from age 1 day to 56 days. There is no indication of any inflection points in the curve or interphase periods, as is seen in mammals (rat,[13] rabbit,[14] dog,[15] mouse[16]). Kobayashi[16] has suggested that the interphase period may be an indicator of the maturation level of the brain in mammals, because several parameters, particularly the electrical activity of the brain, achieve mature development conjointly with the appearance of the interphase period. Although cortical activity in the chicken is well developed at hatching,[17] appreciable development takes place thereafter in terms of complexity of rhythms and increases in amplitude, which may indicate the divergence of this species from a normal mammalian maturation pattern.

Feeding 5% L-phenylalanine for 4 weeks caused a marked retardation in the growth of the chicks, yet the brain weight was proportional to the body weight in these animals (Fig. 1). Similarly, when the chicks receiving the phenylalanine were returned to a basal diet and had recovered most of their growth potential by 8 weeks, their brain weight was still proportional to their body weight. The only animals not fitting the log–log relationship were those in which feeding of the diet containing the amino acid was instituted at 4 weeks of age. They lost appreciable amounts of body weight, yet their brain continued to grow slightly.

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Erythrocyte membrane stabilization by steroids and alcohols; a possible model for anesthesia

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It is known that an extremely wide variety of drugs will stabilize erythrocytes against hypotonic hemolysis.[1–3] This stabilization of the erythrocyte membrane may be in some respects similar to the stabilization of the nerve cell membrane that occurs in anesthesia. In order to see whether erythrocyte stabilization would serve as a useful model for the nerve membrane stabilization of anesthesia it was decided to obtain and compare the values for erythrocyte stabilization by alcohols and steroids with the anesthetic values available in the literature. It is known that some steroids will reduce the degree of hemolysis induced by mechanical stress, by sulfhydryl inhibitors, by prolonged

storage of blood,[4, 5] and on immune and hypotonic hemolysis.[6] Traube[7] had noticed that amyl alcohol protected against hypotonic hemolysis.

MATERIALS AND METHODS

The following were obtained from Eastman Organic Chemicals, Rochester, N.Y.: 1-hexanol, 1-heptanol, 1-decanol, 1-undecanol and L-menthol. The following were obtained from Fisher Scientific Co., N.Y.: 1-propanol, 2-propanol, 1-butanol, *n*-amyl alcohol, tertiary amyl alcohol, 1-octanol, thymol, and β-naphthol. The following steroids were obtained from Steraloids, Inc., N.Y.: pregnanolone (5 β-pregnan-3 β-ol-20-one); epipregnanolone (5 β-pregnan-3 α-ol-20-one); etiocholanolone (5 β-androstan-3 α-ol-17-one; stereoisomer of androsterone); testosterone (4-androsten-17 β-ol-3-one); androsterone (5 α-androstan-3 α-ol-17-one); stilbestrol (diethylstilbestrol); pregnandione (5 β-pregnan-3,20-dione). Cortisone alcohol (4-pregnen-17 α, 21 diol-3,11,20-trione) was obtained from Sigma Chemicals Co.; progesterone was from Mann Research Laboratory. Merck Sharp & Dohme, Division of Merck & Co., graciously donated samples of Decadron (dexamethasone 21-phosphate disodium) and Hydrocortone (hydrocortisone 21-phosphate disodium).

The stock suspension of human erythrocytes was made from a freshly drawn blood sample (heparinized) by removing the plasma and buffy coat and suspending the cells in 154 mM NaCl, 10 mM sodium phosphate buffer, pH 7, as previously described;[1] 0·1-ml aliquots of this stock suspension were added to 1·5 ml hypotonic test solution (66 to 69 mM NaCl, pH 7) containing the test drug. After 5 min of hemolysis at 21–23°, the unhemolyzed erythrocytes were centrifuged, and the optical density of the released hemoglobin was measured in a Beckman DU spectrophotometer at 543 mμ. The final erythrocyte concentrations were between 2 and 2·5 × 10^7 cells/ml. Because of the low aqueous solubility of the steroids and the higher alcohols, most of these compounds were dissolved in absolute ethanol in various dilutions. Before testing the osmotic fragility, an aliquot of 0·05 ml of the ethanolic concentrated drug solution was added to 1·5 ml of the buffered hypotonic NaCl solution; 0·1 ml erythrocytes was then added. Experiments with the alcohols, 1-butanol, 1-pentanol, 1-hexanol, 1-heptanol and 1-octanol, were done in two ways, with concentrated ethanolic solutions as just mentioned, or else by dissolving the alcohols directly in aqueous solution; qualitatively identical results were obtained. All the steroids except cortisone were prepared in ethanolic solutions. The experiments were always in duplicate or triplicate and results agreed within 5 to 8 per cent.

RESULTS

Erythrocyte stabilization by alcohols. Thirteen alcohols were tested for their ability to protect or stabilize erythrocytes against hypotonic hemolysis: three aromatic alcohols, L-menthol, thymol, and β-naphthol, and ten aliphatic alcohols. As shown in Fig. 1 all the alcohols at low concentrations

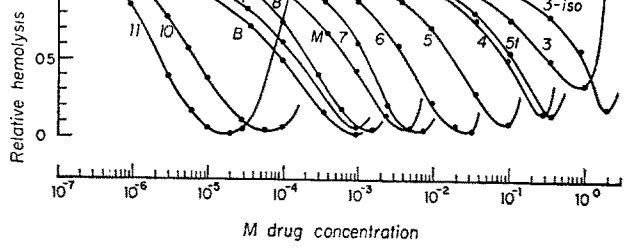

FIG. 1. Low concentrations of aromatic and aliphatic alcohols stabilize human erythrocytes against hypotonic hemolysis. Higher concentrations of these substances hemolyze the cells. For the sake of clarity the points indicating lysis are omitted except those for 1-undecanol and 1-propanol; the lytic phases are merely represented by an upturn of the curve; 11 is 1-undecanol, 10 is 1-decanol, B is β-naphthol, T is thymol, 8 is 1-octanol, M is menthol, 7 is 1-heptanol, 6 is 1-hexanol, 5 is 1-pentanol, 4 is 1-butanol, 5t is tertiary amyl alcohol, 3 is 1-propanol, and 3-iso is isopropanol. A relative hemolysis of 1·0 represents an absolute hemolysis of about 65 per cent.

protected the cells from hypotonic hemolysis while higher concentrations caused lysis. The values for relative hemolysis were obtained by dividing the amount of hemolysis in the presence of the drug by the amount of hemolysis in the absence of the drug. Note that the lines for lysis by the alcohols have been omitted for the sake of clarity; only lysis by 1-propanol and 1-undecanol are drawn in, the others being parallel to these two lines and merely indicated in Fig. 1 by an upturn.

Despite the fact that very high concentrations (10^{-2}M to 1 M) were used, it may be assumed that an extremely rapid equilibration occurred during the first few seconds, and that these high concentrations did not therefore, protect or stabilize the cells by virtue of an osmotic effect. If these concentrations only protected by an osmotic action then stabilization should not persist as long as it does (which is many hours[1, 3]).

Erythrocyte stabilization by steroids. The stabilizing effects of nine steroids are shown in Fig. 2 For the sake of clarity, in Fig. 2 the experimental points representing lysis or hemoglobin precipitation

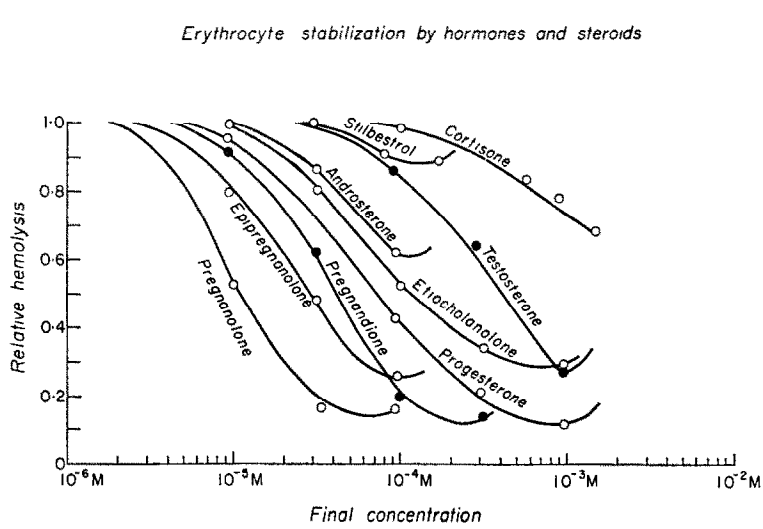

FIG. 2. Low concentrations of steroids protect or stabilize human erythrocytes from hypotonic hemolysis; higher concentrations of the steroids hemolyze or precipitate the erythrocytes. A relative hemolysis of 1·0 represents an absolute hemolysis of about 65 per cent in the absence of any drug.

have again been omitted, these effects being indicated by an upturn of the right-hand part of the stabilization curve. The two water-soluble disodium salts of hydrocortisone-21-phosphate and prednisolone-21-phosphate elicited no more stabilization of the erythrocytes than did equiosmolar concentrations of NaCl or sucrose.

It will be convenient to refer to the concentration that causes 50 per cent stabilization as C_{50}. If the C_{50} values for the alcohols (taken from Fig. 1) are plotted against the molarities required for the reversible suppression of the reflex response of tadpoles,[8] one finds that there is an almost 1 : 1 correlation over a 1,000,000-fold concentration range. This correlation is shown in Fig. 3 which also contains a correlation of the C_{50} values with the minimal blocking concentration of the alcohol on frog sciatic nerve.[9]

The anesthetic effect of certain steroids is now well documented.[10] As an index of erythrocyte stabilization (to see whether there is a correlation to steroid anesthesia) it was necessary to use the amount of relative hemolysis that occurred at a steroid concentration of 7×10^{-5}M, since many of the steroids at higher concentrations precipitated the hemoglobin rather than leading to a translucent lysis. This value for relative hemolysis was plotted versus the AD_{50} (anesthetic dose 50 per cent) for the steroid as calculated by P'an and Laubach.[10] This correlation is shown in Fig. 3 and, although the correlation is only approximate, it does extend over a 100-fold concentration range of AD_{50}.

If the analogy between erythrocyte membrane stabilization and anesthetic membrane stabilization holds, the erythrocyte may serve as a convenient model for testing a theory of anesthesia. According to the ideas put forth by Ferguson[11] and Brink and Posternak,[12] equal degrees of narcosis should occur at equal thermodynamic activities. A modification of these ideas was later made by Mullins,[13] who stated that "equal degrees of narcosis occur with equal volume fractions of narcotic within the membrane."

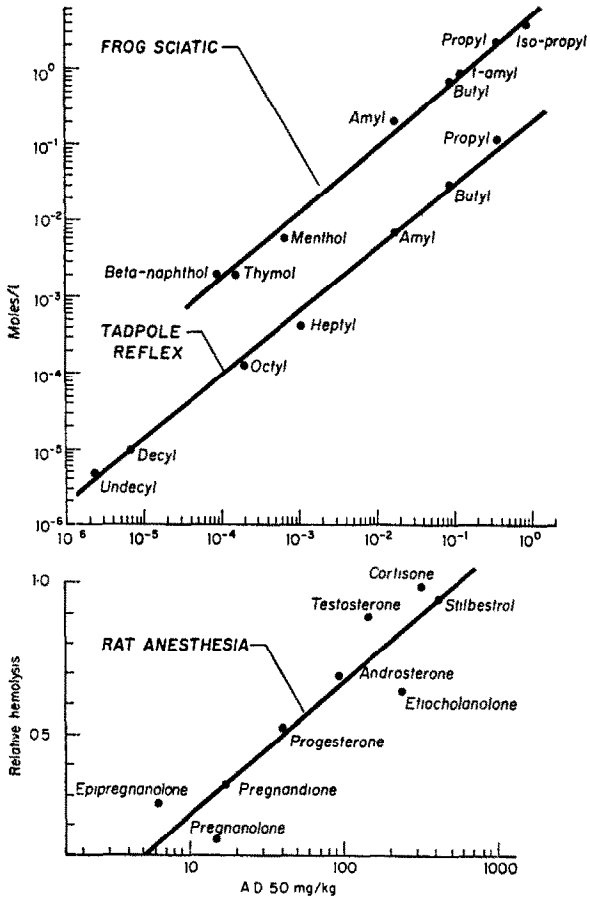

FIG. 3. A correlation is found between C_{50} (top abscissa in moles/l.; data taken from Fig. 1), the concentration at which the relative hemolysis is 0·5, and the concentration producing threshold conduction block of the frog sciatic nerve by the alcohols (top ordinate; data taken from Skou[9]). A correlation is also found between C_{50} and the molarity required for reversible suppression of the reflex response of tadpoles by alcohols (top ordinate; data from Meyer and Hemmi[8]).

The anesthetic doses of steroids (AD_{50}; lower abscissa in mg/kg; taken from P'an and Laubach[10]) correlate with the amount of erythrocyte stabilization that occurs at a steroid concentration of 7×10^{-5}M (data from Fig. 2).

With the experimental values of C_{50} and the activity coefficients for the corresponding alcohol the thermodynamic activities of the alcohols, A_{50}, were calculated ($A_{50} = C_{50} \cdot f$). The activity coefficients (f) of the aliphatic alcohols were obtained from the paper of Brink and Posternak;[12] those for the aromatic alcohols menthol, thymol, and β-naphthol were obtained by calculating the reciprocal of the solubility (as outlined by Brink and Posternak[12]). The thermodynamic activity

required for 50 per cent stabilization, A_{50}, was then plotted versus the number of carbon atoms of the alcohol, and this is shown in Fig. 4. It is seen that the A_{50} (in dimensions of mole fraction) is not quite constant but falls progressively with the higher alcohols. To apply the correction for molecular volume, as suggested by Mullins, each A_{50} value was multiplied by V_m, the apparent molal volume, as calculated directly from the experimental determinations of Traube.[14] The plot of $V_m A_{50}$ is shown at the top of Fig. 4 and is seen to be constant over the complete range of the anesthetic alcohols.

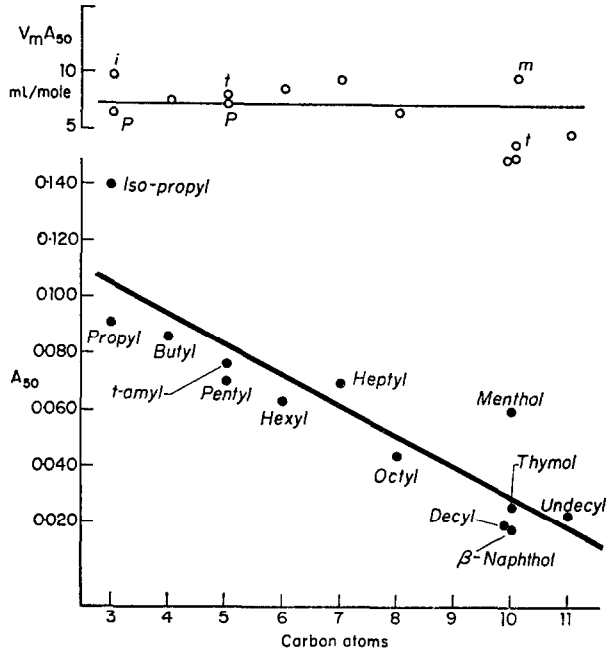

FIG. 4. Some experimental support for the idea that "equal degrees of narcosis occur with equal volume fractions of narcotic in the membrane" (Mullins[13]). A_{50} represents the thermodynamic activity of the alcohol that confers 50 per cent stabilization of the erythrocytes against hypotonic hemolysis. A_{50} was calculated from C_{50}, by means of the activity coefficients (taken from Brink and Posternak[12]) or calculated from the solubility. A_{50} is not constant with increasing number of carbon atoms; however, if A_{50} is corrected for the apparent molal volume (Traube[14]), a constant value is found which is independent of the number of atoms of the alcohol.

The results in Fig. 4 lend some experimental support to the idea that equal-volume fractions of narcotic within the membrane lead to equal degrees of narcosis.

It is hoped that other investigators, particularly those working with the noble gases and volatile anesthetics, will be encouraged to test the erythrocyte in this way.

SUMMARY

Low concentrations of aromatic alcohols, aliphatic alcohols, and steroids protect or stabilize human erythrocytes against hypotonic hemolysis; higher concentrations emulsify the cells. The values for stabilization by the alcohols exhibit a correlation with the molarities required for the reversible suppression of the tadpole reflex and also with the minimal blocking concentrations of the alcohols on frog sciatic nerve. The anesthetic doses of the steroids correlate with the amount of erythrocyte stabilization that occurs at a steroid concentration of 7×10^{-5}M. The data appear to support the idea that the volume fraction of the narcotic within the membrane is important.

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Sensitivity to norepinephrine of isolated atria from scorbutic guinea pigs

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IT IS well documented that tissues with postganglionic sympathetic innervation, including the heart, can take up norepinephrine from the circulating blood or from the surrounding fluid.[1] This uptake is probably an important mechanism of rapid inactivation and very likely determines to a large extent the intensity and duration of the effects of norepinephrine. Cocaine efficiently blocks the uptake of exogenous norepinephrine by tissues.[2, 3] Inactivation of norepinephrine is therefore delayed and tissues become more sensitive to it, since norepinephrine may reach higher concentrations at the receptors of the effector organs. Trendelenburg[4] has further suggested that "prevention by cocaine of the uptake of an amine causes a supersensitivity whose magnitude is proportional to the rate of uptake of that amine."

Similarly, chronic denervation of sympathetically innervated organs also causes supersensitivity to norepinephrine. When the nerves degenerate the organ is unable to take up catecholamines. On the basis of these observations some workers have proposed that any procedure, chemical or surgical, which interferes with the uptake of norepinephrine will cause supersensitivity.

It must be realized that uptake is a complex phenomenon, which consists of two steps: (1) transport into the nerve terminals and (2) subsequent binding in the norepinephrine stores.[5] Pretreatment with reserpine leaves the first step intact and prevents the second, while cocaine seems to prevent the transport into the nerve terminal. The first step appears to be important as a factor limiting the concentration of norepinephrine at the receptors of the effector organ; procedures that interfere with it cause supersensitivity to norepinephrine.

It has been demonstrated that the induction of scurvy in guinea pigs is associated with hyperresponsiveness to the pressor and cardiac inotropic effects of injected catecholamines.[6] To the contrary, the present study shows that isolated atria from scorbutic guinea pigs are normal in their response to norepinephrine.