Aust. J. Biol. Sci., 1977, **30**, 559–72

Ultrastructural Changes in the Adenohypophysis, Adrenal Gland Activity, and Desynchronization of the Oestrous Cycle following Unpredictable Stress in the Rat

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Abstract

An ultrastructural study of the adenohypophysis, after exposure of female Wistar rats to a signalled unpredictable 5- and 15-day stress regimen, is described. Cellular activity of the adenohypophysis correlated well with the circulating levels of corticosterone. Intense secretory activity was observed in all tropic cell types at 5 and 15 days although the observed differences generally were greater in the 5-day stressed group. It was observed that the oestrous cycles of 40 and 100% of the rats became desynchronized over the 5- and 15-day stress period respectively.

Introduction

Stress in its broader aspects is a difficult subject to study experimentally. Although it is now generally accepted that the hypothalamic–hypophyseo–adrenal response plays the key role in regulating the response of an animal to stressors, the degree to which other endocrine systems are involved is still more obscure. In the majority of cases the animal is able to adjust its physiological responses to changing environmental conditions without any overt changes in behaviour.

Pollard *et al.* (1975) demonstrated that exposure of female Wistar rats to a signalled unpredictable stress regimen induced extreme plasma corticosterone elevation at all four stages of the oestrous cycle. It was also observed that the oestrous cycles of 50% of the rats became desynchronized over a 5-day stress period. Subsequently Pollard *et al.* (1976) demonstrated, by a morphological and ultrastructural study of the adenohypophysis, that an initial intense hyperactivity in response to stress was not only confined to the corticotrope but also affected the somatotrope, the FSH and LH gonadotrope, thyrotrope and luteotrope cells in the male rat. This enhanced cellular activity correlated well with circulating levels of corticosterone. After 10–20 days of stress, however, the cellular morphology showed a return to the control condition in all the tropic cell types excepting the luteotrope, the activity of which continued to increase. A similar adaptation occurred in adrenal function as reflected in plasma glucocorticoid levels, which returned to near control levels.

In the present paper a morphological study of the adenohypophysis of the female rat following exposure to signalled, unpredictable footshock is described. The ultrastructure of the adenohypophyseal cells is correlated with changes in the oestrous cycle as determined by daily vaginal smearing during the period of stress and with changes in adrenal function as reflected in plasma glucocorticoid levels.

Materials and Methods

Experimental Animals

Experimentally naive virgin female Wistar rats, 100–130 days old, were used in the experiments described. All animals were housed under identical conditions in groups of three, with food and water provided *ad libitum*, and subjected to a natural day–night lighting regimen. The experiments to be described were conducted in early winter (June).

Oestrous Cycle

Only rats with regular 4-day oestrous cycles, as determined by vaginal smearing, were chosen for experimentation. This was determined by daily smearing through three cycles prior to the experiment. Rats from each oestrous stage were randomly assigned to both stress and control groups. During this time the regimen was as follows: at 0900 h both experimental and control groups were handled and vaginal smears taken. The control animals were returned immediately to their home cages. The animals to be stressed were placed individually into an automated 1-way avoidance box as described by Potts and McKowen (1969). The whole stress procedure was completed before midday. No oestrous desynchrony occurred in the control rats.

Experimental Design

Two groups each of five rats at each of the four stages of the oestrous cycle were stressed for 5 and 15 consecutive days using a daily treatment session of 35 min during which they received eight randomly placed irregular signalled shocks each of 2 mA. The apparatus consisted of a grid box with an escape platform located at one end above the grid floor. This regimen of irregular, signalled footshock with the possibility of escape maximized the psychological component of the stress and the procedure is described in detail by Bassett *et al.* (1973).

Plasma 11-Hydroxycorticosterone Assay

Immediately following the last stress session the animals were killed by cervical dislocation, exsanguinated and plasma samples were frozen for assay. Pooled plasma samples over the entire oestrous cycle were assayed for corticosterone by the fluorimetric method of Mattingley (1962), which is specific for free 11-hydroxycorticosterone.

Electron Microscopy

The pituitaries were treated as described in detail by Pollard *et al.* (1976). Pituitaries from five stressed and two control animals per group were rapidly removed and placed in 4% glutaraldehyde in 0·1 M sodium cacodylate buffer for 1·5 h at 5°C. The tissues were then post-fixed in 2% osmium tetroxide for 1·5 h, dehydrated, and embedded in Araldite. Silver sections from the mid-central region of the adenohypophysis were stained by the triple stain method modified from Soloff (1973). The sections, which were mounted on copper grids, were treated for 2 min with a 0·9% (w/v) $KMnO_4$ solution buffered at pH 6·5 with phosphates. They were then treated with uranyl acetate saturated in 50% (v/v) ethanol for 3 min, followed by 0·2 (w/v) lead citrate solution for 3 min. This method of staining produced increased electron density, particularly of the membrane systems, which improved cellular definition. The grids were examined using a Hitachi HS8 electron microscope.

Results

Plasma 11-Hydroxycorticosterone

The corticosterone values over the entire oestrous cycle for both stressed and control groups were obtained in order to monitor adrenal function during stressing. Stress significantly ($P<0·001$) elevated the plasma corticosterone levels in both the 5- and 15-day groups irrespective of whether desynchrony of the oestrous cycle occurred. The mean control ($\pm$ standard error) corticosterone value of $20\pm3·5$ μg/100 ml plasma rose to a mean stressed value of $72\pm3·7$ μg/100 ml plasma at 5 days. At 15 days of stress the corticosterone value fell to a level of $55·0\pm4·0$ μg/100 ml plasma. This new level was significantly less than the initial extreme value observed after 5 days of stress ($P<0·05$).

Oestrous Cycle Desynchrony

It was found that over the 5-day period of stress 40%, and over the 15-day period of stress 100%, of the rats with normal oestrous cycles developed desynchronized cycles.

The desynchrony consisted of the prolongation of a stage for 2–3 days, or the acceleration of a stage which consequently was not observed by daily smearing. The oestrous cycles of 15-day stressed rats all became desynchronized within the first 8 days of stress. Towards the end of the 15 days the vaginal smears became extremely difficult to stage. They contained mostly small nucleated epithelial cells with varying numbers of disintegrated cornified cells and leucocytes, cellular debris, and mucus.

Ultrastructure of the Cells of the Adenohypophysis

The ultrastructure of the control pituitaries was similar to that described in the literature. There is an extensive cytological and histological literature concerning each of the six specific cell types of the anterior pituitary (see Costoff 1973 for further details). In order to simplify the presentation of the observed findings, first the morphological changes seen throughout the oestrous cycle and then the changes caused by exposure to the stress regimen are described. The findings are illustrated by typical electron micrographs (Figs 1–13).

Observed changes in the FSH and LH gonadotrope cells during the oestrous cycle were similar to those previously described in the mouse (Barnes 1962) and the rat (Roos 1968). The FSH cells were moderately degranulated of secretory granules during the di-oestrous and pro-oestrous stages of the cycle. This could be related to the follicular growth that takes place at this time. Considerable degranulation of the LH cells occurred during pro-oestrus and early oestrus. The gonadotropes were observed to regranulate again at oestrus and metoestrus. Concomitant with degranulation other cytological changes indicative of actively synthesizing cells, such as hyperactivity of the endoplasmic reticulum and Golgi complex, were also observed. Apart from degranulation of the luteotrope cell at oestrus, no particular changes were observed in the other tropic cell types throughout the oestrous cycle.

Stress, however, was shown to cause great cytological changes in all the six tropic cell types. These changes were most marked in the corticotropes, the FSH and LH gonadotropes, and the luteotropes. Changes in the somatotropes and thyrotropes were less marked. Observed differences were also greater in the 5-day stressed group than the 15-day group. In general the pituitary cells of both the 5- and 15-day stressed groups were enlarged and vacuolated with wider intercellular spaces and an increased capillary blood supply than those seen in the control groups.

The corticotrope cell at 5 days of stress was very hyperactive. Extensive, but not complete, degranulation of the secretory granules with hypertrophied endoplasmic reticulum and Golgi complex was seen. There was also an increase in the numbers of ribosomes and lysosomes and the mitochondria were swollen (Figs 1 and 2). At 15 days of stress the changes were similar to the above although a proportion of the cells seemed to have become regranulated and the cytoplasmic changes seemed to have begun to revert back to the control condition. An increase in the number of chromophobes was also seen.

The FSH cell at 5 days of stress showed remarkable modification. The extensive hypertrophy of the endoplasmic reticulum formed large vacuoles in the cytoplasm.

Most cells were moderately degranulated with a concomitant decrease in the number of amorphous bodies. The Golgi complex was swollen and there was an increase in the numbers of ribosomes present (Figs 3 and 4). At 15 days of stress there was a slight reversal of some of the cytoplasmic vacuolation although the cells were still hyperactive.

The LH cell at 5 days of stress also showed modification although the changes were not as marked as those for the FSH cell. The LH cell became hyperactive with hypertrophy of the endoplasmic reticulum forming a vacuolated cytoplasm, and an active Golgi complex (Figs 5 and 6). At 15 days of stress there was no observable change from that seen at 5 days.

The luteotrope or prolactin cell, however, seems to be an exception in which, unlike the degree of adaptation seen for the other tropic cells by 15 days of stress, luteotrope activity increased throughout the duration of the stress procedure. At

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Fig. 1. A control corticotrope (*C*), irregular in shape and well granulated (*S*). A compact rough endoplasmic reticulum (*ER*), rod-shaped mitochondria (*M*) and lysosomes (*L*) are present.

Fig. 2. A typical corticotrope cell (*C*) from a 5-day stressed pituitary. The stressed cell has an enlarged endoplasmic reticulum (*ER*), swollen mitochondria with broken cristae (*M*) and increased numbers of lysosomes (*L*). Typically 5-day stressed cells show degranulation although most cells still have secretory granules present (*S*) and an extensive Golgi complex (*G*).

Fig. 3. A control FSH-gonadotrope (*FSH*) at pro-oestrus. Normally this cell is round or oval in shape with an eccentrically placed nucleus and an abundance of secretory granules (*S*). The endoplasmic reticulum consists of a series of dilated sacs of irregular shape (*ER*), the Golgi complex is well developed (*G*),the mitochondria circular in shape (*M*), and amorphous bodies which are unique to the FSH-gonadotrope are present (*B*).

Fig. 4. An FSH-gonadotrope (*FSH*) from a 5-day stressed pituitary at pro-oestrus. The stressed cell has an enlarged and dilated endoplasmic reticulum in which adjacent sacs coalesce to form large cytoplasmic vacuoles (*ER*), increased numbers of ribosomes (*R*), swollen mitochondria with broken cristae (*M*), and extensive Golgi apparatus (*G*). There is also an extensive degranulation of the secretory granules (*S*) and amorphous bodies (*B*).

Fig. 5. A control LH-gonadotrope (*LH*) at di-oestrus. The endoplasmic reticulum consists of flattened profiles oriented parallel to the outline of the nucleus (*ER*), and well granulated cytoplasm (*S*). Mitochondria with discontinuous cristae (*M*) and lysosomes (*L*) are also present.

Fig. 6. An LH-gonadotrope (*LH*) from a 5-day stressed pituitary at di-oestrus. The cytoplasm is vacuolated (*V*) with swollen mitochondria (*M*) and an increase in lysosomes (*L*).

Fig. 7. A control luteotrope or prolactin cell (*LTH*) with intensive electron-dense and irregularly shaped secretory granules (*S*), an abundance of endoplasmic reticulum arranged as flattened lamellae (*ER*), and round or rod-shaped mitochondria (*M*).

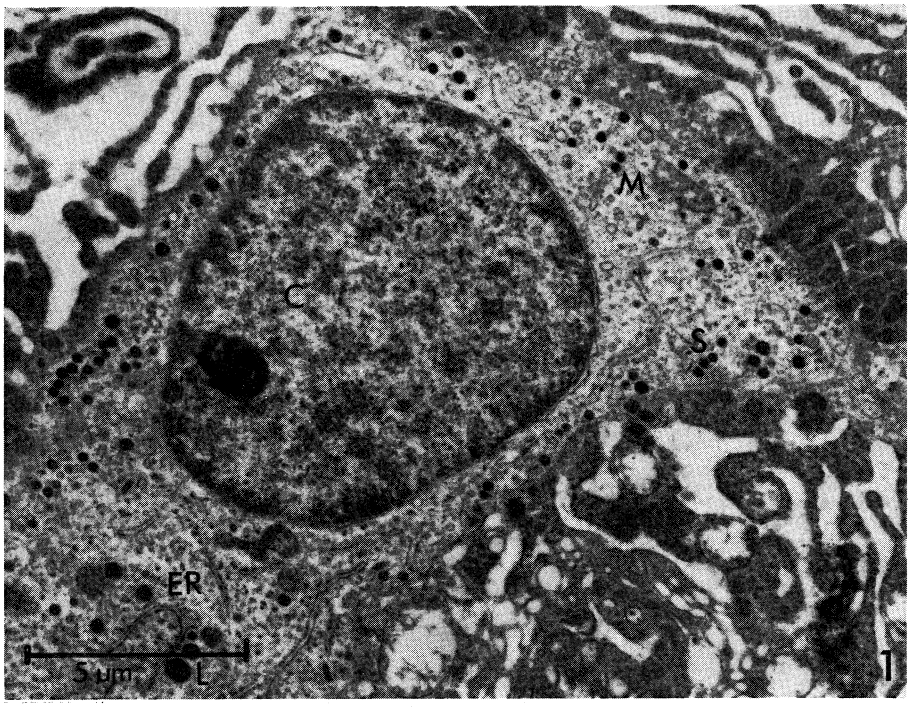
Fig. 8. A luteotrope cell (*LTH*) from a 5-day stressed pituitary. The stressed cell has a greatly enlarged endoplasmic reticulum (*ER*), with increased numbers of ribosomes (*R*), swollen mitochondria (*M*), and an extensive Golgi complex (*G*) with granules (*S*) in various stages of formation.

Fig. 9. A 'storage' somatotrope (*GH*) in which an abundance of densely staining granules (*S*) is obscuring most of the endoplasmic reticulum (*ER*). Mitochondria (*M*) and a Golgi complex (*G*) are present.

Fig. 10. An 'active' somatotrope (*GH*) in which secretory granules (*S*), a lamellar endoplasmic reticulum at one pole of the cell (*ER*), an extensive Golgi complex (*G*), and mitochondria with discontinuous cristae (*M*) are present.

Fig. 11. A control thyrotrope (*T*), a small cell with secretory granules (*S*) found near the cell membrane, a poorly developed endoplasmic reticulum (*ER*), and normal-appearing mitochondria (*M*).

Fig. 12. A typical hypertrophied thyrotrope (*T*) from a 5-day stressed pituitary. The cell is enlarged with increased numbers of mitochondria (*M*), vacuolated and enlarged endoplasm reticulum (*ER*) and active Golgi complex (*G*).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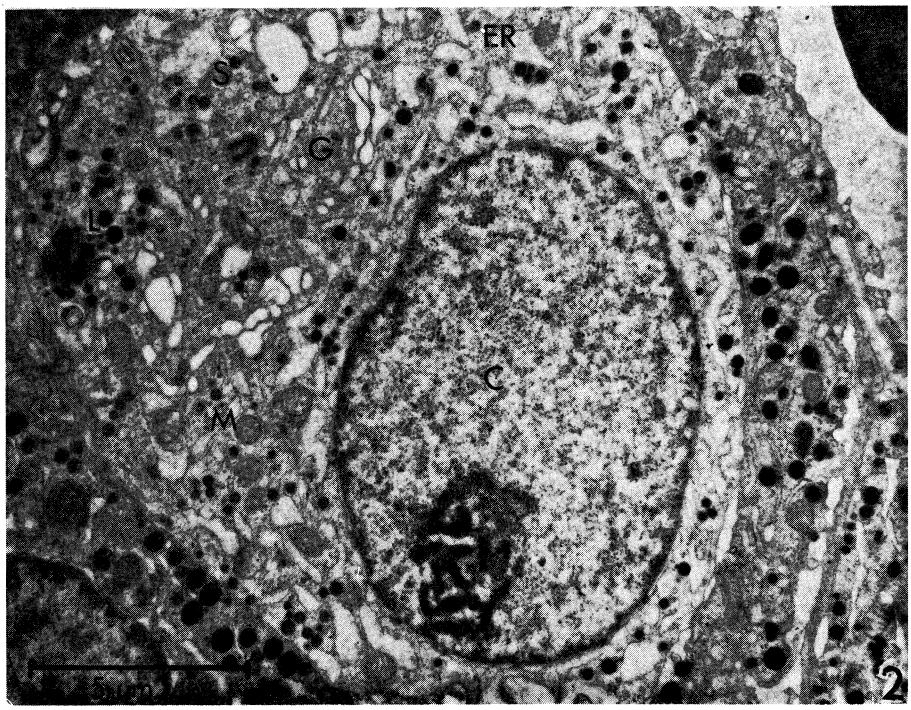
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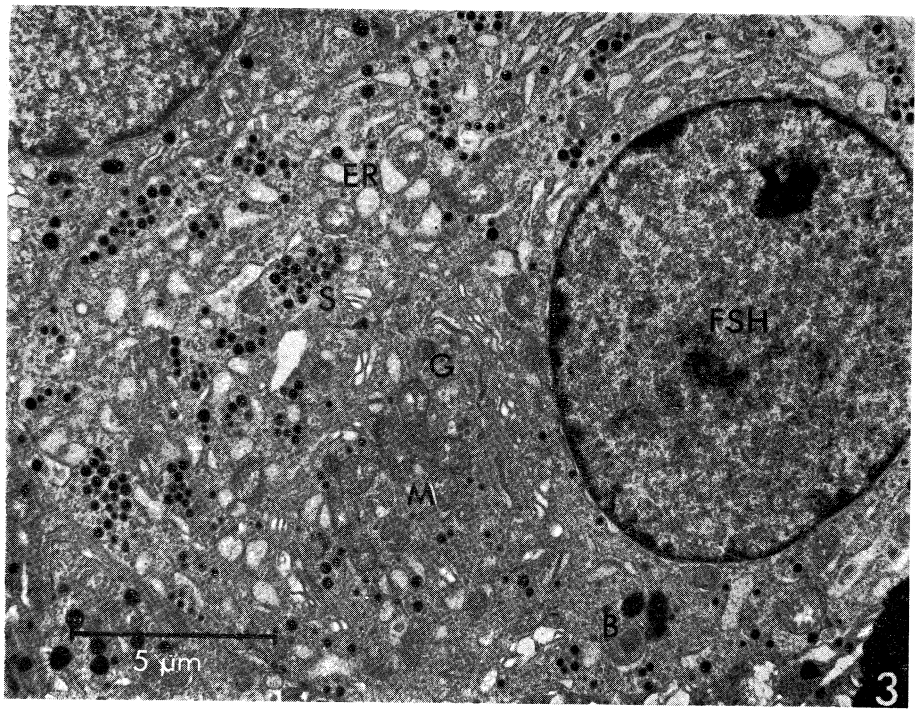
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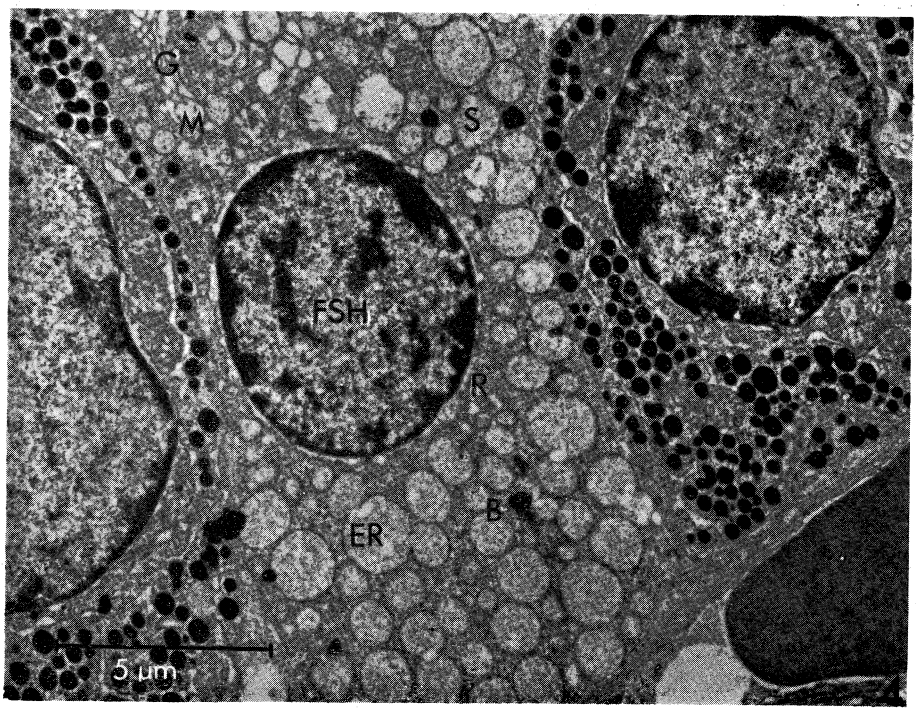
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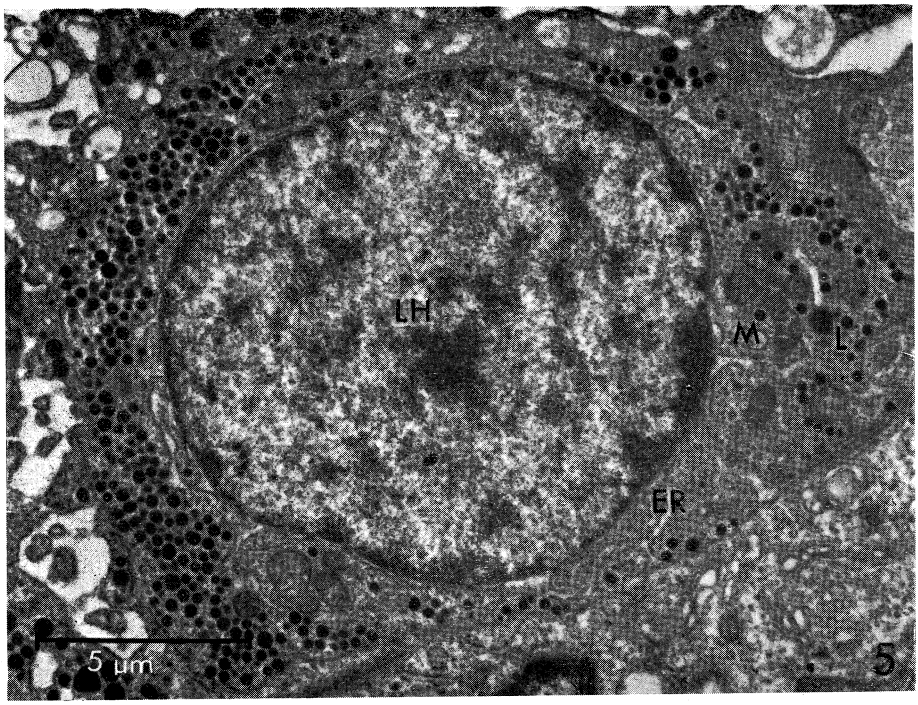
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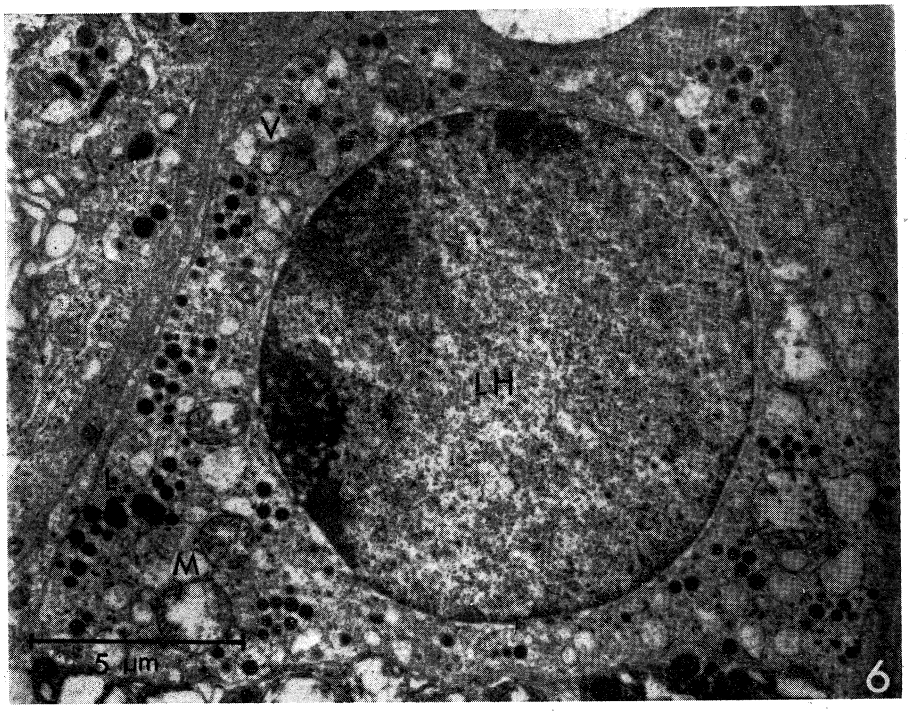
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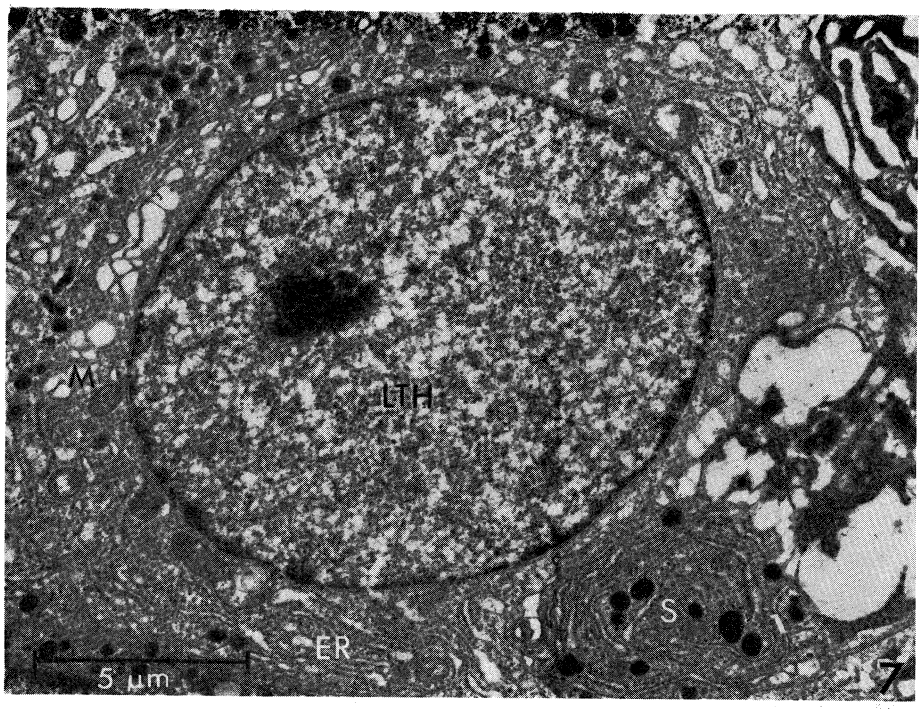
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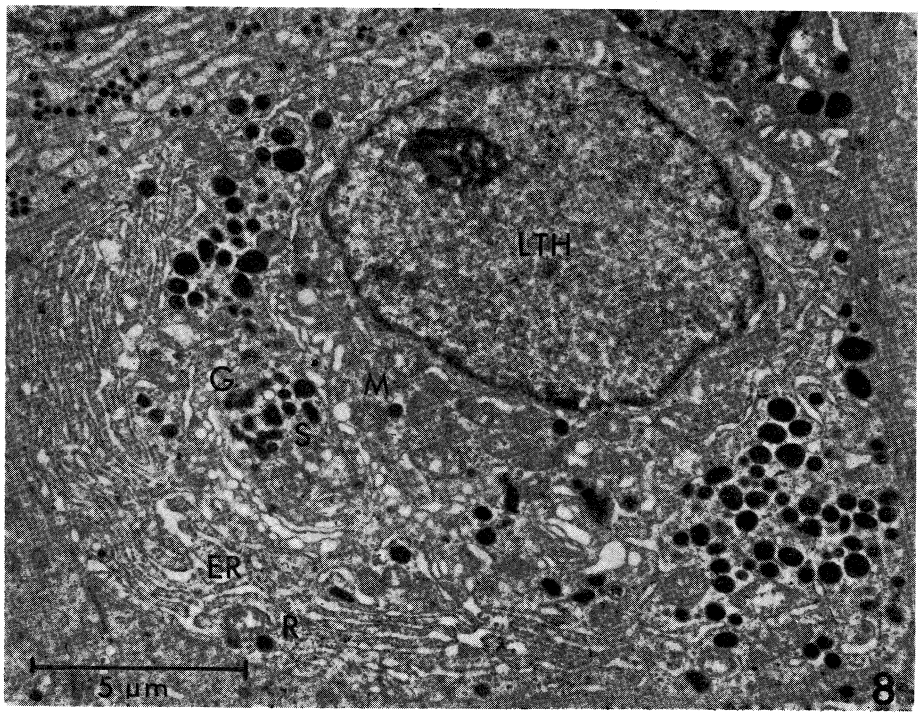
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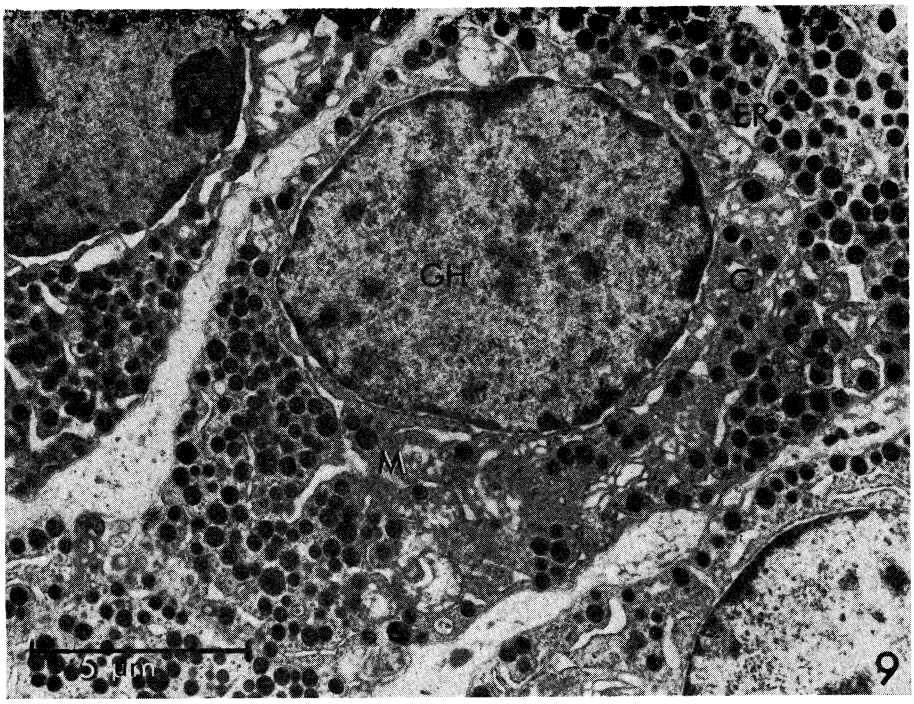
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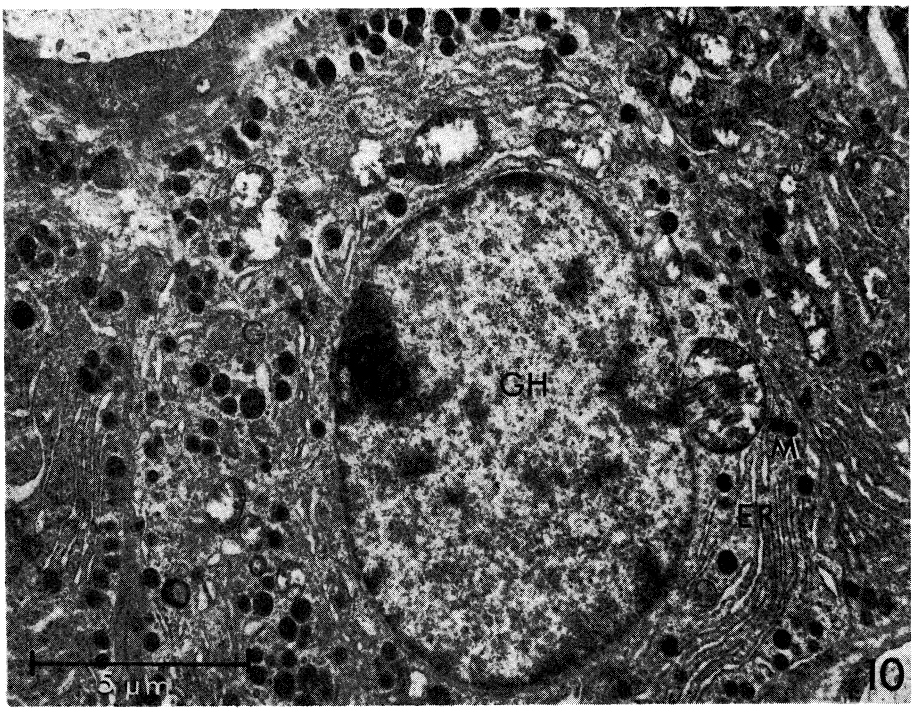
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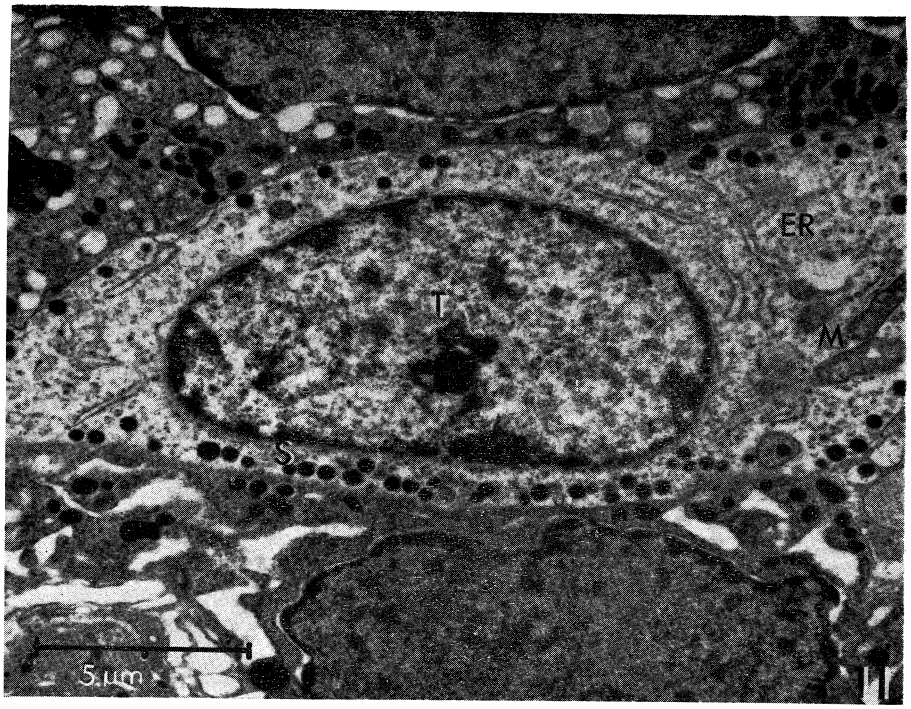
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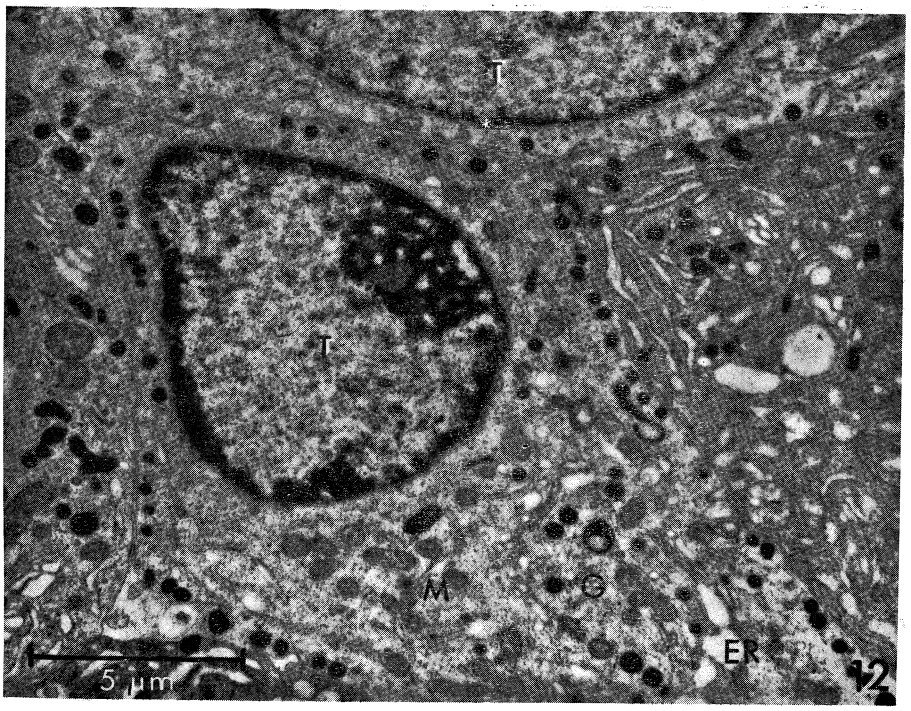
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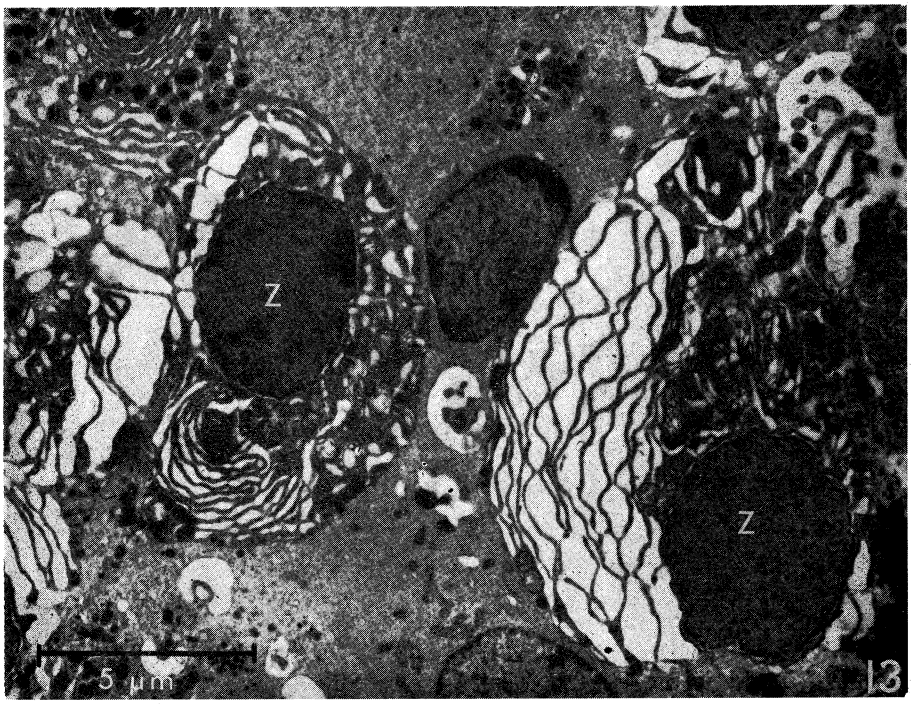

Fig. 13. Dark electron-dense 'castration' cells (*Z*) from a 15-day stressed pituitary. These cells are involuted and exhibit great ultrastructural alterations.

15 days of stress more extensive cellular activity was observed than at 5 days. The rough endoplasmic reticulum was greatly enlarged and well studded with ribosomes. The Golgi complex was extensive, containing granules at various stages of formation. In fact the luteotrope of the stressed cell resembled the luteotrope of pregnancy (Figs 7 and 8).

Somatotrope activity again showed an increase in the stressed groups. Thus at 5 and 15 days of stress an increase in the activity of the active type of somatotrope was seen. No change in the quiescent storage cell type was observed (Figs 9 and 10). Similarly increased secretory activity of the thyrotrope cell was observed at 5 and 15 days of stress (Figs 11 and 12). It seemed that somatotrope and thyrotrope activity showed some return to the control level of activity by 15 days of stress, although this change was marginal.

It was also observed that a larger number of cells showed signs of disintegration in the stressed groups. This was particularly evident in the 15-day stressed animals. Some of these cells resembled the castration cells described by Kovacs and Horvath (1975) with signs of irreversible ultrastructural disintegration (Fig. 13).

Discussion

It was shown that stress caused marked changes in the morphology of the adenohypophysis of the female rat, and these changes also correlated with changes in adrenal function as reflected in plasma glucocorticoid levels. The present observations agree

with the more extensive long-term study made on male rats following exposure to stress where intense secretory activity in all the tropic cells of the adenohypophysis was observed over the first 10 days, after which the cellular morphology showed a return towards the control condition. In both studies an exception was the luteotrope, the activity of which increased throughout the duration of the stress procedure (Pollard *et al.* 1976).

Stress significantly elevated plasma corticosterone levels at 5 and 15 days. The initial high rise to 72 μg/100 ml plasma dropped to a lower level of 55 μg/100 ml plasma by 15 days of stress. Changes in the corticotrope cells indicative of hyperactivity were also most marked at 5 days, and by 15 days demonstrated a return towards the control situation. At 15 days it was also observed that the number of chromophobe cells increased. There is evidence that the chromophobe is a source of ACTH (Sipenstein 1963). If this were the case significant additions to the total amount of ACTH available for release would relieve some of the burden from the corticotrope cells. Despite the continuation of the stress regimen, the ACTH cells reverted to a more normal cellular morphology at 15 days of stress. It would appear, therefore, that the observed drop in plasma corticosterone is not due to the exhaustion of pituitary-stored ACTH but to a decreased release of ACTH in response to the stressor.

In this context, Sakellaris and Vernikos-Danellis (1975) reported that rats subjected to chronic stresses, such as cold, adapted to these stresses in that plasma corticosterone levels returned to their pre-stress levels. These adapted animals, however, were able to respond to additional stimuli by a rise in their corticosterone concentrations, demonstrating that the ACTH synthesis and release had not been inhibited.

With regard to the FSH and LH gonadotropes, the initial stimulatory response at 5 days of stress also showed a degree of adaptation towards the normal condition at 15 days. Other investigators have reported that acute stress elevates serum FSH and LH in both the female (Ajika *et al.* 1972) and male (Euker *et al.* 1975) rat. In contrast, the prolactin or luteotrope cell did not adapt to the stress. Luteotrope activity increased throughout the duration of the stress procedure. That acute stress affects prolactin and the oestrous cycle of the rat has been known for some time (Neill 1970).

It was found that over the 5-day period of stress 40%, and over the 15 day period 100%, of the rats with normal oestrous cycles developed desynchronized cycles. Behavioural studies in rats have shown that prolonged stressors such as overcrowding lead to a lowered fertility in both males and females (Christian *et al.* 1964). It is also interesting to note that a number of stressors are known to induce pseudopregnancy and lactational changes in the female rat (Swingle *et al.* 1951; Nicoll *et al.* 1961). It seems, therefore, that disruptions of reproductive function could be a non-adaptive change associated with a psychological stress response. Kovacs and Horvath (1975) described ultrastructural transformations in the gonadotrope cell stimulated by gonadectomy. The most conspicuous change following gonadectomy was the formation of castration cells which developed due to sustained hypersecretion of gonadotropins. The cells described as 'castration' cells in the 15-day stressed animals (Fig. 13) are indistinguishable from those described by Kovacs and Horvath. The possible formation of such castration cells could lead to an imbalance of gonadotropic secretion and consequently also of prolactin secretion leading to physiological and behavioural changes in the stressed animals.

With regard to the somatotrope and thyrotrope cells, here too stress caused an increase in activity at 5 and 15 days with possibly some reversal towards the normal condition by 15 days. It seems that anxiety is a potent stimulus to growth hormone secretion both in experimental animals and in humans (Mason 1974; Sachar 1975). Interestingly, acute stresses of a physical nature (ether, cold, hypoglycaemia, and sound) have been shown to exert an inhibitory influence on the release of growth hormone as well as thyroxine (Brown and Hedge 1973; Collu *et al.* 1973). The results described for the male rat also indicated an eventual inhibition in somatotrope activity manifested only after an initial stimulation lasting for approximately 10 days (Pollard *et al.* 1976). The initial stimulation in somatotrope and thyrotrope activity is in agreement with the results of Mason (1974) who reported an increased secretion in rhesus monkeys stressed for 3 days using a similar regimen as employed here.

It would thus appear that apart from the well-known adrenal response many other hormonal systems are also highly sensitive to stress. The ultrastructural studies of the adenohypophysis described above revealed that despite hyperactivity of all the tropic cells, some adaptation toward the normal condition was seen by 15 days of stress. Furthermore, repeated stress does not extend the secretory capacity of the adenohypophysis and so adaptation after prolongation of stress possibly reflects a correcting homeostatic process. Concomitant with the endocrine upheaval the oestrous cycle of the stressed animal is markedly desynchronized.

Acknowledgments

We wish to thank Mrs J. M. Gregory for skilled technical assistance in the preparation of tissues for electron microscopy. This research was supported by the Australian Research Grants Committee, Grant No. D1-73/15017.

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Manuscript received 28 April 1977