studies have shown a positive requirement for ammonia in some bacterial species (Bryant & Robinson, 1963) and an ability to utilize branched-chain fatty acids in the synthesis of amino acids such as valine and isoleucine (Allison & Bryant, 1963). Indirect evidence for the synthesis of amino acids has come from work *in vivo* (Clarke, Ellinger & Phillipson, 1966) in which it was demonstrated that an increase in the amount of a number of amino acids occurred after passage of digesta through the sheep rumen.

The following experiments were performed to examine the extent to which carbon from a simple carbohydrate source was used by the rumen bacteria in amino acid synthesis. Short incubations *in vitro* of rumen fluid were carried out with [U-¹⁴C]glucose as the carbohydrate source. The bacterial fraction was separated by centrifugation (at 7500g). The cells were disrupted by sonication and the cell walls and unbroken cells were removed by further centrifugation (at 11000g). Trichloroacetic acid-precipitated protein was then freed from lipid and nucleic acid and the ¹⁴C present in each fraction was determined by using a Packard Tri-Carb liquid-scintillation counter. Most of the label found within the cell was in the trichloroacetic acid-soluble fraction. Appreciable amounts, however, were found in both the nucleic acid (1.4%) and protein (1.7%) fractions.

In further incubations the separated bacterial cells were hydrolysed with 6M-hydrochloric acid for 22h. The resultant amino acid mixture was analysed for ¹⁴C content by using a Technicon automatic analyser with a fraction collector attached. The fractions obtained were taken up in scintillator (Bray, 1960) and counted.

Approx. 0.06% of the glucose utilized by the bacteria appeared in the amino acid fraction. ¹⁴C was found in most of the amino acids, but the degree of labelling varied. Aspartic acid and alanine each contained approx. 22%, glutamic acid 7.0%, glycine 5%, tyrosine, phenylalanine, lysine and methionine each 3%, valine, isoleucine, leucine and arginine each 2%, and proline 1%.

In conclusion it appears that the rumen bacteria are synthesizing, at least in part, most amino acids, including many probably required preformed by the host ruminant.

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Studies on the Isolation of Epithelial Growth Factor from Fractions of Mouse Salivary-Gland Extracts

By E. L. FENTON, CAROLINE B. MANN and D. C. EDWARDS. (*Department of Biological Chemistry, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent, U.K.*)

During studies on extracts of the submaxillary gland of adult male mice that were known to have growth-promoting properties for sympathetic nervous tissue, Cohen (1962) described the isolation of a factor that caused precocious eruption of incisors and opening of eyelids when injected into neonatal mice. These effects were correlated with a thickening of the epidermis, and the material responsible for the effects was called 'epidermal growth factor' (EGF*). This substance is now known as 'epithelial growth factor' (Jones, 1966; Turkington, 1969).

To provide sufficient material for further studies on this factor it was necessary to devise a method of isolation for a larger scale of operation than the Cohen (1962) procedure and that would in addition allow us to use the by-products of the Varon, Nomura & Shooter (1967) method used by us as a routine for the preparation of nerve growth factor.

The assay of EGF by injection into mice proved too time-consuming and certain fractions were toxic at concentrations necessary to detect the EGF. A small quantity of EGF was prepared by Cohen's (1962) method. It was found to be homogeneous in the ultracentrifuge and had a molecular weight of approx. 7000 (B. E. C. Banks, unpublished work). This material was used to immunize rabbits. The ability of the anti-EGF serum to form a precipitate with its antigen was utilized to form an assay that was both rapid and sensitive. Fractions from the different stages in the process were diluted serially with 0.85% NaCl. and diffused against undiluted antiserum in 2% saline agar. By comparison with an EGF standard and observing the last dilutions at which the single precipitation line could be seen, the amount of EGF present in the crude fractions could be estimated.

Gel filtration of centrifuged homogenates of glands was used by Varon *et al.* (1967) as a step in the purification of nerve growth factor. The agar double-diffusion test located EGF in two fractions eluted from the Sephadex G-100 column after the nerve growth factor. The first of these fractions also contained the haemoglobin. The second fraction was in a position more characteristic of a low-molecular-weight protein.

Cohen (1962) removed haemoglobin from crude extracts by boiling and centrifugation, a step that we have shown to liberate the EGF from the

* Abbreviation: EGF, epithelial growth factor.

haemoglobin complex. The liberated EGF then appears in the exclusion profile in a position corresponding to the low-molecular-weight material. A similar release of EGF occurred when the haemoglobin was removed on cation-exchanger SE-Sephadex C-50.

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Biological Effects of Epithelial Growth Factor and its Antiserum in Neonatal Rats

By CAROLINE B. MANN and E. L. FENTON. (*Department of Biological Chemistry, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent, U.K.*)

Neonatal rats have been used to investigate the biological activity of various preparations of EGF* isolated from the submaxillary glands of adult male mice (Fenton, Mann & Edwards, 1970). Observations on the precocious opening of eyelids and eruption of teeth have confirmed those described by Cohen (1962). Comparisons have been made with cortisone acetate and tri-iodothyronine, both known to elicit similar responses in neonatal animals. With cortisone acetate and tri-iodothyronine the eye-opening effect is due to exophthalmos, but with EGF it appeared to be a direct effect on keratinization of the eyelid skin.

In an attempt to acquire more quantitative information on the specific response of animals to various doses of EGF, we adopted the technique described by Angeletti, Salvi, Chesanow & Cohen (1964). Five-day-old rats injected with EGF daily from birth were killed by decapitation and standard-size pieces of skin were cut from the back region. The epidermis was removed by treatment with 2% trypsin, washed, dried and weighed. The level of response varied from litter to litter, but with any one litter there was an increase in epidermal weight up to a maximum response at a dose of 3 µg of EGF/g body wt. per day. An increase in dose produced no further response and often had an inhibitory effect. A more striking effect than the increase in thickness of the epidermis is the decrease in thickness of the dermis. This may merely be a reflection of the overall decrease in growth rate often observed in EGF-treated animals, but the possibility that EGF has a specific effect either inducing fat mobilization or causing epidermal growth at the expense of the dermis has not been eliminated.

Antiserum to EGF has been prepared by repeated

* Abbreviation: EGF, epithelial growth factor.

monthly injections of 1 mg amounts in Freund's complete adjuvant. Its use as a screen for improving purification methods based on its precipitating properties has been described (Fenton *et al.* 1970). Its biological effects are difficult to explain, since it appears both to neutralize the EGF and mimic its effects.

Histological evidence in support of these results is described elsewhere (Farebrother & Mann, 1970).

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The Histological Effects of Epithelial Growth Factor and its Antiserum in the Neonatal Rat

By D. A. FAREBROTHER and CAROLINE B. MANN. (*Department of Pathology and of Biological Chemistry, The Wellcome Research Laboratories, Beckenham, Kent, U.K.*)

Cohen & Elliott (1963) showed histological evidence for thickening of the skin in neonatal rats when treated with a highly purified extract of the submaxillary gland of adult male mice. We have extended the histological observations to cover other tissues. All previous studies on epithelial tissues other than skin have been carried out in organ culture (Jones, 1966; Grossman *et al.* 1969; Turkington, 1969).

Neonatal rats were dosed daily with purified EGF* at 0.5–6 µg/g body wt. per day and killed by decapitation after dosing on the fifth day. Tissues were removed and fixed in 10% formalin buffered to pH 7.2. In addition, some pieces of skin were fixed in 70% ethanol. All tissues were embedded in paraffin wax, sectioned at 5 µm, stained with haematoxylin-eosin and with the Alcian Blue Schiff-periodate method. The fluorescent staining method of Jarrett, Spearman & Hardy (1959) was used to see whether there was any alteration in the type of keratin formed.

Tissues affected were kidney capsule, pericardium, liver, oesophagus, tongue and skin. The liver showed bile-duct proliferation.

The most noticeable effects were seen in the skin, the granular layer and the keratin showing increased thickness. (These findings also applied to the oesophagus and tongue.) The thickness of the dermis was decreased and this was accompanied by a decrease in fat. Histological findings supported the effects of dosing noted by Mann & Fenton (1970),

* Abbreviation: EGF, epithelial growth factor.