90°C in 15 minutes and two washes) contained 800 count/min, and the total chloroform-soluble lipids contained 9000 count/min.

The lipid-soluble radioactivity can rapidly enter and leave the lipid fraction and, on the basis of its partition behavior between aqueous and nonaqueous solvents, cannot be in the free amino acid form. Hydrolysis liberates the radioactivity in the form of the administered amino acid (alanine and phenylalanine). Furthermore, the chloroform-soluble lipid fraction contains relatively large amounts of bound amino acids, which upon hydrolysis appear to represent most or all of the common amino acids.

During the past 7 years there have been a number of reports of amino acid and peptide components of lipid fractions (*11*). Since peptides are noticeably absent in the aqueous part of the cell, it is unlikely that they are carried over into the lipid fraction as an artifact. Folch and Lees (*12*) have described a class of proteolipids characterized by their occurring as a white fluff when a solution of a chloroform-methanol extract of tissue is allowed to equilibrate with water. Material having similar characteristics accounts for a major portion of the lipid-soluble radioactivity obtained when hen oviduct is incubated with radioactive amino acid. In the past several years many intensive efforts have been made to find protein precursor material presumably of peptide nature. These efforts have generally met with failure. Since lipid-soluble materials were eliminated in most of these attempts, it would seem worth while to reconsider the possibility that such precursor material may be concentrated in this hitherto ignored fraction.

Several theoretical aspects would make the consideration of a lipid participation in protein synthesis seem worth while. The microsomal membranes present an extensive oriented lipid surface within the cytoplasm. Since it would appear that amino acids may occur in a lipid-soluble complex, it would seem that an efficient means of rapid amino acid accumulation at sites of synthesis may be accomplished by the structure. Furthermore, the energy considerations in the condensation of two amino acids to form a peptide bond with the concomitant splitting out of water would favor a medium of low water concentration.

The present arguments do not in any way detract from the apparent relationship of ribonucleic acid and protein synthesis but are intended to show another possible aspect in the over-all problem.

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Measurement of the Permeability of the Two Surfaces of a Living Membrane

Recent studies have demonstrated active sodium transport by the isolated toad bladder (*1*). Histologically, toad bladder consists of a single layer of mucosal cells supported on a thin layer of connective tissue containing the blood supply to the tissue and occasional small bundles of smooth muscle. Most of the outer bladder surface has a serosal cover.

When this tissue is mounted as a membrane separating two halves of a Lucite chamber, it can be shown that sodium is actively transported from the solution bathing the mucosal surface to that bathing the serosal surface—that is, in the direction of reabsorption from the bladder urine. Under anaerobic conditions, active sodium transport continues, although at a lesser rate than in the presence of oxygen, and this transport is associated with glycolysis. The lactic acid formed from the glycogen within the membrane does not distribute itself equally about the two surfaces of the membrane, but, regularly, much more accumulates in the medium bathing the serosal surface than in the medium bathing the mucosal surface. This distribution of endogenous lactate is not influenced by the *p*H of the medium on the two sides of the membrane or by the transmembrane electrical potential. This distribution is not dependent on the concommitant transport of sodium ions, for it occurs even when the medium is a choline or magnesium Ringer devoid of sodium. A similar distributon of lactate has been demonstrated about the mucosa of the rat intestine in vitro by Wilson (*2*).

Although more lactate accumulates in the serosal bathing medium than in the mucosal medium, the concentration of lactate is even higher in the tissue water than in the bathing medium. The simplest explanation for the distribution of lactate in the bathing medium is that the mucosal surface is less permeable to lactate than the serosal surface and hence more lactate diffuses out through the latter than through the former.

Figure 1 is a schematic representation of a segment of the bladder wall. The two parallel vertical lines represent the mucosal and serosal surfaces of the membrane, respectively (actually, probably the opposite faces of the mucosal layer of cells); k_1 and k_2 are the respective permeability coefficients of these two surfaces; C_o, C_m, and C_i are the concentrations in mucosal medium, membrane water, and serosal medium of any substance whose permeation is being studied —in this case, lactate.

The generalized equation for the unidirectional flux, M, per unit time for a substance whose concentration on one side is C, across a unit area of membrane containing n separate diffusion barriers is:

$$M = C \frac{k_1 \cdot k_2 \cdot k_3 \cdot \ldots k_n}{(k_1 + k_2)(k_2 + k_3) \ldots (k_{n-1} + k_n)} \quad (1)$$

The requirement for passive diffusion across any barrier is that the respective value of k be the same in both directions. If any process in the membrane facilitates the movement of the test substance in any manner in one direction across the diffusion barrier, this distinction must be recognized, and the resulting different values of k for the two directions must be used in this equation.

In the case of lactate formed within the membrane, there must exist at least the two diffusion barriers depicted in Fig. 1. The net flux, N, of lactate from mucosal to serosal surface is simply

$$N = (C_o - C_i) \frac{k_1 \cdot k_2}{k_1 + k_2} \quad (2)$$

However, there must also exist a transmembrane permeability coefficient, K_{trans}, such that

$$N = (C_o - C_i) K_{trans} \quad (3)$$

Therefore,

$$K_{trans} = \frac{k_1 \cdot k_2}{k_1 + k_2} \quad (4)$$

By adding a tracer amount of radioactive C^{14}-labeled lactate to the medium bathing one surface, let us say the mucosal side, and measuring its rate of appearance on the opposite side, K_{trans} can be directly evaluated over successive periods. After several periods during which the constancy of flux rate indicates that a steady state has been achieved, the experiment is terminated, and the membrane is rapidly weighed and ho-

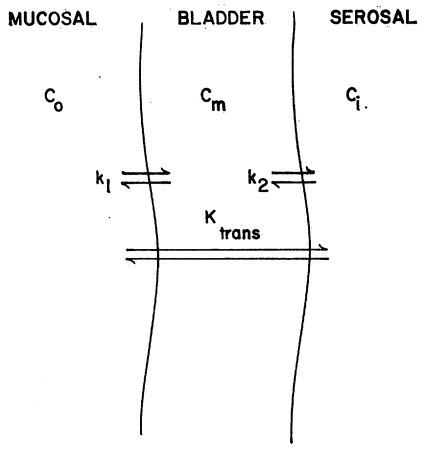

Fig. 1. Schematic representation of a cross section of bladder wall with vertical lines denoting the mucosal and serosal surfaces, respectively.

mogenized in 10 percent trichloroacetic acid to extract the lactate from the tissue. The concentration of C^{14}-labeled lactate in tissue water (*3*) can thus be obtained. From the concentration of C^{14}-labeled lactate within the membrane water and its rate of appearance in the serosal bathing medium, k_2 can likewise be directly evaluated. By substituting values for K_{trans} and k_2 into Eq. 4, k_1 may then be calculated. Because of the relatively low permeability of the membrane to lactate, C_o will remain so much larger than C_i during an experiment that back diffusion may be ignored. The values for k_1 and k_2 may, of course, be evaluated as readily in the opposite direction.

Table 1 shows the values obtained for k_1 and k_2 when measurement was made from mucosal to serosal sides in nine 30-minute periods in three experiments, and from serosal to mucosal surface in twenty 30-minute periods in eight experiments. In every period, k_2 was found to be significantly larger than k_1. Note that any C^{14}-labeled lactate from the medium adherent to the mucosal surface of the bladder will be measured as tissue lactate and tend to make k_2 falsely too low while medium C^{14}-labeled lactate adherent to the serosal surface will result in underestimation of k_1. In spite of this expected limitation in the experimental procedure, satisfactory agreement was found for the values of k_1 and k_2, respectively, when measurement was made in the two directions. This difference in permeability of the two surfaces of the isolated toad bladder is sufficient to account for the observed asymmetrical distribution of lactate about this membrane.

Table 1. Mean values for permeability coefficients for C^{14}-labeled lactate through the isolated toad bladder. All values are means plus or minus the standard error of the mean and are expressed as cm/sec $\times 10^{-7}$. The figures in parentheses give the number of 30 minute periods upon which each value is based.

k_1	k_2	K_{trans}
A. C^{14}-labeled lactate placed on serosal side initially		
5.11 ± 0.59 (22)	94.4 ± 17 (20)	4.62 ± 0.47 (22)
B. C^{14}-labeled lactate placed on mucosal side initially		
4.86 ± 0.67 (9)	95. ± 20 (9)	4.65 ± 0.62 (9)

Since all permeability measurements were made across the short-circuited membrane (*4*)—that is, with no electrical or chemical gradients across the membrane except those of the added radioactive isotopes—the expectation for passive ion movement through the membrane is an equal permeability coefficient as measured in the two directions across the membrane. The mean values and standard errors of the mean shown in Table 1 for lactate for K_{trans} k_1, and k_2 indicate no significant differences in permeability whether the values were obtained during measurements of mucosal to serosal or serosal to mucosal flux. Considering that the measurements in the two opposing directions were of necessity done on different membranes, the agreement is surprisingly good. Thus the passive nature of the lactate movement (simple diffusion) through this membrane is demonstrated.

The permeability coefficients are expressed in centimeters per second instead of square centimeters per second as is customary. This is necessary because the thickness of the diffusion barriers is as yet unknown. We do not know whether the difference between k_1 and k_2 is attributable to a difference in structure of the opposite surfaces of the membrane, to a difference in thickness of diffusion barriers of similar structure, or even to a difference in effective surface area of the two faces of the bladder. This limitation does not affect the relative functional magnitude of k_1 and k_2, which is what has been measured simultaneously in the same membrane.

By analogy with lactate, it is suggested that the main barrier to passive diffusion of sodium is also at the mucosal surface. Such a situation would necessitate that at least part of the active sodium transport mechanism (moving sodium from the mucosal to serosal side) be located on the mucosal surface and function to admit sodium into the mucosal cells at a rate more rapid than can be accounted for by passive diffusion alone (*5*).

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Ability of Bats to Discriminate Echoes from Louder Noise

The dependence of bats upon echolocation suggests a vulnerability to interference by loud noises. Yet hundreds fly together in the darkness of caves, and artificial jamming sounds have remarkably little effect in the laboratory (*1*). Continuous thermal or "white" noise covering the entire frequency range of the orientation sounds of the long-eared bat *Plecotus* (*Corynorhinus*) *rafinesquii* does increase slightly the figure for minimum detectable size of wire obstacles. This finding permits measurements of the bat's ability to discriminate echoes from noise (*2*).

Across the central part of a 32- by 12- by 8-ft room, 28 vertical wires were arranged in four staggered rows, so that a bat had to fly a zigzag course to dodge the wires at more than the chance level of about 40 percent misses. With the more skillful individual *Plecotus,* the smallest size wire detectable in the quiet was one of diameter between 0.2 and 0.5 mm, well below the wavelengths of the bats' orientation sounds (8 to 14 mm). Thermal noise was generated by two banks of 35 electrostatic loud-speakers, which faced the array of wires from opposite ends of the room. In almost the whole space where wires were detected and dodged, the over-all sound-pressure level was between 80 and 90 db above the standard reference level of 0.0002 dyne/cm^2, and even at the lowest points it exceeded 70 db. The spectrum level of the noise varied no more than ±5 db from 15 to 55 kcy/sec, but fell off sharply beyond these limits; thus, the noise level per cycle of band width was usually more than 34 db and always exceeded 24 db. Figure 1 includes all data for the five individual *Plecotus* whose flight was most consistent during 5 days when they were at the peak of their flying skill and avoided 1- to 1.5-mm wires in 80 to 100 percent of the flights, in quiet and in noise. In the control tests,