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Bacterial Ribonucleic Acid Synthesis in Frog Organs after Intraperitoneal Injection of Bacteria

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Plant or animal organs dipped in a suspension of bacteria synthesize bacterial RNA (Stroun *et al.*, 1969; Stroun & Anker, 1971a,b; Anker & Stroun, 1972). This phenomenon, which we called 'trans-cession', is due to the spontaneous release of DNA from bacteria (Borenstein & Ephrati-Elizur, 1969) into cells of higher organisms.

In the present work we show that the same phenomenon can take place in more natural conditions, such as occur, for instance, in living animals after a bacterial infection.

Adult frogs were injected intraperitoneally with 1 ml of a suspension (10^9 bacterial/ml) of *Escherichia coli* (strain B) or *Agrobacterium tumefaciens* (strain B₆). Then 1–72 h later 1 mCi of [³H]uridine was injected, and 3 h later the RNA was extracted from the brains, the heart auricles, the ovaries and the skin. In control animals the suspension of bacteria was characterized by hybridization *in vitro* of [³H]RNA extracted from the animals with bacterial DNA. Samples of each organ were taken for radioautography and sterility tests.

Specific bacterial RNA characterized by RNA–DNA hybridization was found in all organs, even as late as 3 days after infection. The largest amount is found in the brain, which in fact is protected by natural barriers against the bacteria we have used. No bacteria were detected by radioautography. The sterility tests showed no bacteria in the brain and none or few (in too small an amount to be detected by radioautography) in other organs. The number of contaminating bacteria in auricles, ovaries and skin would not yield more than 1 part in 10^6 of the bacterial RNA extracted from these organs. Thus these bacteria cannot account for the extent of hybridization between [³H]RNA extracted from the infected frogs and the bacterial DNA. When auricles of injected frogs were extracted and treated with antibiotics before labelling *in vitro*, bacterial RNA was also found in the same amount. This last result suggests that the bacterial RNA extracted from the animal tissues has been synthesized directly in the

cells and does not come from [³H]RNA released from labelled bacteria.

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Biosynthetic Studies of Glycocalyx-Bound Glycoprotein and Glycolipid Components in Isolated Brush Borders of the Rabbit Small Intestine

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Intestinal brush borders can be regarded as highly differentiated plasma membranes that fulfil a specialized role in the digestion and absorption of foodstuffs. These functions are closely dependent on the intactness of the brush-border structure and hence may be extensively disturbed by factors that change the structure or its rate of biosynthesis. The membranes bear, on their luminal surface, a filamentous structure, the glycocalyx, which is a zone of localized digestive enzyme activity and which has a strongly positive periodic acid–Schiff histological reaction indicative of the presence of carbohydrate-containing material. A considerable body of evidence suggests that these carbohydrates form part of glycocalyx-bound enzymically active glycoproteins, which can be solubilized by papain (Eicholz, 1968) or butanol (Gitzelmann *et al.*, 1964). The present investigation is concerned with the identification of glycolipids of the glycocalyx and with their isotopic labelling, in comparison with glycoprotein components.

The presence of glycolipids in whole intestinal tissue has been demonstrated by Suzuki *et al.* (1968) and Vance *et al.* (1966), although the exact cellular origin does not appear to have been established. Brush borders have been isolated from rabbit small-intestinal scrapings by graded fragmentation in sucrose–5 mM-EDTA (D. S. Parsons, unpublished work) in 60–80% yield [in terms of invertase (β -fructofuranosidase) recovery] and with an eight- to ten-fold enrichment in invertase and lactase activities. Lysis of the brush borders in chloroform–methanol (2:1, v/v) resulted in release of glycolipid (Folch *et al.*, 1957) and denaturation of the bound enzyme. T.l.c. on silica gel G (Brady & Mora, 1970) showed the presence of five glycolipids including trisialoganglioside (GT) and disialogangliosides (GDI₁) (Svennerholm, 1963). The glycolipid fraction was shown

by g.l.c. to contain glucosamine, galactosamine, galactose, glucose and sialic acid. Glycoproteins and enzymes were released from brush-border preparations by treatment with an equal volume of ice-cold butanol. In the soluble fraction invertase was obtained in 80% yield with a fourfold increase in the specific activity. Hydrolysis and g.l.c. showed the presence of bound galactosamine and glucose in these macromolecular components. The biosynthesis of the above brush-border components was examined after pre-injection of rabbits with D-[1-¹⁴C]glucosamine (20 μ Ci/1½ kg animal) 3 h before isolation of the brush borders. The glycoprotein components of isolated brush border appear to become labelled considerably faster than the glycolipids.

The very marked difference in the rates of labelling of the glycoprotein and glycolipid components of the brush border suggests that separate biosynthetic pathways may operate. The results are consistent with the view of a rapid continuous biosynthesis of the glycoproteins and a relatively low rate of turnover of the glycolipids that is possibly more closely related to the rate of cellular renewal.

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The Cyanogen Bromide Fragments of Rabbit Skeletal-Muscle Tropomyosin

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A repeating pattern of hydrophobic residues in tropomyosin has been predicted to account for its 'coiled-coil' structure (Crick, 1953; Cohen & Holmes, 1963). The presence of at least two non-identical chains in tropomyosin has been indicated by the results of polyacrylamide-gel electrophoresis in concentrated urea and in sodium dodecyl sulphate (Weber & Osborn, 1969; Sender, 1971; Cummins & Perry, 1972). Similarly, a larger number of unique cysteine-containing sequences than would be predicted from the cysteine content of the protein has been demonstrated (Hodges & Smillie, 1970, 1972a). However, the numbers of unique methionine-containing and histidine-containing sequences are

consistent with a high degree of similarity in the amino acid sequences of these chains (Hodges & Smillie, 1970, 1972b). Fragments derived from CNBr treatment of the S-carboxymethylated protein were separated into small and large components by gel filtration on Sephadex G-50. Sequence analysis of the smaller fragments, combined with previous information (Hodges & Smillie, 1972b), has increased the number of unique methionine-containing sequences to a minimum of seven, a result still consistent with very similar polypeptide chains. From the mixture of the larger CNBr fragments a component was isolated by ion-exchange chromatography on QAE-(quaternary amino ethyl) Sephadex in 8M-urea at pH 7.5. In aqueous buffers it had a molecular weight of 35000 and circular dichroism indicated an α -helical content of about 60%. In 8M-urea the molecular weight was decreased to 17000. Since it contained both histidine residues of the intact protein, one of which is known to be near the C-terminus (Hodges & Smillie, 1972b), it was derived from the C-terminal half of the molecule. Chymotryptic digestion of this fragment and sequence analysis of the resulting peptides have produced an extended sequence (33 residues) about the two homologous cysteine residues previously described (Hodges & Smillie, 1972a). In this sequence nine positions with single amino acid substitutions and one position with three substitutions were observed, indicating a minimum of four different polypeptide chains in our tropomyosin preparation. No simple pattern of repeating hydrophobic residues was evident. The complete primary structure of this CNBr fragment will hopefully provide information on the relationship between the unique structural features of this protein and its amino acid sequence.

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