Brit. J. Pharmacol. (1960), 15, 185.

UNSPECIFIC DRUG ACTION. THE EFFECTS OF A HOMOLOGOUS SERIES OF PRIMARY ALCOHOLS

BY

H. P. RANG

From the Department of Pharmacology, University College, London

(RECEIVED NOVEMBER 26, 1959)

Experimental results relating to the unspecific depressant action of normal primary alcohols from methanol to octanol on four separate biological systems are presented. It was found that the log-concentration action curves of alcohols on all four systems were straight over most of their range and, for any one system, parallel throughout the series. With arithmetic increase in the alcohol chainlength the concentration required to produce a given effect diminished logarithmically. The rate of this decrease varied in different biological systems, and was always less than the rate of decrease of solubility with chainlength. In two of the systems investigated alcohols beyond octanol failed to show any activity (cut-off phenomenon). The implications of these findings are discussed, with reference to the mechanism of action of unspecific depressants. Ferguson's principle of using thermodynamic activity instead of concentration as an index of activity was applied to the present results. In an appendix, the results are compared with predictions according to Mullins' hypothesis of narcotic action, and found not to agree well.

A wide range of simple organic compounds can exert qualitatively identical depressant actions on many different organisms. The striking absence of chemical specificity in the compounds tested led to the theory that physical rather than chemical properties govern the activity of the compounds. This approach resulted in the formulation of two theories of narcotic action, namely the Overton-Meyer theory and the Traube theory.

Meyer, in 1899, proposed that narcosis results from the drug dissolving in a fatty phase within the cell. This theory predicts that the narcotic potency of a drug will depend on its fat/water partition coefficient, and also that lipoid-rich cells will be most readily affected. Overton (1901) published data showing a striking correlation between narcotic potency (in tadpoles) and olive-oil/water partition coefficient for a wide range of compounds. In 1935 K. H. Meyer and Hemmi found a correlation between narcotic potency of vapours in mice and the oleic-acid/water partition coefficient of the compound. This was held to support the Overton-Meyer theory. Traube (1904) correlated narcotic potency with the activity of lowering surface tension at an air/water interface. Meyer and Hemmi (1935) found that this correlation does not hold with vapours. On the other hand, Warburg (1921) considered that Traube's air/water interface was a rather unlikely model of any cell component, and measured the adsorption of narcotics at a charcoal/water interface, and found a good correlation with narcotic activity. He postulated inhibition of an enzyme by adsorption of narcotic at some cellular interface as a mechanism of narcosis.

Concurrently with these investigations of narcosis, investigations of the bactericidal activity of organic compounds were carried out, mainly by Tilley and Schaffer (1926, 1928), who investigated the relationship between bactericidal potency and chainlength in homologous series. They found that with an arithmetic increase in chainlength, the equitoxic concentration fell logarithmically up to a certain chainlength (the cut-off point) at which it rose rapidly and very soon reached the solubility limit. The logarithmic fall in equitoxic concentration ran approximately parallel to the fall in solubility with chainlength, but was always slightly less steep. Tilley and Schaffer did not seek to correlate potency with any particular physical property.

Also at this time, new insecticides were being sought. Holt (1916) and Moore (1917) related insecticidal potency with boiling point, and Moore put forward the generalization that potency increases with boiling point up to a certain boiling point, above which compounds cease to be toxic. Boiling point is a way of expressing the distribution of a compound between its liquid and vapour phases.

Ferguson (1939) suggested that the use of distribution coefficients be avoided by measuring potency in terms of the thermodynamic activity of the depressant agent in the solution, instead of its concentration. Since the thermodynamic activity of a substance is by definition equal in all phases of a system at equilibrium, the problem of distribution coefficients between numerous different phases within a cell, any of which might be the biophase in which the drug exerts its pharmacological effects, is by-passed. Using this new approach, Ferguson recalculated the data of earlier workers, and also measured the insecticidal potency of many different vapours. A number of generalizations emerged. For nearly all the compounds tested the thermodynamic activity required to produce a narcotic or toxic effect was 0.1 or more. In a few striking exceptions the activity required was much lower (e.g., ammonia, hydrocyanic acid), and the pharmacological action was also quite different. Hence these compounds may be excluded from the wide group of unspecific depressants. Ferguson also found that within an homologous series the thermodynamic activity required to produce a given effect increases steadily with chainlength until it reaches unity (the activity of a saturated solution). Beyond this point the effective thermodynamic activity is unattainable and the cut-off noticed by earlier workers is thereby explained without having to postulate a sudden change of properties at a particular chainlength.

On the other hand Brink and Posternak (1948), investigating depression of conduction in isolated nerve preparations by homologous series of narcotics, found that the equipotent thermodynamic activity showed no progressive rise throughout a homologous series, but rose sharply to unity at a certain chainlength.

Mullins (1954) devised a more direct approach to the problem. He started with the hypothesis that narcotics act by dissolving in some particular phase of the cell (probably the membrane), and that equal narcotic effects are produced when an equal volume fraction of this phase is occupied by narcotic molecules. By making a number of assumptions and approximations concerning the physico-chemical properties of the biophase and its interaction with the narcotic, Mullins was able to predict equipotent thermodynamic activities within a homologous series, and he presents experimental results which show reasonable agreement with these predictions.

The present work was undertaken with the following aims in view.

1. To gain comparative data on the action of a single homologous series (normal primary alcohols) on several different biological systems. This is the converse of the approach usually adopted in the past, and it was interesting to see whether the slight differences between homologous series patterns were also evident when the same series was used on different systems.

2. To throw more light on the mechanism of cut-off, as argued by Ferguson on the one hand and Brink and Posternak on the other.

3. To try to determine the level of organization at which alcohols exert their action, by investigating a sequence of phenomena ranging from mobility of whole organisms to oxygen consumption of isolated tissues.

4. To provide further material on which to test Mullins' hypothesis.

Methods

Paramecium Immobilization

The concentration of different alcohols required to produce the same degree of immobilization in a population of paramecia was measured.

0.2 ml. of a suspension of paramecia in water (containing 100 to 200 organisms) was placed in a small flat perspex cup. Six such cups fitted into the revolving stage of a low-power binocular microscope, so that each could be swung rapidly into the field of view. At a noted time a volume of alcohol solution (0.05 to 0.2 ml.) calculated to give a suitable final concentration was added from a syringe. At known intervals after addition of alcohol, the cups were examined in turn, and counts of the number of immobile organisms (which sink conveniently to the bottom and are quite easily distinguishable) were made. Counts of each cup were made at 5 min. intervals, until the count became steady. When such an equilibrium had been reached (usually after about 15 min.) a drop of strong phenol solution was added to each cup. This immobilized all the organisms and the total number in each cup was counted. The fraction of the total number of organisms immobilized at equilibrium was taken as the response to a particular alcohol concentration.

Gut Contractility

Experiments were carried out on the guinea-pig ileum, suspended in oxygenated Tyrode solution in a 1 ml. organ bath maintained at 32 to 34°.

Contractions elicited by additions of acetylcholine to the bath were recorded by means of a light frontal-writing lever exerting a tension of about 0.3 g. on the ileum. Alcohols dissolved in the Tyrode washing fluid were applied to the preparation and the assays carried out with an automatic assay apparatus (Boura, Mongar and Schild, 1954).

In one set of experiments the ileum was stimulated electrically. The stimulus was supplied from a square-

pulse stimulator, and applied to the preparation between its attachments, which were made with fine wire in place of thread.

Tissue Oxygen Consumption

Experiments were carried out on chopped guinea-pig lung. The animal was killed by a blow on the back of the neck, and the lungs removed and washed in Tyrode solution. The lungs were then chopped into particles 1 mm. square in cross section, using a MacIlwain tissue chopper. 0.2 ml. samples were prepared, using the sampler described by Mongar and Schild (1957).

A few samples were dried and weighed. Their dry weight was about 3 mg. and varied very little between samples from the same animal.

Oxygen consumption was measured by Warburg's direct method. The main compartment of each flask contained 3.0 ml. of Tyrode solution (containing phosphate buffer in place of bicarbonate) and the lung particles, and, in most of the experiments, the alcohol. The central well contained 0.4 ml. of 10% sodium hydroxide solution absorbed in a filter paper roll. In some experiments with water-miscible alcohols, the alcohol was placed in the side-arm, and added only after the control rate of oxygen consumption had been established. In most cases, however, it was necessary to add the alcohol to the main compartment right from the start, and to compare the oxygen uptake with that of separate control flasks.

Anaphylactic Histamine Release

The method described by Mongar and Schild (1957) was used.

Guinea-pigs were sensitized by injecting a solution of 2% egg albumen and 0.5% phenol in normal saline, 1 ml. being given subcutaneously and 1 ml. intraperitoneally. The animals were used 3 to 10 weeks later.

The lungs were chopped as before, and washed for at least 20 min. in 500 ml. of Tyrode solution, in order to remove as much as possible of the histamine released from damaged cells. 0.2 ml. samples were prepared as before, and each sample was placed in a 5 ml. beaker containing 1 ml. of Tyrode solution, or Tyrode plus inhibitor, and a round glass bead was added to help stirring. The beakers were fitted into a shaking rack in a water-bath at 37°, and left for 15 min. 0.1 ml. of 1% egg albumen solution was then added to each (with the exception of the blanks, to which Tyrode was added) and after a further 15 min. shaking the supernatant was sucked off with a filter-tipped pipette. The supernatant solutions were placed in a boiling water-bath for 2 min. in order to destroy histaminase and acetylcholine.

Histamine was assayed on the guinea-pig ileum, using an automatic assay apparatus (Boura, Mongar and Schild, 1954).

Calculation of Thermodynamic Activity

The activities of alcohols in aqueous solution were calculated from the activity coefficients given by

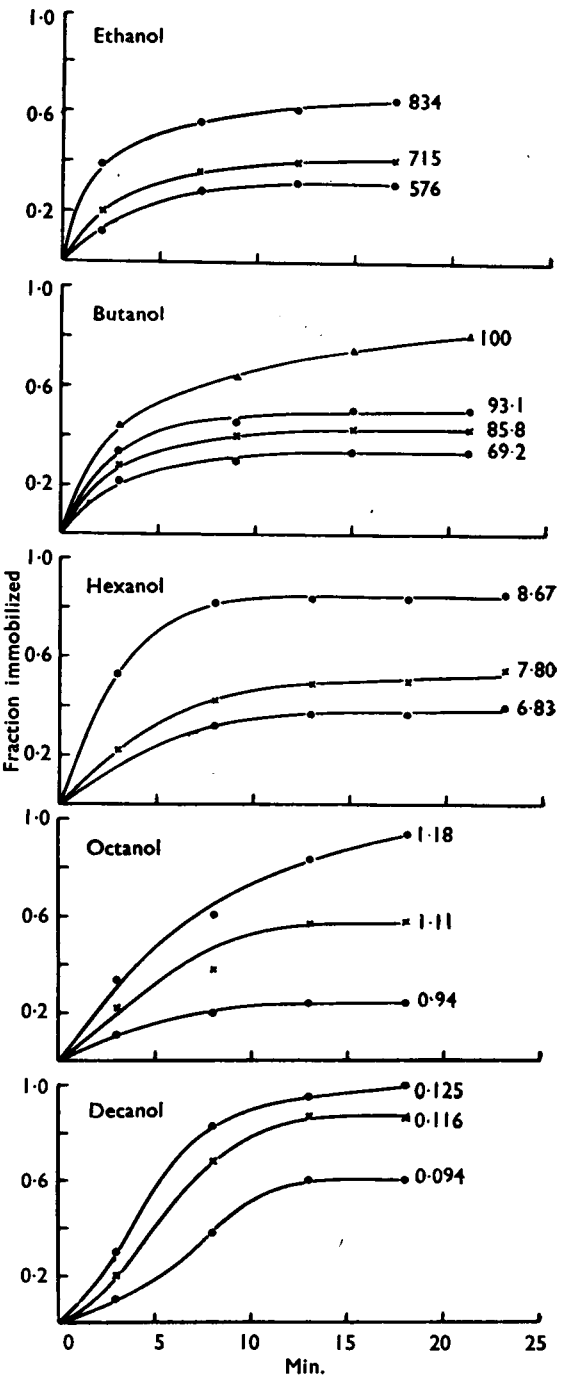

FIG. 1.—Time-action curves of alcohols on paramecium mobility. The figures appended to the curves show the alcohol concentration (mM). Each point represents the mean of three or four observations.

Butler, Thomson and MacLennan (1933), and the mole fraction of alcohol in solution, using the relation:

$$\text{Thermodynamic activity} = \text{Activity coefficient} \times \text{Mole fraction}$$

For alcohols dissolved in Tyrode solution, no measured activity coefficients are available and certain approximations were necessary. Lindenberg (1948) found that the activity of ketones in sodium chloride solution behaved according to the equation:

$$\log (\gamma_s/\gamma_0)/\mu = K = cN$$

where γ_s, γ_0 = activity coefficients in salt solution and water respectively.
μ = ionic strength of salt solution.
K = displacement constant, peculiar to salt and organic solute.
c = proportionality constant, peculiar to organic solute.
N = molecular refraction of organic solute.
For ketones c = 6·3.

In the present measurements, this value of c was used and the ionic strength of Tyrode solution calculated to be 0.159. The correction was, in fact, small (10% for octanol, progressively less for lower alcohols).

Results

Paramecium Immobilization

The qualitative effects of alcohols on paramecium followed a fixed pattern. Paramecia normally move smoothly, rotating continuously about a longitudinal axis and occasionally changing direction abruptly. The first effect of alcohols is to cause their path to become spiral (coinciding with the rotation). The forward movement then becomes progressively slower and eventually stops. At this stage the anterior end is stationary while the posterior end moves in a circle. This movement gradually subsides.

These stages were clearer and the sequence took place more slowly with higher than with lower alcohols.

Such a sequence is unlikely to be the result of overall depression of the cell, and more probably represents a depression of ciliary action passing over the membrane in a fixed pattern.

Fig. 1 shows time-action curves of alcohols on paramecia for alternate members of the homologous series tested. In most cases a clear equilibrium was reached, the time taken for equilibration varying from about 8 min. with ethanol to about 13 min. with decanol. In a few cases (for instance, with the highest concentration of butanol and octanol) no clear equilibrium was established. Where this complication arose the percentage immobilization at the time of equilibrium with lower alcohol concentrations was used.

In other similar toxicity studies, various measures have been used as criteria of activity, such as percentage mortality after a standard time, time taken to kill 50%, etc. In view of the steadily increasing time taken for equilibration

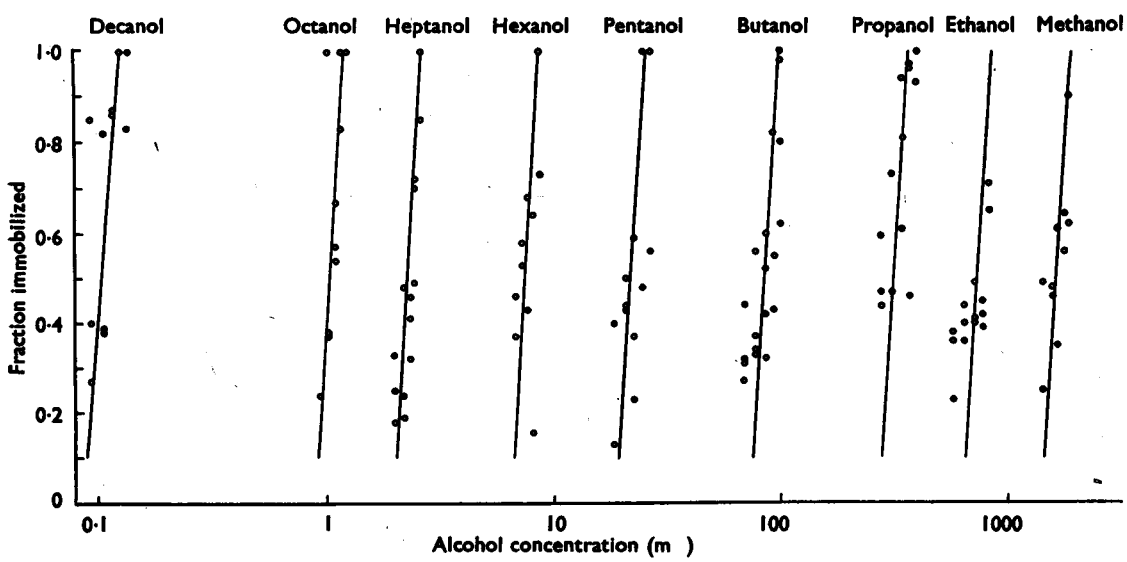

FIG. 2.—Concentration-action curves of alcohols on paramecium mobility. Each point represents a single observation. Parallel regression lines are drawn through the points.

observed in the homologous series of alcohols, it was felt that the criterion of activity must be independent of time relations. Hence percentage immobilization at equilibrium was used as a measure of pharmacological activity.

Fig. 2 shows the fraction of organisms immobilized at equilibrium plotted against alcohol concentration (on a logarithmic scale) for the series of alcohols from methanol to decanol (nonanol excluded). Each point represents a single determination. Since straight lines fit the points well the use of a probit transformation was considered unnecessary. The lines have all been plotted in accordance with a common regression coefficient, judged by eye. In no case does the regression line conflict with the trend of the points.

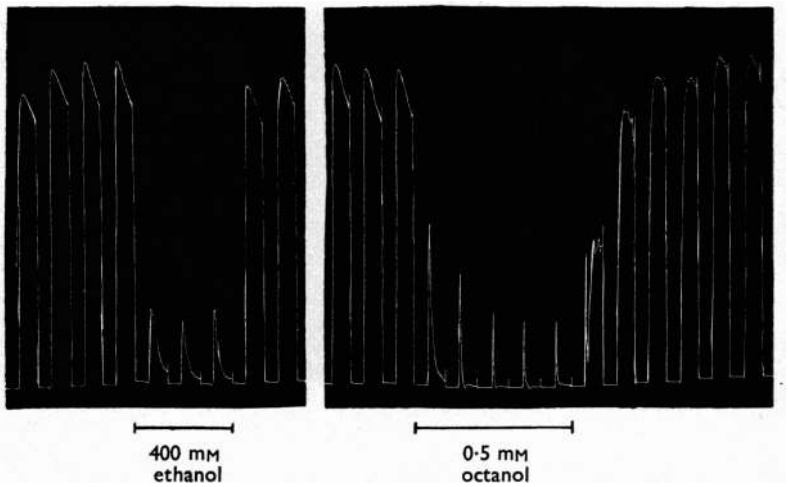

FIG. 4.—Effect of ethanol and octanol on maximal responses of the guinea-pig ileum to 1 μg./ml. acetylcholine.

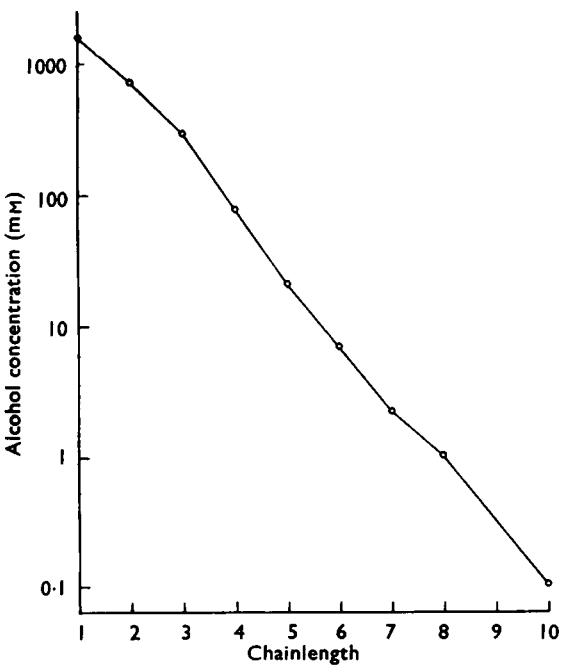

FIG. 3.—Relationship between alcohol chainlength (that is, the number of carbon atoms) and the concentration required to immobilize 50% of a population of paramecia. The concentrations are taken from the regression lines of Fig. 2.

Since these logarithmically-plotted regression lines are parallel, the ratio of concentrations of different alcohols required to immobilize a given fraction of organisms is independent of the fraction selected. Hence it is valid to use equiactive concentrations as a measure of the relative potencies of different alcohols.

The concentration of alcohol causing 50% immobilization at equilibrium has been used as a criterion for comparison. This concentration may be obtained from the regression lines in Fig. 2. The precision of these estimates may be expressed by the coefficient of variation, $\lambda(=0.039)$.

Fig. 3 shows the concentration of alcohol required for 50% immobilization plotted logarithmically against the number of carbon atoms in the chain. It shows a linear logarithmic decrease in equiactive concentration with increasing chainlength. Certain deviations from linearity are seen, namely a flattening at the top of the graph, and a flattening between heptanol and octanol. The flattening at the top has been found also in other experiments in the present series. The kink between heptanol and octanol, however, has not been observed elsewhere.

Depression of Gut Contractility

The action of alcohols is rapidly and completely reversible. This is shown in Fig. 4. The rate of recovery is somewhat less than the rate of development of the effect. If a series of equal doses of agonist are given, and then alcohol is added, the response declines in an exponential

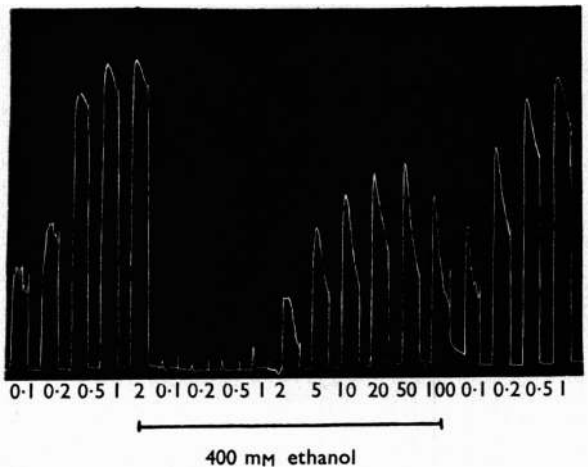

FIG. 5.—Effect of ethanol on the responses of the guinea-pig ileum to acetylcholine. Acetylcholine concentration in μg./ml. is shown under each contraction.

manner, eventually flattening off to a steady lower value. Recovery after removal of alcohol is similarly exponential. The time required for equilibration with alcohols increased with chain-length from about 1 min. for methanol to about 5 min. for octanol. Decanol was found not to reach equilibrium after 20 min. exposure and recovery of the response after its removal was incomplete.

The specificity of the action of alcohols was tested by comparing their effects on contractions produced by histamine, acetylcholine, and electrical stimulation. In one experiment, equivactive doses of histamine and acetylcholine were given alternately, and alcohol was added. Alcohol affected both responses equally and simultaneously. In another experiment, maximal doses of histamine were alternated with maximal electrically-induced contractions (which were slightly smaller than the histamine contractions). Alcohol affected both these responses equally, the electrically-induced contractions remaining smaller than the histamine contractions by the same fraction. Hence the action of alcohols shows no specificity.

It was found that alcohols diminished both the gradient and the maximum of agonist log-concentration action curves. Fig. 4 shows that there is a qualitative modification of the contraction by alcohols. The normal response of smooth muscle to addition of acetylcholine is an immediate rapid contraction, followed by a slower phase which reaches a peak and is followed by gradual relaxation in the presence of acetylcholine. In the presence of a strongly depressant concentration of alcohol, relaxation sets in immediately after the rapid phase of contraction. There is no slow phase of contraction, and relaxation is often complete before the stimulant is washed out. This strongly suggests that alcohols interfere with the contractile mechanism itself rather than with the receptors. The simplest

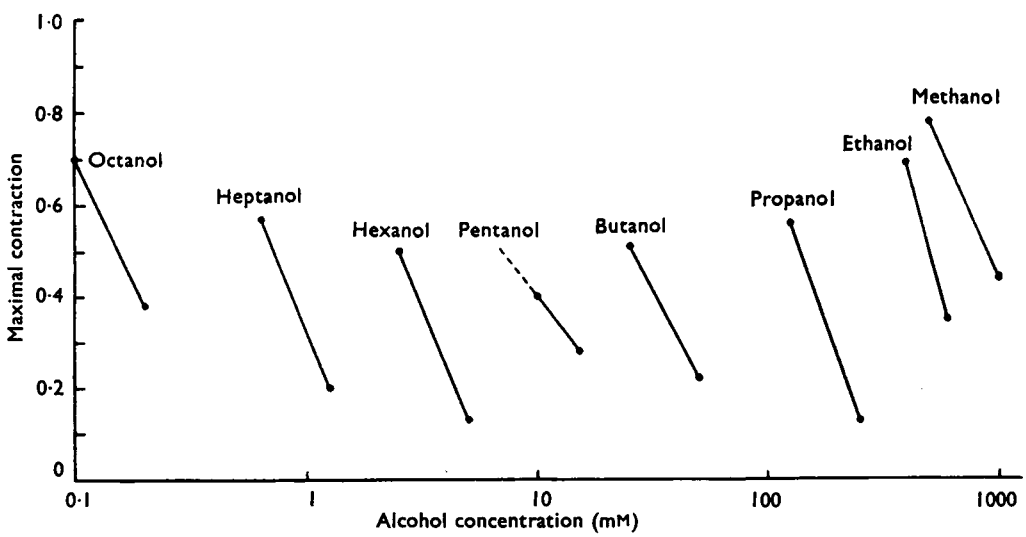

FIG. 6.—Concentration-action curves of alcohols on the responses of the guinea-pig ileum to acetylcholine. The ordinates represent the ratio of the maximal contractions in the presence and in the absence of alcohol. Each point represents a single determination and all were obtained on a single preparation.

way to measure such effects is by diminution of the maximal contraction (Arunlakshana and Schild, 1959).

In practice this was made difficult by the fact that, in the presence of depressants, the maximal contraction tends to diminish with successive doses of stimulant, as shown in Fig. 5. The acetylcholine dose was successively doubled in the presence of alcohol. The contractions increased in size up to a dose of 50 μg., but the response to the following dose of 100 μg. was considerably smaller. Increasing the dose interval reduced this effect, but considerably increased the error. The procedure adopted was to use a standard dose interval of just over one minute, and to take the largest response in a successively doubling series as the maximal response in the presence of depressant.

Preliminary experiments with alcohols showed that the relation between log-concentration of depressant and action in depressing the maximal contraction followed a sigmoid form, approximately linear between 20% and 80% effect, and tailing off symmetrically at either end. In the quantitative tests, therefore, each alcohol was applied in two concentrations, selected to give effects larger and smaller than 50% and to lie within the 20–80% range. The concentration required for a 50% effect, which was used as a criterion for comparison of potency, was obtained by linear interpolation.

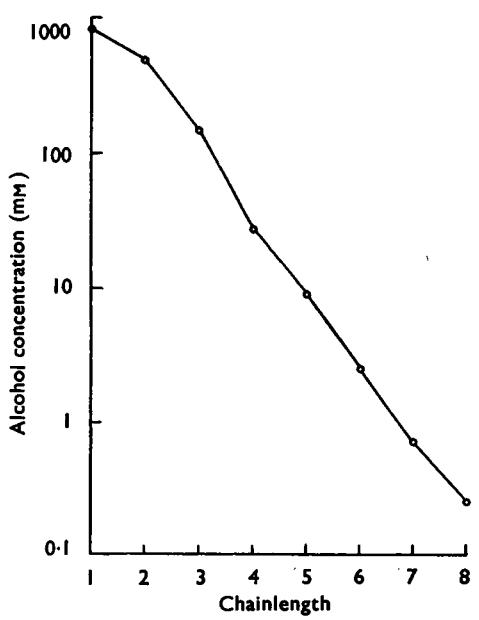

FIG. 7.—Relationship between alcohol chainlength and the concentration required to cause 50% depression of the maximal contraction of guinea-pig ileum to acetylcholine. The points plotted are the means of all the determinations.

Fig. 6 shows a series of two-point comparisons of the alcohols. Each point represents the mean of three or four observations on different ileum preparations. It was found that different preparations varied considerably in their sensitivity to alcohols, but the relative potencies of different alcohols varied much less. The coefficient of variation, λ, was 0.252, indicating an error about nine times that of the paramecium test. The parallel nature of the regression lines and their regular spacing in Fig. 6 is obvious. Fig. 7 shows the linear logarithmic decrease in the concentration required for 50% depression of maximal contraction (taken from the regression lines in Fig. 6) with chainlength. As observed with the paramecium immobilization experiments, there is a slight flattening between methanol and ethanol, but no other marked irregularity.

Depression of Lung Oxygen Consumption

Under the conditions of the experiment, oxygen uptake by lung particles in Tyrode solution continued at a steady rate for about 2½ hr. after placing the flasks in the Warburg apparatus. The rate of uptake in Tyrode solution, expressed as qO_2 (μl. absorbed per min. per mg. dry weight of tissue) varied from 2.5 to 5 in different animals, but samples from one animal gave consistent values.

In the presence of strongly depressant alcohol concentrations, the oxygen uptake frequently slowed and stopped after about 1½ hr. This was, however, always preceded by a period of steady uptake, from which the qO_2 was calculated. This cessation of oxygen uptake was probably due to the death of the tissue resulting from profound depression of its function.

As in the experiments on gut contractility, preliminary experiments showed that the log-concentration action curve was sigmoid in form, with a linear middle region, and two-point comparisons were again used. Fig. 8 shows the series of results obtained on the alcohol series. Each point represents a single experiment, and the straight lines in cases where more than two concentrations were used were drawn by eye. In the lower part of the series, from methanol to hexanol, the results are very similar to those obtained in the previous experiments in that the lines are all parallel and fairly regularly spaced,

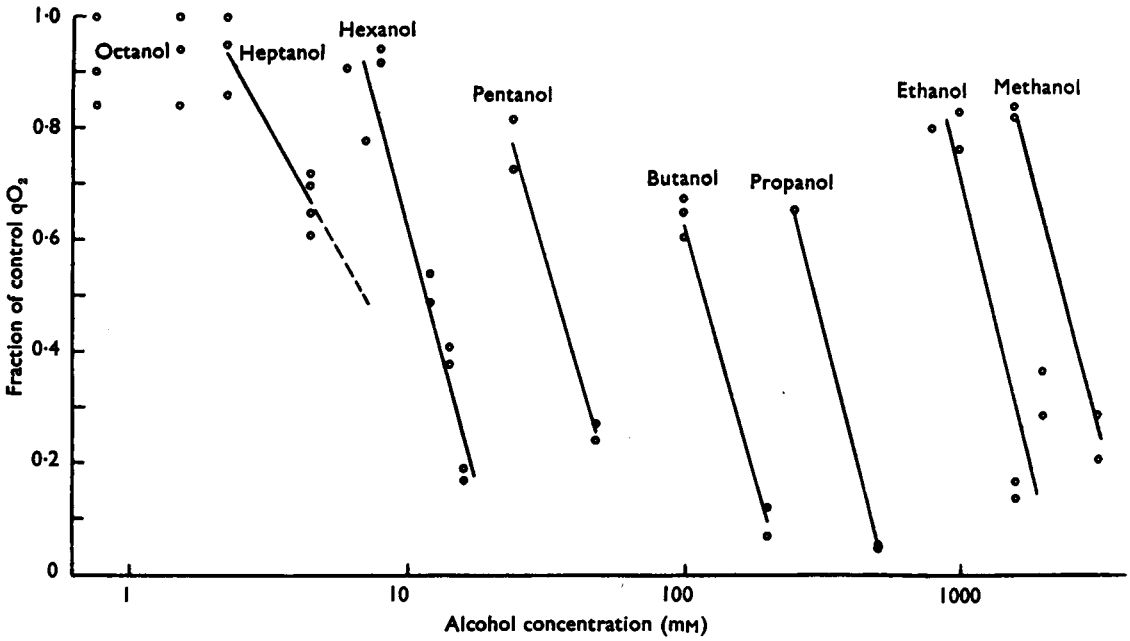

FIG. 8.—Concentration-action curves of alcohols on lung oxygen consumption. The ordinates represent the ratio of oxygen consumptions in the presence and in the absence of alcohol. Each point represents a single determination.

with the exception of methanol and ethanol. Above hexanol, however, the pattern changes completely. Heptanol shows a regression line less steep than the lower members, while octanol showed no significant activity at all. With both these alcohols the highest concentration used was close to saturation.

For the lower members of the series (hexanol downwards) the coefficient of variation, λ, was 0.182. Thus the variability was about six times that encountered in the paramecium tests, due partly to the lower gradient of the regression lines and partly to the greater scatter of the points.

The alcohol concentration required for 50% depression of oxygen consumption is shown plotted against chainlength in Fig. 9. This shows that, as far as hexanol, the pattern is the same as that found in the previous tests. The point for heptanol is taken from the extrapolated regression line of Fig. 8 and represents an unattainable concentration.

Throughout the series, the concentration required for 50% depression of oxygen consumption was approximately three times that required for 50% immobilization of paramecia and approximately 1.5 times that required for 50% depression of gut contractility.

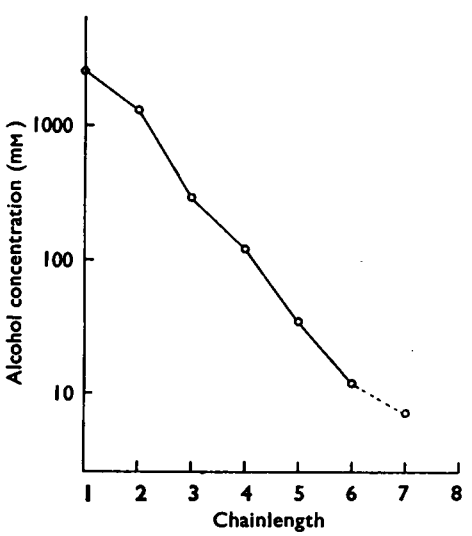

FIG. 9.—Relationship between alcohol chainlength and the concentration required to cause 50% depression of lung oxygen consumption. The value for heptanol was obtained by extrapolation of the regression line in Fig. 8, and represents an unattainable concentration.

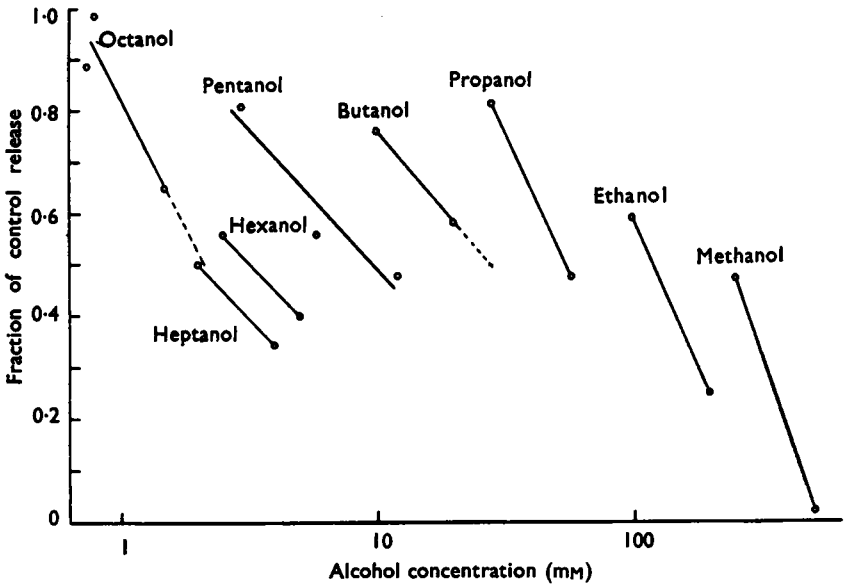

FIG. 10.—Concentration-action curves of alcohols on anaphylactic histamine release. Each point is the mean of all determinations at a particular alcohol concentration.

Depression of Lung Histamine Release

The amount of histamine released by antigen from chopped lung particles in the absence of any inhibitor varied considerably between preparations from different animals. Different samples from the same animal also showed some variation. The mean histamine release from fifty-three 0.2 ml. lung samples obtained from 18 animals was 0.96 μg. Expressed as a percentage of the total histamine, the variation was much less, and the mean value was 26%. In the absence of antigen under the same conditions the mean release was 3%.

As in the previous experiments, it was found that the log-concentration action curves for all these types of compounds were sigmoid in shape, with a middle linear region, and 2-point comparisons were again used.

The results obtained with the alcohol series are shown in Fig. 10. The deviations from parallelism are not significant. The coefficient of variation, λ, was 0.321, that is, the variability, was approximately ten times as great as that encountered in the paramecium experiments.

The spacing of the regression lines is fairly regular as far as hexanol, but the interval between hexanol and heptanol is less, and between heptanol and octanol there is no interval. The highest concentration of octanol used was close to saturation, and did not produce 50% inhibition. Thus, just as in the experiments on oxygen consumption, a cut-off point is reached, beyond which chainlength compounds show no activity. However, in the oxygen consumption experiments, heptanol showed a reduced gradient and an anomalously high equiactive concentration, and octanol showed no activity, while in these experiments both heptanol and octanol show the same gradient as the lower alcohols although their equiactive concentrations are anomalously high.

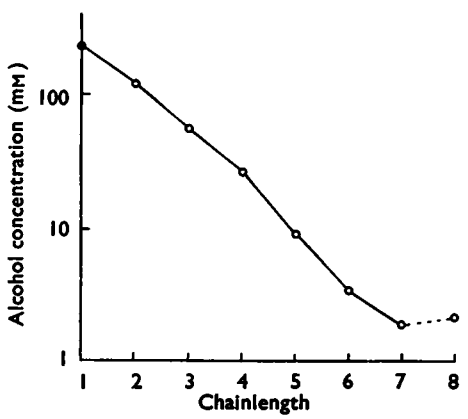

FIG. 11.—Relationship between alcohol chainlength and the concentration required to cause 50% depression of anaphylactic histamine release. The value plotted for octanol was obtained by extrapolation of the regression line in Fig. 10, and represents an unattainable concentration.

Fig. 11 shows alcohol concentration required for 50% inhibition of histamine release plotted against chainlength. Up to hexanol, the graph is approximately linear (no flattening between methanol and ethanol is apparent). The cut-off with octanol is clearly shown.

Discussion

The Log-Concentration Action Relationship

For each system investigated, a set of log-concentration action curves for the series of alcohols has been presented, and it is interesting to compare these figures. In each case the regression lines plotted were parallel over most of the range of alcohols investigated, but the gradient differs between different systems. Thus, in the tests on paramecium toxicity, the regression coefficient was 720, in the tests on gut contractility 90, in the tests on lung oxygen consumption 174, and in the tests on lung histamine release 59. In the paramecium toxicity test, the measurement was quantal, whereas in the other experiments it was almost certainly quantitative (one cannot be absolutely certain that these other effects were not due to the all-or-nothing depression of a certain proportion of cells, but this seems most unlikely). The coefficient given for the tests on paramecium toxicity is therefore not comparable with the other coefficients since it represents only the variability of the population, whereas the regression coefficient in quantitative experiments has other significance.

The constancy of gradient among different members of the homologous series indicates that the order of the reaction is constant. The difference in gradient between different biological systems may be interpreted in two ways. First, the reaction order may vary, which would suggest that the biophase shows fundamentally different properties in the different systems, and that the mode of action of alcohols in the different systems is different. In view of the characteristic pattern of activity in different members of the homologous series, it seems unlikely that the mode of action in different systems is as unrelated as this theory would suggest. Second, the discrepancies of gradient may be explained on the basis of a common mode of action if one supposes that the dependence of the different biological phenomena investigated on the single system which is affected by alcohols shows different quantitative relationships. Thus, the lung oxygen consumption (which has the steepest gradient) may show the complete range of inhibition, from 0 to 100%, over a narrow range of inhibition of the system which is the primary site of action of alcohols, whereas inhibition of histamine release takes place over a much wider range. This explanation appears to be more plausible than the first, since it preserves the unity of the action of unspecific depressants, which is the most striking feature evident in these results.

The differences which are observed in the higher and lower members of the homologous series are more difficult to explain than those of gradient between different systems. Methanol in most of the systems investigated showed a steeper gradient than the succeeding members of the series. It also showed an anomalous potency, greater than would be expected from its chainlength. Ferguson (1939) disposes of the anomalies of methanol by assuming that its physical toxicity is complicated by its chemical reactivity. This is a convenient explanation, but, without definite evidence that its mode of action differs from that of higher alcohols, it does not seem entirely justified. The fact that methanol shows similar anomalies in many of its physical properties (density, refractive index, etc.) does not dispose of the need to explain the anomalies in its biological action. Indeed, it would seem that if its biological distinctiveness could be explained in terms of its physical distinctiveness this would be a very useful step in the elucidation of the mechanism of action of unspecific depressants.

In the two systems investigated in which a cut-off in activity occurred in alcohols lower than octanol, an interesting discrepancy is evident. In the tests on depression of histamine release, no change in gradient of log-concentration action curves was evident before the cut-off activity. In contrast, the tests on depression of oxygen consumption showed that heptanol gave a regression line which was markedly less steep than that of the lower alcohols. Octanol showed no activity whatever. At present, no explanation is available to account for this.

Validity of the Concept of Unspecific Drug Action

The term " unspecific " implies that drugs of this class exert actions on a wide range of biological phenomena, and also that the relative potency of two different drugs is independent of the system on which they are tested. The investigations have verified the first implication, but the second is found not to hold. Fig. 12 is a composite diagram showing the effect of chainlength on equiactive alcohol concentration for the living

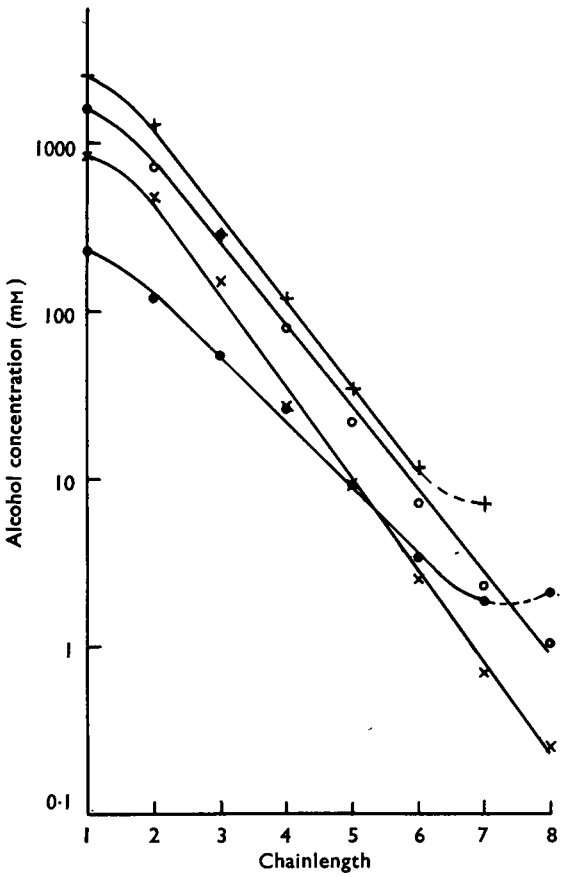

FIG. 12.—The relationship between alcohol chainlength and the concentration required for 50% depression of gut contractility (X), paramecium mobility (O), lung oxygen consumption (+), and lung histamine release (●). The curves have been smoothed out for clarity.

systems investigated. These lines are clearly not parallel. Thus equal concentrations of hexanol are required to cause 50% depression of both gut contractility and anaphylactic histamine release, while with ethanol the concentration required to depress gut contractility is about four times that required to inhibit histamine release. In this strict sense, therefore, the action is not truly unspecific, as ethanol might be said to be specific in depressing histamine release. Similarly, the phenomenon of cut-off means that alcohols completely lose their activity on one system, while retaining it on another. This again confers a type of specificity in certain higher alcohols. It seems, therefore, that the concept of completely unspecific drug action is untenable.

Ferguson's distinction of physical and chemical drug action seems to describe better the obvious differences between these classes of drugs. However, certain obstacles arise even with this distinction. There is the difficulty of deciding exactly what is meant by physical and chemical action. Ferguson and Hawkins (1949) have endeavoured to resolve this difficulty by investigating the toxic actions of very simple inorganic gases, including some inert gases, which are incapable of entering into chemical reactions. They found that these compounds exert the same actions as other narcotics acting by the alleged physical mechanism, and the thermodynamic activities required to produce a certain effect are in accordance with those found for other substances. Thus it is clear that no chemical process involving electron transfer takes place, but it would be difficult to demonstrate such a process taking place in the action of alleged chemically-acting drugs, whose action is entirely reversible. Indeed, it would seem quite possible that drugs acting at thermodynamic activities less than those compatible with Ferguson's criterion for physical activity might be doing so, not on account of any electron-transferring chemical process, but by virtue of some special molecular configuration which renders their physical interaction with the biophase much more disturbing than that produced by the wide range of undistinguished molecules which form Ferguson's physically-acting group.

We therefore have to turn to less direct criteria, of which the most illuminating is the thermodynamic activity of the compound required to produce an effect. Ferguson and Pirie (1948) found that the activities of compounds required to kill grain weevils fell into two clear groups. Those acting at activities between 0.1 and 1 they termed physical agents, and those acting at lower activities (usually very much lower) were termed chemical agents. Such a distinction separates the two groups very nicely if a single biological activity is considered, but different biological phenomena show quite wide variations in the thermodynamic activities of physically-acting drugs required to produce a given effect. Thus in the present investigation the thermodynamic activities of lower alcohols required to depress anaphylaxis were below 0.01, and in no case was an activity greater than 0.1 required with ethanol. This is obviously explained by the smaller disturbance of function which was used as a

TABLE I

CONCENTRATIONS (mM) AND THERMODYNAMIC ACTIVITIES OF ALCOHOLS REQUIRED FOR 50% DEPRESSION OF FUNCTION IN THE BIOLOGICAL SYSTEMS INVESTIGATED

Alcohol	Paramecium Mobility		Gut Contractility		Lung Oxygen Consumption		Lung Histamine Release	
	Concentration	Thermodynamic Activity	Concentration	Thermodynamic Activity	Concentration	Thermodynamic Activity	Concentration	Thermodynamic Activity
Methanol	1,600	0·0466	821	0·023	2,500	0·071	230	0·0065
Ethanol	730	0·0588	476	0·032	1,320	0·085	120	0·0077
Propanol	300	0·0694	149	0·035	298	0·070	56	0·0132
Butanol	81	0·0838	26·6	0·024	121	0·117	26·5	0·0234
Pentanol	21·8	0·105	9·0	0·038	34·4	0·145	9·5	0·0400
Hexanol	7·6	0·150	2·5	0·044	11·9	0·208	3·5	0·0613
Heptanol	2·3	0·178	0·71	0·049	7·1	0·497	1·9	0·133
Octanol	1·05	0·279	0·25	0·061			2·2	0·537

criterion of potency. In Ferguson and Pirie's investigation, the response observed was death.

Gavaudon, Dodé, and Poussel (1944), investigating the thermodynamic activities of compounds required to narcotize aquatic animals, found, similarly to Ferguson, that the activities required fell into two groups, but they suggested that the upper limit of activity for a chemically-acting compound was about 0.04. Clearly this limit cannot be set at any fixed value, since it will depend on the system investigated. The distinction between the activities of the two types of drug on any one system is, however, a useful criterion, and is good evidence that separate mechanisms of action are involved.

Relation Between Chainlength and Thermodynamic Toxicity

The term "thermodynamic toxicity" will be used to denote the inverse of the thermodynamic activity of a compound required to produce a certain effect. No theoretical significance attaches to the phrase.

Ferguson (1939) found, in general, a decrease of thermodynamic toxicity with chainlength within a homologous series, but Brink and Posternak (1948) found that it was constant. This is a question of some theoretical interest, and one which should have a definite answer.

Table I shows the thermodynamic activities of alcohols required for 50% depression of function in the four living systems investigated, and Fig. 13

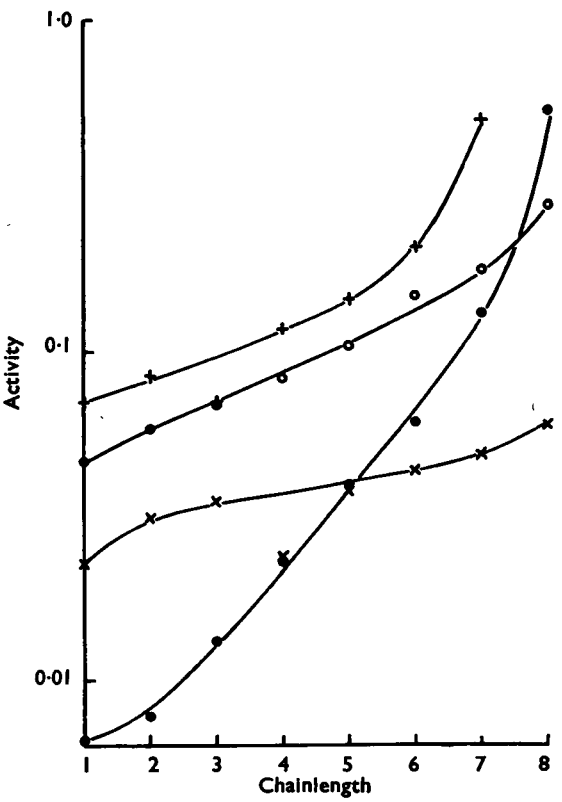

FIG. 13.—Relationship between alcohol chainlength and the thermodynamic activity required for 50% depression of gut contractility (X), paramecium mobility (O), lung oxygen consumption (+), and lung histamine release (●).

shows this plotted logarithmically against chain-length.

In the curves in Fig. 13, it is noticeable that the two cases in which no cut-off occurred, namely, in the tests on paramecium and gut contractility, the curves approximate to a straight line (a large deviation at butanol is evident in the gut contractility curve; this is probably error), while the other two curves in which cut-off was observed show a marked steepening at the top. It must be remembered that both of those tests were carried out in Tyrode solution, and are subject to approximations in the calculation of thermodynamic activity. It is not felt, however, that this can account entirely for the difference.

According to Ferguson's hypothesis, cut-off results from a steady decrease in thermodynamic toxicity with chainlength. Thus at a certain chainlength the required thermodynamic activity exceeds unity and is unattainable. According to this view, there is no reason to anticipate a sharp increase in the required thermodynamic activity immediately preceding the cut-off. However, the present results show that just before the cut-off the thermodynamic activity required is greater than would be predicted from the trend of the thermodynamic toxicity of preceding members of the series. Hence these results agree with Ferguson in that they show a decrease of thermodynamic toxicity with chainlength, and with Brink and Posternak in that they indicate a more rapid decrease of thermodynamic toxicity immediately preceding the cut-off. This conclusion is in agreement with most of the previous quantitative investigations of homologous series, in which it is found that complete cut-off is preceded by reduced thermodynamic toxicity.

It is thus necessary to explain not only the steady fall in thermodynamic toxicity with chainlength but also the more rapid fall which precedes the cut-off. At present no explanation is available for either of these observations, and an explanation will probably be found only when it is possible to test a concrete hypothesis of the mechanism of action of physically-acting drugs by comparing predictions with experimental results.

One feature of the results of Clark (1930) on heart muscle which defies explanation by Ferguson's hypothesis is that in some experiments the higher alcohols were applied in a concentration exceeding that of a saturated solution, in the form of what Clark calls a "colloidal suspension," obtained by prolonged shaking. Clark found that the concentration-action relationship showed no anomaly when such suspensions were used, yet their thermodynamic activity cannot exceed that of a saturated solution.

Mechanism of Action

Mullins (1954) suggested the cell membrane as the site of action, envisaging that the interstices in the lattice structure, which normally confer properties of permeability on it, become blocked up by inert foreign molecules, and the resulting decrease in permeability is responsible for the depression of function. It is indeed difficult to formulate any other mechanism by which the mere presence of inert molecules in a cell can depress its function, and in spite of the inadequate experimental proof of this hypothesis (see appendix) it seems the most probable explanation. It is perhaps an over-simplification to assume that the external cell membrane is the seat of this action: it seems just as likely that some internal barrier, such as the membrane enveloping the mitochondria or other cytoplasmic inclusions, is the site of action, or possibly many different membranes may be involved.

It seems likely that physically-acting depressants in sufficient concentration will depress any aspect of cell function. The question thus arises whether all the functions are depressed in turn as a result of progressive depression of one single system (possibly an enzyme system or decreased permeability of the outer cell membrane), or whether they are depressed individually by these drugs. This question cannot at present be answered. Experiments on the action of unspecific depressants on viable sub-units of the cell, such as mitochondria, might prove illuminating. If it were found that mitochondria were depressed by alcohols in the same way as whole cells, this would be evidence of independent depression of the different units of a living cell, but, if the action on mitochondria differed from that characteristic action on whole cells, then one might assume that depression of some single unit of the cell was responsible for the secondary depression of its many different functions.

Another line of research which might possibly prove constructive in elucidating the mechanism of action is the investigation of the action of alcohols on some synthetic non-living membrane. Thus it would probably prove possible to produce a monolayer of orientated protein molecules, and to study its permeability and the effect of alcohols on it. In this way it might be possible to test Mullins' theory on a system in which it is known that there are no enzymes, and whose physical chemistry should be much easier to work out than that of a cell membrane.

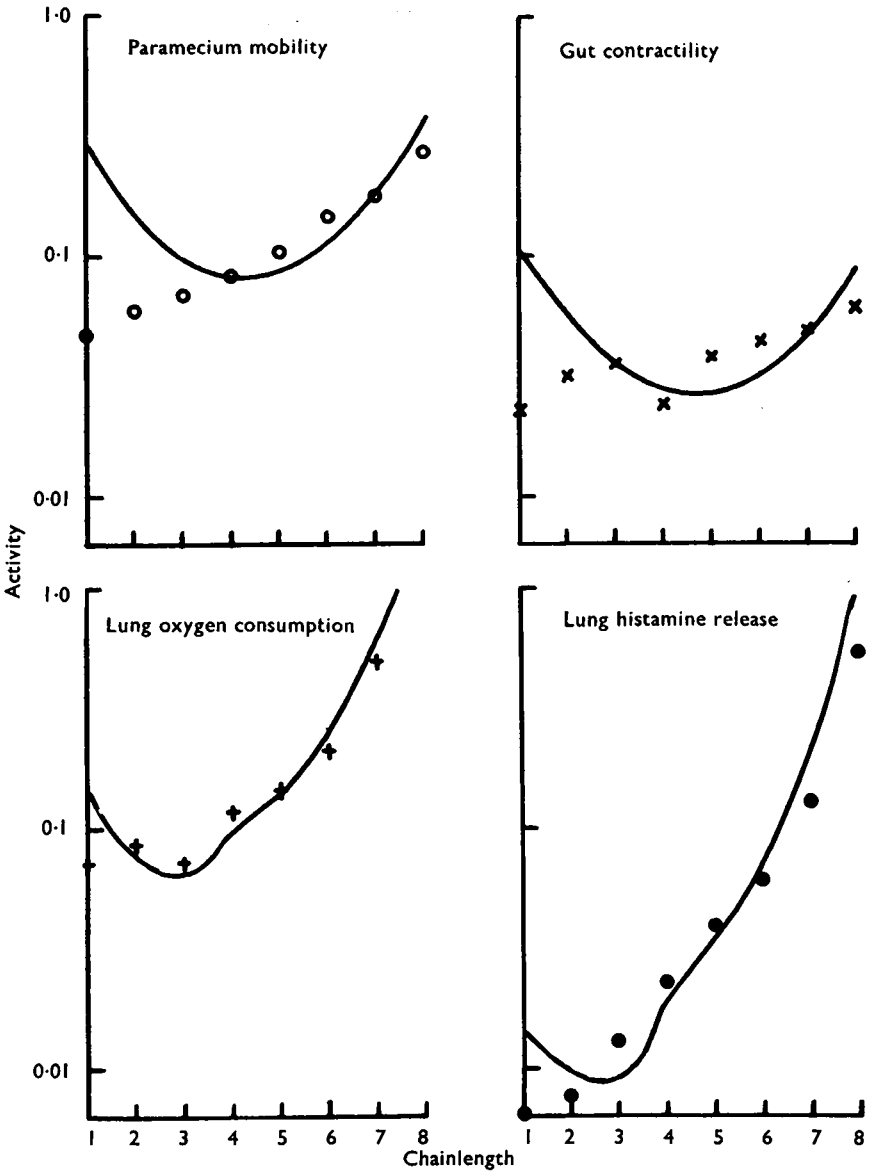

FIG. 14.—Comparison of experimental results with predictions based on Mullins' theory of narcosis. The points are experimental results. The curves are the best-fitting obtainable from Mullins' hypothesis. The constants used in constructing the curves are given in the Appendix.

APPENDIX

Application of Mullins' Hypothesis

Mullins (1954) suggested that narcotics act by dissolving in some phase within the cell (probably a membrane) and that equal narcotic action occurs when an equal volume fraction of the membrane is occupied by narcotic molecules, regardless of their chemical structure.

In order to test this hypothesis, it is necessary to estimate the concentration of narcotic in the biophase. Mullins attacked this problem by estimating the activity coefficient of narcotic dissolved in the biophase, using principles put forward by Hildebrand and Scott (1950). He thus derived the following relationship:

$$\ln\gamma_{nar} = \frac{V_m(\delta_{nar}-\delta_{mem})^2}{RT} \quad \ldots\ldots\ldots\ldots(1)$$

where γ_{nar} = activity coefficient of narcotic in membrane.

V_m = molar volume of narcotic.

δ_{nar}, δ_{mem} = solubility parameter of narcotic and membrane respectively.

This equation is based on theories applying to mixtures of non-polar solvents. A large error may be incurred by applying it in the present circumstances.

The volume fraction of narcotic in the membrane is given by the expression, $X_{nar}V_m$, where X_{nar} is the mole fraction of narcotic in the membrane.

$\gamma_{nar}X_{nar}$ = A_{nar}, the thermodynamic activity required for narcosis.

$\therefore A_{nar}V_m = \gamma_{nar}X_{nar}V_m$.

According to Mullins' hypothesis, $X_{nar}V_m$ is constant.

$\therefore A_{nar}V_m \propto \gamma_{nar}$ .....(2)

In order to calculate γ_{nar} from equation (1), δ_{mem} must be known. This cannot be measured directly. A series of curves of log γ_{nar} against chainlength for different values of δ_{mem} were plotted according to equation (1) using values of V_m and δ_{nar} given by Mullins. According to Mullins' hypothesis (equation 2), one of these curves should be the same shape as the experimental plot of log $A_{nar}V_m$ against chainlength. Thus from each of the four biological systems investigated a value of δ_{mem} giving the best fit between experimental data and theoretical prediction was selected. Fig. 14 shows the experimental data (points) compared with the best fitting theoretical curves. The values of δ_{mem} and $X_{nar}V_m$ used in fitting the curves are as follows:

	$X_{nar}V_m$	δ_{mem}
Paramecium mobility	7·34	11·8
Gut contractility	2·52	11·5
Lung oxygen consumption ..	5·25	12·9
Lung histamine release ..	0·63	13·5

With the paramecium and gut contractility experiments the fit is very poor, but it is much better in the experiments on histamine release and oxygen consumption. However, the striking feature of the theoretical predictions—the minimum at a certain chainlength—is entirely lacking in the experimental results. Some of Mullins' results, relating to depression of nervous tissue, do in fact show this minimum, and the fit is much more convincing. Mullins found that the membrane solubility parameter giving the best fit was about 11, while in the present case it was about 13 in the only cases in which a possible fit was obtained. A parameter of 11 corresponds to a molecule with approximately the same ratio of hydrophilic and hydrophobic groups as butanol, which corresponds roughly with what is known of the cell membrane. A parameter of 13 corresponds to the hydrophilic/hydrophobic ratio of ethanol, which is most unlikely to correspond with the cell membrane.

The present results do not, therefore, support Mullins' hypothesis. However, the theoretical predictions are based on doubtful assumptions, and allow no firm conclusions to be drawn.

My sincere thanks are due to Dr. H. O. Schild, whose advice and encouragement were invaluable.

The work was supported by a grant from the Medical Research Council.

References

Arunlakshana, O., and Schild, H. O. (1959). *Brit. J. Pharmacol.*, **14**, 48.

Boura, A., Mongar, J. L., and Schild, H. O. (1954). Ibid., **9**, 24.

Brink, F., and Posternak, J. M. (1948). *J. cell. comp. Physiol.*, **32**, 211.

Butler, J. A. V., Thomson, D. W., and MacLennan, W. H. (1933). *J. chem. Soc.*, p. 674.

Clark, A. J. (1930). *Arch. int. Pharmacodyn.*, **38**, 101.

Ferguson, J. (1939). *Proc. roy. Soc. B.*, **127**, 387.

—— and Hawkins, S. W. (1949). *Nature (Lond.)*, **164**, 963.

—— and Pirie, H. (1948). *Ann. app. Biol.*, **35**, 532.

Gavaudon, P., Dodé, M., and Poussel, H. (1944). *Mem. Serv. Chim. Etat. (Paris)*, **31**, 384.

Hildebrand, J. H., and Scott, R. (1950). *The Solubility of Non-electrolytes.* New York: Reinhold Publishing Corp.

Holt, J. J. H. (1916). *Lancet*, **5**, 1136.

Lindenberg, B. A. (1948). *C.R. Soc. Biol. (Paris)*, **226**, 721.

Meyer, H. H. (1899). *Arch. exp. Path. Pharmak.*, **42**, 119.

Meyer, K. H., and Hemmi, H. (1935). *Biochem. Z.*, **277**, 39.

Mongar, J. L., and Schild, H. O. (1957). *J. Physiol. (Lond.)*, **135**, 301.

Moore, W. (1917). *J. Agric. Res.*, **10**, 365.

Mullins, L. J. (1954). *Chem. Rev.*, **54**, 289.

Overton, E. (1901). *Studien uber die Narkose.* Jena: Fischer.

Tilley, F. W., and Schaffer, J. M. (1926). *J. Bact.*, **12**, 303.

—— —— (1928). Ibid., **16**, 279.

Traube, J. (1904). *Pflüg. Arch. ges. Physiol.*, **105**, 541.

Warburg, O. (1921). *Biochem. Z.*, **119**, 134.