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# Urethane anaesthesia and pituitary-adrenal function in the rat

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Urethane anaesthesia produced a prolonged hypersecretion of ACTH as shown by plasma and adrenal corticosterone and adrenal ascorbic acid changes. There was also a concurrent depletion of the adrenaline content of the adrenal gland. Adrenal demedullation did not prevent the steroid changes induced by the anaesthetic. Hyperactivity of the pituitary-adrenal axis for 24 hr in the urethane-anaesthetised rats did not prevent further alteration of steroid levels in response to a stress.

PENTOBARBITONE treatment has been shown to depress the secretion of ACTH (Egdahl, 1961) whereas ether anaesthesia has been shown to stimulate the secretion of ACTH (Royce & Sayers, 1958). Sayers (1957) found that prolonged ether anaesthesia induced an initial secretion of ACTH in the adrenalectomised rat which was followed by a decline to non-detectable levels. It has been suggested that the transient stimulation of ACTH is compatible with the excitation during the induction period (Sayers & Royce, 1960), but Barrett & Stockham (1963) found that continuous ether anaesthesia produced a continued high level of steroid in the blood. These findings prompted the investigation of the influence of prolonged anaesthesia on the steroid secretion of the adrenal cortex. The effect of urethane anaesthesia was investigated since it is a good anaesthetic in the rat, producing a stable and safe level of anaesthesia.

Urethane has been reported to induce hyperglycaemia (Seuffert & Ullrich, 1925), depression of inflammatory responses (Peng, 1930), leuococytopenia (Stein, 1949) and an increase in the basal metabolic rate (Aub, Bright & Forman, 1922), all conditions which may be associated with hyperactivity of the adrenal gland. The adrenaline content of the adrenal medulla, as well as the plasma and adrenal corticosterone and adrenal ascorbic acid, have been estimated to establish if the cortex and the medulla of the adrenal gland were affected by the anaesthetic and whether there was any inter-relationship.

## Methods

Wistar male rats weighing 150–250 g were kept in a room at a constant temperature of 23 $\pm$ 2° and stored in single cages. They were fed on a diet of cubes (diet 41B, Lane-Petter & Dyer, 1952) and allowed water *ad lib.* Adrenal demedullation was carried out by the method of Ingle & Griffiths (1942) and the animals were left for 12 weeks before use. Blood samples were obtained from the abdominal aorta and centrifuged at 3,000 rpm for 10 min, and the plasma was analysed for corticosterone. The adrenal glands were removed, cleaned and weighed; one was assayed

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for ascorbic acid content by the method of Roe & Keuther (1943), whilst corticosterone analysis on half of a 0·01 N HCl homogenate was made by the method of Barrett & Stockham (1963). The remaining portion of the adrenal homogenate was estimated for its adrenaline content by the method of Shore & Olin (1958).

Urethane as a 50% w/v solution in water was given intraperitoneally in a dose of 1·5 g/kg. Phenobarbitone sodium in 0·9% w/v saline was given intraperitoneally in a dose of 200 mg/kg. ACTH (Cortrophin, Organon) dissolved in 0·9% w/v saline was given subcutaneously in a dose of 5 units/kg. Some animals were also exposed to ether vapour for 1 min to stimulate the discharge of ACTH from the pituitary.

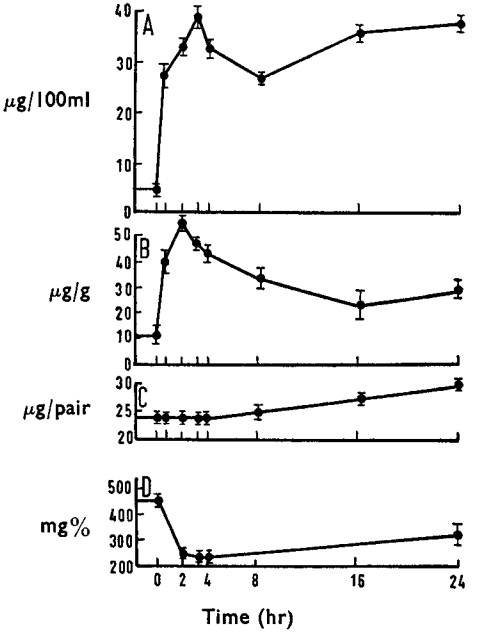


FIG. 1. Changes of indices of pituitary-adrenal cortex function during urethane anaesthesia. Each point is the mean of 6 to 15 observations (± s.e.). A, plasma corticosterone; B, adrenal corticosterone; C, adrenal weight; D, adrenal ascorbic acid.

## Results

### THE ADRENAL ASCORBIC ACID, CORTICOSTERONE AND ADRENAL WEIGHT CHANGES AFTER URETHANE

The results are summarised in Fig. 1. The plasma and adrenal corticosterone levels were elevated and the adrenal ascorbic acid levels were depleted throughout the period of anaesthesia. The adrenal glands increased in weight by 25%. These changes suggested that there was prolonged hypersecretion of ACTH from the pituitary. The corticosterone in the plasma reached a maximum concentration of 39·4 μg/100 ml in the

first 3 hr and subsequently declined to 26·9 $\mu$g/100 ml at 8 hr. The adrenal corticosterone changes exhibited a similar pattern during the first 8 hr. After 8 hr the plasma corticosterone again increased significantly ($P < 0{\cdot}01$) whereas the adrenal level continued to fall. During this period the adrenal ascorbic acid concentration recovered from the minimum level of 250 mg% at 3 hr to 340 mg% at 24 hr. After 8 hr there was marked haemoconcentration.

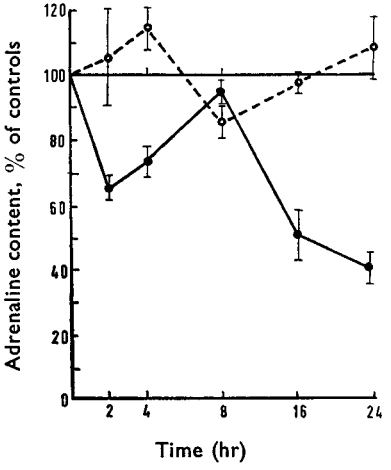


FIG. 2. Adrenal adrenaline levels during either urethane (continuous line) or phenobarbitone (broken line) anaesthesia. Each point is the mean of 6 to 9 observations. Adrenaline content expressed as percentage of control values (± s.e.).

### THE ADRENALINE CONTENT OF THE ADRENAL GLANDS DURING URETHANE ANAESTHESIA

Fig. 2 shows that there was a triphasic variation of the adrenaline content during urethane anaesthesia. There was a significant depletion during the first 4 hr which was followed by a significant recovery to normal at 8 hr. Subsequently there was another very large depletion of adrenaline, reaching over 50% at 24 hr. Although blood adrenaline levels were not determined, it is reasonable to suggest that during the first 4 hr and subsequent to 8 hr of urethane anaesthesia there are elevated levels of adrenaline in the blood.

### COMPARISON OF PHENOBARBITONE AND URETHANE ANAESTHESIA WITH REGARD TO CORTICOSTERONE AND ADRENALINE CHANGES

To study whether the hormonal changes described for rats under urethane anaesthesia were features of general anaesthesia irrespective of the anaesthetic, the steroid and adrenaline changes were investigated after phenobarbitone. Fig. 3 shows that during the first 4 hr of phenobarbitone treatment, there was only a slight alteration of the plasma level and no alteration of the adrenal steroid level. Fig. 2 shows there was no

alteration of the adrenal adrenaline for the first 4 hr, but subsequently there was a depletion of the amine content. After 8 hr there was an increase of the plasma and adrenal steroid concentrations.

Thus whilst phenobarbitone had little effect on the plasma and adrenal steroid levels and adrenal adrenaline content during the first 4 hr, urethane had a marked effect. Phenobarbitone caused an adrenaline depletion after 4 hr, whereas during urethane anaesthesia the amine level showed a return towards the control value. After 8 hr the picture was even more complicated; however, both urethane and phenobarbitone caused a marked elevation of the plasma steroid concentration. At the same time urethane induced a significant depletion of adrenaline whereas phenobarbitone had no effect.

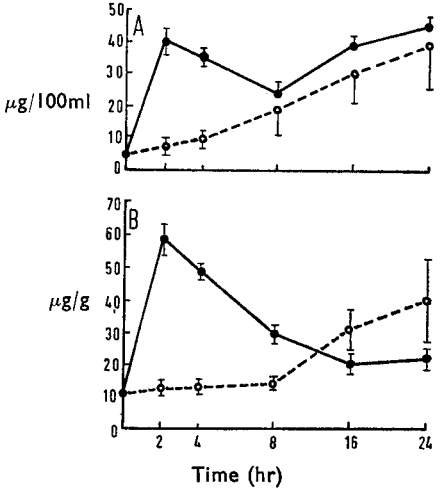


FIG. 3. Changes of plasma and adrenal corticosterone concentrations during either urethane (continuous line) or phenobarbitone (broken line) anaesthesia. Each point is the mean of 4 to 15 observations (± s.e.). A, plasma corticosterone; B, adrenal corticosterone.

CORTICOSTERONE LEVELS DURING URETHANE ANAESTHESIA IN INTACT AND ADRENAL DEMEDULLATED RATS

To determine whether the secretion of adrenaline was responsible for the changes of plasma and adrenal corticosterone, the effects of urethane in adrenal-demedullated rats were examined. There was an initial increase of plasma and adrenal corticosterone in the demedullated rats for the first 4 hr followed by a significant fall at 8 hr. This result was similar to that found in the intact rat (Fig. 4) and indicated that the release of adrenaline from the adrenal medulla played no major role in the release of corticosterone during the first 8 hr of urethane anaesthesia. Subsequent to 8 hr, the fall in plasma level continued in the demedullated rats whereas the intact rats showed a further rise. However, the adrenal corticosterone concentration returned towards the control level in both groups.

THE EFFECT OF A STRESSFUL STIMULUS AFTER 24 HR URETHANE ANAESTHESIA

The effects of injection of ACTH and exposure to ether vapour after prolonged activation of the pituitary-adrenal axis were investigated (Fig. 5). Both stimuli caused significant ($P < 0{\cdot}05$) increases in both the plasma and adrenal corticosterone concentrations. Thus 24 hr of

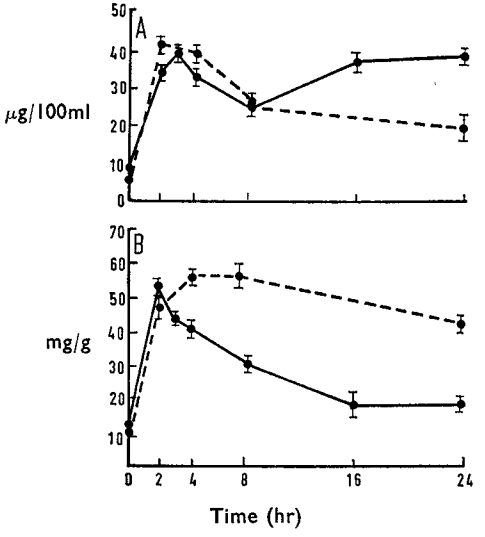


FIG. 4. Corticosterone changes in intact (continuous line) and adrenal demedullated (broken line) rats during urethane anaesthesia. Each point is the mean of 4 to 15 observations (± s.e.). A, plasma corticosterone; B, adrenal corticosterone.

urethane anaesthesia did not prevent the release of ACTH from the pituitary or prevent the adrenal gland from synthesising or releasing the steroid. However, neither ACTH nor the ether stimulus depleted the adrenal ascorbic acid level despite this having recovered by 100 mg% from the maximally depleted value. This suggested that the sensitivity of the adrenal ascorbic acid index to ACTH during the repletion phase was altered and therefore not suitable as a measure of pituitary-adrenal activation.

## Discussion

Urethane anaesthesia produced a remarkably prolonged hypersecretion of ACTH from the pituitary gland as measured by all the indices of pituitary-adrenal activity used. Hermansky, Pudlach & Dienster (1955) also showed that urethane induced a release of adrenocortical hormones as indicated by the peripheral lytic effect on leucocytes.

As continuous anaesthesia had been shown to cause adrenocortical activation (Barrett & Stockham, 1963) it was possible that the hormonal changes were due to general anaesthesia. Phenobarbitone anaesthesia did not change the plasma or adrenal corticosterone level during the first 8 hr, but after this period there was an increase in toxicity, 16 out of

32 died by 8 hr and 47 out of 51 by 24 hr, and the animals which survived showed a rise in plasma and adrenal steroid concentrations. Gorby, Leonard, Ambrus & Harrison (1953) reported that corticoids increased the toxicity of phenobarbitone. The change in adrenal corticosterone was very variable and there was no significant increase in adrenal weight. These factors suggested that the hormonal changes after 8 hr treatment were related to the toxic effect of the drug. These results cast doubt on the validity of using a rise in plasma corticosterone as an index of ACTH release in these circumstances. It was concluded that the steroid changes during urethane anaesthesia were not simply resultant of general anaesthesia.

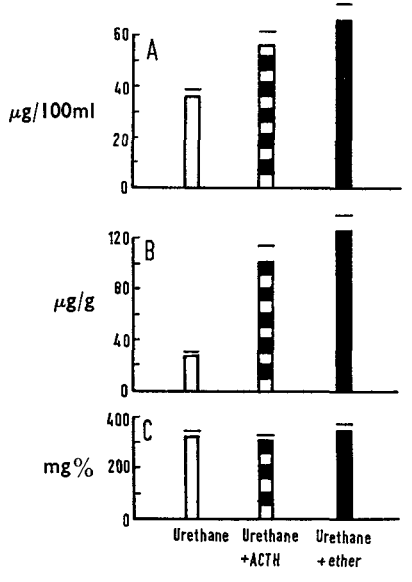


FIG. 5. The effect of the ether stimulus or ACTH on corticosterone levels (A, plasma; B, adrenal) and adrenal ascorbic acid levels (C) after 24 hr urethane anaesthesia. Each value is the mean of 7 to 11 observations. The horizontal bars represent one standard error.

No direct evidence for a central action of urethane on the anterior pituitary was presented since the drug was extremely toxic in hypophysectomised rats. Urethane was also toxic in adrenalectomised rats.

Urethane activated the adrenal medulla, which was in agreement with Kodama (1930), who recorded that the secretion of amines was prevented by section of the splanchnic nerves. Aub, Bright & Forman (1922) showed that the hyperglycaemia during urethane anaesthesia was due to the secretion of adrenaline from the adrenal gland, and De (1946) reported that this persistent hyperglycaemia was reduced after section of the hypothalamus. This evidence implied that there was a central excitation under the anaesthetic which induced the secretion of adrenaline from the adrenal gland.

The changes in plasma and adrenal corticosterone were concurrent with the depletion of adrenal adrenaline for both urethane and phenobarbitone anaesthesia. The stimulation of the adrenal cortex may have followed an increase in blood levels of adrenaline. However, the increments in plasma and adrenal corticosterone under urethane anaesthesia were not altered by adrenal demedullation. Therefore it was likely that the activation of both divisions of the adrenal gland was of central origin. Additional evidence for a central effect on the anterior pituitary was presented by Fuhrmann (1950) who described an increased release of pituitary gonadotrophins.

It was probable that the changes in the plasma steroid level were not mediated by one mechanism since the response was triphasic in nature. During the second rise in the plasma level after 8 hr there was no concomitant increase in adrenal corticosterone concentration and the adrenal ascorbic acid concentration recovered by 100 mg%, but there was a further adrenaline depletion from the gland. After 8 hr there was a pronounced haemoconcentration and Doljanski & Rosin (1944) reported that the liver underwent serious degenerative changes with extended anaesthesia. These observations indicated that the second rise in the plasma corticosterone was not due to a further release of ACTH from the pituitary but was mediated by general deterioration of the animal.

24 hr after injection of urethane the rats were still anaesthetised, the plasma corticosterone was 960%, and the adrenal corticosterone was 230% above the control values. Both ACTH injection and the exposure to the ether vapour produced significant increases in the plasma and adrenal corticosterone levels. Therefore prolonged hyperactivity of the pituitary-adrenal system had not reduced the capacity to respond to a stress. It was of interest that a high plasma corticosterone level over a long period had not depressed the pituitary or adrenal gland by a feedback mechanism. The increments of adrenal corticosterone concentrations for both stimuli were significantly ($P < 0{\cdot}01$) greater than in the previously untreated rat, which may be related to the 25% increase in adrenal weight over 24 hr. However, the increments in the plasma corticosterone were 20% smaller than in the untreated rat. The reason for this is not clear, but recently Kolthoff, Macchi & Wyman (1963) demonstrated that rats with regenerated adrenal glands, although synthesising corticosteroids in response to a stress in a normal manner, failed to exhibit a large rise in plasma steroid level due to adrenal circulatory changes. The hypersecretion of adrenaline with urethane anaesthesia may therefore have affected the circulation of blood through the adrenal gland and thus modified the response to a stress.

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