A Dissociation between the Adrenal and Extra-Adrenal Actions of Corticotrophin by Periodate Oxidation

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In previous reports evidence has been presented that corticotrophin is capable of influencing metabolic processes in extra-adrenal tissue both *in vivo* and *in vitro* (Engel, 1957; Engel, 1961; White & Engel, 1958). The validity of this evidence has depended on the demonstration that these biological actions of corticotrophin occur in the absence of the adrenal cortex and that they are not due to contamination of the corticotrophin with known pituitary factors. Perhaps the most convincing support for the capacity of corticotrophin to act on a tissue independently of adrenal-cortical stimulation is the demonstration that the hormone promotes the release of free fatty acids from adipose tissue and influences the uptake and utilization of glucose by this tissue *in vitro* (White & Engel, 1958; Verner & Engel, 1959; Lynn, McLeod & Brown, 1960). Of significance is the finding that Ca^{2+} ion is required to be present in the incubating medium to support this response (Lopez, White & Engel, 1959; Freinkel, 1961), just as it is necessary for the optimum steroidogenic response of adrenal-cortex slices to corticotrophin *in vitro* (Birmingham, Elliott & Valère, 1953; Péron & Koritz, 1958).

The evidence that the recorded extra-adrenal actions of corticotrophin are not due to contamination of the hormone preparations with other biologically active peptides is less direct. In this Laboratory it has depended on the use, where possible, of highly purified samples of corticotrophin and of the application of chemical and immunological procedures to identify the active peptide as corticotrophin (Kostyo & Engel, 1960). Of significance is our finding (unpublished) that the synthetic nonadecapeptide corticotrophin of Li, Meienhofer, Schnabel, Tung-bin-lo & Ramachandran (1960) and the tricosapeptide corticotrophin of Hofmann, Yajima, Yanaiharu, Liu & Laude (1961) have lipolytic activity on adipose tissue *in vitro*. Particular advantage has been taken of the reversible inactivation of corticotrophin by hydrogen peroxide and cysteine (Dedman, Farmer & Morris, 1955). To our knowledge only the melanocyte-stimulating hormones (Dixon, 1956*a*) and parathyroid hormone (Rasmussen, 1959) share this property.

Dixon (1956*b*) reported that periodate oxidation of corticotrophin eliminated its ability to deplete the adrenal cortex of ascorbic acid. Geschwind & Li (1954) had used this procedure to identify the *N*-terminal serine residue in corticotrophin. Geschwind & Li (1958) and Evans, Rosenberg & Simpson (1961) have confirmed the loss of adrenal-stimulating activity of periodate-treated corticotrophin, both adrenal-ascorbic acid depletion and weight maintenance of the adrenal cortex being used as criteria. In view of our previous studies of the extra-adrenal actions of chemically inactivated corticotrophin, it became of particular interest to test the periodate-treated corticotrophin for these actions. Dr H. B. F. Dixon, Cambridge, generously gave us samples of corticotrophin A_1 treated with periodate by the method he described in 1956, in which the reaction was stopped by the addition of sulphite. Preliminary results revealed that the periodate-treated corticotrophin had ketogenic activity in the rat. Furthermore, it retained the ability to stimulate lipolysis in adipose tissue *in vitro* (White, Lopez & Engel, 1959) and altered glucose uptake by adipose tissue. Steelman & Smith (1960), in a joint study with us, found that the periodate-treated corticotrophin retained adipokinetic activity in the mouse. Assays of adrenal-stimulating activity by the adrenal-ascorbic acid-depletion and plasma-corticosterone methods by Dr R. Guillemin confirmed the loss of potency with respect to the adrenal cortex.

In the foregoing paper, Dixon (1962) describes the preparation of periodate-treated corticotrophin by a new method involving the subsequent use of borohydride and yielding a product in which the *N*-terminal serine residue is presumably replaced with a glycolyl residue. The present report concerns the biological properties of this modified corticotrophin. It confirms and extends the preliminary results with the earlier periodate-treated corticotrophin, demonstrating that the chemical modification of the *N*-terminal serine of corticotrophin yields a peptide with a marked decrease of adrenocorticotrophic activity, as judged by conventional assays *in vivo*, but retention of proportionately greater extra-adrenal activity.

MATERIALS AND METHODS

Hormones

Pig corticotrophin A_1 and pig periodate-borohydride-treated corticotrophin A_1 , lots no. 59/36, C 479 and C 601, were used.

The technique for the preparation of periodate-borohydride-treated corticotrophin and its properties are described in the preceding paper (Dixon, 1962). The corticotrophin A_1 and treated product were freely soluble in water. Standard solutions were prepared and either used immediately or quickly frozen by immersion in solid carbon dioxide and acetone and stored in the deep-freeze. The final dilutions were made just before use. Initial experiments established that the original potencies of the peptides were maintained over long periods under these conditions. Reversible inactivation with H_2O_2 and cysteine was carried out according to a modification of the methods of Dixon (1955) and of Dedman, Farmer & Morris (1957), as previously described (Engel & Engel, 1958).

Corticotrophin assays

These were kindly performed for us by Dr R. Guillemin, Department of Physiology, Baylor University, Texas, using the plasma-corticosterone method in 24 hr.-hypophysectomized rats for the assay *in vivo* (Guillemin, Clayton, Smith & Lipscomb, 1958; Guillemin, Fortier & Lipscomb, 1959) and a modified Saffran & Schally (1955) technique for assessment of steroidogenic potency *in vitro*.

Additional assays *in vitro* by a modification of the technique of Saffran & Schally (1955), in which corticosterone production was measured by H_2SO_4 fluorescence, were also performed in this laboratory.

Extra-adrenal activities

Because of limitations in the quantity of treated corticotrophin available, only those extra-adrenal activities previously demonstrated to be elicited with small quantities of corticotrophin were examined. Two techniques *in vitro* and two techniques *in vivo* were used.

Production of free fatty acids by rat epididymal adipose tissue *in vitro*. Portions (50–60 mg.) of epididymal adipose tissue were excised under pentobarbital anaesthesia from 250–300 g. male albino Wistar rats, kept without food overnight. The tissue was incubated at 37° for 3 hr. in 0.9 ml. of Krebs–Ringer bicarbonate medium containing 2% of bovine serum albumin in 10 ml. stoppered Erlenmeyer flasks, gassed with $O_2 + CO_2$ (95:5). The flasks were shaken in a Dubnoff metabolic incubator at 70–80 oscillations/min. Hormone solutions were added in 0.1 ml. volume; 0.1 ml. of water was used as a control, except where indicated. The free fatty acid contents of tissue and medium were measured by the method of Dole (1956) and expressed as μ moles of free fatty acids/100 mg. of adipose tissue (initial wet wt.). When the potencies of peptides were compared, tissue and medium free fatty acids were measured and net production of free fatty acids was expressed as the difference between total free fatty acids released from the hormone-treated adipose tissue and that from untreated tissue from the same animal.

Glucose uptake by rat epididymal adipose tissue *in vitro*.

The system *in vitro* described above was used except that the medium contained 1 mg. of glucose/ml. and the bovine serum albumin concentration was increased from 2 to 4 g./100 ml. This was done because of the finding that the stimulating effect of corticotrophin and epinephrine on glucose uptake by adipose tissue may be masked or blocked by excessive accumulation of free fatty acids within the tissue when the albumin in the medium is insufficient to accept all the free fatty acids released by lipolysis (Verner, Blackard & Engel, 1962). The glucose uptake was determined from initial and final concentrations of glucose in the medium, estimated by a glucose-oxidase method (Saifer & Gerstenfeld, 1958) and expressed as mg. of glucose abstracted/g. of adipose tissue in 3 hr.

Adipokinetic activity. Liver fat content was determined 4 hr. after intraperitoneal injection of 0.1 ml. of the test solution into 20–25 g. female mice which had been removed from their feeding cages 1 hr. previously (Engel, Engel & McPherson, 1957). The results are expressed as percentage of liver fat by wet weight.

Hypoglycaemic activity. Male albino mice (20 g.) that had been kept without food for 12 hr. were injected intraperitoneally with 0.1 ml. of the hormone dissolved in 0.9% NaCl solution. Before, and 1 hr. after, injection, blood-sugar determinations were performed on $Ba(OH)_2$ - $ZnSO_4$ -treated blood filtrates by the glucose-oxidase method (Saifer & Gerstenfeld, 1958). The results are recorded as the mean difference in blood glucose from the pre-injection level. It has been shown previously that corticotrophin preparations induced hypoglycaemia in both intact and adrenalectomized mice (Westermeyer & Raben, 1954) and rats (Engel & Engel, 1954; Engel, Fredericks, Lopez & Albertson, 1958).

Fig. 1. Seasonal variation in net lipolytic response of rat epididymal adipose tissue to corticotrophin A_1 . Results are expressed as the ratio of the response to 0.5 μ g. of corticotrophin/ml. to that of the unstimulated control. Numbers in parentheses represent the number of observations at each point.

Table 1. Net release of free fatty acid from rat epididymal adipose tissue

The net release of free fatty acids (μ moles/100 mg. of adipose tissue/3 hr.) is the amount released both into the medium and tissue from pieces (50–60 mg.) of rat epididymal adipose tissue stimulated by corticotrophin or periodate-treated corticotrophin in excess of the amount produced by a comparable piece of unstimulated tissue from the same animal. Control tissue released $0.80 \pm 0.053 \mu$ mole/100 mg. in 3 hr. (46 observations). Numbers in parentheses represent the number of observations.

Dose (μ g./ml.)	Corticotrophin A ₁		Periodate-borohydride-treated corticotrophin		
	Lot 1	Lot 2	Lot 59/36	Lot C 479	Lot C 601
5.0	—	—	2.67 ± 0.351 (4)	—	—
1.0	—	—	—	—	1.14 ± 0.170 (5)
0.5	2.33 ± 0.277 (9)	—	0.45 ± 0.142 (4)	0.42 ± 0.133 (11)	0.50 ± 0.133 (6)
0.1	0.83 ± 0.171 (13)	0.95 ± 0.128 (15)	0.17 ± 0.125 (4)	0.02 ± 0.075 (14)	0.26 ± 0.100 (15)
0.05	—	0.33 ± 0.179 (9)	—	—	—
0.01	0.38 ± 0.171 (13)	0.22 ± 0.068 (17)	0.05 ± 0.094 (4)	0.13 ± 0.098 (15)	0.19 ± 0.094 (11)
0.001	—	0.08 ± 0.118 (12)	0.12 ± 0.090 (4)	—	0.18 ± 0.129 (11)

RESULTS

Corticotrophin assay. By the plasma-corticosterone method *in vivo*, in hypophysectomized rats (Guillemin *et al.* 1958), the various lots of corticotrophin A₁ assayed between 65 and 70 units/mg. Lot 59/36 of periodate-borohydride-treated corticotrophin assayed < 0.1 unit/mg., but the other lots (C 479 and C 601) assayed 1.0 unit/mg. By contrast, the potencies by the steroidogenic assay *in vitro* (Saffran & Schally, 1955) were 49 units/mg. for corticotrophin A₁ and 5 units/mg. for periodate-borohydride-treated corticotrophin (lot 59/36). Preliminary assays of lots C 479 and C 601 of the treated corticotrophin likewise indicate that they exhibit approximately one-tenth of the potency of corticotrophin in the assay *in vitro*. The possible significance of the differences in assay results by the techniques *in vivo* and *in vitro* is discussed below.

Lipolytic activity. In a preliminary study in which the lipolytic activity of corticotrophin A₁ was compared with that of lot 59/36 of the treated corticotrophin, it was reported that both peptides induced significant degrees of lipolysis by adipose tissues exposed to concentrations of the peptides as low as 0.1μ g./ml. (Engel, Boright, White & Kostyo, 1960). Hollenberg, Raben & Astwood (1961) have reported comparable activity of corticotrophin on adipose tissue.

These data implied that corticotrophin A₁ and periodate-borohydride-treated corticotrophin have comparable activities on adipose tissue. However, subsequent studies indicated that it was not justified to use these data for estimation of the relative potencies of the two peptides, since they were pooled from a number of animals and collected over several months. Extensive experience with the measurement of free fatty acid release by adipose tissue *in vitro* has shown that not only may there be considerable variation in the lipolytic response of adipose tissue from different animals

but also there may be gross variations in the sensitivity of adipose tissue during different seasons of the year. In general, sensitivity is lowest in mid-winter. Fig. 1 illustrates this seasonal variation in net lipolysis expressed as the ratio of the response of tissue stimulated with corticotrophin A₁ to the control unstimulated tissue. Since inadequate attention was paid to these details in the original experiments of Engel *et al.* (1960), the following study in which these variables were more rigidly controlled was carried out.

Table 1 presents data on net lipolysis by adipose tissue stimulated by corticotrophin A₁ and the treated peptide. In this experiment the activity of the two peptides was tested on the samples of the same adipose tissue and expressed as net lipolysis, as defined in the Methods section. Note that the responses of the adipose tissue to the different lots of periodate-borohydride-treated corticotrophin were similar at any given dose and that approximately 10 times as much of the treated corticotrophin was required to give the same response as produced by corticotrophin A₁. This ratio of activity has now been repeatedly confirmed with different batches of peptides, regardless of the seasonal variation in the sensitivity of the adipose tissue.

The lipolytic action of corticotrophin as well as other adrenal and extra-adrenal effects of the peptide are reversibly inactivated by the hydrogen peroxide and cysteine treatment (White & Engel, 1958). This procedure leads to the reversible oxidation of methionine, the fourth amino acid of corticotrophin, with the formation of the methionine sulphoxide (Dedman, Farmer & Morris, 1961). Table 2 gives results for the reversible inactivation of the lipolytic activities of the two corticotrophins, demonstrating that they behave identically in this regard.

Stimulation of glucose uptake by adipose tissue *in vitro*. In Table 3 are found data showing that both

peptides stimulate glucose uptake by adipose tissue and that this property of periodate-borohydride-treated corticotrophin may be reversibly abolished by hydrogen peroxide and cysteine treatment. No attempt was made to establish the minimal effective dose in this study.

Adipokinetic activity. Five micrograms of each peptide elicited comparable increases in liver fat, whereas 1 μ g. was ineffective (Table 4). Dr M. S.

Raben, Boston, Massachusetts, had previously tested a sample of periodate-borohydride-treated corticotrophin supplied by Dr Dixon and found it to have adipokinetic activity (personal communication.)

Hypoglycaemic activity. In 21 control mice the blood sugar did not change in 1 hr. ($+5 \pm 3$ mg./100 ml.). After intraperitoneal injection of 20 μ g. of corticotrophin A_1 , the blood sugar fell 43 ± 5 mg./

Table 2. *Reversible inactivation of corticotrophin A_1 and periodate-borohydride-treated corticotrophin with hydrogen peroxide and cysteine hydrochloride*

Release of free fatty acids from rat epididymal adipose tissue into incubation medium: 100 μ g. of the peptides was dissolved in 1 ml. of 0.2M-phosphate buffer, pH 6.7; 0.05 ml. of 30% H_2O_2 was added and allowed to stand for 30 min. at room temperature. Excess of H_2O_2 was removed by addition of 0.05 ml. of catalase solution containing 1500 units/ml. The solution was diluted to 5 ml. with water and 2.5 ml. was rapidly frozen with acetone-solid carbon dioxide and saved for later assay. For reactivation, 2.5 ml. of the above solution was incubated with 0.1M-cysteine-HCl at 78° for 19 hr., at pH 4.1. Hydrogen peroxide and cysteine 'blanks' were prepared for controls. Corticotrophin A_1 and periodate-borohydride-treated A_1 , untreated, inactivated and reactivated, were added to flasks at a concentration of 1 μ g./ml. N.S., Not significant.

Peptide preparation	Corticotrophin A_1			Periodate-borohydride-treated A_1		
	No. of observations	Medium fatty acids (μ moles/100 mg.)	P	No. of observations	Medium fatty acids (μ moles/100 mg.)	P
Control (water)	4	0.16 ± 0.10	—	4	0.06 ± 0.14	—
Reagent control	10	0.26 ± 0.14	N.S.	2	0.39 ± 0.02	N.S.
Untreated peptide	8	2.26 ± 0.20	<0.01	8	2.00 ± 0.16	<0.01
H_2O_2 -treated peptide	10	0.32 ± 0.26	N.S.	10	0.22 ± 0.09	N.S.
H_2O_2 -cysteine-treated peptide	8	2.00 ± 0.16	<0.01	10	1.91 ± 0.18	<0.01

Table 3. *Influence of corticotrophin A_1 and periodate-borohydride-treated corticotrophin A_1 on glucose uptake by rat epididymal adipose tissue in vitro*

Conditions of incubation were the same as those given in Tables 1 and 2, except that the medium contained 1 mg. of glucose/ml. and 4% of bovine serum albumin. Reversible inactivation with H_2O_2 -cysteine was carried out as described in Table 2. Glucose uptake is expressed as mg./g. of adipose tissue/3 hr. (means $\pm$ S.E. are given). N.S., not significant.

Peptide treatment	Dose (μ g./ml.)	Corticotrophin A_1			Periodate-borohydride-treated A_1		
		No. of observations	Glucose uptake (mg./g./3 hr.)	P	No. of observations	Glucose uptake (mg./g./3 hr.)	P
Control	—	11	0.86 ± 0.11	—	11	0.86 ± 0.11	—
Untreated	5.0	8	2.40 ± 0.27	<0.01	9	1.86 ± 0.19	<0.01
Untreated	1.0	9	1.69 ± 0.09	<0.01	12	1.93 ± 0.21	<0.01
Control (water)	—	—	—	—	6	0.53 ± 0.14	—
Reagent control	—	—	—	—	11	0.95 ± 0.16	N.S.
Untreated	1.0	—	—	—	12	2.24 ± 0.23	<0.01
H_2O_2 -treated	1.0	—	—	—	12	0.70 ± 0.15	N.S.
H_2O_2 -cysteine treated	1.0	—	—	—	12	1.75 ± 0.21	<0.01

Table 4. *Adipokinetic effect of corticotrophin A_1 and periodate-borohydride-treated corticotrophin in female mice*

See text for description of technique. N.S., not significant.

Dose of peptide (μ g./mouse)	No. of mice	Corticotrophin A_1		Periodate-borohydride-treated A_1	
		Percentage of liver fat	P	Percentage of liver fat	P
Control	5	3.57 ± 0.14	—	3.57 ± 0.14	—
5.0	5	6.34 ± 0.14	<0.01	6.18 ± 0.12	<0.01
1.0	6	2.97 ± 0.25	N.S.	3.42 ± 0.11	N.S.

100 ml. compared with 45 ± 4 mg./100 ml. with 20 μ g. of periodate-borohydride-treated corticotrophin. Injection of 10 μ g. of the peptides did not result in a significant decline in blood sugar.

DISCUSSION

The present studies establish that periodate oxidation greatly reduces the adrenal-stimulating activity of corticotrophin as judged by the assay *in vivo* without comparably altering its potency with respect to certain extra-adrenal systems. The potency of corticotrophin was reduced from 65–70 units to 1 unit/mg. by oxidation with periodate. When tested on adipose tissue *in vitro*, periodate-borohydride-treated corticotrophin retained at least 10% of its original activity. No decline in potency of corticotrophin was detectable by the liver fat and hypoglycaemia tests after oxidation of the hormone with periodate.

In contrast with periodate, hydrogen peroxide treatment of corticotrophin reduces adrenal and extra-adrenal activities equally (Engel & Engel, 1958). The potency of corticotrophin treated with hydrogen peroxide on the adrenal cortex *in vivo* is quite comparable with that of periodate-treated corticotrophin, but the latter still maintains its extra-adrenal activity. This activity is lost when the periodate-borohydride-treated corticotrophin is further treated with hydrogen peroxide. These comparisons emphasize the dissociation between adrenal and extra-adrenal activities resulting from periodate oxidation.

On the basis of both the assays and the chromatographic separation of the periodate-treated corticotrophin from unchanged corticotrophin, it seems most unlikely that the effects of the former are attributable to residual contamination with unaltered corticotrophin. Since relatively large doses of this hormone are required for most of the extra-adrenal effects, it would be surprising for such contamination to escape detection in the adrenal assay and yet appear in the less-sensitive extra-adrenal systems. This argument is particularly relevant to the adipokinetic and hypoglycaemic effects of the two peptides, where the effective and ineffective doses were both large and differed from each other by factors of only 5:1 and 2:1 respectively in the two systems.

Even less likely is the possibility that both peptides are equally contaminated by a known pituitary hormone, which, in turn, is responsible for the so-called extra-adrenal actions of corticotrophin. So far, only growth hormone shares with corticotrophin a significant number of metabolic properties comparable with those of corticotrophin on extra-adrenal tissues (Engel, 1957). Quantitatively, however, the potency of growth hormone

in these systems has generally differed from that of corticotrophin. For example, it is considerably less potent in its effects on adipose tissue (White & Engel, 1958; Knobil & Greep, 1959; Engel & White, 1960). Growth hormone resists periodate oxidation (Geschwind & Li, 1958), but, unlike corticotrophin, it is not inactivated by hydrogen peroxide (Kostyo & Engel, 1960). The behaviour of periodate-borohydride-treated corticotrophin with hydrogen peroxide treatment effectively eliminates growth hormone as a contaminant.

Thyrotrophin shares some actions with corticotrophin on extra-adrenal tissue (Engel, 1957; White & Engel, 1958). However, unlike corticotrophin (Lopez *et al.* 1959) and periodate-borohydride-treated corticotrophin (unpublished work), there is no Ca^{2+} requirement for the effect of thyrotrophin on adipose tissue *in vitro* (Freinkel, 1961). Periodate treatment abolishes the thyroid-stimulating action of thyrotrophin (Geschwind & Li, 1958), but no information is available on the influence of this treatment on the action of the hormone on extra-thyroidal tissues. The melanophore-stimulating hormones and vasopressin have been largely inactive in the systems studied (Engel & Engel, 1958; White & Engel, 1958; Steelman & Smith, 1960).

Finally, in terms of the chemical separation of corticotrophin and its periodate-oxidized derivative, Dixon (1962) has indicated the great improbability of contamination by a known or hitherto unrecognized biologically active peptide. This peptide would have to be chromatographically inseparable from corticotrophin under the conditions used preparatively, either because of chemical similarity or because of the formation of a relatively stable complex with corticotrophin, and after periodate treatment it would have to be similarly inseparable from periodate-borohydride-treated corticotrophin, which, however, is easily separated from corticotrophin. The activity of the contaminating peptide would also have to withstand periodate treatment. We consider this alternative unlikely.

The metabolic actions of the treated corticotrophin described in this report represent the first clear evidence that a dissociation between the adrenal and extra-adrenal actions of corticotrophin is possible. This dissociation is presumably based on the structural alteration of the peptide, which, according to Dixon (1962), consists solely of modification of the *N*-terminal serine residue, probably to a glycolyl residue. Before, all chemical or enzymic procedures altering the corticotrophin molecule appeared to affect adrenal and extra-adrenal activities (exclusive of melanophore-stimulating activity) comparably, at least on a qualitative level (see Engel, 1961, for review).

Dixon & Weitkamp (1961) have further modified periodate-borohydride-treated corticotrophin to yield a corticotrophin with a *N*-terminal glycine. Preliminary studies in this laboratory indicate that this latter corticotrophin likewise retains proportionately greater extra-adrenal than adrenal activity. On the other hand, acetylation of the *N*-terminal serine residue of corticotrophin significantly reduces its capacity to stimulate the adrenal cortex, but increases its melanophore-stimulating activity (Waller & Dixon, 1960). The same is true of the periodate-borohydride-treated corticotrophin (Dixon, 1962). Studies in this Laboratory indicate that *N*-acetylcorticotrophin has very little lipolytic activity. A free amino group on the *N*-terminal amino acid thus appears to be necessary for the full action of corticotrophin on the adrenal cortex *in vivo*.

From the present work it appears that the complete *N*-terminal serine is not essential for the action of corticotrophin on extra-adrenal tissues, but it seems that an identical amino acid sequence following the *N*-terminal serine is required for the metabolic stimulation of both adrenal and extra-adrenal tissues. Harris (1959) and Waller & Dixon (1960) have suggested that a free *N*-terminal serine may inhibit one extra-adrenal action of corticotrophin, the melanophore-stimulating action. From our results with corticotrophin, periodate-borohydride-treated corticotrophin and the melanophore-stimulating hormone, it is apparent that the structural requirements for the melanophore-stimulating activity of corticotrophin are not identical with those for the other extra-adrenal actions.

These considerations impute to the *N*-terminal serine a highly specific function with respect to the adrenal cortex and, indeed, suggest that it may be responsible for the specificity of corticotrophin for its target gland. One possibility is that the serine serves to bind corticotrophin to the adrenal cortex, permitting an essential peptide sequence thereafter to initiate the characteristic metabolic events which culminate in steroidogenesis. This peptide chain, exemplified by periodate-borohydride-treated corticotrophin, would then represent a relatively non-specific metabolic stimulant which is capable of influencing metabolic processes in a variety of tissues without the degree of discrimination which enables corticotrophin to affect the adrenal cortex quite selectively.

The role suggested above for the *N*-terminal serine of corticotrophin might also be used to account for the discrepancy in potency of periodate-borohydride-treated corticotrophin compared with corticotrophin as measured by the steroidogenic assays *in vivo* and *in vitro*. In the assay *in vivo* the potency of corticotrophin A_1 was 70 times that

of periodate-borohydride-treated corticotrophin, whereas in the test *in vitro* it was only 10 times as great, which in turn is comparable with the relative potencies of these peptides on adipose tissue *in vitro*. If the *N*-terminal serine of corticotrophin endows the hormone with a specific affinity for the adrenal cortex but is not involved in the actual stimulation of the gland, it would be reasonable to expect that the modified corticotrophin would exhibit low adrenal-stimulating activity *in vivo* but would still be capable of acting on adrenal tissue *in vitro*. An alternate explanation is that the modified corticotrophin is more readily metabolized *in vivo*. The similar adipokinetic and hypoglycaemic potencies of periodate-borohydride-treated corticotrophin and corticotrophin argue against this interpretation. These considerations do not account for the differences in potency of corticotrophin A_1 in the assays *in vivo* and *in vitro*. Guillemin (1960) has discussed this problem in relation to other corticotrophin peptides and their degradation products.

SUMMARY

1. The adrenal and extra-adrenal activities of corticotrophin A_1 and periodate-borohydride-treated corticotrophin A_1 have been compared *in vivo* and *in vitro*.

2. Periodate-borohydride treatment of corticotrophin leads to marked loss of adrenal-stimulating activity as assayed by the plasma-corticosterone response in hypophysectomized rats, but to a less complete loss of activity as judged from stimulation of steroidogenesis by adrenal slices *in vitro*.

3. Periodate-borohydride-treated corticotrophin retains reduced but significant activity compared with corticotrophin A_1 when tested for four extra-adrenal activities: stimulation of fatty acid release from, and glucose uptake by, rat epididymal adipose tissue *in vitro*, adipokinetic effect in female mice and hypoglycaemia in starved male mice.

4. The significance of the dissociation between adrenal and extra-adrenal actions of corticotrophin by modification of the *N*-terminal serine residue of corticotrophin is discussed in relation to the structure and function of the peptide.

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Studies on Experimental Thiamine Deficiency

2. TISSUE BREAKDOWN AND GLYOXYLIC ACID FORMATION*

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Since glyoxylic acid was detected in the blood, urine and tissues of rats suffering from thiamine deficiency and also in rats after prolonged feeding of restricted low-fat diet containing added thiamine (Liang, 1962), it was suggested that the formation of glyoxylic acid is related to excessive tissue

breakdown. It was also noted that creatinuria is a notable sign of thiamine deficiency; it is attributed to the tissue breakdown and can be correlated with loss of body weight (Sure & Dichek, 1941; Sure & Ford, 1942). Creatine synthesis apparently depends on the availability of glycine, arginine and methionine from the common metabolic pool contributed by body tissues and diet (Bloch & Schoenheimer, 1940). Studies have shown that urinary creatine was derived from liver creatine

* Part 1: Liang (1962).

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