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Note on the *in vitro* assay of corticotrophin

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Factors affecting the precision of the *in vitro* assay of corticotrophin using quartered adrenals of rats have been examined. Satisfactory results were obtained only by using tissue from healthy adult male Wistar rats which had been kept quiet and at a constant temperature for several days before the assay. Greater precision was obtained from rats first anaesthetised with pentobarbitone and then decapitated. The assay is not considered to be an adequate procedure for checking commercial injections of corticotrophin.

SAFFRAN, Grad & Bayliss (1952) and Saffran & Bayliss (1953) have shown that incubation of rat adrenal tissue *in vitro* with corticotrophin resulted in the release of corticosteroids into the medium. This response of the isolated adrenal gland has been used as the basis for an assay procedure for corticotrophin. To obtain reproducible results it was necessary to use quartered adrenals equally among the dose groups and to introduce a pre-incubation period which reduced the initial output of corticosteroids to a more or less constant base level (Saffran & Schally, 1955; McKerns & Nordstrand, 1955b). van der Vies (1957) pointed out that the test animals in this assay method had to be kept under carefully controlled conditions in order to improve the precision of the assay.

We have examined various factors which might increase the precision of the assay.

Experimental methods

The *in vitro* method used was essentially that of Saffran & Schally (1955) as modified by van der Vies (1957). Male albino rats, 150–200 g, born and raised in the animal colony of the Food and Drug Directorate and derived from an inbred Wistar strain, were acclimatised for two weeks at a constant temperature of 76° ± 1° F. After killing the animals by decapitation or pentobarbitone anaesthesia or decapitation after pentobarbitone anaesthesia, the adrenals were carefully removed and weighed. The glands were then cut into quarters and subjected to the preincubation period in the Warburg apparatus as described by Saffran & Schally (1955). In our hands, the quartering of the adrenals was more uniform when a razor blade was used instead of fine scissors which is in agreement with Kitay, Holub & Jailer (1958).

Results and discussion

Exposure of the donor rats to continuous darkness for periods up to one week did not change the response of the isolated adrenal tissue to corticotrophin *in vitro*. However, a change of more than 2° F in the temperature of the animal room usually produced a large variation in the

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production of corticoids by the adrenal tissue. This sensitivity to environmental temperature confirmed the observations of McKerns & Nordstrand (1955a) and Kitay & others (1958).

Although quartered adrenal tissue obtained from Sprague-Dawley and Wistar rats from our colony responded equally well to corticotrophin, the Wistar rats were employed routinely in this study because they were usually the easier to handle. The age of the donor rat had no significant effect on the response of the adrenal cortex to stimulation with corticotrophin. However, like Tanaka (1957), we found adrenal tissue from male rats to be more responsive than that from female rats. A typical set of data shown in Table 1 indicates that the glands from donor male rats usually gave a steeper slope and a larger mean response than those from females.

TABLE 1. EFFECT OF THE SEX OF THE DONOR RAT ON THE *In Vitro* ADRENAL RESPONSE TO CORTICOTROPHIN

Sex of the donor rat	Dose of corticotrophin (mU)	Response (μg/100 mg tissue)	Slope log dose response line
Female	10·0	32·6 ± 1·0*	19·7
	30·0	42·0 ± 1·1	
Male	10·0	47·4 ± 2·4	28·3
	30·0	60·9 ± 3·7	

* Average of 4 values ± standard error.

Adrenal glands from rats with an upper respiratory infection were unable to distinguish between the low and high dose levels. The fact that the output of corticoids was in the lower part of the normal range suggested that the adrenal tissue from these infected rats could not be further stimulated by exogenous corticotrophin.

The results in Table 2 demonstrate that adrenal glands from rats that were anaesthetised with pentobarbitone sodium and then decapitated provided a steeper log dose response line than adrenal tissue from rats that were killed by either decapitation or pentobarbitone sodium anaesthesia alone.

TABLE 2. INFLUENCE OF THE METHOD OF KILLING OF THE DONOR RATS ON THE RESPONSE OF THE ADRENAL TISSUE TO CORTICOTROPHIN

Treatment	Dose of corticotrophin (mU)	Response (μg/100 mg tissue)	Slope log dose response line
Pentobarbitone sodium	10·0	43·3 ± 3·2*	25·2
	30·0	55·3 ± 1·3	
Decapitation	10·0	46·3 ± 0·8	27·7
	30·0	59·5 ± 1·0	
Pentobarbitone sodium and decapitation ..	10·0	44·1 ± 1·6	42·5
	30·0	64·4 ± 2·9	

* Average of 4 values ± standard error.

When halved adrenal glands were used in each flask, and the number of rats in each assay was increased from 8 to 16, the variation within dose groups was as large as that between dose groups. However, when

quartered adrenals were employed and tissue from each donor rat was included in each dose group, valid assays were obtained. This confirms the work of Saffran & Schally (1955); and McKerns & Nordstrand (1955b). This finding limits the number of dose groups for each assay to two, unless the adrenal glands were divided into sixths or eighths. However, it was very difficult to divide the adrenal tissue evenly when fragments were smaller than quarters. Since each of the eight flasks in the assay contains tissues from each rat, the variation within dose groups should be a minimum, and could be attributed to errors in sectioning of the glands, weighing, extraction and estimation of the corticosteroids produced, as well as variation in the viability of the individual quarters. Consequently, the standard error of such an assay was probably not dependent on biological variation, but rather could be attributed to mechanical and chemical manipulations. According to Bangham, Mussett & Stack-Dunne (1962) the estimates of error obtained from the collaborative assay of the Third International Standard for corticotrophin by the *in vitro* method were unreliable and led to weights which showed large variations between assays. In their opinion the tests of statistical validity in this assay procedure may have very little meaning. The data in Table 3 show that the potency of commercial preparations assayed by

TABLE 3. THE DETERMINATION OF THE POTENCY OF COMMERCIAL PREPARATIONS OF CORTICOTROPHIN BY THE *In Vitro* METHOD

Preparation	Labelled route of administration	Labelled potency (I.U.)	Found potency *in vitro* (I.U.)
A	Intramuscular	25·0	17·3 (10·2–29·6)
		25·0	14·0 (4·6–42·8)
		25·0	19·9 (11·6–33·9)
B	,,	40·0	22·4 (8·4–60·0)
		40·0	14·4 (10·3–43·9)
C	,,	40·0	27·6 (17·6–43·2)
		40·0	19·6 (9·3–41·2)
D	Intramuscular and intravenous	25·0	12·7 (5·3–30·6)
	,,	25·0	7·6 (3·5–16·7)

the *in vitro* procedure varied from approximately 30 to 80 % of the labelled value. However, the wide fiducial limits for each assay usually included the labelled potency. In assays of this type, an unweighted mean potency may provide a more reliable estimate than one in which weights for individual potency ratios are taken into consideration. The finding that the *in vitro* assay gave a lower potency than that stated on the label was not unexpected since the commercial preparations were assayed by the manufacturer by a method involving subcutaneous administration of the corticotrophin.

CONCLUSION

Satisfactory results with the *in vitro* method of assay of corticotrophin were obtained in our laboratory only by employing quartered adrenal tissue from healthy adult male Wistar rats which were kept as quiet as possible and at a constant temperature for several days before the assay.

In addition, the use of adrenal glands from rats which were anaesthetised with pentobarbitone sodium before decapitation, usually resulted in an assay with increased precision since the log dose response lines tended to be steeper.

According to our data the *in vitro* assay does not provide an adequate procedure for checking the potency of commercial samples of corticotrophin in pharmaceutical form which have been labelled for intramuscular or subcutaneous use.

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