control. In all results cited the differences between treated and control animals were statistically significant ($P < 0.05$). It is apparent therefore that the castrated animals were more sensitive to the action of the drug, when given at low doses than the intact animals.

In contrast with the 50% decrease in incorporating ability and the lower polyribosome content of ribosomes from skeletal muscle of castrated immature rats (Breuer & Florini, 1965), we could find only a slight decrease (92% of intact control) in the incorporating ability of ribosomes from castrated adult rats and virtually no differences between the sucrose density gradients of ribosomes from intact and castrated animals.

These results are consistent with the idea that the susceptibility of muscular protein synthesis to the antianabolic action of anti-inflammatory glucocorticoids is, in spite of previous findings (Bullock, Peters & White, 1969), to some extent moderated by androgens.

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Effect of Cortisone on Transfer Ribonucleic Acid and Aminoacyl-Transfer-Ribonucleic Acid Synthetases in Rat Liver

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Prior RNA synthesis is consistently given as the explanation for the subsequent induction of selected enzymes by glucocorticoids in mammalian liver. Because of the central role of tRNA in translation of the genetic message and because of the known quantitative and/or qualitative changes in these parameters under a variety of pathophysiological syndromes (reviewed by Baliga, Borek, Weinstein & Srinivasan, 1969), including the changes brought about by two animal hormones [oestrogen on chick oviduct (O'Malley, Aronow, Peacock & Dingman, 1968) and thyroxine on rat liver (Yang & Sanadi, 1969)] and a plant hormone [6-benzyladenine (Anderson & Cherry, 1969)], it was important to determine whether the 20–30% increase in

tRNA synthesis (with orotic acid as the precursor), observed here as early as 30 min after cortisone administration, reflected general increase in all tRNA species or stimulation of selected tRNA species.

Co-chromatography of doubly labelled tRNA, prepared from saline- or cortisone-injected rats, in presence of [^{14}C]- and [^3H]-orotic acid respectively, on a benzoylated DEAE-cellulose column, where each type of tRNA is eluted as a sharp peak at a characteristic position, revealed a constant $^3\text{H}/^{14}\text{C}$ ratio over the entire range of u.v.-absorbing material, indicating that all tRNA species were uniformly labelled. Similarly, the amino acid-acceptance capacities were unaltered after cortisone administration when tested with synthetases both from normal and from hormone-injected rats. Possible alterations in selected isoaccepting species (see references) were next investigated. Co-chromatography on an RPC-Freon column of tRNA^{Leu} or tRNA^{Tyr}, obtained from saline- or cortisone-treated rats and aminoacylated *in vitro*, did not reveal qualitative differences in the isoaccepting species for these two tRNA species. Modification of tRNA function by cortisone through attachment or incorporation into tRNA was investigated by density-gradient centrifugation. No binding to tRNA of [^3H]cortisone administered *in vivo* or [^3H]cortisol *in vitro* was evident.

From these and other studies it seems that the general increase in liver tRNA synthesis effected by cortisone is likely to be a prelude to, and part of, the hepatomegaly that follows hormone administration, and is probably not related to the induction of specific enzymes by the glucocorticoid.

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Incorporation of ^{14}C from [^{14}C]Glucose into Amino Acids by Rumen Bacteria

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The extent to which the rumen microbial population can synthesize amino acids from carbohydrate and simple nitrogen sources and thus supplement the low amounts of amino nitrogen in the ruminant diet is largely unknown. However, pure culture

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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

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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.