ethyl alcohol containing 10 percent HCl (wt./vol.). This solution was concentrated to dryness on a water bath, redissolved in a small amount of 6*N* HCl, and heated at 100°C for 1 hour to hydrolyze the conjugate. The resulting solutions were subjected to the $BaNO_3$ test (*8*) for sulfate ion. This test was consistently positive with the compound believed to be the azo pyrryl sulfate, and negative with the azo pyrryl glucuronide from bile. After hydrolysis of the glucuronide followed by paper chromatography of the reaction mixture and elution with ethanol-HCl (*9*), glucuronic acid was demonstrable by Dische's method (*10*).

Apart from strictly chemical considerations, a bilirubin sulfate is of special interest in the demonstration of another conjugate of bilirubin with an acid radical characterized by a change in the van den Bergh reaction from indirect to prompt direct, of the type observed in the bile and in blood serum from patients with "regurgitation" jaundice. Billing *et al.* (*5*) observed that the conjugated bilirubin from bile is readily converted to free bilirubin in 0.06*N* NaOH at room temperature. They noted, however, that a small fraction was stable to this treatment, behaving as though it were a different type of conjugate. The present bilirubin sulfate is also unaffected by this treatment. Nevertheless, an azo pyrryl compound with $R_f$ corresponding to the sulfate has not thus far been encountered in bile or samples of blood serum, and thus it cannot be stated that the small fraction of alkali-stable, direct-reacting bilirubin observed by Billing is a sulfate. The fact that the bilirubin sulfate is alkali-stable, as compared with the glucuronide, may indicate that the conjugation is through the OH rather than the COOH groups, the latter being conjugated in the glucuronide (*6*).

Despite the failure, at least thus far, to observe the $R_f$ of bilirubin sulfate on paper chromatograms of natural material (bile and blood serum), it is recognized that at least under certain circumstances complexes of bilirubin with acid radicals other than glucuronic acid might be responsible for a prompt, direct diazo reaction (*11*).

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## Possible Involvement of Lipids in Protein Synthesis

Since the pioneering observations of Caspersson (*1*) and Brachet (*2*) some 17 years ago on the coincidence of location of ribonucleic acid and protein-synthesizing ability, an impressive number of correlative observations has accumulated (*3*). Because of the sheer weight of this information, one is compelled to recognize that, in general, the maintenance of protein synthesis is dependent upon the integrity and, in some cases, upon the dynamic state of the ribonucleic acid.

The number of papers appearing in the literature pertaining to the possible role of ribonucleic acid in protein synthesis has followed an almost autocatalytic curve, such that in the first decade after the initial observations comparatively little was done or said about the relationship. The emphasis, however, has continued to grow at an increasing rate, so that at present, practically all work in the field includes some observation which is interpreted in terms of the anticipated close relationship. At present ribonucleic acid is given the role of template (*4*), amino acid carrier (*5*), and activator (*4*).

The danger in this trend is in the possibility that it may tend to overshadow and obscure other aspects of the problem of protein synthesis. The fact that microsomal material of a wide variety of cells appears to be the most active component with respect to protein synthesis (amino acid incorporation) and that the major part of the cellular ribonucleic acid is also contained in this structure is often cited in favor of the ribonucleic acid–protein relationship. It is often overlooked, however, that most of the cytoplasmic lipid is also in this structure and, interestingly enough, accounts for about 5 times as much of the total weight as the ribonucleic acid (*6*).

A substantial group of observations supporting a ribonucleic acid–protein relationship is based on the fact that preliminary NaCl extraction or ribonuclease treatment strongly inhibits amino acid incorporation in cells and cell fragments (*3*). It has been similarly observed that in the hen oviduct, purified lecithinase A (*7*), lysolecithin (*7*) and deoxycholate have a very potent inhibitory effect on amino acid incorporation (Table 1).

Coenzyme A and cytosine triphosphate have been shown to be actively involved in the biosynthesis of lipids (*8*). Stimulation of incorporation of amino acids into protein has been observed in the hen oviduct upon the addition of CoA and cytosine triphosphate (*9*). These findings may be the result of a quite indirect relation in the over-all metabolism of the cell, as is also true in most of the observations linking ribonucleic acid to protein synthesis. In experiments reported in this paper (*10*) it has been observed that after a few minutes' incubation the chloroform-soluble lipid fraction of hen oviduct contains relatively large amounts of radioactivity compared with the amount which is entering the proteins or is associated with the nucleic acids. For example, after a 10-minute incubation of a hen oviduct mince with phenylalanine, in which the total cold trichloroacetic acid soluble radioactivity within the cell was 390,000 count/min, the total proteins contained 11,200 count/min, the total nucleic acids (soluble in 5 percent trichloroacetic acid at

Table 1. Effect of lipolytic agents and ribonuclease on phenylalanine-3-$C^{14}$ incorporation in hen oviduct mince.

| Conditions for 5-min pretreatment | Medium concentration* | Total count/min incorporated in 10 min | Percentage inhibition† |
|---|---|---|---|
| Control (no additions) | | 17,200 | |
| Lecithinase A | 70 μg/ml | 9,000 | 48 |
| Lysolecithin | 210 μg/ml | 7,150 | 59 |
| Deoxycholic acid | 0.5%‡ | 4,600 | 73 |
| Ribonuclease | 210 μg/ml | 12,600 | 34 |

* These figures are based on the total liquid volume in the incubation and do not indicate the internal concentration of the cells.
† These effects are not due to decreased ability of cells to take up amino acid.
‡ Since the solubility of deoxycholic acid in water is only 0.025 percent, the actual external concentration was much lower.

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-