Table 1. Trials to criterion for six subjects in five experiments.

Experiment				
I	II	III	IV	V
110	50	30	70	140
30	40	20	40	140
80	50	30	60	140
90	60	20	30	140
130	70	20	50	140
60	50	20	50	140
		Means		
83	53	23	50	140
		S.D.		
32.5	9.4	4.7	12.9	0

forced odor. In this experiment all subjects reached criterion within 70 trials; the mean number of trials to criterion was 53, with a standard deviation of 9.4.

Males and females provided the odor stimuli to be discriminated in experiment III: two male C57B1 *Mus musculus* in odor-box 2 supplied the reinforced odor; two such females were the source of unreinforced odor. All subjects reached criterion within 30 trials; the mean number of trials to criterion was 23, with a standard deviation of 4.7. Each of two subjects required 30 trials; the remaining four required 20 trials each.

For experiment IV the discrimination to be made was between the odors of two male C57B1 *Mus musculus*; the two were neither litter mates nor litter mates of the test animals. All subjects reached criterion within 70 trials; the mean number of trials to criterion was 50, with a standard deviation of 12.9. Individual scores ranged from 30 to 70 trials.

The data from experiments II, III, and IV suggest that the mice may have had an initial preference for the rewarded odor stimuli; the question of whether or not such preferences existed is irrelevant to the purpose of this study. Ability in odor discrimination is demonstrated equally well whether performance results from reinforcement of natural preferences or from development of new ones.

In experiment V the six subjects followed the same procedure as for the previous four experiments, with the blower on, except that no odor stimuli were placed in the odor boxes. This procedure was designed to control for extraneous cues emanating from the odor boxes: for example, auditory or residual odor characteristics peculiar to one of the odor boxes. If such cues were significant the mice should have demonstrated discrimination in this experiment. The results show that after 140 trials none of the subjects reached criterion or showed any consistent improvement. The possibility that discrimination in experiments II–IV was based on sounds produced by the mice in the different boxes can be excluded on the basis of two considerations: First, since adult mice produce noise only sporadically, the probability was extremely small that these cues sufficed to enable the subjects to reach the 90-percent criterion of performance. Second, the noise produced by the blower was very loud and covered a wide range of frequency, probably masking any animal sounds.

These results indicate that, under appropriate experimental conditions, mice can readily discriminate closely related species (experiment II) and sexes (experiment III) on the basis of odor cues alone. This ability in discrimination can be interpreted in terms of its functional and adaptive value for the social behavior of mice. Ability to discriminate the odors of conspecific from odors of extraspecific animals probably serves as part of the isolating mechanism, ensuring the survival and integrity of a species by keeping it separated from sympatric and adjacent species. Similarly, sexual recognition facilitates the distant location of sex partners, making reproduction in this semisolitary animal a more efficient process.

Functional interpretation of the individual-discrimination ability found in mice (experiment IV) is more difficult. Individual recognition may be important to ambulatory pups, aiding them in locating the proper nest. However, there is no real support for the implication of individual discrimination in this and similar behaviors, since other environmental cues may guide them equally well. An alternative hypothesis for the existence of this highly developed individual-discrimination capacity is that it evolved only as a by-product of the necessity to discriminate between their own and closely related species. Interspecies discrimination requires the development of an extremely acute olfactory sense, because an animal often shares part of his range with genetically similar species probably having very similar odors.

One should not assume from the results of this study that individual recognition occurs in nature, or that olfaction is necessarily the primary modality employed for individual recognition; however, the knowledge that odor cues, and the capacity to use them, do exist should prove useful in future investigations of the behavior of wild mice.

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References and Notes

1. A. S. Parkes and H. M. Bruce, *Science* **134**, 1049 (1961).
2. W. J. Carr and W. F. Caul, *Anim. Behav.* **10**, 20 (1962); J. Chanel and E. Vernet-Maury, *J. Physiol.* **55**, 121 (1963); R. E. Moore, *Dissertation Abstr.* **23**, 4032 (1963).
3. H. M. Bruce and O. M. V. Parrott, *Science* **131**, 1526 (1960).
4. W. C. Allee, *ibid.* **95**, 289 (1942); W. Etkin, *Social Behavior and Organization Among Vertebrates* (Univ. of Chicago Press, Chicago, 1964), p. 15.
5. Dispensed from a standard medicine dropper; each contained approximately 0.05 ml.
6. L. W. Gellerman, *J. Genet. Psychol.* **42**, 207 (1933).
7. We thank E. Valenstein, E. Simmel, O. Dent, and B. Morrison for critical review of this report.

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Osmotic Mechanism and Negative Pressure

Scholander's conclusion that a solvent attains a negative hydrostatic pressure in solution (*1*) is a logical consequence of his assumption that the interaction between solute and solvent is localized to the near vicinity of the solute molecule. It does not depend upon the solute bombardment mechanism for osmotic pressure which he presents. He makes this assumption explicitly in his earlier paper (*2*) by the statement ". . . the water fabric, as it were, although *interrupted* by the solute molecules, evidently is not otherwise altered." This assumption essentially restricts any changes in the activity of the water in a solution to changes in hydrostatic pressure. If the only way the activity of water in a solution can change is through its hydrostatic pressure, at equilibrium the hydrostatic pressure of water must be the same in all parts of a system. Since it is an experimentally proved fact that the hydrostatic pressure of pure water in equilibrium with a solution through a semipermeable membrane is negative with respect to that of the solution, it follows from the assumption stated above that the hydrostatic pressure of the water within the solution must also be negative with respect to that of the solution.

Thus, the whole argument of whether or not the hydrostatic pressure of water in a solution is negative reduces to an argument of the validity of the assumption that the structure of water in a solution is essentially unaltered by the solute molecules. From the work of Choppin and Buijs (*3*) (and other references cited therein), it would appear that this question is not yet closed. It is still possible that the activity of water in a solution could be lowered by interaction with the solute independently of the hydrostatic pressure of the water. It is also possible that the same thing could occur by interaction of water with solid surfaces. It is the neglect of this possibility that Gardner and Rawlins (*4*) raised in criticism of an earlier article by Scholander *et al.* (*5*).

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References and Notes

1. P. F. Scholander, *Science* **156**, 67 (1967).
2. ———, *Proc. Nat. Acad. Sci. U.S.* **55**, 1407 (1966).
3. G. R. Choppin and K. Buijs, *J. Chem. Phys.* **39**, 2042 (1963).
4. W. R. Gardner and S. L. Rawlins, *Science* **149**, 920 (1965).
5. P. F. Scholander, H. T. Hammel, E. D. Bradstreet, E. A. Hemmingsen, *ibid.* **148**, 339 (1965).

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Scholander (*1*) has revived the old explanation of osmotic pressure by analogy with gas pressure, as follows: "When a mole of gas fills a liter flask at 20°C, it exerts a pressure of +24 atm on the bottle. . . . Similarly, when a mole of solute molecules is confined within a liter of water, the molecules exert a pressure of +24 atm on the retaining boundary." No attempt is made to justify this conclusion, which must, therefore, be considered as dogma. Let us examine the analogy, on which this dogma is based.

The original investigations of Van't Hoff on osmotic pressure led to the conclusion that the gas laws applied literally to osmotic pressure (*2*). The equation for the gas laws was actually applied as follows:

$$P = NRT/V \text{ for gas pressure} \qquad (1)$$

where N is the number of moles; P is the pressure; R is the gas constant; T is the absolute temperature; and V is the volume.

Since the molar concentration (C) of a solution can be expressed as

$$C = N/V \qquad (2)$$

the equation for osmotic pressure was modified to

$$P = CRT \qquad (3)$$

For dilute solutions, this equation was adequate. It was soon found, however, that the equation holds only for *very* dilute solutions of solutes such as sucrose. (The calculated value for a volume molar solution is only 70 percent of the measured value.)

Better agreement was obtained by including the properties of the solvent as well as the solute in the equation:

$$P = \ln \frac{N_1 + N_2}{N_1} \cdot \frac{RT}{V^1} \qquad (4)$$

where N_1 is the number of moles of solvent; N_2 is the number of moles of solute; and V^1 is the volume of 1 mole of solvent.

Finally, since according to Raoult's law:

$$p/p^0 = N_1/(N_1 + N_2) \qquad (5)$$
$$P = (RT/V^1) \ln (p^0/p) \qquad (6)$$

where p^0 is the vapor pressure of pure H_2O; and p is the vapor pressure of the solution.

At high concentrations, where the osmotic pressure calculated by the simple gas laws is in error by more than 30 percent, the values calculated from Eq. 6 check with the observed values within less than 1 percent (*3*).

The important conclusion is that when the equation is expressed in terms of the *solute*, the calculated values are in error; when it is expressed in terms of the *solvent*, the calculated values are correct. Obviously, then, the osmotic pressure produced *must* depend on the properties of the solvent and not on those of the solute. That osmotic pressure can be generated without the presence of "solute pressure" can be demonstrated as follows:

A 10 percent gelatin gel is prepared. It is then enclosed in a semipermeable membrane and immersed in an infinite volume of stirred, molar dextrose solution. Osmosis will occur from the gelatin gel into the dextrose solution, until the gelatin attains the same vapor pressure as the dextrose solution. The shrunken gel is now placed in an osmometer, and its osmotic pressure (due to matric forces) is determined in the standard way by the pressure required to stop osmosis. When the gelatin is removed and replaced by some of the molar dextrose solution, *exactly the same value will be obtained for its osmotic pressure as for that of the gelatin.*

This follows from the fact that the gelatin and dextrose have the same vapor pressures when transferred to the osmometer. Therefore, the same pressure must be applied to raise their vapor pressures to that of pure water. Since the gelatin molecules are part of a rigid structure, and are held permanently in place by intermolecular bonds between the molecules, no "solute pressure" can possibly occur when its osmotic pressure (from matric forces) is measured. Since the molar dextrose registers the same osmotic pressure as the gelatin and possesses the same vapor pressure, it must also exhibit zero "solute pressure." Therefore, the dogma of osmotic pressure due to "solute pressure" is invalid.

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References

1. P. F. Scholander, *Science* **156**, 67 (1967).
2. G. W. Scarth and F. E. Lloyd, *Elementary Course in General Physiology* (Wiley, New York, 1930).
3. F. H. Getman and F. Daniels, *Outlines of Theoretical Chemistry* (Wiley, New York, 1937).

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In his discussion of the mechanism of osmosis, Scholander (*1*) concludes that an inconsistency exists between two thermodynamic expressions related to the chemical potential of water: the Poynting equation and Raoult's law. However, when the fundamental relation between these two equations is examined, it does not appear that this conclusion is correct.

Scholander states that the lowering of vapor pressure for a solution is proportional to the solute mole fraction, according to Raoult's law, while the vapor pressure reduction for pure water is proportional to the solute mole ratio, according to the Poynting equation. Since the vapor pressures of a solution and pure water must be identical when they are in equilibrium across a semipermeable membrane, Scholander concludes that there is obviously an inconsistency between Raoult's law and the Poynting equation.

In Raoult's law

$$dp_g = p_0 \cdot N_s/(N_s + N_w), \qquad (1)$$

where dp_g is vapor pressure change, p_0 is vapor pressure at standard pressure

(1 atm), N_s is the number of moles of solute and N_w is the number of moles of water, there is a clear dependence of vapor pressure change on the solute mole fraction.

However, in the Poynting relation

$$dp_gV_g = dP_lV_l, \qquad (2)$$

where dP_l is liquid pressure change, V_g is gas molal volume and V_l is liquid molal volume, the solute concentration is not a basic part of the equation. Thus the source of Scholander's statement of the dependence of the Poynting equation on the solute mole ratio is not clear. In fact, if the pertinent thermodynamics are examined closely, it appears that Raoult's law and the Poynting equation are not inconsistent, but are in fact functionally related to each other as indicated below. In the Poynting equation vapor pressure change is a function of both liquid and gas molal volumes as well as liquid pressure change. Thus in determining a vapor pressure change the effect of any molal volume changes must be incorporated. While liquid volume can be considered a constant for all but extreme pressure changes, gas volume changes are significant and must be evaluated through the equation of state for an ideal gas

$$V_g = RT/p_g, \qquad (3)$$

where R is universal gas constant and T is absolute temperature. If Eq. 3 is substituted for V_g in the Poynting relation, Eq. 2, we obtain the following equation

$$RT\ dp_g/p_g = V_l\ dP_l$$

which can be integrated for the change in vapor pressure with either a positive or negative change in liquid pressure from standard atmospheric pressure.

$$RT \int_{p_0}^{p} \frac{dp_g}{p_g} = V_l \int_{0}^{P} dP_l$$

The result of this integration

$$P = (RT/V_l)\ln(p/p_0) \qquad (4)$$

is the familiar relation between the chemical potential of water and its vapor pressure (*2*). This equation can also be considered to be the Poynting equation incorporating the functional dependence between gas volume and its pressure.

If Raoult's law, Eq. 1, is rearranged to the following form

$$p/p_0 = N_s/(N_w - N_s)$$

we see immediately that the Poynting equation and Raoult's law are not inconsistent. Rather they are functionally related by the basic equation for water potential, Eq. 4, through the equation of state for an ideal gas. Thus Scholander's numerical evaluation of the Poynting equation, which differed from the evaluation of Raoult's law, must be incorrect.

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References

1. P. F. Scholander, *Science* **156**, 67 (1967).
2. R. O. Slatyer, *Plant Water Relationships* (Academic Press, New York, 1967), p. 21.

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The basic argument is that whenever solute molecules are confined in a solvent they exert pressure on the boundary by being reflected back. When a semipermeable membrane takes the stress, this empirically is the solute (equal to osmotic) pressure. When the boundary is the free solvent surface, simple balance of forces requires that the hydrostatic solvent pressure now becomes lowered by the solute pressure. Empirically this shows up by a lowering of the vapor pressure in accordance with Poynting's relation (*1*). Similarly, when water has been removed from an elastic gel, by pressure or evaporation, the matrix has become compressed and balance of forces requires that the water (which holds the gel together) becomes stressed by the same amount. In either case therefore, an expanding force acts on the free water surface. In Levitt's experiment he simply balances matrix pressure against solute pressure across a semipermeable membrane. The lowering of the vapor pressure in both components reflects quantitatively the lowering of the hydrostatic pressure of the water.

The numerical relation between osmotic pressure and concentration is an old battleground, and it still remains for Knoerr to show how Raoult's empirical mol fraction relation in the solution can give the same value for the vapor pressure as the related but different Poynting's equation for the solvent compartment.

We have verified important data from earlier workers. Thus, we find that a dextran solution with an osmotic pressure of 40 cm-H_2O pulls water through a dialyzing membrane at the same rate (within ±2 percent) as water of −40 cm hydrostatic pressure (*2–4*). Also, the flux of water is at least a thousand times greater than a diffusive flow would give (*3, 4*). For, at the same gradient, the volume of air going through our wet membrane per minute is approximately the same as that of liquid water going through. In terms of activity gradient the discrepancy would be vastly greater. Our 0.002 molal dextran solution pulls through a 0.1 molal sugar solution, or a 0.2 molal NaCl solution, at a rate of 90 to 95 percent of that of water. Thus, the flow goes against the osmotic pressure and is not driven by the water potential (*4*).

The mechanism for osmotic flow in the above system seems to be that the nonpermeant molecules pressing on the free surface strive to enlarge the space available for them, and hence pull through hydraulically all molecules that can penetrate the membrane. Imbibition and osmotic flux are generated, respectively, by matrix or solute pressure against a fluid boundary which is free to expand. The water itself, or the permeant solution, is a passive mediator like the oil in a hydraulic system. The hydrostatic pressure under which water operates in a truly semipermeable system is given away by its vapor pressure.

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References and Notes

1. It is a pleasure to acknowledge that Professor Karl Herzfeld of the Catholic University of America in a letter points out that 30 years ago he published an essentially similar idea [*Phys. Z.* **28**, 58 (1937)].

2. Earl of Berkeley and E. G. Hartley, *Proc. Roy. Soc. London, Ser. A* **82**, 271 (1909).
3. A. Mauro, *Science* **126**, 252 (1957)
4. G. Meschia and I. Stenikar, *J. Gen. Physiol.* **42**, 429 (1958).

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