blue shift of the Soret band and a loss of the 695 nm. band, and the Soret band of the ferro form increased in extinction by 15% without shift. Product III probably had a much looser crevice structure than product I, as product III was autoxidizable and not reduced by NADH-cytochrome *c* reductase. The *pK* values for the high-spin transition for native cytochrome, product I, and product III were 2.5, 2.7 and 4.3 respectively. The ferro form of product III, unlike product I, was partly in the high-spin state at a neutral pH and was converted into a low-spin state by imidazole. A ligand change from sulphur to an oxygen atom after methionine oxidation, unlike carboxymethylation, may result in only a small disruption of the haem environment.

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Biochemical Changes in Skin and Muscle after Thermal Injury

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The concentrations of various enzyme systems have been examined in homogenates of skin and muscle taken from the hind limbs of cats after thermal injury, to compare changes in the enzymic activities of the tissues with those previously found in the lymph draining the tissues (Lewis, 1967). A mean decrease of 14% was found in the lysosomal enzyme acid phosphatase in muscle and skin, and a second lysosomal enzyme, β -glucuronidase, was decreased by 15% in muscle and 50% in skin. However, no increase in either of these enzymes was found in the lymph draining the injured limb. Mean increases were observed in the skin cytoplasmic enzymes lactate dehydrogenase and creatine phosphokinase of 100% and 35% respectively, in spite of the facts that up to 30% of the total skin lactate dehydrogenase appeared in the lymph during 4 hr. after injury, and that overall protein-synthetic activity measured by the incorporation of labelled precursors *in vivo* and by the activity of skin ribosomes *in vitro* (White & Worthington, 1968) was decreased by about 50%. Further, administration of the inhibitors cycloheximide and actinomycin failed to affect the increased concentrations of cytoplasmic enzymes in skin and

lymph. Therefore the changes in these enzymes cannot be due to increases in their respective rates of synthesis but might be attributed to some form of activation. Examination of the isoenzyme patterns of lactate dehydrogenase by isoelectric focusing in polyacrylamide gels (Dale & Latner, 1968) indicated that formation of isoenzyme LDH-1 (H_4) was stimulated after thermal injury both in skin and in lymph even after the administration of cycloheximide.

As early as 30 min. after thermal injury, there was a decrease in skin and muscle glycogen of approx. 50% and, after 4 hr., an average increase in skin DNA of 43%. These changes, taken together with formation of isoenzyme LDH-1, which favours pyruvate oxidation, may well constitute a part of the mechanism leading to tissue repair.

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Changes in the Cell-Wall Composition of a Strain of *Bacillus subtilis* grown in a Chemostat

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The cell-wall composition of strains of *Bacillus subtilis* has been shown to change qualitatively and quantitatively when the organisms are grown under different growth-limiting conditions in a chemostat. Thus, strains that contain a teichoic acid (a phosphorus-containing polymer) in their walls lose this polymer when grown under phosphorus-limiting conditions (Ellwood & Tempest, 1967, 1968).

A laboratory strain of *B. subtilis* was found not to contain any teichoic acid in its wall, and to have a wall composition similar to that of *Bacillus polymyxa* (Goundry, Davison, Archibald & Baddiley, 1967; Nermut & Murray, 1967), suggesting the presence of an outer coat of lipoprotein.

It was of interest to discover what changes occurred in the composition of the cell wall of this organism with respect to the lipoprotein coat, when it was grown with different limiting substrates in a chemostat.

Cell walls from this *B. subtilis* grown in several different limiting media were prepared by disintegration of heavy suspensions of washed organisms in a Braun M.S.K. disintegrator. Chromatographic analyses of the cell walls after acid hydrolysis showed the presence of ten amino acids and

three sugars (one of which was glucose), the same components being found in all cases. However, quantitative analyses for protein (Lowry, Rosebrough, Farr & Randall, 1951), carbohydrate (Dubois, Gilles, Hamilton, Rebers & Smith, 1956), and free lipid (extraction with chloroform-methanol, 2:1, v/v) showed large differences. The cell walls of organisms grown under magnesium-limitation contained 40% protein, 12% carbohydrate and 30% lipid, whereas the walls from nitrogen-limited organisms contained 20%, 30% and 11% respectively. Cell walls from organisms grown for comparison in a complex medium in a shaken-flask batch culture and harvested in mid-exponential phase had 20% protein, 23% carbohydrate, and 5% lipid. There were also large differences in the amount of lipoprotein material that could be removed from cell-wall preparations by extraction with sodium dodecyl sulphate.

These results show that the lipoprotein coat of the cell wall of this strain of *B. subtilis* varied in amount when the organism was grown in a chemostat in different limiting media. This demonstrates once again the importance of defining the growth conditions of bacteria in interpreting cell-wall analytical results.

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Insulin-like Action of 3-Hydroxybutyrate in the Ewe

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The relationship between the blood ketone-body concentration and the plasma non-esterified fatty acid (NEFA) concentration in non-lactating ewes, reported by Reid & Hinks (1962), suggests the possibility that blood ketone-body concentration may influence the rate of NEFA release in the sheep. It has been shown in the dog and in man that infusion of ketone bodies lowers plasma NEFA and blood glucose concentrations (Mebane & Madison, 1964; Senior & Loridan, 1968) and also inhibits the

rise in the plasma NEFA concentration induced by noradrenaline administration (Björntorp & Schersten, 1967).

In the present study it was shown that 3-hydroxybutyrate has an anti-lipolytic action in the ewe. Infusion of 3-hydroxybutyrate at 46 or 115- μ moles/kg. body wt./min. for 20 min. resulted in a fall of 28% in the plasma NEFA concentration from the mean resting value of 380 μ equiv./l. In addition, the effect of infusion of noradrenaline (3.0 nmoles/kg. body wt./min. for 20 min), which normally results in a sixfold rise in plasma NEFA concentration, was virtually abolished by infusion of the higher dose of 3-hydroxybutyrate for 20 min. before, and during, the period of noradrenaline infusion.

3-Hydroxybutyrate infusion also influenced the plasma glucose concentration. Infusion of 3-hydroxybutyrate (115 μ -moles/kg. body wt./min. for 20 min.) significantly lowered plasma glucose concentration from 5.03 mM to 4.32 mM. The 50% rise in plasma glucose concentration normally induced by noradrenaline (3.0 nmoles/kg. body wt./min. for 20 min.) was diminished when 3-hydroxybutyrate was infused for 20 min. before, and during, the 20 min. infusion of noradrenaline.

These results indicate that 3-hydroxybutyrate has an insulin-like action in the ewe. It has been inferred that the anti-lipolytic and hypoglycaemic effects of 3-hydroxybutyrate in the dog may result from increased insulin secretion induced by 3-hydroxybutyrate (Mebane & Madison, 1964). However, Manns, Boda & Willes (1967) have shown that in the sheep infusion of 3-hydroxybutyrate does not stimulate insulin secretion. Thus the possibility that the insulin-like action of 3-hydroxybutyrate is mediated through the release of insulin seems unlikely in this species.

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