tions from pregnant rats is due to a decrease in the content of cytochrome *P*-450 and not to inhibition by progestogens.

We are indebted to the Sir Halley Trust Fund for the financial support given to M.G.N. for this work.

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Microsomal Dehydrogenation of *cis*- and *trans*-Acenaphthene-1,2-diol to Acenaphthenequinone

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1,8-Naphthalic acid has been isolated after the oxidation *in vivo* and the microsomal oxidation of *cis*- and *trans*-acenaphthene-1,2-diol (Hopkins & Young, 1966; Hopkins, 1968). It was suggested by Hopkins (1968) that the oxidation might involve an initial dehydrogenation of the diols to produce aldehydic or ketonic compounds. The presence of acenaphthenequinone in microsomal incubation mixtures of the diols has now been demonstrated by thin-layer- and gas-liquid-chromatographic methods, and the compound has also been isolated.

cis- or *trans*-Acenaphthene-1,2-diol (1.5 m-moles) in 0.1 M-phosphate buffer, pH 7.4, was incubated at 37° for 2 hr. in open flasks with NAD⁺ (0.15 m-mole), nicotinamide (4.5 m-moles), and a rat liver microsomal preparation. Acenaphthenequinone was isolated from the incubation mixture, and was characterized by comparison with the authentic compound and by conversion into the 2,4-dinitrophenylhydrazone.

The formation of carbonyl material was followed during microsomal incubations of each diol. Samples of the incubation mixture were treated at timed intervals with 2,4-dinitrophenylhydrazine and the increase in *E*₄₅₀ in the absorption curve of the resultant 2,4-dinitrophenylhydrazone was measured. No increase was observed in the absence of microsomes or in the presence of microsomes inactivated by heat. In the absence of NAD⁺, the rate of increase was much less. The appearance of the absorption curve resembled closely that of authentic acenaphthenequinone 2,4-dinitrophenylhydrazone.

The possibility arises that naphthalic acid, isolated by Hopkins (1968) from microsomal incubations of diols, was formed from acenaphthenequinone during the isolation procedure. In that procedure, the incubation mixture was left at room temperature overnight at pH 11, before adjustment

to pH 2. In the present work, acenaphthenequinone was kept overnight in the presence of a rat liver microsomal preparation at pH 11 and then the mixture was adjusted to pH 2. Naphthalic acid could readily be detected by gas-liquid chromatography, whether or not the microsomal preparation had been inactivated by heat. Only trace amounts of naphthalic acid could be detected, however, if the quinone had stood overnight in the microsomal preparation at pH 7.4, before adjustment to pH 2. Further aspects of this will be discussed.

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Modification of Methionine in the Haem Environment of Cytochrome *c*

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It has been suggested that the spectral changes when horse heart ferricytochrome *c* is modified by *N*-bromosuccinimide (NBS) are caused by tryptophan modification (Yonetani, 1968; Stellwagen, 1968). However, if methionine is a haem-coordinating ligand (Harbury *et al.* 1965), oxidation of methionine to sulfoxide (O'Brien, 1966) is more likely to explain the spectral changes. Formation of the NBS-modified product (I) resulted in a 4 nm. blue shift of the Soret band and disappearance of the 695 nm. band of the ferri form; the ferro form underwent a 2 nm. red shift of the Soret band and a decrease of the α - and β -bands. Tryptophan is probably not involved, as similar spectral changes occurred after reaction with NBS at pH 7 although less tryptophan was modified at this pH. At pH 3.0, NBS modified the cytochrome to a high-spin product (II) with a high-spin and mixed-spin-type spectrum up to pH 7.5. The high-spin type had a Soret band of 391 nm. whereas that of native cytochrome *c* was 394 nm. The spectrum of the ferro form was similar to reduced produce I.

A haem peptide isolated from a tryptic hydrolysate of cytochrome *c* formed low-spin haemochromes with imidazole, methionine and methionine sulfoxide. The α -bands for these haemochromes were 550 nm., 552 nm. and 547 nm. respectively. If these band positions correspond to the electronegativity of the ligand atom, the atoms involved may be nitrogen, sulphur and oxygen respectively. Methionine sulphone and the carboxymethylsulphonium salt of methionine did not form haemochromes.

Carboxymethylation of both methionine residues of cytochrome *c* (product III) resulted in a 2 nm.

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.

I thank the National Research Council of Canada for financial support.

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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