that the metal may be removed by dialysis against dithionite *plus* dipyridyl or phenanthroline[10,11].

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Preparation of an adrenal steroid 21-hydroxylating system exhibiting activity in the absence of the carbon monoxide-binding pigment, cytochrome P-450

Cytochrome P-450 has been implicated as the oxygen-activating component in adrenal steroid hydroxylation using various mitochondrial and microsomal preparations[1–3]. We have been unable to confirm the necessity for the presence of cytochrome P-450 in active, purified preparations of steroid 21-hydroxylase (EC 1.14.1.8).

Sheep adrenal glands were processed, fractionated and assayed for 21-hydroxylating activity, using 17-hydroxyprogesterone as substrate, as previously described[4]. The most active dialyzed ammonium sulfate precipitate (0.3 S) was frozen and thawed once, and centrifuged for 20 min at 10 000 × *g*. About 50% of the activity remained in the supernatant, which was further fractionated by gel filtration on a column (5 cm × 100 cm) of Sephadex G-200 at 4°. The column was equilibrated with 0.1 M phosphate buffer (pH 6.8) containing reduced glutathione, 1 mg/ml, and

eluted with the same buffer with an upward flow rate of 20 ml/h. The applied material was resolved into five components of which only the first fraction, traveling with the the front, exhibited hydroxylating activity. Several different homogenates which had been stored for various lengths of time up to 6 months at −20° were processed in this way. Some of these active preparations were concentrated by ultrafiltration to yield protein concentrations of from 1.88 to 11.5 mg/ml (ref. 5). These preparations were capable of 21-hydroxylating 17-hydroxyprogesterone to 17,21-dihydroxy-4-pregnene-3,20-dione with an average production of 58 mμmoles/mg protein per h. These active preparations were analyzed for the presence of the carbon monoxide-binding pigment, cytochrome P-450, by the methods of ESTABROOK, COOPER AND ROSENTHAL[1] and OMURA AND SATO[6]. The difference spectra were obtained with a Bausch and Lomb 505 recording spectrophotometer. There was no evidence for the presence of cytochrome P-450 in any of these active preparations regardless of protein concentration. Identical results were obtained with preparations stored as such, or in the presence of 50% glycerol, at −20°. The absence of P-450 in active preparations obtained by fractionation in the presence of reduced glutathione and subsequent storage in glycerol is all the more pertinent since ICHIKAWA AND YAMANO[7] recently reported the reversal of P-420 to P-450 by treatment with reduced glutathione and the stabilizing influence of glycerol on P 450.

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