proportions, with a standard deviation per residue of less than 5%.

Amino acids were also released quantitatively from bradykinin and [5-valine]-angiotensin II amide.

Incubations of two D-residue analogues of [5-valine]-angiotensin II amide ([6-D-histidine]- and [2-D-arginine]-angiotensin II amide) showed that bonds adjacent to D-amino acid residues were not cleaved.

Enzyme digestion was applied to Synacthen that had been partially racemized by alkali treatment (Lee & Buettner-Janusch, 1963). The results indicated where extensive racemization had probably occurred.

Incubation of performic acid-oxidized ribonuclease showed that aspartic acid and glutamic acid could be detected separately from asparagine and glutamine. Glutamine and methionine sulphone were not released quantitatively, and the amounts of these amino acids did not increase when the time of incubation was increased.

Hydrolysis by bound peptidases might be a useful method both as a purity check for synthetic peptides and in the elucidation of peptide and protein amino acid sequences.

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Model of an Insulin Dimer and Tetramer, and a Hypothetical Proinsulin

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Revised X-ray co-ordinates for insulin giving data for both the main chain and R-group atoms have become available (Blundell *et al.* 1971), and from these a number of atomic models of the monomer have been built. However, as the molecule crystallizes as a hexamer composed of three identical dimer pairs, I have constructed a Biobits model (Smith & Smith, 1970; Smith, Smith & Roberts, 1970) illustrating various points that will not be seen from the atomic monomer model.

First, a complete insulin dimer showing both α - and β -chains together with all R groups was constructed. Secondly, a single-backbone-only mono-

mer was built on each side of the complete dimer, thus enabling a tetramer to be examined from all sides. The two zinc atoms are included so that a complete idea of the hexamer can be visualized.

The model is extremely lightweight and stable so that it can be turned and examined from all directions. Possible peptide chain interactions can be considered as well as R-group interactions and, because each R group is represented by a single bit, such groups can be readily inserted or removed without disturbing the basic molecular structure. It is also possible to insert the proinsulin intermediate peptide and to hypothesize on the three-dimensional structure of this molecule, on the different immunological and other observed reactions between the two etc.

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Antimicrobial Proteins in Bovine Neutrophils

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Milk of cows with mild mastitis has greater antimicrobial activity than has normal milk (Derbyshire, 1964). The phagocytic activity of the cellular factor has been described (Elek, 1959), but there has been less definition of the antimicrobial humoral factors in mastitis milk. One of the humoral factors described is a basic protein fraction with antimicrobial activity isolated from the cells in bulk milk samples (Hibbitt, Brownlie & Cole, 1971). It was stable to heating at 70°C for 30 min. The present work indicates that an acid-extractable fraction is obtainable from the cells of mastitis milk, consisting of 95–99% neutrophils, with activity against streptococci and both coagulase-positive and coagulase-negative staphylococci.

A sterile mild mastitis was induced in the cow by infusion of 1 μ g of an *Escherichia coli* lipopolysaccharide endotoxin through the teat canal and the milk was collected 16h later. Cells were removed from the milk by centrifuging at 400g_{av}. and washed three times in a cold iso-osmotic saline. The cells were homogenized in ice-cold 0.2M-hydrochloric acid in a Waring Blendor at full speed for 2min, then centrifuged at 20 000g_{av}. for 10min. The clear supernatant was dialysed against five changes of double-glass-distilled water, and any

euglobulin precipitate was removed by centrifugation before the supernatant was freeze-dried. To check the possibility of contamination with serum components, this fraction was checked against anti-(bovine serum), but no lines of identity were formed. From this cell fraction, a basic protein subfraction was isolated after passage straight through a DEAE-cellulose column at pH 7.5 in a 10mM-phosphate buffer. The eluate was bound to a CM-cellulose column at pH 4.6 in an 0.1M-acetate buffer and was removed with 0.2M-hydrochloric acid as a single peak. This basic fraction had a spectrum of antimicrobial activity similar to that possessed by the original fraction, and was used to produce an antiserum.

The antiserum gave three lines of identity against this fraction, and was used to estimate semi-quantitatively the basic protein fraction that had been released from milk cells into milk whey. A technique with a double-diffusion precipitin assay was used (Feinberg, 1959). The increase in the basic protein fraction isolated from milk was closely related to the increase in the antimicrobial activity in the milk whey from cows with an induced mild mastitis.

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Biosynthesis of Yeast Mitochondria: Effects of Rifampicin

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The *petite* mutation in *Saccharomyces cerevisiae* involves an irreversible loss of the ability of the yeast to assemble functional mitochondria. Williamson (1970) has suggested that *petite* mutants might interfere with the action of a mitochondrial DNA-dependent RNA polymerase in its synthesis of a repressor of a nuclear gene coding for an initiator of mitochondrial DNA replication. We have shown that rifampicin, an inhibitor of prokaryotic (Ezekiel & Hutchins, 1968), chloroplast

(Surzycki, 1969) and rat liver mitochondrial (Gadaleta, Greco & Saccone, 1970) RNA syntheses, considerably enhances the *petite* mutagenic effect of ethidium bromide treatment and depresses the mutagenic effects of acriflavine or nitrogen-source starvation treatments, though having no mutagenic effect alone.

Rifampicin, however, is shown to have no effect on the growth rate or respiratory activity of yeast cultured with glycerol as a carbon source, which implies that under these conditions rifampicin does not appear to interfere with transcription of mitochondrial DNA (see also Williamson, 1970). The possibility remained that conditions of glucose repression, under which the ethidium bromide and acriflavine experiments were carried out, might cause a breakdown of a mitochondrial permeability barrier to rifampicin. This possibility was eliminated by the demonstration that rifampicin did not affect the ability of the yeast to recover respiratory activity on transfer from anaerobic culture with glucose as a carbon source to aerobic culture with glycerol as a carbon source.

Wintersberger (1970) also showed that RNA polymerase isolated from yeast mitochondria is not sensitive to rifampicin. Nor is cytoplasmic RNA synthesis in yeast significantly affected by the antibiotic (Dezelee, Sentenac & Fromageot, 1970).

If the effects of rifampicin on the *petite* mutation do result from inhibition of RNA synthesis, then its action must be on a polymerase, possibly extra-mitochondrial, involved in the maintenance of functional mitochondria and representing only a small fraction of total RNA polymerase activity.

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