therefore, evident from the present results that cyclic AMP mediates the activity of the β-receptor in the acinar parotid gland (*10*).

It has been suggested that the α-receptor exerts its effect by decreasing the amount of cyclic AMP in the cell (*10*). In the present work, propranolol, which blocks cyclic AMP production, had no effect on the α-adrenergic response (Fig. 1). Therefore, it seems unlikely that the α-receptor operates by either decreasing or increasing the cyclic AMP in the cell. Furthermore, the data shown in Fig. 1 indicate that the action of one receptor is not mediated by the inhibition of the other. However, since both receptors are present in the same cell (Fig. 2), it would not be surprising if the action of one receptor indirectly affects the other. As the α-adrenergic response in parotid gland and several other tissues results in K^+ release, a modulation of Na^+, K^+ adenosine triphosphatase might be involved (*11*).

The release of K^+ in nonglandular tissues has been studied by Ellis *et al.* (*3*) and others (*2, 12*), who concluded that the process is mediated by an α-receptor. In rat liver perfused in situ, K^+ release may be mediated by a β-receptor (*13*). This response occurs after some delay, compared to the rapid K^+ release caused by activation of α-receptors in other systems (*2, 13*). In experiments with whole animals, it may sometimes be difficult to determine which receptor is responsible for the K^+ release. We found that isoproterenol causes vacuole formation in the rat parotid gland in vivo (*5*). However, isoproterenol fails to do so in slices of the gland incubated in vitro. It is assumed that the relatively large dose of isoproterenol injected in vivo acted indirectly by displacing norepinephrine from nerve terminals. The endogenous catecholamine thus released presumably caused the observed α-receptor response.

Since norepinephrine released from nerve terminals appears to be the natural stimulant in the parotid system (*14*), under physiological conditions both α- and β-receptors will be activated simultaneously. Thus K^+ release and enzyme secretion will be concurrent. Ion movement, if it results in water transport, would facilitate enzyme secretion in the intact gland.

SAMUEL BATZRI
ZVI SELINGER, MICHAEL SCHRAMM
Department of Biological Chemistry, Hebrew University of Jerusalem, Jerusalem, Israel

References and Notes

1. B. Katz, *Nerve, Muscle and Synapse* (McGraw-Hill, New York, 1966), pp. 81, 86.
2. E. E. Danile, D. M. Paton, G. S. Taylor, J. Hodgson, *Fed. Proc.* **29**, 1410 (1970).
3. E. Ellis, B. L. Kennedy, A. J. Eusebi, N. H. Vincent, *Ann. N.Y. Acad. Sci.* **139**, 826 (1967).
4. A. S. V. Burgen and N. Emmelin, *Physiology of the Salivary Glands* (Arnold, London, 1961), pp. 148, 206.
5. S. Batzri, A. Amsterdam, Z. Selinger, I. Ohad, M. Schramm, *Proc. Nat. Acad. Sci. U.S.* **68**, 121 (1971).
6. I. R. Innes and M. Nickerson, in *The Pharmacological Basis of Therapeutics*, L. S. Goodman and A. Gilman, Eds. (Macmillan, New York, 1965), p. 477.
7. A. Bdolah and M. Schramm, *Biochem. Biophys. Res. Commun.* **18**, 452 (1965).
8. H. Babad, R. Ben-Zvi, A. Bdolah, M. Schramm, *Eur. J. Biochem.* **1**, 96 (1967).
9. M. Schramm and E. Naim, *J. Biol. Chem.* **245**, 3225 (1970).
10. G. A. Robison, R. W. Butcher, E. W. Sutherland, *Ann. N.Y. Acad. Sci.* **139**, 703 (1967).
11. B. Belleau, *ibid.*, p. 589.
12. D. H. Jenkinson and I. K. M. Morton, *Nature* **205**, 505 (1965); D. G. Haylett and D. H. Jenkinson, *ibid.* **224**, 80 (1969).
13. N. Friedmann, A. V. Somlyo, A. P. Somlyo, *Science* **171**, 400 (1971).
14. B. C. R. Strömblad and M. Nickerson, *J. Pharmacol. Exp. Ther.* **134**, 154 (1961); M. Schramm, *Biochim. Biophys. Acta* **165**, 546 (1968).
15. A. Amsterdam, I. Ohad, M. Schramm, *J. Cell Biol.* **41**, 753 (1969).
16. Supported by NIH grant 5 R01 10451-05 BIO.

15 June 1971; revised 4 August 1971 ■

Unstirred Water Layers in Intestine: Rate Determinant of Fatty Acid Absorption from Micellar Solutions

Abstract. *Bile acid and fatty acid uptake from micellar solutions by intestinal cells fails to reflect the incremental free energy changes expected for permeation that is rate limited by cell membranes. However, altering the size of the diffusing particle or the thickness of the unstirred water layer does change uptake. These observations show that the unstirred water layer is rate limiting for intestinal absorption of lipids from micellar solutions.*

While the presence of unstirred water layers adjacent to biologic and artificial membranes has been known for some years, only recently has their importance to the study of transport processes across these membranes become widely recognized (*1, 2*). We now present data indicating that such unstirred layers in the intestine are the major determinant of the rate of passive bile acid and fatty acid absorption from micellar solutions by the jejunal mucosal cells.

Bile acids and fatty acids are passively absorbed across the proximal small intestine (*3*). Theoretically, both the unstirred water layer adjacent to the luminal surface of the mucosal cell and the lipid cell membrane itself contribute to the resistance encountered by a molecule as it is absorbed from the bulk mucosal solution into the cell interior. Under these conditions flux of solute molecules across the unstirred layer will equal

$$(D/d)\ (C_1 - C_2) \qquad (1)$$

where D is the diffusion coefficient for the molecule, d is the thickness of the unstirred layer and C_1 and C_2, respectively, are the concentrations of the solute in the bulk phase and in the unstirred layer immediately adjacent to the luminal surface of the cell membrane. Flux across the cell membrane, on the other hand, will equal

$$P(C_2 - C_3) \qquad (2)$$

where P is the permeability coefficient for the solute molecule, and C_3 is its concentration at the interior surface of the cell membrane. Thus, the passive flux (J) of a solute into the jejunal cell is described by the equation

$$J = (D/d)\ (C_1 - C_2) = P(C_2 - C_3) \qquad (3)$$

Two limiting situations exist where either the unstirred layer or the cell membrane may become the major resistance to overall absorption. First, if P is very large relative to D/d, then diffusion across the unstirred layer becomes primarily rate limiting, and the effective concentration gradient for the solute equals that which exists between the bulk phase and the cell interior; thus,

$$J = (D/d)\ (C_1 - C_3) \qquad (4)$$

Since D for solute molecules of low molecular weight (M) is approximately inversely proportional to $M^{0.5}$, in this situation the product $JM^{0.5}$ (or in $JM^{0.5}$) for a series of solutes measured under identical conditions equals a constant (*4*).

In the second limiting situation where D/d is very large relative to P, permeability of the lipid cell membrane is rate limiting and essentially

$$J = P(C_1 - C_3) \qquad (5)$$

Further, P is proportional to $e^{\Delta F}{}_{w \to 1}$ where $\Delta F_{w \to 1}$ is the free energy change necessary to move 1 mole of the solute molecule from water into the cell membrane (*2*). Since P is rate limiting in

this situation the product ln $JM^{0.5}$ for different solute molecules will not equal a constant; instead, the quantity ln $JM^{0.5}$ will vary in a regular manner that is determined by the increment in $\Delta F_{w\to 1}$, that is, $\delta\Delta F_{w\to 1}$, that exists for absorption of different solute molecules of a homologous series. As was reviewed by Diamond and Wright (*2*), the addition of a particular substituent group to a molecule changes its rate of permeation by a constant factor. With the use of this factor it is possible to calculate the values of $\delta\Delta F_{w\to 1}$ for specific substituent groups (*2*).

These theoretical considerations provide the basis for the first line of evidence suggesting that under physiological conditions the rate of bile acid and fatty acid absorption from micellar solutions is determined primarily by the unstirred water layer. The removal of an -OH group (in the case of bile acid monomers) or the addition of a $-CH_2-$ group (in the case of fatty acid monomers) enhances passive absorption. The increase in J is not accounted for by changes in M so that ln $JM^{0.5}$ also increases in value. From these data the apparent incremental free energy values ($\delta\Delta F^*_{w\to 1}$) of +338 cal/mole and −285 cal/mole, respectively, were obtained for the addition of a -OH group to the bile acid molecule and a $-CH_2-$ group to the fatty acid molecule. These effects of -OH and $-CH_2-$ are in the usual direction for the effects of these groups on solute permeation limited by cell membrane in other biological structures (*2*). Thus, during absorption of these substances from monomer solutions D/d must be relatively greater than P, so that the cell membrane primarily is rate limiting to uptake.

In contrast, when fatty acid is dissolved in a bulky micelle (Table 1), J does not increase with chain length, ln $JM^{0.5}$ is constant, and the incremental free energy ($\delta\Delta F^*_{w\to 1}$) expected for the addition of a $-CH_2-$ group is not observed. These findings are consistent with the view that diffusion of the large micelle across the unstirred layer is rate limiting, so that P for the fatty acid must be larger than D/d for the movement of the micelle.

A second line of evidence involves a comparison of relative rates of tissue uptake for a bile acid from monomer and micellar solutions. The rate of taurocholate absorption, for example, equals 10.7 nmole min^{-1} mM^{-1} per 100 mg of tissue and 5.1 nmole min^{-1} mM^{-1} per 100 mg of tissue, respectively, from solutions containing monomers and micelles. Rates of 29.2 nmole min^{-1} mM^{-1} per 100 mg of tissue and 9.5 nmole min^{-1} mM^{-1} per 100 mg of tissue were obtained under comparable conditions with taurodeoxycholate. Thus, the ratio of the rate of the tissue uptake from micellar solution to the rate from monomer solution equaled 0.48 for taurocholate and 0.33 for taurodeoxycholate. If, for micellar structures of high molecular weight, D is inversely proportional to $M^{0.33}$, the diffusion constants of the micelles of these two bile acids relative to their respective monomers can be calculated to approximately equal 0.58 for taurocholate and 0.37 for taurodeoxycholate (*5*). Hence, there is close agreement between the decreased rate of absorption of these bile acids from micellar solutions and the expected lower rates of diffusion of the micelles across the unstirred layer—0.48 and 0.58, respectively, for taurocholate and 0.33 and 0.37, respectively, for taurodeoxycholate. Thus, this finding also is consistent with the view that the unstirred layer is rate limiting for absorption from micellar solutions.

Finally, reduction in the thickness of the unstirred layer, d, by vigorous stirring significantly enhances the rate of absorption from micellar solutions but not from solutions containing monomers (Table 2). Tissue uptake of fatty acid 6:0 and 8:0 (*6*) and of taurocholate from monomer solutions essentially is unaltered by stirring of the bulk solution—a finding that is consistent with the view that membrane penetration is rate limiting for the absorption of these solutes. In contrast, absorption of the fatty acid 16:0 and taurodeoxycholate from micellar solutions is increased by 114 and 125 percent, respectively, by stirring—a finding that again demonstrates the marked resistance offered by the unstirred layer to the movement of large structures such as micelles.

Our studies demonstrate that the rates of passive absorption of the bile acid and fatty acid constituents of the micelle do not manifest the relationship of $\delta\Delta F^*_{w\to 1}$ of substituent groups expected if membrane penetration were rate limiting. Further, these rates are inversely related to the size of the micelle and to the thickness of the unstirred layer. Taken together, these observations constitute the first strong evidence that during absorption of lipids contained in micelles the unstirred water layer adjacent to the cell and not the lipid cell membrane itself pri-

Table 1. Rates of uptake of bile acid and fatty acid from monomer solutions and of fatty acid from micellar solutions across the rat jejunal brush border. The rates of uptake J were determined from bulk solutions containing 0.2 to 0.5 mM concentrations of the test molecules, but the data are normalized to a concentration of 1.0 mM. The term ln $JM^{0.5}$ represents the natural logarithm of the product of J and the square root of the molecular weight of the test molecule, while $\delta\Delta F^*_{w\to 1}$ equals the apparent incremental free energy change for the addition of a substituent group in each series calculated as described in reference (*2*), that is, $\delta\Delta F^*_{w\to 1} = (\ln J^+/J^\circ)\ (-\ RT)$ where J^+ and J°, respectively, are the passive fluxes for the molecules with and without the specific substituent group.

Test substance	J†	ln $JM^{0.5}$	$\delta\Delta F^*_{w\to 1}$‡
A. From monomer solutions			
Taurodeoxycholate	26 ± 2	6.4	
Taurocholate	15 ± 3	5.8	+ 338
Fatty acid 7:0	36 ± 3	6.0	
Fatty acid 8:0	58 ± 4	6.5	− 294
Fatty acid 9:0	91 ± 5	7.0	− 277
B. From micellar solutions			
Fatty acid 14:0	18 ± 2	5.6	
Fatty acid 16:0	18 ± 3	5.6	0
Fatty acid 18:0	17 ± 2	5.6	0

† Everted jejunum of the rat was exposed to Krebs bicarbonate buffer (*p*H 7.4, 37°C) containing a labeled test substance and inulin for 3 to 4 minutes. After correcting for adherent mucosal fluid contamination, rates of absorption across the jejunal brush border (J) were calculated and are expressed as the mean (± 1 S.E.M.) number of nanomoles taken up per minute per 100 mg (dry weight) of tissue per 1 mM concentration of test substance in the bulk buffer solution (*7*). ‡ $\delta\Delta F^*_{w\to 1}$ is expressed as calories per mole. The bile acid and fatty acid in experiment A were dissolved in buffer alone while the long chain fatty acids in experiment B were dissolved in buffer in the presence of 20 mM taurodeoxycholate.

Table 2. Effect of stirring of the bulk buffer phase on the uptake of bile acid and fatty acid from monomer and micellar solutions. Test substances in experiment A were dissolved in Krebs buffer at 1.0 mM. In experiment B the fatty acid 16:0 was dissolved in buffer in the presence of 20 mM taurodeoxycholate, and uptake of taurodeoxycholate was determined from a 20 mM solution. The "stirred" incubation solution was agitated with a magnetic stirrer running fast enough to produce a distinct vortex within the incubation vessel. In other experiments it was shown that the measured adherent mucosal fluid volume was unaltered by stirring.

Test substance	J		Increase (%)
	Unstirred	Stirred	
A. From monomer solutions			
Fatty acid 6 : 0	30 ± 3	28 ± 2	−7
Fatty acid 8 : 0	50 ± 3	58 ± 4	16
Taurocholate	15 ± 3	14 ± 2	−7
B. From micellar solutions			
Fatty acid 16 : 0	21 ± 5	45 ± 8	114
Taurodeoxycholate	87 ± 6	196 ± 14	125

marily determines the overall rate of absorption. Thus, the reasons for the marked differences in rates of absorption of different lipid substances in the same species or of the same lipid in different species may be explained in terms of differences in the geometry or physical characteristics of this unstirred layer rather than in differences in the cell membrane.

FREDERICK A. WILSON
VERNEY L. SALLEE, JOHN M. DIETSCHY
Department of Internal Medicine, University of Texas Southwestern Medical School at Dallas, 5323 Harry Hines Boulevard, Dallas 75235

References and Notes

1. J. Dainty, in *Advances in Botanical Research*, R. D. Preston, Ed. (Academic Press, London, 1963), pp. 279–3 → E. M. Wright and J. M. Diamond, *Proc. Roy. Soc. London Ser. B* **172**, 203 (1969).
2. J. M. Diamond and E. M. Wright, *Annu. Rev. Physiol.* **31**, 582 (1969).
3. J. M. Dietschy, *J. Lipid Res.* **9**, 297 (1968); J. M. Johnston and B. Borgström, *Biochim. Biophys. Acta* **84**, 412 (1964).
4. W. D. Stein, *The Movement of Molecules across Cell Membranes* (Academic Press, New York, 1967), pp. 65–85.
5. D. M. Small, *Advan. Chem. Ser.* **84**, 31 (1968).
6. Number of carbon atoms: the number of double bonds.
7. V. L. Sallee, F. A. Wilson, J. M. Dietschy, *J. Lipid Res.*, in press.
8. This work was supported by PHS training grant TO1-AM-5490 and research grant HE-09610 and by a grant from the John and Mary Markle Foundation.

26 May 1971; revised 16 August 1971 ■

Desiccation-Tolerant Flowering Plants in Southern Africa

Abstract. *The South African flora contains a unique abundance of higher plants which withstand virtually complete desiccation. Water potentials of field-dry leaves corresponded to 30 to 40 percent relative humidity. Mature leaves survived from 15 to approximately 0 percent relative humidity. Known examples were increased from 4 to 15 species, which for the first time included grasses.*

Extremely few angiosperms ("flowering plants") possess mature foliage that is desiccation-tolerant. Of the ten species reported in the literature, four (*Chamaegigas intrepidus, Craterostigma plantagineum, Myrothamnus flabellifolia,* and *Xerophyta humilis*) occur in southern Africa, together with a number of reputedly drought-tolerant ferns. However, the drought tolerance (*1*) of these species has not been determined. No survival tests have been applied to establish the revival of the foliage beyond resumption of a superficially healthy appearance on rehydration, except for *Myrothamnus*.

In order to investigate these questions further, foliage was collected in a dry condition from the field during the dry season in 1970. Survival of tissue was judged by a combination of tests [regain of turgor on rehydration, neutral red uptake (*2*), exclusion of Evans blue (*3*), photosynthesis in chlorophyllous leaves (*4*), and formation of chlorophyll in nonchlorophyllous leaves], insofar as they were applicable to a particular species. It was found that the four African species survived desiccation under field conditions to water potentials equivalent to 33 percent relative humidity and less [determined by the gravimetric vapor exchange method (*5*); see Table 1]. Moreover, it became apparent that a number of other flowering plants and ferns growing in similar habitats were equally as hardy (see Tables 1 and 2).

The data for *Chamaegigas* (Table 2) was particularly striking; the basal "submerged" leaves of the minute water plant had a drought tolerance of 0 to 5 percent, its floating leaves 5 percent when folded together in the bud, but 96 percent when mature! Although drought tolerances of 0 percent have been reported for mosses and lichens (*6*), the lowest reliable value of drought tolerance previously reported for flowering plants was 85 percent relative humidity for plants of *Hordeum vulgare* 'Kearney' which had been hardened by exposure to low temperatures for several weeks (*7*). Preliminary tests on a number of other flowering plants indicated that they also were endowed with extreme drought tolerance, but time did not allow the tolerance to be measured (for example, *Xerophyta equisetoides* Baker, *X. squarrosa, X. villosa* = *Vellozia villosa* Baker).

The fact that four drought-tolerant grasses were found is particularly important, as no member of this economically important family has previ-

Table 1. Water stress of leaves (given as the relative humidity of air in equilibrium with the leaves at 28°C) collected in a dry condition in the field. Drought tolerance levels were determined by allowing field dry foliage to equilibrate to air of various constant relative humidities, maintained by saturated solutions in equilibrium with solid solute at 28°C (*8*). Mixtures of water and sulfuric acid were employed for relative humidities below 15 percent, and concentrated sulfuric acid was used for establishing approximately 0 percent relative humidity (*8*). Equilibration was extremely slow; in general, 4 to 8 weeks were required for constant weight to be attained. The dry-land ferns tested possessed drought-tolerance values ranging from 15 percent relative humidity for the least hardy species, *Doryopteris concolor* (Langsd. & Fisch.) Kuhn (a value equal to the tolerance found for the American fern *Selaginella lepidophylla*), down to approximately 0 percent relative humidity for *Mohria caffrorum* (L.) Desv. Virtually all of the "resurrection" angiosperms tested possessed drought tolerances of approximately 0 percent relative humidity, that is, 50 percent or more of the leaf tissues survived equilibration over concentrated sulfuric acid (see Table 2).

Plants	Relative humidity (%)
Craterostigma wilmsii Engl.	51
Coleochloa setifera (Ridley) Gilly	39
Xerophyta retinervis Baker	37
Oropetium capense Stapf	33
Myrothamnus flabellifolius Welw.	33
Xerophyta humilis (Baker) Dur. & Schinz	32
Xerophyta viscosa Baker	31
Chamaegigas intrepidus Dinter ex Heil	30
Craterostigma plantagineum Hochst.	15
Xerophyta squarrosa Welw. ex Baker	7

Table 2. Drought tolerance of mature foliage, expressed as the equivalent relative humidity at 28°C.

Plant	Relative humidity (%)
Scrophulariaceae	
Craterostigma wilmsii	0 to 15
C. plantagineum	Approx. 0
Chamaegigas intrepidus	
Submerged leaves	0 to 5
Immature floating leaves	5
Mature floating leaves	96
Myrothamnaceae	
Myrothamnus flabellifolia	Approx. 0
Velloziaceae	
Xerophyta clavata Baker	5
X. viscosa	Approx. 0
X. elegans Baker	Approx. 0
X. retinervis	Approx. 0
X. humilis	Approx. 0
Cyperaceae	
Coleochloa setifera	Approx. 0
Poaceae	
Oropetium capense	Approx. 0