## The oxidation and reduction of corticotropin $A_1$

Adrenocorticotropic hormone (ACTH) activity, measured by the ascorbic-acid depletion test[1], is lost when oxidizing agents act on preparations of the hormone. Reactivation of material oxidized by air by treating it with hydrogen sulphide has been reported[2], but reactivation of material treated with hydrogen peroxide was not observed under these conditions[3,4]. DEDMAN, FARMER AND MORRIS[3] discovered conditions for reactivating peroxide-inactivated material when they heated partially inactivated oxycellulose concentrates of ACTH with thiols in acid solution.

DIXON AND STACK-DUNNE[4] demonstrated that a physico-chemical alteration occurred in corticotropin $A_1$ (the predominant form of ACTH extracted from pig pituitaries) when it was inactivated with hydrogen peroxide. The treatment was with 0.8 *M* $H_2O_2$ in phosphate buffer of pH 6.7 for 30 minutes, after which less than 0.5% of the original activity remained. The alteration observed was a fall in retention volume of the substance on chromatography on the carboxylic ion-exchange resin Amberlite IRC-50. The partition coefficient was changed by about 15%. An artificial mixture of the active and inactive substances was separated by ion-exchange chromatography.

DIXON AND STACK-DUNNE[4] successfully reactivated a hydrogen-peroxide inactivated preparation of corticotropin $A_1$, following the method of DEDMAN *et al.*[3]. It was therefore of interest to learn whether the reactivation was accompanied by a reversal of the change in partition coefficient between resin and buffer observed on inactivation. To avoid hydrolysis of corticotropin $A_1$ which might produce active components held more strongly by the resin[5], the treatment with a thiol was carried out under less acid conditions than DEDMAN *et al.*[3] used. Table I shows the results of the experiments.

TABLE I

RETENTION VOLUMES OF PEAKS DERIVED FROM CORTICOTROPIN $A_1$

| *Experiment and column dimensions* | *Column number* | *Treatment* | *Retention volume in ml per 100 ml resin bed* |
|---|---|---|---|
| 1 | 240 | $H_2O_2$ 0.73 *M* 30 min pH 6.7 | 249 |
| | 241 | a. $H_2O_2$ 0.73 *M* 30 min pH 6.7 | |
| 39.5 cm × 0.68 $cm^2$ | | b. Cysteine 0.1 *M* 18 h 69–75° pH 4.1 | 288 |
| | 242 | None | 292 |
| 2 | 252 | $H_2O_2$ 0.84 *M* 30 min pH 6.7 | 257 |
| | 253 | a. $H_2O_2$ 0.84 *M* 30 min pH 6.7 | |
| 42.4 cm × 0.68 $cm^2$ | | b. Thioglycollate 0.2 *M* | |
| | | 18.5 h 72–75° pH 3.4–3.5 | 296 |
| | 254 | None | 298 |

The chromatography followed the details of DIXON AND STACK-DUNNE[4] and their finest resin sample was used to obtain columns of high resolving power.

In experiment 1 corticotropin $A_1$ (15.6 mg) was dissolved in the sodium phosphate buffer used for chromatography (3.5 ml) (0.2 *M* phosphate, $Na^+$ 0.3 *N*, pH 6.70 containing 0.2% phenol), and hydrogen peroxide (0.3 ml 9.2 *M*) added. After 30 minutes catalase solution (0.06 ml 0.01 *M*) was added.

A sample (1.75 ml) was chromatographed (C240, Table I and Fig. 1) and to another sample (1.75 ml) cysteine hydrochloride (63 mg), NaOH solution (0.6 ml 1.0 *N*), glacial acetic acid (0.1 ml) and water (1.5 ml) were added (final volume 4.0 ml, pH 4.1, cysteine concentration 0.1 *M*). The solution was heated in air saturated with water vapour at 69°–75° for 18 hours. The product was recovered by extraction into phenol and removal of the phenol with ether[4]. The yield was 6.6 mg and 5.8 mg were chromatographed (C241, Table I and Fig. 1). The peak, though decreased in size, appeared in the position of corticotropin $A_1$. Some ninhydrin-positive unretarded or slightly retarded material was present (cystine or cysteine?).

In experiment 2 corticotropin $A_1$ (11.6 mg) was dissolved in the buffer (2.1 ml) and $H_2O_2$ solution (0.21 ml 9.2 *M*) added. After 30 minutes catalase solution (0.013 ml 0.01 *M*) was added in portions and the solution stirred until bubbling ceased. To each of two 1.0 ml samples thioglycollic acid (0.016 ml) was added (final concentration 0.2 *M*, pH 3.4–3.5). One was heated to 72°–75° for 18.5 hours and both were chromatographed after neutralizing the acid with sodium hydroxide (0.67 ml 0.3 *N*, to maintain the sodium ion concentration at 0.3 *N*) and adding 0.33 ml

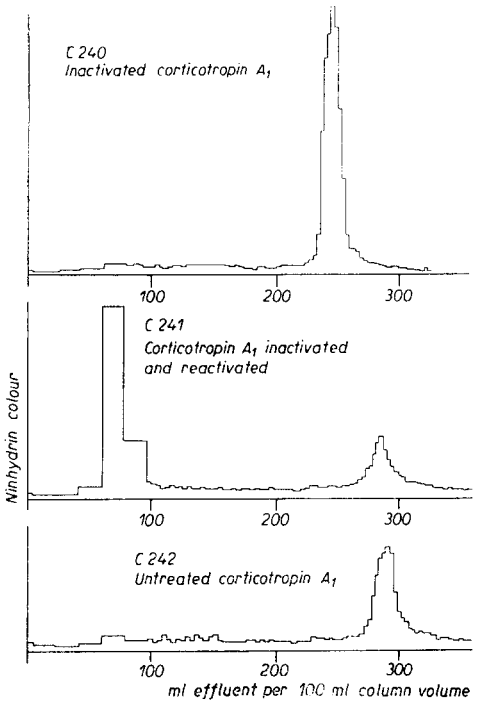


Fig. 1. Comparison of chromatograms of corticotropin $A_1$ after different treatments. Column of 39.5 cm × 0.68 cm² packed with finely ground carboxylic ion-exchange resin Amberlite IRC-50 in equilibrium with the eluent, 0.2 *M* sodium phosphate buffer (pH 6.7, sodium ion concentration 0.3 *N*). C240: Corticotropin $A_1$ treated with hydrogen peroxide; C241: Corticotropin $A_1$ treated with hydrogen peroxide and subsequently with cysteine; C242: Untreated corticotropin $A_1$.

of the phosphate buffer. 5 ml samples of effluent from the column emerging after one free volume were within 0.02 units of the correct pH.

Table I shows that the chromatographic properties of the original corticotropin $A_1$ were restored on heating the inactivated substance with cysteine or thioglycollic acid. The yield appeared low in experiment 1 but the phenol desalting of a small sample made the significance of this doubtful. In experiment 2 the ninhydrin-colour recoveries of the peaks of C252, C253 and C254 were 75%, 29% and 52% (expressed in the case of the first two in terms of the oxidized material before the thioglycollic acid was added). Roughly the same loss, 2,800 and 2,660 arbitrary ninhydrin-colour units, occurred in columns C252 and C254 (corticotropin $A_1$ exhibited a colour yield of 1,600 units per mg). If a similar loss occurred on chromatography in C253, the yield of corticotropin $A_1$ from the inactivated form was 59%. A larger yield, 38%, corrected on the same assumption to 74%, was obtained under the same conditions (thioglycollate 0.2 *M*, pH 3.4–3.5, 71°–81°) when the heating was shortened to 7 hours, and no trace of the faster peak was detectable. The uncertain baseline and the spreading of corticotropin peaks at low loads[4] make the measurement of recoveries unsatisfactory.

DEDMAN, FARMER AND MORRIS[3] discovered that heating peroxide-inactivated oxycellulose concentrates of ACTH with a thiol restored their activity. DIXON AND STACK-DUNNE[4] confirmed this with purified corticotropin $A_1$, and showed that the complete inactivation was accompanied by a change in chromatographic behaviour. The reactivation is now shown to be associated with restoration of the original chromatographic properties. This evidence is strong support for the theory that ACTH activity is an intrinsic property of corticotropin $A_1$ and not merely due to its contamination with a minor component which possesses the biological activity.

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