Identification of Phenazocine, a Potent New Analgesic

THE synthesis of a new analgesic of remarkable potency, phenazocine (2-hydroxy-5,9-dimethyl-2-phenethyl-6,7-benzomorphan), has recently been announced[1]. This compound, of which the (−) isomer has an analgesic effect twenty times as great as that of morphine, is now undergoing clinical trials in the United States.

The absolute identification of a compound of this type is of considerable importance, as all synthetic analgesics previously described have been shown to be habit forming, and have therefore been placed under international control. Phenazocine may be identified by both colour and crystal tests[2]. It gives a brown colour with the formaldehyde/sulphuric acid reagent (Marquis) and a bright blue turning to yellow green with the ammonium molybdate/sulphuric acid reagent. It resembles the morphine alkaloids in giving a yellow colour followed by orange with Vitali's test, while with the micro-diazo test[3], when coupled with diazotized *p*-nitroaniline, it gives a brown colour, turning to bluish grey as the test drop dries. These tests, however, do not serve to differentiate between the racemic and the optically active forms of phenazocine, nor to distinguish this compound from 2-hydroxy-2,5,9-trimethyl-6,7-benzomorphan, which also has analgesic properties[4]. Nevertheless, this may readily be done by means of crystal tests. With potassium iodide solution (±) phenazocine gives oily rosettes, the (−) isomer an oily amorphous precipitate, and the trimethyl compound no precipitate at all. With sodium carbonate solution they give bunches of irregular prisms, fans of oily needles, and dense rosettes of prisms respectively. The first two crystallize slowly and incompletely, while crystals of the last form in a few minutes. With picrolonic acid (±) phenazocine gives an oily precipitate, (−) phenazocine shell-like rosettes, and the trimethyl compound curving blades that are highly characteristic.

Run as a paper chromatogram, using the butanol-citric acid system described by Curry and Powell[5], phenazocine has an R_F value of 0·80 and 2-hydroxy-2,5,9-trimethyl-6,7-benzomorphan a value of 0·45

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Corticosterone Inhibition of Pyridine Nucleotide Oxidase from Heart Sarcosomes

IT has recently been found in this laboratory that corticosterone is present in higher concentrations in heart tissue than in plasma[1]. Since steroid hormones are known to interfere with tissue oxidations, presumably at the level of the flavine enzymes[2], the relative abundance of corticosterone in heart tissue suggested a study of the effect of this steroid on heart tissue oxidations *in vitro*. Reduced diphosphopyridine nucleotide was chosen as substrate in order to get the flavine enzymes involved as directly as possible. The enzyme preparation used in the experiments was made from isolated pig heart sarcosomes. These were ground with alumina oxide, suspended in dilute tris-buffer *p*H 7·4 and centrifuged for 20 min. at 25,000 *g*. The opalescent supernatant contained an active reduced diphosphopyridine nucleotide-oxidase[3] with a specific activity of about 0·1 μmole reduced diphosphopyridine nucleotide oxidized per min. per mgm. protein at 25° C.

The time course of the oxidation of reduced diphosphopyridine nucleotide is presented in Fig. 1. Addition of corticosterone to a final concentration of 10^{-4} *M* produces an instantaneous fall in the rate of oxidation as measured by the decrease in optical density at 340 mμ of the reaction mixture. The reaction product was diphosphopyridine nucleotide also when corticosterone was present. This could be shown by the restoration of optical density to the initial value following addition of the diphosphopyridine nucleotide-specific alcohol dehydrogenase and ethanol.

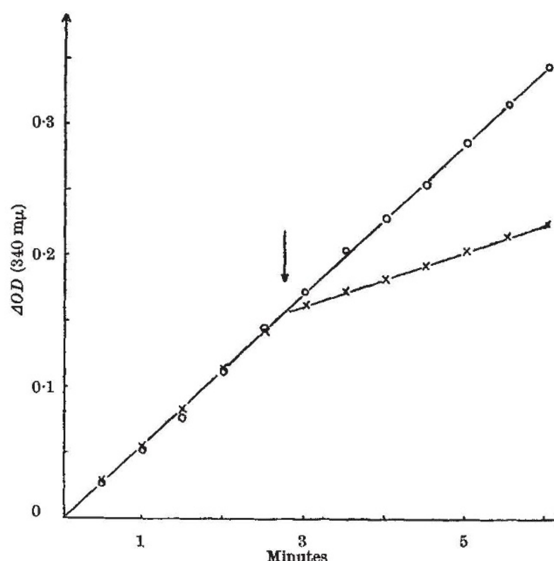

Fig. 1. Corticosterone inhibiton of reduced diphosphopyridine nucleotide-oxidase. Each cuvette contained 25 μmole *tris*/hydrochloric acid, 0·25 μmole reduced diphosphopyridine nucleotide and enzyme in a total volume of 2·5 ml. *p*H 7·4, 25° C. The reaction was started by addition of the enzyme. The arrow indicates addition of 0·25 μmole corticosterone dissolved in 10 μl. dioxane to the experimental, and of 10 μl. dioxane to the control cuvette. Each point represents the mean of changes in optical density recorded in two separate runs. O—O, control; ×—×, corticosterone

Cytochrome *c* is commonly agreed to be a component of the reduced diphosphopyridine nucleotide-oxidase system[4], linked with the dehydrogenation of reduced diphosphopyridine nucleotide by the cytochrome *c* reductase. The effect of corticosterone on the reduction of cytochrome *c* was therefore compared with the effect on the complete oxidase system. A comparison was also made with the effect of corticosterone on the diaphorase activity of the enzyme preparation. The results are given in Table 1. They show that cytochrome *c* reductase is inhibited to about the same extent as the reduced diphosphopyridine nucleotide-oxidase within the range of corticosterone concentrations used. The diaphorase activity, however, is almost unimpaired by the addition of the steroid. These results thus agree with the above-mentioned suggestion that the site of action of the steroid hormones in the respiratory chain lies between the flavoproteins and cytochrome *c*.

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