extracted and examined (Skipski, Smolowe, Sullivan & Barclay, 1965). Up to 70% of the phospholipids were hydrolysed. Although hydrolysis of diacylglycerophospholipids by phospholipase C releases 1,2-diglyceride, this does not accumulate and a major product appeared to be unesterified fatty acid. This suggested that a lipase active against 1,2-diglyceride might be present in the membranes.

Erythrocytes were therefore incubated with [^3H]palmitate to label their phospholipids (Shohet, Nathan & Karnovsky, 1968), and the transfer of radioactivity from phospholipids to other lipids was followed during phospholipase treatment. Labelling of 1,2-diglyceride was unchanged, and the unesterified fatty acid and monoglyceride fractions together accumulated radioactivity equal to that lost by the phospholipids.

The total lipids of both unlabelled and labelled membranes were isolated, emulsified and treated with phospholipase C. Under these conditions the only major product was 1,2-diglyceride, both by inspection of chromatograms and by transfer of radioactivity from phospholipids to other lipids.

The difference in products seen after treatment of membranes and of emulsified lipids was further confirmed by measuring the acyl ester and unesterified fatty acid content of these preparations after phospholipase treatment. There was a loss of acyl esters and a concomitant increase in unesterified fatty acids only when membrane-bound lipid was hydrolysed.

The observed hydrolysis of 1,2-diglyceride appeared to be a property of the membranes rather than of a contaminant in the phospholipase C preparations. It was, however, still possible that the commercial phospholipase C preparations contained a lipase that would act only on membrane-bound diglyceride. The products formed during treatment of two other membrane preparations, human erythrocyte membranes from cells stored in acid-citrate-glucose medium and rat cerebral-cortical myelin (fraction $\text{ML}_{0.8}$ described by Rodrigues de Lores Arnaiz, Alberici & De Robertis, 1965) were therefore examined. In each case the only major product that appeared was 1,2-diglyceride.

These results indicate that the membranes of rat erythrocytes contain a lipase that is highly active against 1,2-diglyceride at pH 7.4 with 0.75 mM- Ca^{2+} . Further characterization of this activity will initially be aimed at defining conditions under which externally added substrates are hydrolysed.

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Cytoplasmic Redox Control of Protein Synthesis

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Partial hepatectomy initiates biochemical events causing a greatly increased rate of protein synthesis (Bucher, 1963). The purpose of this investigation was to derive a system *in vitro* that would accurately reflect the known changes in protein-synthetic activity *in vivo*.

A technique (Scornik, Hoagland, Pfefferkorn & Bishop, 1967) for removing lysosomes from rough endoplasmic reticulum (microsomal fraction) has been used here to obtain microsomal preparations from normal and regenerating rat livers. By using a low concentration (0.01 mM) of GTP and transfer factors and measuring the initial rate of amino acid transfer from aminoacyl-tRNA to peptide it was shown that the microsomal preparation from normal liver has only 50% the rate of transfer carried out by the preparation from regenerating liver. Raising of the GTP concentration to 0.4 mM eliminates these differences, largely by stimulating the microsomal preparation from normal liver, there being relatively little effect on the preparation from regenerating liver, GSH and other thiols also have a relatively larger stimulatory effect on the former microsomal preparation than on the latter.

A marked decrease in the initial rate can be produced by adding GSSG, and again the inhibitory effect is more dramatic on the microsomal preparation from normal liver than on that from regenerating liver, thus increasing the difference between the activities of the two preparations. Rat livers were briefly perfused with the oxidizing agent $\text{NNN}'\text{N}'$ -tetramethylazofornamide (diamide) (O'Brien, Weitzman & Morris, 1970). The microsomal preparation derived from livers of animals treated with this drug had a lower initial rate of synthesis, greater stimulation by thiols and markedly diminished sensitivity to inhibition by GSSG compared with the control preparation.

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We postulate that a membrane-associated control factor exists that can have an oxidized or a reduced form. The results show that GTP is also involved. The control factor, X, may have to exist in a GTP complex, X-GTP, in order for protein synthesis to occur. This complex is readily obtained if factor X is reduced, but can be derived from oxidized factor X only in response to high GTP concentrations. The ratio of oxidized factor X to reduced factor X present is thus postulated as being critical. According to this model, the normal liver has a greater ratio than the regenerating one. The results from the diamide experiments, in which oxidation occurs, agree with this model, and lend strong support to its validity.

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The Site and Nature of Transferase II-Guanosine Triphosphate Complex Attachment to Mammalian Ribosomes

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In mammalian protein synthesis, transferase I binds the incoming aminoacyl-tRNA to the acceptor site on the ribosome. This is followed by peptide-bond formation at the acceptor site, and the peptidyl-tRNA is then translocated to the donor site by the action of transferase II. This enzyme requires GTP and its action is inhibited by cycloheximide. We are now able to add detail to the mechanisms involved.

With the use of either Millipore filters or Sephadex columns, complex-formation between [¹⁴C]GTP and purified transferase II has been demonstrated. This reaction is not impeded by cycloheximide. Both the isolated complex and a mixture of GTP and enzyme have been used to study the binding of this complex to the ribosome and its subsequent fate.

As shown by using labelled GTP, binding of the enzyme-nucleotide complex occurred very rapidly when the peptidyl-tRNA was in the donor position, but less extensively when the peptidyl-tRNA was in the acceptor position. With prolonged incubation the latter type of ribosomes eventually bound

amounts comparable with those bound by the donor type; reaction with puromycin demonstrated that this was due to transfer of the peptidyl-tRNA to the donor site during prolonged incubation. It was concluded that the presence of peptidyl-tRNA at the acceptor site occludes binding of the complex.

When cycloheximide was added to the reaction mixture it decreased the amount of labelled GTP bound. When GTP was first bound to the ribosomes in the presence of transferase II, and cycloheximide was subsequently added, the bound GTP was expelled in a form that passed through a Millipore filter and was thus no longer attached to the enzyme. It was concluded that the association of GTP with the enzyme is altered on binding and becomes susceptible to the action of the inhibitor.

Studies are presently being undertaken with labelled transferase II to determine whether cycloheximide similarly decreases the amount of enzyme bound and whether addition of the inhibitor to ribosomes already bearing labelled transferase II causes release of the enzyme at the same time as the GTP is expelled.

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Biosynthesis of Tryptophanase by Polyribosomes and by Ribonucleic Acid and Ribosomes Isolated from *Escherichia coli*

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We have previously reported (Parish, Khairul Bashar & Brown, 1971) the biosynthesis of tryptophanase (EC 4.2.1.e) by using polyribosomes isolated from induced cultures of *Escherichia coli* M.R.E. 600. We now report the synthesis of a protein with the same chromatographic properties as tryptophanase by using RNA from induced cells and an S-30 fraction from repressed cells. The properties of proteins synthesized by the polyribosome system and the RNA-S-30 fraction system are compared.

RNA was isolated from induced cells by the method of Parish (1968) and 'preincubated S-30 fraction' was isolated from repressed cells by the method of Nathans (1968). Mixtures of RNA, S-30 fraction, cofactors necessary for protein synthesis, 19 unlabelled amino acids and one radioactive amino acid were incubated at 37°C. The incorporation of [³H]leucine into a protein that co-chromatographed with tryptophanase on DEAE-cellulose was demonstrated. The incorporation was shown