methionine and ATP are added. *S*-Adenosyl-methionine can replace methionine, but ATP is still required. The formation of protoporphyrin is inhibited by *S*-adenosylethionine and *S*-adenosyl-homocysteine.

The aerobic reaction has an optimum at about pH 7, whereas the anaerobic reaction has an optimum at about pH 8.5. A number of compounds markedly inhibit the anaerobic reaction but have no effect on the aerobic one. 1,10-Phenanthroline (0.17 mM) inhibits by 90%, $\alpha\alpha'$ -bipyridyl (1.5 mM) by 77%, riboflavin, FMN and FAD (30 μ M) all by about 95% and 2,4-dinitrophenol (0.7 mM) by 90%.

With a crude extract there is no requirement for a hydrogen acceptor under anaerobic conditions, although nicotinamide nucleotides stimulate slightly. An extract freed from small molecules by passage through Sephadex G-25 or G-50 has no activity unless nicotinamide nucleotides are added as well as *S*-adenosylmethionine and ATP. NAD⁺ or NADH partially restore activity, and NADP⁺ or NADPH are slightly more effective; maximum activity, equal to that of crude extract, is obtained with NADH plus NADP⁺. Sephadex-treated extracts lose activity rapidly, but addition of succinate prevents this. The succinate is probably oxidized via the succinoxidase system, thus keeping the extract free of oxygen.

The enzyme responsible for the aerobic reaction seems similar to that described in liver mitochondria (Sano & Granick, 1961; Batlle, Benson & Rimington, 1965) where O₂ is the hydrogen acceptor, and no cofactors are required. The results suggest that in the anaerobic reaction a flavoprotein and iron, either as a component of the flavoprotein or as a non-haem-iron protein, are involved in the removal of hydrogen from coproporphyrinogen, and that NAD(P)⁺ is the final hydrogen acceptor. The inhibition by 2,4-dinitrophenol suggests that ATP is required for energy-linked reverse electron transfer (cf. Sewell & Aleem, 1969). *S*-Adenosyl-methionine does not seem to be metabolized during the reaction and thus it may simply activate the enzyme. Whatever its role, its involvement explains why *R. sphaeroides* excretes coproporphyrin when illuminated anaerobically under conditions of methionine deficiency caused by adding ethionine or threonine to the medium (Gibson, Neuberger & Tait, 1962).

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Multiple Activities of Penicillin Acyltransferase of *Penicillium chrysogenum*

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Natural penicillins are thought to be formed by substitution of the L-aminoadipyl group of isopenicillin N by various acyl groups (Demain, 1966). Three enzyme activities have been detected in *Penicillium chrysogenum* that could be involved in this substitution, namely: (a) penicillin acyltransferase, which catalyses isotopic exchange of ³⁵S between penicillins and 6-aminopenicillanic acid (Pruess & Johnson, 1967); (b) 6-aminopenicillanic acid acyltransferase, which synthesizes penicillins from acyl-CoA and 6-aminopenicillanic acid (Spencer, 1968; Gatenbeck & Brunsberg, 1968); (c) penicillin acylase, which hydrolyses penicillins to 6-aminopenicillanic acid and the side-chain acid (Vanderhaeghe, Claesen, Vlietinck & Parmentier, 1968). A fourth relevant activity now observed in *P. chrysogenum* 51-20F3 extracts is: (d) the hydrolysis of phenylacetyl-CoA. The optimum conditions for the assay of these activities in 0.05 M-sodium phosphate buffer containing 2 mM-dithiothreitol and 1 mM-EDTA were: (a) 2 mM-[³⁵S]penicillin G and 7.5 mM-6-aminopenicillanic acid, pH 7.8; (b) 1 mM-6-aminopenicillanic acid (K_m 5×10^{-4} M) and 7.5 mM-[¹⁴C]phenylacetyl-CoA (K_m 6.7×10^{-3} M), pH 7.4; (c) 0.25 mM-[³⁵S]penicillin G (K_m 1.67×10^{-4} M), pH 7.9; (d) 7.5 mM (not optimum)-[¹⁴C]-phenylacetyl-CoA (K_m 5×10^{-3} M), pH 8.0.

When *P. chrysogenum* was transferred from a glucose to a lactose medium, the appearance of each of the four activities in mycelial extracts showed a similar pattern. Disruption of the mycelial extracts in the French press, precipitation with (NH₄)₂SO₄ and fractionation on Sephadex G-100 and DEAE-cellulose gave a 130-fold purification of each of the activities. Apart from the crude extract, in which non-specific enzymes hydrolyse the phenylacetyl-CoA, the ratios of the activities remained constant throughout the various purification stages. The fractionation patterns on DEAE-cellulose and Sephadex G-100 were identical, and on polyacrylamide-gel electrophoresis all four activities were found in the same narrow band. The pH and temperature inactivation curves were the same. Complete inhibition was given by 2 mM-N-ethylmaleimide and activities were restored by addition of excess of dithiothreitol. CoA did not enhance the rate of exchange activity (a), nor was there an absolute requirement for dithiothreitol since, after its removal by using Sephadex G-100 more than 50% of each of the activities was retained.

The four activities are explainable on the basis of a single thiol-dependent enzyme and a Ping Pong Bi Bi mechanism with an alternate hydrolytic step.

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Changes in Polyribosome Metabolism in *Escherichia coli* K12 after Adsorption of Colicin E3 to Sensitive Cells

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Fixation of colicin E3, a simple protein, to specific receptors in the surface of sensitive bacteria rapidly results in the complete inhibition of protein synthesis. RNA synthesis, including mRNA synthesis, continues without inhibition, but 70S ribosomes extracted from bacteria, after prolonged colicin E3 treatment, show greatly decreased activity *in vitro* deriving from some specific alteration of the 30S subunit (Konisky & Nomura, 1967).

We have now shown that inhibition of protein synthesis induced by colicin E3 is soon followed by the fairly rapid loss of polyribosomes to yield equal proportions of both 70S and 50S+30S ribosomal subunits. This effect cannot be ascribed to increased nuclease activity. Moreover, before the onset of polyribosome degradation, abnormal polyribosome complexes can already be detected, which, unlike normal polyribosomes, dissociate at low Mg^{2+} concentrations. Thus within 1 min of the complete cessation of protein synthesis at least 40% of polyribosomes are demonstrably unstable when the Mg^{2+} concentration is lowered to 1 mM.

'Pulse-chase' experiments *in vivo* indicate that the release of nascent polypeptide chains from polyribosomes is inhibited in colicin-treated cells. In contrast, other experiments indicate that protein synthesis is blocked as a result of inhibition of some initiation step. The changes in ribosome function provoked by the adsorption of colicin E3 may therefore affect a step in the protein-biosynthetic cycle, common to both polypeptide-chain extension

and initiation, as has been suggested for streptomycin (Modolell & Davis, 1969).

Colicin E3 has no effect on ribosomes *in vitro* (Konisky & Nomura, 1967) and like other colicins it does not apparently penetrate the bacterial cell surface. The colicin acts extracellularly, presumably by promoting a specific alteration of the cytoplasmic membrane. Inhibition of ribosome function is not, however, dependent on continued RNA or protein synthesis. The surface-bound colicin must therefore trigger the 'activation' (or inhibition) of some normal cell constituent, presumably membrane-bound, which then effects modification of the 30S ribosomal subunit.

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Activity of Skeletal-Muscle Ribosomes from Intact and Castrated Rats treated with Triamcinolone Acetonide

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Triamcinolone acetonide was administered at doses of 20, 5 and 0.2mg/kg by intraperitoneal injection into intact adult male rats (250g, Carworth CFHB Strain) or similar rats castrated 10 days previously. In each experiment ribosomes were isolated from five control animals (intact or castrated) and five drug-treated animals 12h after the administration of the drug. The amino acid-incorporating ability *in vitro* of the ribosome preparation from each animal was measured over a 20 min incubation period in the presence of diluted liver cell sap and energy (Bullock, White & Worthington, 1968). The method for the isolation of ribosomes was essentially as described previously (Peters, Richardson, Small & White, 1970) except that 0.1M-tris buffer was used in the homogenizing medium and treatment with amylase was omitted.

The means of the amino acid-incorporating activities of ribosomes from intact animals at triamcinolone doses of 20 and 5mg/kg were both 79% of control, and in castrated animals were 73 and 79% of control, respectively. However, at 0.2mg/kg the mean activity of ribosomes from intact animals was 93% of control, whereas that from castrated animals was 77%. When castrated animals were pretreated daily for 7 days with testosterone (2.0mg/kg per day) the mean ribosomal activity 12h after administration of triamcinolone acetonide (0.2mg/kg) was elevated to 90% of