conditions the complicating reactions of the host cells are removed.

A report is now in preparation describing analogous experiments in our laboratories, in which sulfanilamide was employed instead of PABA. Sulfanilamide is similar to PABA not only in structure but in that similar resonant structures exist in which the positions *ortho* to the amino group possess a formal negative charge. Sulfanilamide inhibitions similar to our already described PABA inhibition have been observed with Gram-positive and Gram-negative bacteria as well as with cell-free preparations of rickettsiae. As in the case of PABA, these inhibitions are relieved by the addition of DPN+. Andrewes *et al.* (*10*) found that *p*-sulphonamidobenzamidine and *p*-sulphonamidobenzamidoxime were potent antirickettsial agents. It is of interest to note that both of these compounds can give rise to resonant structures bearing formal negative charges in the *ortho* positions, which should permit addition to the *para* position of DPN+. Among other things, Andrewes *et al.* (*10*) proposed that nuclear-substituted sulfonamides have "the specific power of adding on to some unknown structure in the enzyme and thus affecting its activity." They further state "it is significant that a free amino group is essential in all drugs derived from sulphanilamide for activity." The latter statement can now be reexamined in light of reference (*1*). Cell permeability has been found in our laboratories to play a significant role in the antibacterial property of sulfanilamide against rickettsial infections.

The evidence presented suggests a simultaneous explanation for both the inhibition of rickettsiae by PABA and the DPN+ reactivation phenomenon of Bovarnick (*4*). Both the Snyder and Bovarnick phenomena seem to have DPN+ involvement as a common factor, the PABA inhibition phenomenon being due to inactivation of rickettsial DPN+ while Bovarnick's reactivation phenomenon restores the DPN+ to rickettsiae in which DPN+ has been eliminated by physical methods. The implications of the data presented, insofar as they offer an additional mode of sulfonamide action, are apparent and emphasize the biological significance of the chemical reactivity of DPN+.

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Color Reaction of Bilirubin with Sulfuric Acid: a Direct Diazo-Reacting Bilirubin Sulfate

A color reaction of bilirubin with the Liebermann-Burchard reagent was first observed in 1936, but was not reported or studied in detail at that time. It was noted then, however, that following this reaction the bilirubin became soluble in water. Unfortunately the significance of this change in relation to the diazo reaction was not appreciated at that time.

This reaction has now been studied in some detail and has been found to be of special interest because it yields an anionic complex of bilirubin which exhibits a prompt, direct diazo reaction. Except for a brief reference in a recent paper of mine (*1*), no mention of the color reaction has been found in the literature. The reaction occurs more slowly with bilirubin and sulfuric acid alone, and it is evident that the acetic anhydride in the Liebermann-Burchard reagent serves only as a vehicle to bring the sulfuric acid into rapid contact with the bilirubin. The addition of 1 ml of the acetic anhydride-sulfuric acid (10:1) reagent to 5 ml of a chloroform solution of bilirubin is followed promptly by a dark red color, the reaction mixture then exhibiting a strong absorption band at maximum 540 mμ. If the solution is shaken in a separatory funnel with distilled water, the red color at once changes to brown-yellow, and it is evident that much of the pigment has now become water soluble, this fraction entering the aqueous phase.

The remainder of the bilirubin in the chloroform phase may be converted to the water-soluble type by successive addition of small amounts of the Liebermann-Burchard reagent and shaking with water. It is evident that in the course of the reaction small fractions of the bilirubin are converted to a dark brown pigment, probably bilifuscin, which precipitates at the interphase. Biliverdin is also formed in considerable amount, and it continues to form, so that the aqueous, initially yellow or brownish yellow soon becomes greenish brown.

After separation of the pooled aqueous fraction from any entrained chloroform, by filtration, the van den Bergh reaction is prompt and direct in type. Unlike the behavior of starting bilirubin, its behavior is polar, as is shown by reverse phase chromatography on siliconized *kieselguhr*, by the method of Cole and co-workers (*2*). The substance corresponds in chromatographic behavior to their pigment II. The polar pigment is readily separated from biliverdin and other impurities by adsorption on a column of aluminum oxide. The diazo-reacting pigment is held at the top of the column, the impurities coming off with the effluent. The pigment is then readily eluated with 0.1*N* sodium hydroxide solution. It retains its polar character and is not extracted by chloroform upon acidification of the eluate.

Thus far it has been impossible to crystallize this prompt, direct diazo-reacting compound. It is quite labile, readily undergoing oxidation to biliverdin and bilifuscin. Due to its polar behavior and water solubility, it is difficult to transfer to organic solvents, although it is extracted in part by *n*-butanol at *p*H 4 to 5.0. The polar, prompt and direct-reacting pigment is readily diazotized in the initial aqueous solution after the *p*H is adjusted to 4.0. The azo compound is extracted by *n*-butanol and may then be compared with the azo dipyrryl compounds of free bilirubin and of the conjugated bilirubin of human bile, which, according to recent evidence, is mainly an ester diglucuronide (*3–6*). The crude bilirubin glucuronide used in the present study was prepared by Malcolm Campbell (*7*). For the present comparison, Schmid's paper chromatographic method (*3*) was used. The concentrated butanol solutions of the three azo compounds were run on the same strip (Whatman 3 MM), ascending chromatography being achieved with *n*-propionic acid, methyl ethyl ketone, and water (25:75:30). With a solvent front of about 30 cm, the R_f for the azo pigment from crystalline bilirubin is 0.55, that of the glucuronide 0.40. The bilirubin sulfate regularly exhibits two zones, 0.30 (red) and 0.22 (purple). The basis for this has not been determined and is receiving further study.

It has been possible to show that the azo pyrryl compound, after paper chromatography as described, contains sulfate, while that of the glucuronide, under the same conditions, does not. For this demonstration (*8*) a considerable amount of each azo pigment was chromatographed, then eluted from the paper by

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.