}$, $\frac{1}{27}$ and $\frac{1}{81}$ all depressed the height of ascent of water in a capillary tube by equal amounts. The same law of capillary activity held also for homologous series of alcohols and fatty acids. However, the later results of Führer[4] disagree with Traube's hypothesis, for he found that the average increment of activity of successive alcohols on the fish Phoxinus is a fourfold one, and not threefold. Again, on comparing the activities of ethyl and heptyl alcohols on numerous organisms of different species, he found that other vertebrates likewise gave an average quotient of about four. Invertebrates gave quotients varying from three to four, the lowest members of the animal series, as the protozoon Noctiluca and the cœlenterate Cydippe, having the lowest quotients.

The experiments to be described below were all made upon tadpoles, and they show that the narcotising concentrations of homologous

[1] Führer. *Arch. f. exp. Path.* LII. p. 69. 1904.

[2] Traube. *Arch. f. d. ges. Physiol.* CV. p. 541. 1904; CXXIII. p. 419. 1908.

[3] Overton. *Studien über Narkose*, Jena, 1901.

[4] Führer. *Ztschr. f. Biol.* LVII. p. 465. 1912.

alcohols, esters, ketones and other substances vary very considerably in the same organism at different stages of its growth and development. The quotients, so far from being constant, varied between the extremes of 2·1 and 6·3 for alcohols, and between even wider limits for urethanes, so they entirely disagree with Traube's hypothesis of the dependence of narcotic action on capillary activity.

Method. The tadpoles used in the experiments were obtained from naturally fertilised spawn of *Rana temporaria.* Three days after hatching out, the larvæ were separated from the jelly of the ova, and were put in tanks and large glass jars with Spirogyra and other weed. The weed and water were frequently changed, and 30 to 40 days after hatching, the tadpoles were in addition given fresh frog's muscle and liver every few days. The temperature was about 12° C. for the first month of development, and 14° afterwards.

In each experiment, six tadpoles were put in a stoppered bottle containing 20 or 40 c.c. of the narcotising solution, the larger volume being used in the experiments with the 40 and 80 day tadpoles, and in all of those with very volatile narcotics as chloroform and ether. The bottles stood on a plate in a big glass jar containing water at 18° C. and the temperature was easily kept constant by the radiation from a glow lamp placed a suitable distance away. The tadpoles were not considered narcotised until every sign of movement had completely disappeared. Tadpoles less than a week old are very sluggish in their movements, so it is only after frequent observation that one is justified in assuming that absence of movement means complete narcosis.

In the earlier experiments the concentrations required to produce complete narcosis within 30 minutes were determined, as it was found that there was little or no further effect produced after longer intervals. For instance, 21 day tadpoles put in ·024 gm. mol. butyl alcohol moved 4 min.; put in ·022 M alcohol moved 12 min., whilst in ·020 M alcohol they moved for six or more hours. However, the reaction to methyl and ethyl alcohols is not so sharp as this, whilst the reaction of 40 and 80 day tadpoles is less sharp for all the alcohols, so the time of narcotisation has to be varied in accordance with the size of the tadpoles. In Fig. 1 are shown some of the results obtained by narcotising tadpoles of different ages with ethyl alcohol. Ordinates indicate the concentration of alcohol in gram molecular weights, and abscissæ the time required to produce complete narcosis. Judging from the form of the curve, the newly hatched (·5 day) tadpoles must have practically reached narcotisation equilibrium in a little over 20 minutes, whilst the two day tadpoles must have reached it in 30 minutes. The 21 day tadpoles must have taken about 60 minutes, the 42 day tadpoles about 80 minutes, and the 83-day tadpoles about 100 minutes. No sufficiently complete series of observations were made on tadpoles of more than two and less than 21 days' growth, but probably equilibrium would nearly be reached in about 30 minutes by tadpoles of less than a week's growth, whilst those of a fortnight might need 45 minutes. The necessity for prolonging the narcotisation time was not realised at first, so all the observations described below on tadpoles 20 days or less in age were made for a 30 minute period. Still the error introduced thereby is only small, and for many narcotics non-existent. Thus different narcotics exert their action with very different degrees of rapidity. This is well shown by the curves in Figs. 2 and 3, obtained with 23 day and 83 day tadpoles respectively. In every case the

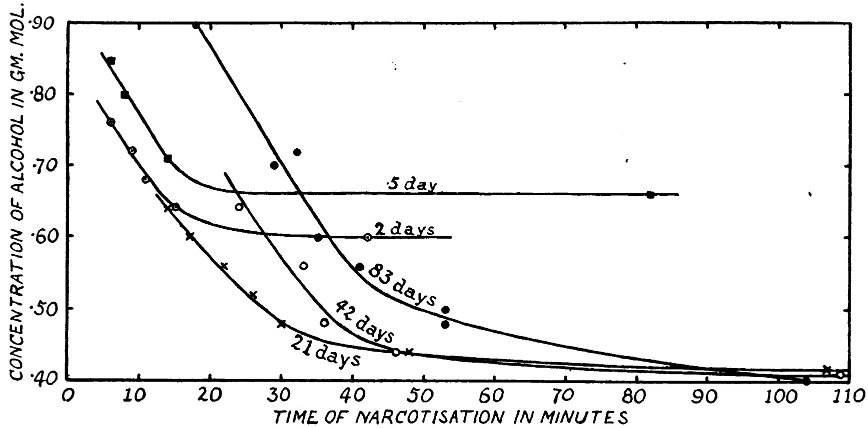

Fig. 1.

concentration of alcohol required to produce narcotisation in 180 minutes is taken as 100, and the concentrations narcotising in shorter times are calculated as percentages on it. We see that in order to narcotise 23 day tadpoles in 60 minutes, the concentration of methyl alcohol required is 12 % greater than that narcotising in 180 minutes, whilst for butyl alcohol it is probably only about 2 % greater. The 83 day tadpoles responded less sharply to ethyl alcohol than to methyl alcohol, so that in order to narcotise in 30 minutes the ethyl alcohol had to be over 80 % more concentrated than that narcotising in 180 minutes, whilst to narcotise in 60 minutes it had to be 22 % more concentrated. Of course these values are only rough approximations, as the narcotisation times were found to vary a good deal with different batches of tadpoles. Another series of observations, made on some 46 day tadpoles, gave a sharper reaction with ethyl alcohol than with methyl, whilst another series, made on some 39 day tadpoles, gave the reverse relationship. Probably, therefore, the tadpoles react to about the same extent to both these alcohols, and certainly they react much

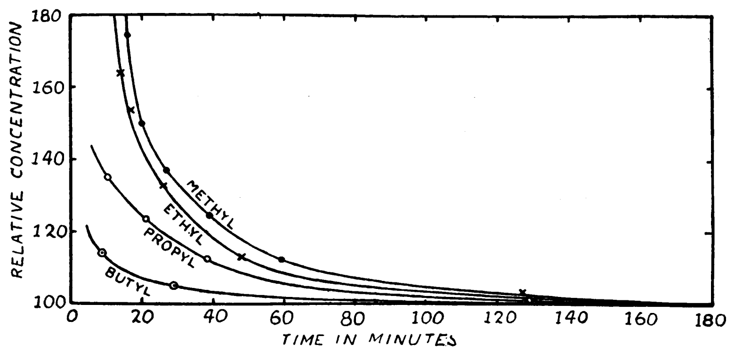

Fig. 2.

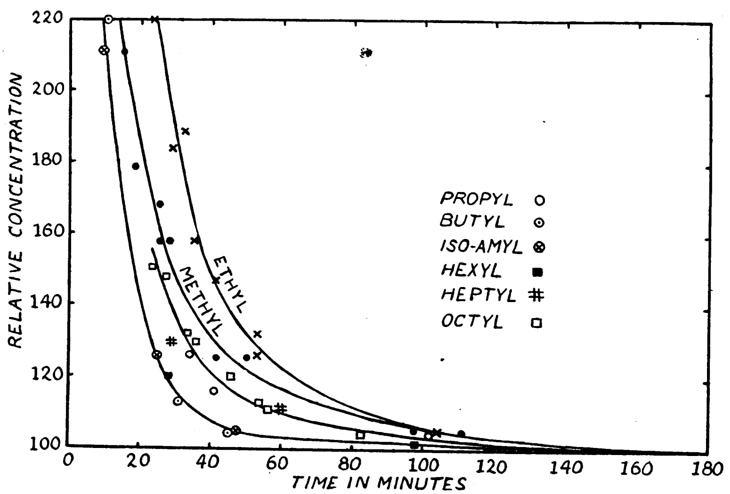

Fig. 3.

less sharply to them than to the higher alcohols. As can be seen from the experimental values given in the figure, propyl, heptyl and octyl alcohols acted in a more or less similar manner on the tadpoles, so a single curve is drawn for all these three alcohols. Butyl, iso-amyl and hexyl alcohols likewise acted similarly, and so are represented by a single curve. From this curve we see that in order to narcotise the tadpoles in 60 minutes, the concentration of these alcohols required was only about 3 % greater than that narcotising in 180 minutes. The cause of these differences in the rapidity of narcotisation effected by the various alcohols is unknown. Probably they are to some extent dependent on the velocity of osmosis of the alcohols into the cells, which as Traube[1] has deduced from Overton's[2] experiments on plasmolysis, is increasingly greater on passing from the lower to the higher alcohols. Still it is difficult to understand why heptyl and octyl alcohols should narcotise more slowly than butyl, iso-amyl and hexyl alcohols. Perhaps it depends on the fact that the narcotising solutions of these alcohols are so extremely dilute (*e.g.* *a* 1 in 55,000 solution of octyl alcohol).

The samples of monohydric alcohols and of most of the other narcotics employed in the experiments were obtained from Schuchardt.

Monohydric Alcohols.

The action of eight of the monohydric alcohols was investigated. Normal amyl alcohol is so insoluble, and so difficult to obtain in a pure state, that the iso body was used instead. Fühner found that the concentration of n-amyl alcohol required to narcotise Phoxinus was 73 % that of iso-amyl alcohol, but I find that my results with the iso body fall into best agreement with the narcotising concentrations of butyl alcohol on the one hand and of hexyl alcohol on the other if it is assumed that the narcotising concentration of n-amyl alcohol is only 50 % that of the iso alcohol. Hence the "amyl" values given in the table are really iso-amyl values which have been halved. Hexyl, heptyl and octyl alcohols are almost insoluble in pure water, so I adopted Fühner's plan of adding some ethyl alcohol to increase their solubility. The hexyl and heptyl alcohols were mixed with nine volumes of ethyl alcohol before dilution, and the octyl alcohol with 19 volumes. A correction had to be made for this added ethyl alcohol, but it is only a very small one.

From the table it will be seen that complete sets of determinations were made with tadpoles of seven different ages, ranging from ·5 day to 83 days. As the newly hatched tadpoles are especially sluggish, two sets of experiments were made with different batches, and it will be seen that the results obtained are in fair agreement. With this exception, the whole of the experiments were made upon tadpoles of

[1] Traube, *l. c.* [2] Overton. *Ztschr. f. physik. Chem.* XXII. p. 189. 1897.

Alcohol	Time of narcotisation— 30 minutes: ·5 day, Narcotising concentration		Q.	2·5 days, Narc. con.	Q.	6 days, Narc. con.	Q.	12 days, Narc. con.	Q.	60 minutes: 22 days, Narc. con.	Q.	80 minutes: 40 days, Narc. con.	Q.	100 minutes: 83 days, Narc. con.	Q.	Mean Q.
Methyl	1·80 1·78	1·78		1·60		1·55		1·37		·98		·99		1·00		
			2·5		2·6		2·8		2·6		2·1		2·4		2·4	2·5
Ethyl	·72 ·67	·70		·61		·55		·53		·46		·41		·41		
			6·2		5·8		5·2		5·0		4·6		3·9		4·1	5·0
Propyl	·12 ·105	·113		·105		·105		·105		·100		·104		·101		
			6·3		5·5		6·2		4·9		4·7		4·7		4·4	5·2
Butyl	·0175 ·0185	·018		·019		·017		·0215		·0215		·0223		·023		
			5·6		5·8		5·2		5·2		5·1		5·3		4·8	5·3
(Amyl)	·0027 ·0036	·0032		·0033		·0033		·0041		·0042		·0042		·0048		
			4·0		4·9		3·5		4·2		4·4		4·7		4·2	4·3
Hexyl	·00077 ·00083	·00080		·00067		·00093		·00098		·00095		·00090		·00115		
			4·4		3·4		3·7		3·6		3·4		2·6		4·0	3·6
Heptyl	·000175 ·00019	·000183		·00020		·00025		·00027		·00028		·00035		·000285		
			3·0		2·7		2·8		2·5		2·6		2·8		2·5	2·7
Octyl	·000065 ·000058	·000062		·000075		·00009		·000107		·000107		·000127		·000115		
Size of tadpoles—		6·0 mm.		8·5 mm.		11·2 mm.		13·7 mm.		14·7 mm.		16·0 mm.		28·0 mm.		

the same original source. The tadpoles used for the first 22 days' experiments were all thrown away after narcotisation, but then, as the stock began to get rather low, they were kept and used again 18 days later, and still again some 43 days later still. The 83 day tadpoles had dwindled so in numbers that only three tadpoles instead of six were used in each narcotisation experiment, but they were so large and easy to observe that probably no appreciable error was introduced thereby. Many of them had developed small hind limbs. A sample of 20 tadpoles was measured on each occasion, and the average lengths are given in the table.

The data in the table show that newly hatched tadpoles were narcotised by 1·78 gm. mol. of methyl alcohol (a 7·15 % solution by volume), or nearly twice as great a concentration as that required by tadpoles 22 or more days old. Tadpoles of intermediate age required intermediate amounts. The reaction to ethyl alcohol changed similarly during growth, but propyl alcohol exerted practically the same narcotic action on tadpoles of all ages. With butyl alcohol we find the beginning of the changed reaction, for we see that the older tadpoles needed a distinctly more concentrated alcohol for narcotisation than the younger ones. With amyl (iso-amyl) alcohol this changed reaction is quite well marked. The hexyl alcohol values, though rather irregular, show a similar increase of narcotising concentration with growth of the tadpoles, whilst heptyl and octyl alcohols show a greater increase still. If a mean be taken of the values obtained with 40 and 83 day tadpoles on the one hand, and of the ·5 day and 2·5 day tadpoles on the other, the ratio between the means works out as follows:

Alcohol	$\frac{40+83 \text{ day}}{\cdot 5+2\cdot 5 \text{ day}}$	Alcohol	$\frac{40+83 \text{ day}}{\cdot 5+2\cdot 5 \text{ day}}$
Methyl	·59	Amyl	1·38
Ethyl	·63	Hexyl	1·40
Propyl	·94	Heptyl	1·66
Butyl	1·22	Octyl	1·77

We see that there is a perfectly regular change in the ratio of the mean narcotising values as we pass from the lowest to the highest alcohol investigated. The extreme ratios vary in the proportion of one to three, or roughly speaking one may say that newly hatched tadpoles need nearly twice as much methyl alcohol for narcotisation as fully grown tadpoles, but only about half as much octyl alcohol.

The difference in the action of the alcohols on the young and old tadpoles is even more strikingly exhibited if the narcotising concentrations of methyl and octyl alcohols be compared. The methyl alcohol value for ·5 day tadpoles is 28700 times greater than the octyl alcohol value, and for 2·5 day tadpoles it is 21300 times greater. On the other hand for 40 day tadpoles it is only 7800 times greater, and for 83 day tadpoles, 8700 times greater. This means that the average of the quotients between the individual alcohol values must differ considerably. It works out at 4·6 for the ·5 day tadpoles and 4·4 for the 2·5 day, but sinks to 3·8 for the 40 and 83 day tadpoles. Führer obtained a mean quotient of 4·0 for small frogs (*R. esc.*) narcotised by ethyl and heptyl alcohols, whilst my experiments with 40 and 83 day tadpoles give, for the same pair of alcohols, mean quotients of 4·2 and 4·3 respectively.

As regards individual quotients, we see no sign of the constancy required by Traube's hypothesis. The quotient between methyl and ethyl alcohols was almost always the smallest; that between ethyl and propyl alcohols was about twice as large; those between propyl and butyl and between butyl and amyl alcohols slightly larger still, and then the quotients began to diminish again, till that between heptyl and octyl alcohols was not much bigger than that between methyl and ethyl alcohols. From the mean quotients given at the extreme right of the table, we see that there was a regular waxing and waning of the quotients on passing from methyl to octyl alcohol. No great stress can be laid on the exact numerical values of these quotients, as one cannot be certain of the purity of the alcohols employed, but at least they leave no room for doubt that there is great variation. Führer observed somewhat similar though less marked variations of quotient in his experiments with Phoxinus. Thus the quotients between the methyl, ethyl, butyl and amyl alcohol values worked out at 2·4, 3·7, 3·9 and 3·8 respectively, and between heptyl and octyl alcohols, at 3·3. Even the laking values for erythrocytes showed a variation of the same type, though smaller in range.

My data for monohydric alcohols differ considerably from those obtained by Overton, though our results with most other narcotics show fair agreement. He worked with *R. temp.* tadpoles 8—15 mm. in length, and he gives the narcotising values for methyl, ethyl, propyl and butyl alcohols as ·57, ·29, ·11 and ·038 M respectively. These values yield quotients of 2·0, 2·6 and 3·0, or disagree with Führer's results almost as much as with mine.

Esters.

In their reaction to acid esters tadpoles behave as they do with propyl alcohol, and show little or no change of response during growth. Experiments were made with four ethyl esters, upon tadpoles of three different ages. The ·1 day tadpoles were just hatching out at the time of experiment, and had to be stirred vigorously to separate them from the jelly of the ova. The values for ethyl acetate and propionate are in fair agreement with Overton's, but my values for the butyrate are smaller than his, and for the valerianate, considerably smaller. Consequently my quotients are greater than his. They average 3·4, 3·6 and 3·0 for the ·1 day, 3 day and 19 day tadpoles, whilst his quotients average 2·5.

	·1 day		3 days		19 days		Overton's values	
Ester	Narc. con.	Q.	Narc. con.	Q.	Narc. con.	Q.	Narc. con.	Q.
Ethyl acetate	·037		·039		·030		·03	
		4·9		4·9		3·9		2·9
Ethyl propionate	·0075		·008		·0077		·0105	
		2·8		3·3		2·8		2·4
Ethyl butyrate	·0027		·0024		·0028		·0043	
		2·6		2·7		2·2		2·3
Ethyl valerianate	·00103		·0009		·0013		·0019	
Size of tadpoles—	5·0 mm.		9·1 mm.		14·5 mm.			

The tadpoles probably do not behave with urethanes in the same way as they do with acid esters. They seem to show a distinct indication of the changing response observed with alcohols. Thus from the data in the table we see that though the concentration of methyl urethane acting on ·5, 6 and 20 day tadpoles was nearly constant, that of ethyl urethane was distinctly greater on the ·5 day tadpoles than on the 6 and 20 day ones. A repetition of the experiment on another batch of ·5 day tadpoles gave a value of ·047 M, instead of the ·048 M value quoted. The narcotising concentrations of propyl and iso-butyl urethanes are probably constant throughout, but that of iso-amyl urethane increases by 70 % on passing from the ·5 day tadpoles to the 20 day ones.

	·5 day		6 days		20 days		Overton's values	
Ester	Narc. con.	Q.	Narc. con.	Q.	Narc. con.	Q.	Narc. con.	Q.
Methyl urethane	·29		·26		·30		·27	
		6·0		7·4		8·6		6·6
Ethyl urethane	·048		·035		·035		·041	
		8·0		7·4		5·3		—
Propyl urethane	·006		·0047		·0066		—	
		(2·0)		(1·5)		(2·1)		—
Iso-butyl urethane	·0030		·0032		·0032		—	
		4·3		3·2		2·7		—
Iso-amyl urethane	·0007		·0010		·00118		—	
Size of tadpoles—	5·4 mm.		11·0 mm.		14·5 mm.			

The quotients between the urethane values are for the most part considerably greater than those observed with other homologous series of narcotics. Between methyl and ethyl urethanes the quotient averages 7·3, or is similar to Overton's value, and between ethyl and propyl urethanes it averages 6·9. The quotient between propyl and iso-butyl urethanes averages only 1·9, which suggests that the narcotising concentration of butyl urethane is if anything less than half that of the iso-butyl urethane. The quotient between iso-butyl and iso-amyl urethanes averages 3·4, or about the normal.

Ketones and other Narcotics.

The reaction of tadpoles to ketones differs from that observed with either alcohols or esters, for all three ketones investigated narcotised tadpoles of increasing size in steadily diminishing concentrations. The diminution was not great, amounting to 15 % for acetone, 22 % for methyl-ethyl ketone, and 32 % for methyl-propyl ketone, on passing from ·5 day to 18 day tadpoles, but the results are so regular that they are probably genuine. The narcotising values obtained with 18 day tadpoles correspond closely with Overton's.

	·5 day		7 days		18 days		Overton's values	
	Narc. con.	Q.	Narc. con.	Q.	Narc. con.	Q.	Narc. con.	Q.
Acetone	·33		·32		·28		·26	
		3·1		3·4		3·4		2·9
Methyl-ethyl ketone	·105		·095		·082		·09	
		3·4		3·5		3·9		4·7
Methyl-propyl ketone	·031		·027		·021		·019	
Size of tadpoles—	5·5 mm.		11·4 mm.		14·6 mm.			

Of the other narcotics investigated chloroform shows the most remarkable changes of all, for its narcotising concentration with ·5 day tadpoles is only a third as great as that with 17 and 78 day tadpoles. A repetition of the experiment with another batch of ·5 day tadpoles gave exactly the same narcotising value as that quoted. Chloral hydrate also acts in smaller concentrations on young tadpoles than on older ones, but the data quoted are to be accepted with reserve, as they merely indicate the concentrations required to produce narcosis in 30 minutes. Overton has shown that chloral hydrate is a very slowly acting narcotic, for he found that 16 mm. tadpoles, which are narcotised in about four hours by ·006 M chloral hydrate, require 1–2 hours to be narcotised by a ·015 M solution.

	·5 day	6 days	17 days	78 days (100 mins.)	Overton's values
Chloroform	·00045	·00075	·0013	·00135	·0012
Chloral hydrate	·019	·029	·034	—	(·015)
Paraldehyde	·033	·028	·018	·022	·025
Nitromethane	·15	·14	·112	·113	·082
Ethyl ether	·045	·045	·032	—	·07
Size of tadpoles—	6·6 mm.	11·6 mm.	14·7 mm.	26·0 mm.	

The other three narcotics investigated all act on tadpoles of different ages in the reverse manner to chloroform and chloral hydrate. The concentration of paraldehyde narcotising ·5 day tadpoles is 83 % greater than that narcotising 17 day tadpoles, whilst the concentration of ethyl ether is 41 % greater, and that of nitromethane 34 % greater. From 17 days onwards to 78 days there is probably little or no change in the reaction of the tadpoles to the three narcotics investigated. The time of narcotisation of the 78 day tadpoles was taken as 100 minutes, as in the corresponding alcohol experiments.

The Fatal Concentrations of Monohydric Alcohols.

In addition to the investigations of the narcotising concentrations of the monohydric alcohols, three sets of determinations were made of their fatal concentrations. After keeping the tadpoles for half an hour in the narcotising solutions, they were transferred to fresh water, and the proportion of them showing any recovery of spontaneous movement within the next few hours was noted. They sometimes took an hour to recover, but seldom more than two hours. The concentration of each alcohol killing 50 % of the tadpoles (or three out of the six employed in each experiment) was determined as nearly as possible. For instance, of six 14 day tadpoles kept half an hour in 1·44 M ethyl alcohol, all recovered: of six in 1·52 M alcohol five recovered, and of six in 1·60 M alcohol, only one recovered, so the fatal concentration was taken to be 1·56 M.

On comparing the fatal concentrations of two day tadpoles with the narcotising concentrations of 2·5 day tadpoles, it is found that the relationships between the two are extraordinarily variable. The fatal concentration of methyl alcohol is only slightly greater than the narcotising concentration: that of the ethyl alcohol is nearly twice as great: that of propyl alcohol four times as great, and so on to the higher alcohols with steady increase of ratio, till finally it appears that the fatal concentration of octyl alcohol is no less than 55 times its narcotising concentration. The next series of experiments was made with

14 and 16 day tadpoles, and on comparing these values with the narcotising concentration of 12 day tadpoles, we find that the range of variation in the ratios is considerably less than before. The fatal concentration of methyl alcohol is now more than twice as great as the narcotising concentration, whilst that of octyl alcohol is only about 23 times as great. This alteration of ratio is chiefly dependent on the great changes in the fatal concentrations of the alcohols. The 15 day tadpoles could stand nearly twice as great a concentration of methyl alcohol as the two day tadpoles, but only a little more than half as great a concentration of heptyl and octyl alcohols.

	Duration of exp. 30 minutes					
Alcohol	2 days Fatal	2·5 days Narcot.	*F/N*	15 days Fatal	12 days Narcot.	*F/N*
Methyl	1·75	1·60	1·1	3·0	1·37	2·2
Ethyl	1·14	·61	1·9	1·56	·53	2·9
Propyl	·43	·105	4·1	·47	·105	4·5
Butyl	·172	·019	9·1	·160	·0215	7·4
Iso-amyl	·09	·0066	13·6	·057	·0082	7·0
Hexyl	·023	·00067	34·3	·014	·00098	·14·3
Heptyl	·0083	·00020	41·5	·0045	·00027	16·7
Octyl	·0041	·000075	54·7	·0025	·000107	23·4
Size of tadpoles	8·1 mm.	8·5 mm.		14·9 mm.	13·7 mm.	

	Duration of exp. 60 minutes			100 minutes		
Alcohol	78 days Fatal	83 days Narcot.	*F/N*	78 days Fatal	83 days Narcot.	*F/N*
Methyl	1·58	1·11	1·4	1·30	1·00	1·3
Ethyl	·90	·47	1·9	·60	·41	1·5
Propyl	·35	·106	3·3	·23	·101	2·3
Butyl	·09	·0235	3·8	·07	·023	3·0
Iso-amyl	·04	·0099	4·0	·03	·0096	3·1
Hexyl	·0062	·00126	4·9	·0049	·00115	4·3
Heptyl	·00177	·00032	5·5	·00146	·000285	5·1
Octyl	·00080	·000123	6·5	·00046	·000115	4·0
Size of tadpoles	25·0 mm.	28·0 mm.		25·0 mm.	28·0 mm.	

The third set of determinations was made on tadpoles of mixed origin, and varying in age from 68 to 88 days. In each experiment eight tadpoles were put in the narcotising solution, and four of them were transferred to fresh water after 60 minutes, and the remaining four after 100 minutes. As can be seen from the table, the differences in the fatal concentrations for these two periods of exposure are considerable, the 100 minute values being only about three-fourths as great as the 60 minute values. Doubtless the fatal concentrations after a 30 minute interval would have been considerably greater, probably quite 50 % greater, than the 60 minute values. They would then, in the case of the lower alcohols, have approximated to the concentrations observed with 15 day tadpoles. However, no such determinations were made, as it would have been misleading to compare them against narcotisation values which had not nearly reached equilibrium. There is not much difference between the narcotising values of the 83 day tadpoles after 60 minutes and after 100 minutes, so probably the 60 minutes data afford the best basis of comparison. The actual ratio between the fatal and the narcotising concentrations of methyl alcohol is lower than that observed with 15 day tadpoles, but this is clearly due to the long period for which the tadpoles were subjected to the alcohol. The range of variation in the ratios obtained with methyl alcohol on the one hand and octyl alcohol on the other is in the proportion of 1 to 4·6, whereas it was as 1 to 10·6 for 15 day tadpoles, and as 1 to 50 for two day tadpoles. This marked alteration is chiefly due to the greatly increased sensitiveness of the tadpoles to the higher alcohols. Thus the fatal concentration of octyl alcohol is less than a third of that for 15 day tadpoles, and a fifth of that for two day tadpoles.

These results suggest that with still older organisms the ratios between killing and narcotising concentrations would become more and more similar, and might finally approximate. This is what one would naturally expect, and what indeed has actually been found to hold to some extent for rabbits. Baer[1] introduced various doses of methyl, ethyl, propyl, butyl, and iso-amyl alcohols into the stomach by means of a sound, and he found that the concentrations of all the alcohols producing a narcosis which lasted days and which was frequently fatal, were on an average nearly double the corresponding concentrations producing mild narcosis.

It should be mentioned that the fatal concentrations of octyl alcohol quoted are only approximations. In order to keep the octyl alcohol in solution, it had to be diluted 100-fold with ethyl alcohol, and so the corrections for this added alcohol are considerable. The heptyl alcohol, however, needed only a 20-fold dilution with ethyl alcohol.

The Cause of the Changing Reaction of Growing Organisms.

There can be little doubt that one of the chief causes of the changes in the reaction of the growing tadpoles to narcotics is the changing composition of the cell lipoids. Their chemical composition undergoes very marked alterations during growth, and probably the relative

[1] Baer. *Arch. f. Physiol.* p. 283. 1898.

solubility of different narcotics in them changes likewise. Raske[1] found that the dried brain of ox embryos contained considerably less lecithin and cholesterin than adult ox brain, and it contained no cerebrin at all. Koch and Mann[2] analysed the brain of children aged six weeks and two years, and of an adult aged 19, and found a considerable and steady increase of the cholesterin and cerebrin with growth. L. Smith and Mair[3] found that whilst the brain of infants contained 0–4 % of cerebroside, that of adults contained 18–21 %. Subsequently they[4] analysed the brain of puppies aged three days, and three, six and twelve weeks, and found that the cerebroside steadily increased with growth from 1·5 % up to 11·5 %, whilst in the brain of a full grown dog it amounted to 21·4 %. Hence all investigators agree in finding a rapid increase in the cerebrin during the growth of mammals, and there can be little doubt that there are similar changes of composition in the lipoids of Amphibia and other vertebrates. No investigations of the relative solubility of narcotics in brain lipoids appear to have been made, but Loewe[5] found that a chloroform solution of kephalin took up considerably more methylene blue from an aqueous solution with which it was mixed than did a solution of cerebroside, whilst cerebroside took up very much more than cholesterin.

Though the changes in the composition of the cell lipoids during growth may be almost entirely responsible for the changes in the narcotising concentrations, it is difficult to believe that they are likewise chiefly responsible for the very great changes in the fatal concentrations of the alcohols observed with tadpoles of different ages. Some other factor must be concerned, which is of relatively more importance with the lower alcohols than with the higher ones. It may be pointed out that the concentration of octyl alcohol which killed two day tadpoles is identically the same as that found by Fühner and Neubauer[6] to lake red blood corpuscles (*i.e.* to rupture their lipoid "plasmahaut"). The fatal concentration of heptyl alcohol, however, is only two-thirds the laking concentration; that of butyl alcohol is about a half; that of ethyl alcohol a third, and that of methyl alcohol, a fourth.

[1] Raske. *Ztschr. f. physiol. Chem.* x. p. 336. 1886.
[2] Koch and Mann. This *Journal,* XXXVI. p. xxxvi. 1907.
[3] L. Smith and Mair. *Journ. Path.* XVI. p. 131. 1911.
[4] L. Smith and Mair. *Ibid.* XVII. p. 123. 1912.
[5] Loewe. *Biochem. Ztschr.* XLII. p. 150. 1912.
[6] Fühner and Neubauer. *Zntrlb. f. Physiol.* xx. p. 117. 1906.

SUMMARY.

Tadpoles of different ages are by no means equally affected by narcotics, especially by the monohydric alcohols. They were tested ·5, 2·5, 6, 12, 40 and 83 days after hatching, and it was found that whilst the narcotising concentration of methyl alcohol steadily dwindled from 1·78 M to ·99 M, and that of ethyl alcohol from ·70 M to ·41 M, that of propyl alcohol remained almost constant throughout. On the other hand that of butyl alcohol increased 22 % on passing from the young to the old tadpoles; that of iso-amyl alcohol increased 38 %; of hexyl alcohol, 40 %; of heptyl, 66 %, and of octyl, 77 %. Hence the ratio between the narcotising concentrations of methyl and octyl alcohols ranged from 28700 to 1 for ·5 day tadpoles down to 8200 to 1 for 40 and 83 day tadpoles.

The quotients between the narcotising concentrations of successive alcohols were by no means constant. They averaged 2·5 between methyl and ethyl alcohols, but rose to 5·0 between ethyl and propyl alcohols. The quotients between the remaining pairs of alcohols averaged 5·2, 5·3, 4·3, 3·6 and 2·7.

The narcotising concentrations of ethyl acetate, propionate, butyrate and valerianate did not vary with the age of the tadpoles, but those of the urethanes varied to some extent like those of the alcohols.

The narcotising concentrations of the three ketones investigated diminished steadily during the growth of the tadpoles. Those of paraldehyde, nitromethane and ethyl ether likewise diminished considerably, but that of chloroform increased threefold on passing from ·5 day to 17 day tadpoles.

The fatal concentrations of the monohydric alcohols (*i.e.* those killing 50 % of the tadpoles in 30 or 60 minutes) vary very greatly with tadpoles of different ages. For two day tadpoles the fatal concentration of methyl alcohol was only 1·1 times the narcotising concentration, but on passing to the higher alcohols it steadily became greater and greater, till the fatal concentration of octyl alcohol was no less than 55 times the narcotising concentration. With 80 day tadpoles, on the other hand, the fatal concentration of octyl alcohol was only 6·5 times the narcotising concentration, whilst that of methyl alcohol was 1·4 times the narcotising concentration.

The great changes in the reaction of growing organisms to narcotics are probably dependent, for the most part, on changes in the composition of the cell lipoids.