CHANGES IN SOME BIOCHEMICAL PARAMETERS INCLUDING CYTOCHROME P-450 AFTER HYPOPHYSECTOMY AND THEIR RESTORATION BY ACTH ADMINISTRATION IN RATS FOUR MONTHS POST HYPOPHYSECTOMY*

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SUMMARY - Following withdrawal of ACTH by hypophysectomy, rat adrenal gland P_{450} content falls to a low level with a half life time of 3.5 days. The content can be restored by subsequent ACTH treatment in animals as long as four months post hypophysectomy with parallel regeneration of in vitro steroid synthetic capacity. The recovery pattern of a number of other gland parameters suggests that the regeneration of P_{450} and steroidogenic capacity in these severely atrophied glands occurs by a mechanism which is largely dependent on cell replication.

INTRODUCTION

Cytochrome P_{450} serves as the terminal oxidase in a variety of NADPH dependent hydroxylation reactions. In mammals it is commonly located in the microsomal fraction except in the case of steroid secreting tissues where it is also found in mitochondria. In liver where P_{450} is confined to the microsomes and involved in the detoxification of many drugs and toxic materials, treatment of the animal with a substrate such as phenobarbitol raises hepatic P_{450} content several fold by an apparent inductive mechanism (1). The factors controling P_{450} levels

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in steroid secreting cells however, are not understood. The situation would appear more complex than in liver due to the presence of P_{450} in the mitochondrial cell compartment and since the various hydroxylases are part of synthetic biochemical pathways. We have utilized the adrenal cortex to study this mechanism(s), since the majority of the cell population is involved with steroidogenesis and since the integrity and functional capacity can be manipulated experimentally by hypophysectomy and subsequent treatment with ACTH.

MATERIALS AND METHODS

Normal and hypophysectomized Sprague-Dawley rats weighing about 150 g. were obtained from Hormone Assay Laboratories, Chicago. Adrenal regeneration was produced by injections of 10 units of ACTH (Acthar, Armour, Co.) given subcutaneously in 0.5 ml 0.9% NaCl at 12 hr. intervals for the time as shown.

The glands were carefully trimmed of surrounding fat prior to weighing. In vitro steroidogenic capacity was determined as described previously (2). DNA and RNA content was determined by the method of Schneider (3). P_{450} level of adrenal gland homogenate was measured by the method of Mitani et al. (4). The level of aa_3 was assayed by reducing with succinate according to the procedure of Cammer et al (5). Protein was determined by the Lowry method.

All measurements on regenerating adrenals were performed 3 hours after the final injection of ACTH at which time the steroidogenic capacity was found to be maximal.

RESULTS AND DISCUSSION

The decay of adrenal cytochrome P_{450} and succinate reducible

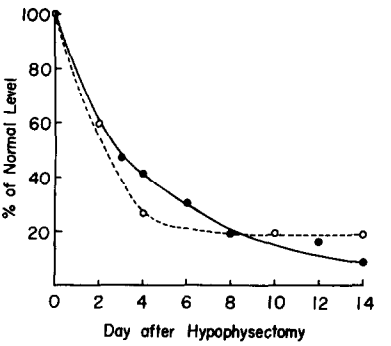

Figure 1 The decay of adrenal P-450 and succinate reducible cytochromes a + a_3 content following hypophysectomy ● - P-450; O - a + a_3. Each point was obtained from 8 adrenals for P-450 and 18 adrenals for a + a_3.

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cytochromes a + a_3 content is shown in Fig. 1. The apparent half life of 3.5 days for P_{450} is in good agreement with that for cholesterol side chain desmolase determined earlier by one of us (6). After approximately two weeks post hypophysectomy a basal level of P_{450} is reached which does not decrease further. This probably represents the P_{450} content of the Zona glomerulosa portion of the gland which is not greatly affected by hypophysectomy (7).

The decay of succinate reducible cytochromes a + a_3 is slightly faster than total P_{450} content. This finding is of interest in view of earlier electron microscope investigations which have emphasized changes in mitochondrial morphology following hypophysectomy (7). The characteristic vesicular form of these mitochondria revert to a more tubular type which closely resembles mitochondria from embryonic adrenal (8). The initial transformation from the tubular to vesicular structure appears to be controlled by ACTH (8). It is then tempting to suggest that the loss of vesicular form represents a dedifferentiation of the mitochondria to a non-

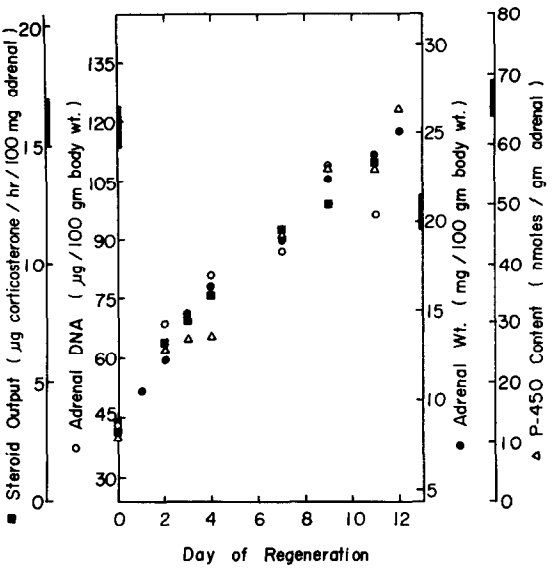

Figure 2 Adrenal regeneration in animals four months post-hypophysectomy. ● - weight, Δ - P-450 content, ■ - steroid output and O - DNA content. Each point represents 8 adrenals except DNA points which are from 4 adrenals. The normal range for each parameter is shown as a bar on its respective axis. The apparent more rapid return of adrenal weight (mg/100 gm body wt) to the normal range is misleading since during the period of regeneration, the average body weight decreased by about 25%. Correcting for this change, the normal value becomes 27 mg/100 g body weight making the recovery of adrenal weight parallel to the other three parameters.

steroid synthetic type. The current results suggest that the mitochondria may also be impaired in their ability to conduct oxidative phosphorylation and render it more difficult to interpret the ultrastructural changes seen in these mitochondria.

The adrenal DNA content of animals 10 weeks post hypophysectomy had fallen to a low level which remained constant for another 18 weeks. The weight, DNA, RNA and protein content could be restored by ACTH treatment. Recovery proceeded at the same rate for animals 10 and 28

weeks post hypophysectomy. Fig. 2 shows the recovery of adrenal weight, P_{450} content, in vitro steroidogenic capacity and DNA content in animals 28 weeks post hypophysectomy. In this experiment all parameters were recovered in a parallel manner indicating that the increases in P_{450} content and steroidogenic capacity were largely a consequence of cell replication. That the newly synthesized P_{450} represents the appearance of functional hydroxylases is demonstrated by the parallel recovery of steroidogenic capacity. ACTH may also increase cellular P_{450} levels concomittantly with its cellular hypertrophic effect. That this may be the case is suggested by the fact that in some experiments the recovery of DNA content in the early stages of regeneration appeared to be slightly faster than the other parameters. A definitive interpretation of this possibility will require further experimentation.

REFERENCES

1. J. Baron and T. R. Tephly, Arch. Biochem. Biophys., 139, 410 (1970).
2. M. K. Mostafapour and T. T. Tchen, Biochem. Biophys. Res. Commun., 44, 774 (1971).
3. W. C. Schneider, Methods in Enzymology, 3, 680 (1957), Academic Press, New York.
4. F. Mitani, and S. Horie, J. Biochem., 65, 269 (1969).
5. W. Cammer and R. W. Estabrook, Arch. Biochem. Biophys., 122, 735 (1967).
6. T. Kimura, Endocrin., 85, 492 (1969).
7. D. D. Sabatini, E. D. P. DeRobertis and H. B. Bleichmar, Endocrin. 70, 390 (1962).
8. A. I. Kahri, Amer. J. Anat. 127, 103 (1970).