The biological activity of corticoid hormones is characterized in particular by their so-called glucotropic and mineralotropic effect, i.e. by an effect on glycogen storage and on retention of sodium and excretion of potassium ions by the organism. Cortisol and cortisone display a marked glucotropic activity while their mineralotropic activity is low. Prednisone and prednisolone, i.e. the 1-dehydro derivatives of cortisone and cortisol exhibit a 3–4 times higher glucotropic activity than the starting cortisone and cortisol. No mineralotropic activity of prednisone and prednisolone has been demonstrated. In view of these properties prednisone and prednisolone are made use of against rheumatoid arthritis.

For introducing a double bond into position 1-2 of the A ring of the pregnene skeleton either purely synthetic or microbiological procedures can be used. Microbial dehydrogenation of cortisone gives higher yields of 1-dehydrocortisone (prednisone) than the synthetic process. For this reason commercial prednisone is prepared nowadays mostly by the microbiological method.

Various microbial strains were used for introducing the double bond into position 1-2 of the cortisone molecule, such as Bacillus subtilis (Lindner et al. 1956), Bacillus sphaericus (Stoudt et al. 1955), Corynebacterium simplex (Nobile et al. 1955), Mycobacterium sp. (Krasilnikov et al. 1959), Didymella lycopersici (Visher, Myestre & Wettstein, 1955) and Nocardia blackwelli (Stoudt et al. 1958).

In the present work our attention was directed to the selection of a suitable microbial strain and to the finding of optimal transformation conditions for dehydrogenating cortisone in position 1-2 of the steroid skeleton.

**MATERIALS AND METHODS**

**Microorganism.** Transformation was investigated in 248 strains of various genera (Fusarium, Actinomyces, Proactinomyces, Nocardia and Mycobacterium) under submerged conditions on a reciprocal shaker. Strains of the genus Fusarium were cultivated for 48 hrs. in 100 ml. wort, the other strains for 24 hrs. in 10 ml. broth. The strains tested were obtained from the collections of microorganisms of the Biological Institute of the Czechoslovak Academy of Sciences, from the Department of Microbiology, Faculty of Natural Science, J. E. Purkyně University, Brno, Department of Technical Microbiology and Biochemistry, Faculty of Chemistry, Slovak Technical College, Bratislava, American Type Culture Collection, Research Institute of Antibiotics, Moscow and Research Institute of Pharmacy and Biochemistry, Prague. They were first evaluated as to their qualitative ability to dehydrogenate cortisone in position 1-2 with the aid of the colour reaction with Lugol’s solution. In strains which displayed the ability to dehydrogenate cortisone to prednisone within 48 hours of fermentation the course of transformation and the rate of transformation of cortisone into prednisone
was investigated quantitatively. Among the strains capable of carrying out the transformation there were marked differences in the rate of the reaction. It was most rapid in the strain *Mycobacterium flavum* 390 (from the Research Institute of Pharmacy and Biochemistry) therefore the named strain was used for studying the optimal conditions of dehydrogenation of cortisone into prednisone.

*Cultivation and transformation of steroids.* The inoculation and the actual cultivation proceeded in 750 ml. Erlenmeyer flasks containing 100 ml. broth, placed on a reciprocal shaker (9 cm. amplitude, 90 rev./min.) at 30°C for 24 hrs. The inoculum used amounted to 2% culture volume. The growth of the culture was followed turbidimetrically (spectrophotometer Coleman) at 575 mμ. For tentative transformation tests cortisone at a concentration of 20 mg. was used while for quantitative colorimetry 40 mg./100 ml. medium was applied. In both cases, cortisone was dissolved in 2 ml. methanol before addition to the medium. After adding cortisone, samples for analysis were removed at various time intervals.

**Analytical methods.** For tentative tests of the dehydrogenating capacity the different colour reaction of cortisone, prednisone and of the 20β-hydroxy epimer of its 20-dihydro derivative (III) with Lugol's solution was used. While cortisone reacts with Lugol, giving rise to a blue colour, prednisone and the above-mentioned 20β-hydroxy derivatives give brown colour with the reagent. Five-ml. samples of the medium were removed after 3, 6, 24 and 48 hours of fermentation and extracted with 10 ml. chloroform. The chloroform was evaporated on a porcelain dish, the residue extracted with 1 ml. chloroform and placed on a disc of chromatographic paper Whatman no. 4 (disc diameter 2 cm.). Drying was effected with an infrared lamp. After application of the sample the paper disc was placed on a dish with Lugol's solution. In the presence of cortisone the paper is coloured blue. If the medium contains less than 25% cortisone and if prednisone or the 20β-hydroxy derivative (III) is formed as the main metabolite the paper disc is coloured yellow-brown. The colour of cortisone, prednisone or of the 20β-hydroxy derivative (III) is particularly well pronounced after removing excess Lugol's solution with water.

The content of the prednisone and of the 20β-hydroxy derivative (III) formed during transformation was estimated by quantitative colorimetry on the basis of a colour reaction with sulphuric and acetic acid. Basically, the method described earlier for estimating prednisone in the presence of cortisone (Kakáč, 1959) was used. In a similar manner, but using ethanol in place of acetic acid, the content of prednisolone during microbial dehydrogenation of cortisol was estimated by Mizsei and Szabó (1961). On applying the present modification (Kakáč, 1962) prednisone produces maximum yellow-brown colour only after 60 min. of heating to 100°C while the 20β-hydroxy derivative reaches its final colour intensity after 3 min. at the same temperature.

20β-Hydroxy derivative (III). Three ml. chloroform extract is evaporated to dryness in a water-bath, 5 ml. dilute acetic acid (10 : 1) is added to the residue and the contents of the test-tube heated in a boiling-water bath for 3 min. Then 1.5 ml. concentrated sulphuric acid is added to the test-tube, the contents are shaken and left standing for 3 min. After this period the test-tube is submerged for 2 min. into a boiling-water bath and the contents are cooled under tap water. After 10 min. of cooling the content of 20β-hydroxy derivative of prednisone is estimated in 1 cm. cuvettes on a Hilger Spekker photocolorimeter against a blue-green filter Ilford H-606.

**Prednisone.** Three ml. chloroform extract is evaporated in a water-bath to dryness and 5 ml. concentrated acetic acid and 1 ml. concentrated sulphuric acid is added to the residue. After shaking the test-tube