

Table 1. EFFECT OF DMI ON FOOD INTAKE

Food deprivation (h)	0									16								
	0			11			0			11								
Water deprivation (h)	0			11			0			11								
DMI dosage ($\mu\text{moles} \times 10^{-4}$)	0			24			90			0			24			90		
Rat No.																		
1	3.4	3.3	1.9	3.4	3.3	3.1	6.8	7.7	7.5	6.8	7.1	8.7						
2	4.3	1.8	2.4	2.1	3.5	1.9	7.1	9.5	9.3	6.9	8.8	6.9						
3	0.5	1.2	2.7	2.1	1.8	1.5	5.2	6.6	7.0	5.6	4.4	5.7						
4	2.0	3.3	3.2	3.0	2.3	3.0	6.5	6.8	6.7	5.0	4.9	5.9						
5	1.4	2.2	2.9	0.8	2.9	1.8	6.7	6.4	7.4	4.6	5.5	5.1						
6	0.8	0.6	1.4	1.5	1.2	2.1	5.8	7.9	9.3	7.2	8.5	8.5						
7	3.0	2.7	0.9	2.1	3.2	1.7	6.3	7.5	6.2	5.7	6.3	6.8						
8	1.9	0.9	1.4	1.8	1.2	1.7	6.6	7.5	6.6	6.6	5.4	7.7						
9	3.0	2.8	2.9	2.2	1.4	1.7	5.3	6.2	7.9	6.2	6.6	5.7						
10	2.6	3.5	2.4	3.3	2.6	3.6	6.0	9.4	9.5	7.6	8.6	7.1						
Mean	2.3	2.2	2.2	2.2	2.3	2.2	6.2	7.6*	7.7*	6.2	6.6	6.8						

Results are expressed as food intake (g) in the hour after injection.

* Difference from placebo significant at $P \leq 0.05$.

of cannulae was checked by testing for noradrenaline-induced eating. Food intake in the hour following injection was measured. No water was available during the test period.

Owing to interaction of the control of food and water intake, four conditions of food and water deprivation were considered. DMI was given at two doses, 24 and 90×10^{-4} μmoles . The solutions of DMI were made isotonic to cerebrospinal fluid with sodium chloride; isotonic saline (0.9 per cent⁹) was used as a placebo. Table 1 shows the effect of the two dose levels of DMI on food intake of ten rats in four states of food and water deprivation.

DMI at both dose levels (24 and 90×10^{-4} μmoles) caused a significant increase in food intake in water-satiated but food-deprived rats. No significant difference between the responses to the two dose levels of DMI was observed. The drug, in food-satiated rats or in food-deprived and water-deprived rats, had no significant effect on food intake. These results demonstrate that, in the food satiated rat, the lateral hypothalamus is unresponsive to a drug known to potentiate the effects of noradrenaline^{10,11}, suggesting that there is little release of noradrenaline. It is not fully understood why DMI failed to potentiate food intake when both the cholinergic and adrenergic systems were activated, although it may have been due to an interaction similar to that demonstrated in the amygdala⁸. After a period of food-deprivation only, however, DMI effectively increased food intake. The present study provides additional evidence to support the concept of noradrenaline release in the lateral hypothalamus during natural hunger.

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Surface Area per Lipid Molecule in the Intact Membrane of the Human Red Cell

MODELS for the structure of the red cell membrane vary according to the assessment of the area occupied by the lipids in the cell surface. In 1925, Gorter and Grendel¹ found the area of a monomolecular film of extracted lipids at an air-water interface to be twice that of the cell surface area and postulated a lipid bilayer in the membrane. Dervichian and Macheboeuf² used similar techniques and obtained a ratio of 1:1. Bar *et al.*³ found that the ratio depends on the surface pressure used in compressing the film: higher pressures lead to a lower area per lipid molecule and hence to a lower ratio of lipid area to membrane area, and lower pressures lead to a higher ratio. There is, however, little basis for deciding which pressure best represents the situation of the lipids in the intact membrane.

I believe that the surface area occupied by lipid molecules in the membrane can be calculated directly without the use of surface area measurements of extracted lipids. The most straightforward approach would be to determine the total number of lipid molecules per cell and then to obtain an area per molecule directly from the cell surface area; however, the different areas for cholesterol and phospholipid molecules would not be obtained. My approach permits calculation of equivalent surface areas for each component and also of a thickness for the hydrocarbon region. I start by determining the total volume occupied by the hydrophobic portions of the lipids and, taking the volume of the hydrophobic part of a given lipid, calculate its equivalent surface area from the total area of the membrane. One assumes that (a) all the lipid of the cell is in the membrane; (b) the area of the membrane is equal to the area of the cell surface as seen in the light microscope; (c) the cholesterol molecule is located in the hydrophobic portion of the lipid; (d) the amount of water in the hydrophobic portion of the lipid is negligible; (e) the volumes of the hydrocarbon chain groups are like those in liquid hydrocarbons; and (f) the hydrophobic region is of uniform thickness over the whole cell surface.

Excluding the first (hydrophilic) carbon, the volume of the average fatty acid or fatty aldehyde chain of n carbons and b double bonds is

$$v = (n - 2b - 2) \text{CH}_2 + \text{CH}_3 + 2b \text{CH}$$

where CH_2 , CH_3 and CH , the volumes of the respective groups, are 54.0, 27.0 and 21.5 \AA^3 (based on liquid hydrocarbons)⁴. The average chain has $n = 17.5$ carbons and $b = 1.26$ double bonds⁴, so $v = 459 \text{\AA}^3$.

The total hydrophobic lipid volume can be calculated from the total mass of lipid per cell, m , knowing the composition of the lipid portion of the membrane and considering a mixture of phospholipids each having two chains of average length. It is

$$V_p = \frac{2v F_p m N}{M_p}$$

where F_p is the weight fraction of phospholipid, M_p the average molecular weight and N the Avogadro number.

Similarly, for the non-cholesterol neutral lipids and glycolipids

$$V_g = \frac{2v F_g m N}{M_g}$$

The volume of cholesterol (including cholesteryl esters in F_c) is

$$V_c = \frac{F_c m}{\rho_c}$$

where ρ_c is the density of cholesterol.

The total hydrophobic volume is then

$$V_h = V_p + V_g + V_c, \\ = 2vmN \left(\frac{F_p}{M_p} + \frac{F_g}{M_g} \right) + \frac{F_c m}{\rho_c}$$

From a large body of data reviewed and summarized by van Deenen and de Gier⁵ we obtain: $M_p=780$; $F_p=0.58$; $F_c=0.25$; $M_g=900$; and $F_g=0.17$; and, assuming that the crystalline density of cholesterol, $\rho_c=1.067$, is appropriate¹⁴, $V_h=0.85 \text{ m cm}^3$.

The area per phospholipid (or glycolipid) is given by the fractional volume occupied by the two hydrophobic chains times the cell area (see assumption *f*)

$$A_p = \frac{2vA}{V_h} = 1.22 \times 10^{-21} \frac{A}{\text{m}} \text{ cm}^2$$

(Errors due to compositional inaccuracies tend to cancel: for example, a 10 per cent error in estimating the average chain volume will only produce about 3 per cent error in the area per molecule.)

Four recent values⁵ for m together with one more recent value⁷ give $m=(4.99 \pm 0.07) 10^{-13} \text{ g/cell}$.

The cell area has been calculated as 145 ± 8 microns² using the analysis of Ponder⁸ with current measurements of human red cell dimensions⁹, so the equivalent area per phospholipid (or glycolipid) is $A_p=35.5 \pm 2.0 \text{ \AA}^2$.

Similarly, the equivalent area for a cholesterol molecule is

$$A_c = \frac{M_c A}{\rho_c V} = 23.0 \pm 1.3 \text{ \AA}^2$$

The thickness of the hydrophobic layer, T_h , can also be calculated

$$T_h = \frac{V}{A} = 26 \text{ \AA}$$

Inaccuracies in estimating the hydrophobic volumes of the lipids do not tend to cancel, however, and the value obtained is consequently less precise.

The combined equivalent area occupied by one cholesterol molecule and one phospholipid molecule would be 58.5 \AA^2 . The area of such 1:1 combinations in monolayers at high compression is 90–105 \AA^2 for human red cell lecithin and cholesterol^{13,10} and, generally, 100–110 \AA^2 for combinations of cholesterol with a variety of phospholipids¹⁰. Furthermore, the area per phospholipid may be compared with the area of two closely packed hydrocarbon chains in the hexagonal phase of a liquid paraffin, which would exceed 40 \AA^2 (ref. 11), and the cholesterol area with the minimum obtained for fully compressed cholesterol films, 35 \AA^2 (ref. 12). Clearly, there is too much lipid to form a smooth monolayer over the surface of the red cell.

If the possibility of a lipid bilayer is considered, the surface area available to the lipids is twice the cell area and we obtain: $A_p=71 \pm 4 \text{ \AA}^2$; $A_c=46 \pm 3 \text{ \AA}^2$; and $A_{p+c}=117 \pm 7 \text{ \AA}^2$. (If the straightforward calculation mentioned in the introduction is made, the surface area for two lipid molecules in a bilayer would be

$$A_{L+L} = \frac{4A}{mN \left(\frac{F_p}{M_p} + \frac{F_c}{M_c} + \frac{F_g}{M_g} \right)} = 124 \pm 7 \text{ \AA}^2$$

Because both A_{p+c} and A_{L+L} are functions of A/m , they do not overlap in consequence of the standard deviations shown. The value of A_{L+L} is somewhat larger than A_{p+c} because less than half of the molecules are cholesterol.)

The area per phospholipid in the case of the bilayer may be compared with areas obtained from X-ray diffraction studies of mixtures of naturally occurring phospholipids. In the lamellar phase and at high water content the area per phospholipid has been found to be 60–72 \AA^2 (ref. 15). For a 1:1 combination of cholesterol and

phospholipid in extracted red cell lipids in the lamellar phase the combined area is about 86 \AA^2 at 0° C (ref. 16); but the area at 37° C would be substantially larger (~10 per cent based on the thermal behaviour of the K⁺ soaps¹⁵). Thus the equivalent surface areas for the lipid components of the red cell are roughly 10–20 per cent larger than those expected for a simple bilayer configuration. The larger areas could be explained in terms of a broadly conceived bilayer model in which proteins penetrate the lipid headgroup layers to make hydrophobic contact with the hydrocarbon chains, thereby increasing the area per molecule¹³, or in terms of a bilayer which is interrupted in places by non-lipid regions, for example, by protein structures extending through the membrane.

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Note added in proof. A similar, but not identical, treatment is in preparation by D. Branton and D. Deamer and will appear in *Protoplasma*.

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Increment Thresholds in Photopic Conditions in the Hooded Rat

It is usually assumed that the rat retina contains few, if any, cones^{1,2}. If this is the case, the rods might saturate in photopic conditions, as in man³ and monkeys⁴. The question is of considerable importance in view of the great amount of experimental work on vision that has been done with these animals, for if rod saturation occurs the level of illumination chosen in different experiments would be crucial.

Increment thresholds were determined in the present experiment using ten hooded rats and a Lashley jumping stand. Stimuli consisted of 10 cm diameter circles, projected onto the backs of the 14 cm square translucent doors of the jumping stand. Backgrounds were provided by a 1,000 W projector mounted behind the jumping platform. Both the background and the stimulus were of tungsten light. Their intensities were controlled by neutral density filters and measured with an S.E.I. photometer.