NARCOSIS AND EMULSION REVERSAL BY INERT GASES*

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INTRODUCTION

This work represents a continuation of a series of studies from this laboratory in which it was shown that nitrogen and other inert gases at sufficient pressures are able to exert a true narcotic effect (1-4). Although this was first demonstrated only in reactions involving a synapse it also occurs with higher pressures in peripheral nerve. The present experiments were initiated in an effort to elucidate further the mechanism of this narcotic effect exerted by a chemically inert molecule. Clowes (5) showed that Ca, alcohols, and certain other narcotics are able to reverse emulsions stabilized with sodium soaps from the oil in water to the water in oil state and Hirschfelder and Serles (6) have used this model to explain the antagonistic action of Ca and Mg. We proposed to determine whether reversal could also be observed with the inert gases.

Experimental Procedure

As suggested by Clayton (7) resistance to current flow was used to determine the state of the emulsion. When water is the continuous phase, the emulsion will conduct a current provided a sufficient concentration of an electrolyte is present, whereas if the oil phase is continuous, little or no current will flow.

The emulsion was prepared in a glass-lined steel chamber which had a volume of 30 ml. Through the wall of the chamber were inserted two platinum electrodes through which the resistance was determined with a Wheatstone bridge and direct current while the emulsion was being shaken. In later experiments we used alternating current and found no essential difference in the values obtained.

The emulsions formed consisted of 5 ml. of olive oil and 5 ml. of 4 mM NaOH solution. The exact volume ratio did not seem to be critical in determining the state of the emulsion. The free fatty acid present in the oil reacted with the NaOH to form soap which stabilized the oil in water emulsion. The shaking of the emulsion was standard-

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1 We are indebted to the Pfaunder Company of Rochester, New York, for their courtesy in applying the glass lining to the chamber.
ized as to time and amplitude with a Burrell wrist action shaker. Before use the various gases were transferred to smaller tanks containing 50 ml. of 1 N NaOH to absorb any traces of CO₂ present.

Controls were alternated with the experimental emulsions. They consisted of emulsions formed in the same way and subjected to exactly the same conditions except for the pressure of gas.

We standardized the procedure as follows: shake at maximum amplitude for 5 minutes; add the gas; shake for 10 minutes; allow to stand for 10 minutes; then shake for 5 minutes. The resistance was measured at definite times during the procedure, and any pressure of gas was considered below the threshold if it did not reverse the emulsion by the end of the last period of shaking. All experiments were run at room temperature.

RESULTS

Prior to any experiments with gases we determined the amounts of CaCl₂ required to reverse oil in water emulsions formed with varying amounts of NaOH. The purpose was to characterize an emulsion which was definitely oil in water, but which required a minimum of CaCl₂ to effect reversal. The data shown on Fig. 1 were obtained from emulsions of two different volumes (4 ml. and 20 ml.) consisting of equal parts of oil and water.

The dotted line shows the chemical equivalence of calcium and sodium. The amount of calcium required to reverse fell somewhat below this line. Since pure olive oil and water when shaken vigorously together give a water in oil emulsion, we felt that a minimum amount of Na was necessary to stabilize an oil in water emulsion and that the reduction of the Na concentration by Ca to this value would cause reversal before all the Na was replaced. Calcium apparently replaces sodium in combination with free fatty acids and when all the soap has thus reacted further addition of sodium is without effect. This explains then the plateau reached in Fig. 1. The fact that the two graphs at two different volumes did not reach a plateau at the same concentration of Ca may possibly be attributed to differences in the intensity of shaking and in the oils used.

As a result of these preliminary experiments we decided to use emulsions stabilized with 0.004 M NaOH, since this was sufficient to prevent auto-reversal without making the emulsion insensitive to reversing agents.

Table I summarizes the effects of the various gases on the state of such emulsions. No reversals were obtained with helium at the highest pressures available. Sixteen experiments with N₂O at pressures up to 53 atmospheres did not cause reversal. Higher pressures were not available since the gas liquefies at 71.8 atmospheres at 21°C. Nitrogen required pressures above 100 atmospheres while argon seemed to be effective at pressures above 60 atmospheres. While the greater effectiveness of argon compared to nitrogen seems well established by these data, the determination of the exact magnitude of the difference would require further experimentation.
Carbon dioxide was very effective in reversing the emulsions. To measure the amounts required we placed emulsions with 30 ml. of oil and 30 ml. of either 4, 8, or 16 mm/liter NaOH in a flask which was then evacuated to a pressure of 30 to 50 mm Hg at room temperature. When CO₂ was introduced into the flask in small amounts the pressure first increased promptly and then fell slowly toward its previous level as CO₂ was absorbed by the emulsion. By recording the initial and final pressures we were able to determine

![Graph showing concentrations of CaCl₂ required to reverse oil-in-water emulsions at different concentrations of NaOH.]

**Fig. 1.** Concentrations of CaCl₂ required to reverse oil-in-water emulsions at different concentrations of NaOH. Two experiments with 4 and 20 ml. of emulsion, respectively.

**TABLE I**

<table>
<thead>
<tr>
<th>Gas</th>
<th>Pressure atmospheres</th>
<th>No. of cases</th>
<th>Water in oil</th>
<th>Oil in water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helium</td>
<td>80-107</td>
<td>0</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7-53</td>
<td>0</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Nitrous oxide</td>
<td>130</td>
<td>5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td>100</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Argon</td>
<td>67-87</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60-80</td>
<td>11</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>33-53</td>
<td>2</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>
the amounts of CO₂ absorbed and the final pressure of the gas. If this amount failed to reverse the emulsion more CO₂ was added and so on until it was visually apparent that the emulsion had reversed.

These data are shown in Fig. 2. The upper points (hollow circles) represent the average total amounts of CO₂ which went into the emulsion, the standard error is represented by the bars. The lower points (solid circles) are the amounts calculated by difference to be in the water phase on the assumption that the oil phase is saturated at the partial pressure of CO₂ remaining after equilibration. The straight line is drawn through the points of chemical equivalence of HCO₃⁻ and Na⁺. It is evident that reversal occurs approximately at the point where all the Na is in the form of NaHCO₃.

**DATA**

**DISCUSSION**

Bancroft's theory (8) of emulsions states that if two immiscible liquids are shaken together the one with the lower interfacial tension will surround the one with the higher. Or in other words, the phase with the higher tension...
will round itself into a sphere—the position with the lowest surface energy—and the other phase will, as a result, surround it. This state can be changed if the interfacial tension of the dispersed phase is lowered below that of the continuous phase.

By occupying space in the surface of either the water or the oil phase, an inert particle could lower the interfacial tension and this would reverse the emulsion if the amount accumulated in the dispersed phase were sufficient to lower its interfacial tension below that of the continuous phase.

The accumulation of an inert gas molecule at the interface would be expected according to Roberts’ postulation that the absorption of a substance at an interface will increase as the ratio of electrical charge/mass for that substance decreases (9).

According to these ideas any inert gas would be expected to reverse an emulsion if it possessed two properties: (1) a tendency to partition itself into the oil phase rather than the water phase, and (2) the ability to lower the interfacial tension of the oil below that of the surrounding water.

Comparison of the solubility characteristics of the gases used is given in Table II.

From column I we would expect the order of effectiveness of gases in reversing an emulsion to be \( \text{Ar} > \text{N}_2 > \text{N}_2\text{O} > \text{He} > \text{CO}_2 \). Argon actually did seem to have a lower threshold than \( \text{N}_2 \). Since the maximum pressure of \( \text{N}_2\text{O} \) available is 53 atmospheres it was not possible to determine whether its emulsion reversal threshold really exceeds those of argon and nitrogen but it was certainly closer to these than would be expected from its oil solubility. The ineffectiveness of \( \text{N}_2\text{O} \) at 53 atmospheres is explainable from the oil/water solubility ratio, but from oil solubility alone \( \text{N}_2\text{O} \) would be expected to be the most effective of the gases used.

### Table II

<table>
<thead>
<tr>
<th>Gas</th>
<th>Oil/water solubility 37°C</th>
<th>Oil solubility Bunsen coefficient 37°C</th>
<th>Emulsion reversal pressure atmospheres</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argon*</td>
<td>5.3</td>
<td>0.14</td>
<td>60</td>
</tr>
<tr>
<td>Nitrogen*</td>
<td>5.2</td>
<td>0.067</td>
<td>100</td>
</tr>
<tr>
<td>Nitrous oxide</td>
<td>3.2‡</td>
<td>1.6‡</td>
<td>53+</td>
</tr>
<tr>
<td>Helium*</td>
<td>1.7</td>
<td>0.015</td>
<td>107+</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sources of data for table:
|| Cotton seed oil at 40°C.
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Helium did not cause reversal at pressures where \( \text{N}_2 \) was effective. Since higher pressures of helium were not available it was not possible to determine whether helium at any pressure will reverse an emulsion, but certainly it is less effective than nitrogen or argon. So far as they go, these data therefore support the parallelism between effectiveness of emulsion reversal and oil/water solubility ratios for \( \text{Ar} \), \( \text{N}_2 \), and \( \text{He} \). The data for nitrous oxide are not in conflict with this theory but are definitely not in accord with the oil solubility order.

The great effectiveness of \( \text{CO}_2 \) we attributed to its chemical action since from the oil/water solubility ratio it should require greater pressures than helium before causing reversal, and from the oil solubility data it should be second to nitrous oxide in effectiveness.

These experiments suggest therefore that narcosis may be produced by accumulation of the inert gases in the lipide in excess of the amount in the watery components of the membrane and in amounts sufficient to render the lipide phases more continuous; thus the membrane becomes stabilized and a region such as the synapse does not conduct an impulse.

CONCLUSION

Investigations of the effect of high pressures of \( \text{N}_2 \) (100 to 130 atmospheres) and of \( \text{Ar} \) (60 to 80 atmospheres) showed that these gases are effective in reversing the phases of an oil in water emulsion. Nitrous oxide did not cause reversal at pressures as high as 53 atmospheres nor did helium as high as 107 atmospheres. We found \( \text{CO}_2 \) most effective in reversing the emulsions and attributed this to its chemical properties. It is suggested that these observations may help to explain the narcotic effects of inert gases.

REFERENCES