BRAIN MECHANISMS RESPONSIBLE FOR ACTH RELEASE IN EXPERIMENTAL BURNS—PART I

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The cerebral cortex of the dog possesses an inhibitory influence over the pituitary-adrenal-axis. Rise in adrenal venous 17-OHCS output occurs after decortication operation. The output further rises after burn trauma. After brain removal up to the level of thalamus there is increased adrenocortical response after burn. In solitary pituitary experiments with supratentorial brain matters removed, increased adrenocortical secretion has been noted. Adrenal venous 17-OHCS output rises further after burn trauma. The hind-brain-factor (HBF) is not very important for the increased adrenocortical secretion in solitary pituitary experiments as the same events have been noted after removal of the infratentorial structures. In this case there is increased adrenocortical activity and 17-OHCS output further increases after burn trauma.

INTRODUCTION

In the second half of the nineteenth century Claude Bernard said that one of the most important features of all living beings is their ability to maintain the constancy of their internal *milieu* in spite of changes in the environment. The different defense reactions to maintain a stable internal environment has been called by Cannon as 'homeostasis'. This is achieved through the excess liberation of adrenalines. In 1915 he mentioned, 'It is a matter of prime importance for further discussion to determine whether the adrenal glands are in fact roused to special activity in times of stress.' He found the evidence that adrenal secretion (adrenin) was increased in emotional excitement and painful stimulation. Selye (1936) attached importance to the pituitary-adrenocortical system. The totality of the damage and the body's adaptive reactions has been called by Selye as stress syndrome or general adaptation syndrome. It has got three stages: (1) the alarm reaction; (2) the stage of resistance; and (3) the stage of exhaustion. The neuroendocrine system is important in maintaining resistance during stress.

The following theories have been put forward to explain the mechanism of adenohypophyseal ACTH release:

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(1) Feed-back mechanism of Sayers (Sayers and Sayers 1947, 1948; Sayers 1950). The circulating level of adrenocortical steroids in blood controls ACTH secretion.

(2) Reflex discharge of adrenaline from the adrenal medulla controls ACTH release (Long 1947, 1952; Gershberg *et al.* 1950; McDermott *et al.* 1950).

(3) ACTH release is controlled by hypothalamic influences through hypophyseo-portal vessels (de Groot and Harris 1950; Harris 1950; Harris and Jacobsohn 1950; Harris and Fortier 1954; and others).

Karplus and Kreidl (1909, 1910, 1912) first described that the hypothalamus had some bodily functions. On stimulation of the walls of the third ventricle there was acceleration of the heart-beat, changes in the movements and secretion of the gut, dilatation of the pupils and other autonomic effects. Cerebral hemispheres were removed from many of their experimental animals for destroying the projection fibres. On stimulation of the wall of the third ventricle in such animals the same reactions were found. Local anaesthesia applied to the part to be stimulated abolished the reactions. They also traced the paths which were essential for the mediation of the response. Cardiac responses depended upon the ventral roots of the upper five or six thoracic segments. Stellate ganglionectomy blocked the response.

Houssay and Molinelli (1925) stated that epinephrin secretion by the adrenal medulla is controlled by the hypothalamus.

Hess (1925, 1932, 1938, 1948, 1954) stressed upon the importance of the hypothalamus and he developed a method for stimulating different parts of the diencephalon in conscious cats by indwelling electrodes. After the stimulation experiments were over, the particular area of the brain was lesioned and the effects were observed.

Bard (1928) stated that the hypothalamus was important for rage reactions in cats. 'Sham rage' in a decorticate cat is not present when the connection between the hypothalamus and the brain stem is disrupted. Thus in the caudal hypothalamus of the cat there is a centre which controls the emotional mechanisms, e.g. the angry behaviour.

Cannon's (1932) work on emergency reactions and homeostasis laid importance on the sympathetic nervous system.

The hypothalamus is not only an important nodal area which is related to behavioural and emotional responses but also it is important as a centre which regulates the endocrinological activities of the body.

Selye (1954) says that the different endocrine glands are important for the elicitation of the General Adaptation-Syndrome. The adaptation also occurs at a higher level through the mediation of the different areas of the central nervous system. Lissák and Endröczi (1960) stresses the importance of the central nervous system in the adaptation activity. Elkes (1962) thinks

that the septal region, the median eminence of the hypothalamus, the peri-aqueductal grey and other unidentified regions act as important areas for maintaining equilibrium. Upward influences come from the brain stem and downward influences project from the cortex and the caudate and lentiform nuclei. The various pathways that are required for appropriate homeostasis are as yet not very clear. These responses include endocrine, somatomotor and visceromotor effect. The role of hippocampus and amygdala in the activity of the pituitary-adrenal-axis is in the process of recent evaluation. This mechanism is important for adaptation activity after stress. Elkes further states, 'Indeed, stress response may be manifest not only as overactivity (resulting from the faltering of a physiological braking mechanism) but also as underactivity (resulting from excessive inhibitory tone fired, in turn, by a runaway excitatory process). It is noteworthy that in the underactivity and withdrawal of extreme depression, the levels of hydroxycorticoid are apt to be high rather than low. Stress response may thus travel in different guices; and it will be well not to take overactivity and underactivity at their face value in judging such a response.'

Richter (1958) thought that Selye's (1950) 'stress concept' did not attach any importance to the nervous system in response to stress. However, in 1954 Selye explained this position. Richter also thought of the possibility that the nervous system might be affected by stress. Very severe stress leads to changes in the hypothalamic nuclei and these lesions explain for the different types and special combinations of the responses to stress. Also these may produce mental and physical diseases with periodic manifestations. For maintaining homeostasis the hypothalamic cells were markedly stimulated. Ultimately there was exhaustion of the hypothalamus with production of lesions. Some swim-stressed rats actually developed pituitary tumours.

Roussy and Mosinger (1946) in their *Traite de Neuro-Endocrinologie* said that they were forced to demonstrate the existence of a reflex hypophyseal neuroregulation as a result of concentrating their study on the sum total afferent nervous paths to the excitosecretory centres of the hypophysis. On page 333 they mention that the cingulate gyrus has to influence the functional role of both the hypophysis and epiphysis.

The adenohypophysis does not get any direct nervous connection of any functional significance with the hypothalamus (Okada *et al.* 1955; Palay 1957; Farquhar and Rinehart 1957; Harris 1958). However, direct functional neural connection to the adenohypophysis has been thought to exist by Vazquez-Lopez and Williams (1952), Metuzals (1956), Smith (1956), and others.

At present extrahypothalamic structures controlling the activity of the pituitary-adrenal-axis are in the process of exploration (Endröczi and Lissák 1960; Endröczi *et al.* 1954; Martin *et al.* 1958, and others).

In this part the results have been presented regarding the influence of

hypothalamic and extrahypothalamic areas on pituitary-adrenal-axis after burn (ablation studies). This also includes the solitary pituitary experiments.

Brain Lesions and ACTH Secretion

Hume and Egdahl (1959) summarized the reports of Egdahl *et al.* (1958) and Egdahl *et al.* (1959) where it was shown that even after removal of the brain the animals could respond to operative injury with rise in adrenocortical secretion. In one group only the cortex was removed and in the other group cortex and thalamus were ablated. Both these groups responded to operative trauma well. In a third group all the brain matters above the inferior colliculus were removed leaving behind the pituitary, cerebellum, pons and medulla. Operative trauma in this group showed marked adrenocortical response on many occasions. In all the groups the resting levels of corticosteroid secretion was high. Hume and Egdahl (1959) concluded that the secretions of ACTH and adrenocorticosteroid after trauma were under the control of a complex neurohumoral mechanism situated in different areas of the brain.

Story *et al.* (1959) showed persistent elevation of corticosteroid secretion up to six hours after removal of large portion of the hypothalamus in some animals.

Egdahl (1960) studied adrenal venous 17-OHCS output and catecholamines in the basal condition and after burn trauma in dogs with isolated pituitary. Up to five days after operation these animals had high resting corticosteroid and two-thirds of the animals responded to the stimulus with increased 17-OHCS output. Low-resting catecholamine levels increased after burns. He postulated a HBF (hind brain factor) which passed through the systemic circulation and acted on the pituitary and thereby released ACTH. Possibility of a spinal cord neurosecretion is also there.

That the hind brain factor is not important is proved by the experiments of Wise *et al.* (1962) and those of Egdahl (1962) himself. He mentioned a period of depressed adrenocortical function after the preparation of isolated pituitary. Following this there was a persistent elevation of the basal secretion. Removal of total brain and spinal cord or removal of posterior pituitary or abdominal viscera did not change the response.

Egdahl (1961*a*) showed that the constriction of the inferior vena cava is a strong stimulus to the isolated pituitary.

Matsuda *et al.* (1963) removed the forebrain anterior to the superior colliculus in rats leaving behind the pituitary intact. Removal of the cerebral cortex and subjacent brain in the rat did not show any sustained rise in peripheral plasma corticosteroid. In the rat, according to the authors, cerebral cortical inhibition does not occur. For pituitary-adrenocortical activation in the rat at least the median eminence-stalk-pituitary complex should be present.

Matsuda *et al.* (1964) used animal preparations (rats) with removal of all forebrain anterior to the superior colliculus, but having median eminence, stalk and pituitary intact. In these animals adrenocortical response was increased after ether anaesthesia with or without trauma equal to that of normal controls. With nembutal anaesthesia trauma did not lead to a rise in adrenocortical secretion unless a large peninsula was left connecting the hypothalamus to the dorsal mesencephalon and remaining hind brain. Spinal cord section in otherwise intact animal under nembutal (but not ether) anaesthesia prevented the rise in adrenocortical secretion in response to a leg-break distal to the cord section. Ether directly stimulates the median eminence leading to increased ACTH release.

Wise *et al.* (1964) studied the effect of brain removal in dogs previously subjected to pituitary stalk section. The hypothalamus and other neural tissues above the midbrain were removed in 11 dogs and the effects on adrenocortical secretion were noted. They found that the response in severely stressed dogs was not only due to CRF from the hypothalamus but it might be also due in part to an ACTH stimulating humoral agent liberated from traumatized tissues.

Endröczi *et al.* (1963) observed an increase of ACTH secretion in cats after cholinergic chemical stimulation of the medial and caudal hypothalamus as well as the posterior hypothalamus and ventral tegmentum. No significant change in ACTH secretion occurred after stimulation of the hypothalamo-pituitary-neurosecretory system. This observation is at variance with the hypothesis that vasopressin or oxytocin plays an important part in the control of pituitary-adrenocortical activity.

Brodish (1963) said that in the rat the entire area of the ventral hypothalamus extending from the optic chiasma to the mammillary bodies is involved in ACTH secretion. A diffuse hypothalamic nucleus or network controls ACTH secretion. The centre is not a localized and discrete one.

Hume and Jackson (1959) observed that for a period of time varying from four hours to seven days after hypothalamic destruction the adrenocortical response to trauma was intact. From seven to 14 days there was loss of normal response to trauma but ACTH response was there. Spinal cord section abolished the response to trauma in the normal dog.

Witt and Keller (1960) performed hypothalamectomy and midbrainectomy on dogs. These preparations survived total pancreatectomy performed two to four weeks after brain lesions and without any hormonal supportive therapy. There was diabetis mellitus. Adrenal cortices were not atrophied, rather in some animals there was varying degree of hypertrophy.

Keller *et al.* (1954) found that ventral hypothalamectomy did not interfere with the eosinopenic response to surgery.

Stimulation of the thalamus or lower posterior hypothalamus (Suzuki

et al. 1960) led to increased adrenocortical function. Stimulation of antero-medial paraventricular region of the hypothalamus had the same result (Mason 1958*a*). Stimulation of the thalamus (Mason 1958*b*) or putamen (Mason 1958*a*, 1958*b*) did not alter the adrenocortical function.

Materials and Methods

Male dogs of 10 to 15 kg in weight were used in this experiment. Intravenous nembutal anaesthesia was administered in a dose of 30 mgm/kg of body weight. Right lumbo-adrenal vein was cannulated after the method of Hume and Nelson (1955). Burn trauma was produced by immersing the extremity in boiling water (100° C) for 30 seconds under nembutal anaesthesia. Adrenal venous 17-OHCS has been estimated after the method of Silber and Porter (1954). Sterile techniques have been used all throughout the procedures except in the very acute experiments. Animals exhibiting intracranial infections were excluded from the series. In brain lesion-experiments the extent of lesion was determined by staining the sections with Nissl stain.

I. *Control experiments for brain operations*

In this group 15 male dogs of 10 to 15 kg in weight were used. Cannulation of lumbo-adrenal vein was done 48 hours before the control brain operation. This operation includes the exposure of the brain after incision over the meninges through the trephine hole. The layers were closed as usual without any brain lesion. Burn trauma to the right hind limb was inflicted at 60 minutes after this procedure in five dogs, at 180 minutes in five dogs and at 24 hours in five dogs. Adrenal venous blood 17-OHCS output was measured at 1/2, 1, 2, 3 and 6 hours after burn. Then intravenous ACTH (0·8 unit/kg) was administered and the adrenocortical response was noted.

II. *Decortication and burn*

Ten male dogs of 10 to 15 kg in weight were used in these experiments. The lumbo-adrenal vein was cannulated 48 hours before the brain operations. Bilateral decortication was carried out in a single stage. Bleeding was controlled by silver clips, gelfoam and ligation as required. Sixty minutes after decortication intravenous ACTH (0·8 unit/kg) was injected. Rest for 24 hours was allowed to the animals. Burn trauma to the right hind limb was applied and the adrenal venous 17-OHCS output was measured at 1/2, 1, 2 and 3 hours after burn. Intravenous ACTH test was repeated.

III. *Animals with brain removed up to thalamo-hypothalamic level*

Five male dogs of 10·5 to 14 kg in weight were used in these experiments. Lumbo-adrenal vein was cannulated 48 hours before the brain removal operations. Removal of brain up to the thalamo-hypothalamic level was carried

out on both sides. Posterior neural connections remained intact. At 30 and 60 minutes after these procedures adrenal venous blood 17-OHCS output was measured. Bleeding was checked as mentioned previously. Standardized burn trauma was inflicted on the right hind limb. Adrenal venous corticosteroid output was measured at 1/2, 1, 2 and 3 hours after burn. Intravenous ACTH was injected after this.

IV. *Animals with solitary pituitary and intact hind brain*

In this group eight male dogs of 10 to 15 kg in weight were used. Lumboadrenal vein was cannulated 48 hours before the brain operations. The dogs were operated under nembutal anaesthesia. Brain removal was carried out through craniotomy openings by methods of section and suction.

During the process of removal of the brain, the middle cerebral arteries stand out in prominence and these require ligature to minimize haemorrhage. In all the experiments, both here and subsequently, where brain removal operations have been performed, bleeding from vessels has been checked by the following procedures as required:

(i) Ligations, (ii) application of Cushing's clips, (iii) cautery and (iv) gelfoam.

Supratentorial neural structures were removed leaving behind the pituitary only. The infratentorial structures were kept undisturbed. Adrenal venous blood was collected at 1/2, 1 and 3 hours after this procedure. Five per cent glucose and five per cent glucose in saline transfusions were carried out during the process of brain removal. The right inferior extremity was burned and corticosteroid output was measured at 1/2, 1, 2 and 3 hours after burn. ACTH (0·8 unit/kg) was administered intravenously. Artificial respiration was carried out in some dogs.

The blood-pressure changes during surgery are shown in Figs. 1–3.

The pituitary was histologically studied after staining the sections with Gomori's CAHP stain and haematoxylin and eosin stain.

V. *Absolutely solitary pituitary with removal of the hind brain*

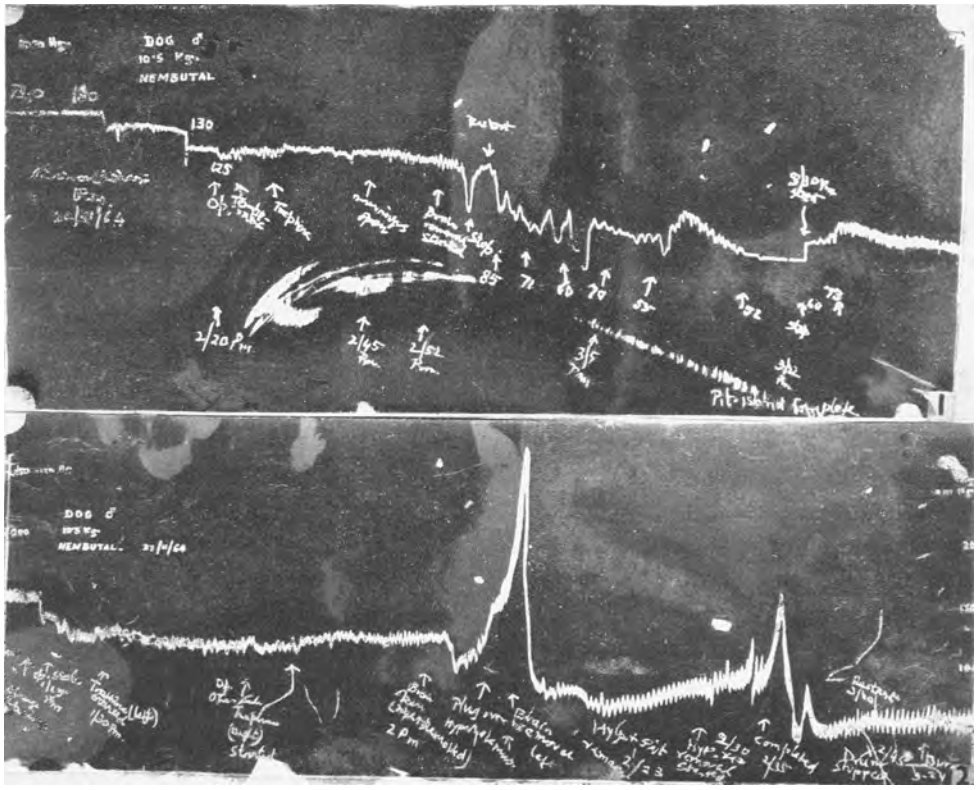
Five male dogs of 10 to 15 kg in weight were used. The procedures were same as in group IV. Removal of infratentorial brain substance was an additional step. The tentorium cerebelli was removed and the cerebellum was extirpated and this facilitated the removal of the hind brain. The pituitary was left alone (solitary pituitary). These dogs required blood (recently drawn blood from hypophysectomized donors), fluid transfusions and artificial respiration. Sixty minutes after the brain operations standardized burn trauma was produced on the right hind limb and corticosteroid output was measured at 1/2, 1, 2 and 3 hours after burn. ACTH response was also noted.

Histological examination of the pituitary was carried out after staining the section with Gomori's CAHP stain and haematoxylin and eosin stain.

RESULTS

I. *Control experiments for brain operations*

In this group adrenal venous 17-OHCS output was high during cannulation operation in comparison to the post-cannulation values (48 hours after cannulation).



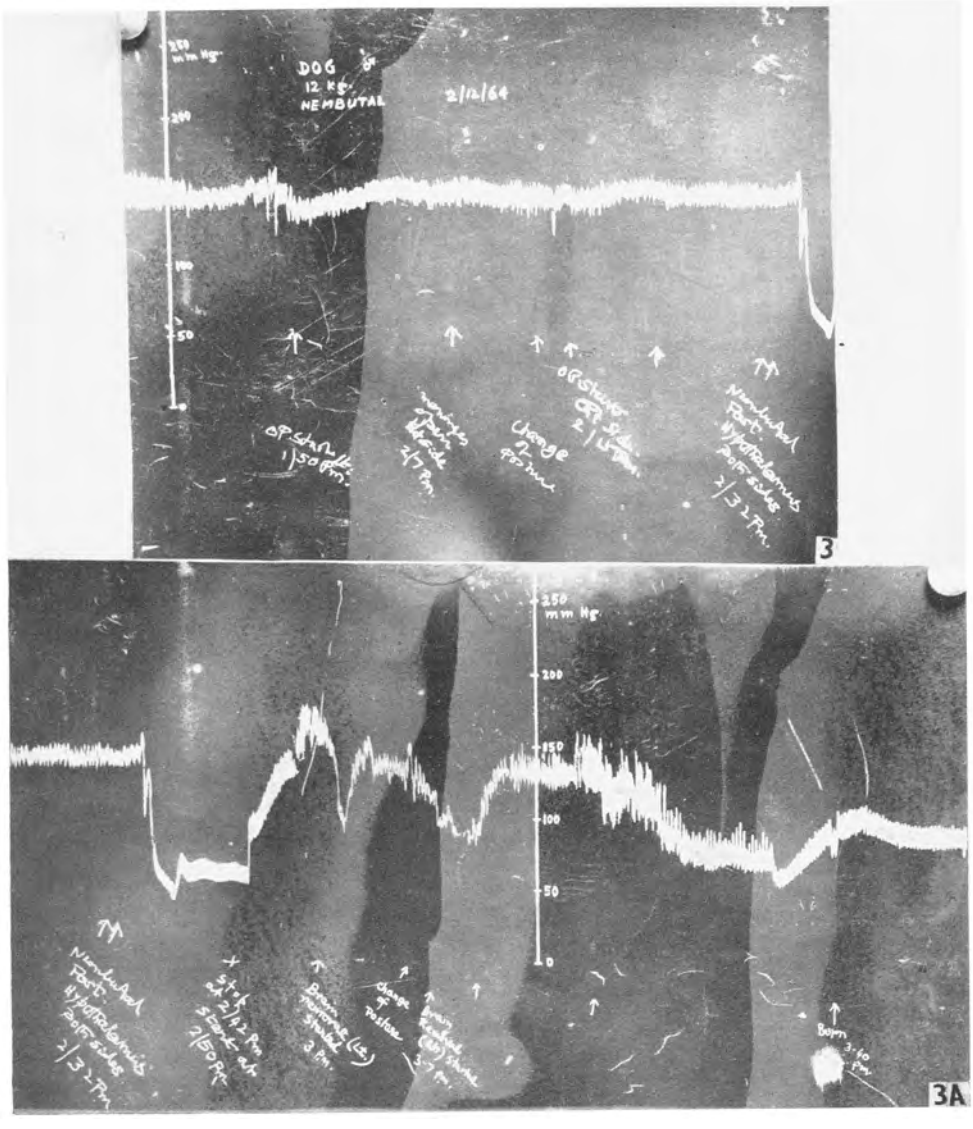
FIGS. 1–2. 1, blood-pressure changes during surgery for isolated pituitary experiment; 2, blood-pressure changes during surgery for isolated pituitary experiment and burn.

Subgroup A (Table I and Statistical Table I).—During the surgery of control brain operations the corticosteroid output was significantly high at 30 and 60 minutes. Thirty minutes after burn the corticosteroid output was significantly high (0·1% level) when compared to the value at 60 minutes after brain operation. At 1, 2, 3 and 6 hours after burn the difference was significant at 1% level. Intravenous administration of ACTH showed good adrenocortical response six hours after burn.

TABLE I

Control experiments for the brain lesioned dogs and burn

Dog numbers		137	138	139	140	141	Mean	S.D.	D.F.
Observation numbers		Adrenal venous 17-OHCS output (gamma/minute)							
1	During adrenal vein cannulation	8·2	7·8	9·5	10·1	11·0	9·320	1·326	4
2	Forty-eight hours after cannulation	1·9	2·6	3·1	2·0	3·5	2·620	0·691	4
3	Thirty minutes post-operative	9·5	10·2	11·6	12·0	12·5	11·160	1·262	4
4	Sixty minutes post-operative	9·0	7·6	11·2	10·5	10·8	9·820	1·494	4
5	Thirty minutes after burn	20·1	25·0	23·7	25·0	20·5	22·860	2·401	4
6	Sixty minutes after burn	18·7	21·3	22·0	21·3	17·0	20·060	2·124	4
7	Two hours after burn	17·2	22·3	20·0	18·5	16·4	18·880	2·349	4
8	Three hours after burn	17·6	19·6	16·7	19·7	15·0	17·720	1·994	4
9	Six hours after burn	15·3	18·5	17·4	16·6	15·6	16·680	1·314	4
10	ACTH (I.V.)	21·0	24·8	21·5	24·0	22·7	22·800	1·611	4



FIGS. 3–3A. Blood-pressure changes during surgery for isolated pituitary experiment and burn. Near the middle of the tracing, effect of injection of nembutal into the posterior hypothalamus on both sides is noted.

STATISTICAL TABLE I

Values of 't' together with D.F. for statistical tests of significance for different types of 'intra-group' paired comparisons regarding adrenal venous 17-OHCS output in dogs (control experiments for the brain lesions)

							D.F.	't'
Observation	No.	1	vs.	Observation	No.	2	4	12·727 †
,,	,,	2	vs.	,,	,,	3	4	−18·852 †
,,	,,	2	vs.	,,	,,	4	4	−11·861 †
,,	,,	4	vs.	,,	,,	5	4	− 9·674 †
,,	,,	4	vs.	,,	,,	6	4	− 8·470 *
,,	,,	4	vs.	,,	,,	7	4	− 5·992 *
,,	,,	4	vs.	,,	,,	8	4	− 5·700 *
,,	,,	4	vs.	,,	,,	9	4	− 6·558 *
,,	,,	9	vs.	,,	,,	10	4	−10·426 †

* Significant at 1% level.
† Significant at 0·1% level or more stringent level.

Subgroup B (Table II and Statistical Table II).—During control brain operations up to three hours the 17-OHCS output was significantly high in comparison to the 48 hours' post-cannulation value. Significantly high 17-OHCS output was noted up to six hours after burn and intravenous ACTH injection showed good response.

STATISTICAL TABLE II

Values of 't' together with D.F. for statistical tests of significance for different types of 'intra-group' paired comparisons regarding adrenal venous 17-OHCS output in dogs (control experiments for the brain lesions)

								D.F.	't'
Observation	No.	1	vs.	Observation	No.	2	..	4	10·897 †
,,	,,	2	vs.	,,	,,	3	..	4	−20·189 †
,,	,,	2	vs.	,,	,,	4	..	4	−10·261 †
,,	,,	2	vs.	,,	,,	5	..	4	−31·584 †
,,	,,	5	vs.	,,	,,	6	..	4	−19·123 †
,,	,,	5	vs.	,,	,,	7	..	4	− 8·608 *
,,	,,	5	vs.	,,	,,	8	..	4	−10·876 †
,,	,,	5	vs.	,,	,,	9	..	4	−18·154 †
,,	,,	5	vs.	,,	,,	10	..	4	− 8·757 †
,,	,,	10	vs.	,,	,,	11	..	4	− 4·607 *

* Significant at 1% level.
† Significant at 0·1% level or more stringent level.

TABLE II

Control experiments for the brain lesioned dogs and burn

Dog numbers		142	143	144	145	146	Mean	S.D.	D.F.
Observation numbers		Adrenal venous 17-OHCS output (gamma/minute)							
1	During adrenal vein cannulation	9·5	8·7	9·6	10·5	7·1	9·080	1·278	4
2	Forty-eight hours after cannulation	3·0	2·4	2·6	1·5	1·9	2·280	0·589	4
3	Thirty minutes post-operative	10·7	11·0	12·6	10·2	9·6	10·820	1·128	4
4	Sixty minutes post-operative	10·3	12·4	9·5	10·0	7·5	9·940	1·756	4
5	Three hours post-operative	9·0	8·5	8·6	7·2	7·0	8·060	0·899	4
6	Thirty minutes after burn	21·8	22·7	24·5	20·1	18·8	21·580	2·210	4
7	Sixty minutes after burn	20·6	24·5	25·0	18·0	15·9	20·800	3·976	4
8	Two hours after burn	17·3	21·7	21·7	16·8	16·8	18·860	2·601	4
9	Three hours after burn	16·2	18·3	17·3	15·1	14·7	16·320	1·501	4
10	Six hours after burn	13·1	16·0	17·0	13·0	14·3	14·680	1·774	4
11	ACTH (I.V.)	24·6	20·1	22·5	19·6	18·3	21·020	2·513	4

Subgroup C (Table III and Statistical Table III).—17-OHCS output was high up to six hours after control brain operations. After 24 hours the output reached a basal level. The corticosteroid output was high up to six hours after burn and intravenous ACTH administration showed a good response.

II. *Decortication experiments* (Table IV and Statistical Table IV)

17-OHCS output reached a basal level 48 hours after cannulation. After 30 and 60 minutes of decortication the corticosteroid output was significantly high. This high output was also noted at 24 hours. Burn trauma stimulated the pituitary-adrenal-axis further and a good response was noted after intravenous ACTH.

STATISTICAL TABLE III

Values of 't' together with D.F. for statistical tests of significance for different types of 'intra-group' paired comparisons regarding adrenal venous 17-OHCS output in dogs (control experiments for the brain lesions)

							D.F.	't'
Observation	No.	1	vs.	Observation	No.	2	4	17·136 †
,,	,,	2	vs.	,,	,,	3	4	−11·424 †
,,	,,	2	vs.	,,	,,	4	4	− 6·257 *
,,	,,	2	vs.	,,	,,	5	4	− 7·024 *
,,	,,	2	vs.	,,	,,	6	4	− 7·685 *
,,	,,	2	vs.	,,	,,	7	4	− 2·749
,,	,,	7	vs.	,,	,,	8	4	−28·108 †
,,	,,	7	vs.	,,	,,	9	4	−22·272 †
,,	,,	7	vs.	,,	,,	10	4	−18·483 †
,,	,,	7	vs.	,,	,,	11	4	−25·125 †
,,	,,	7	vs.	,,	,,	12	4	−19·182 †
,,	,,	12	vs.	,,	,,	13	4	− 7·351 *

* Significant at 1% level.

† Significant at 0·1% level or more stringent level.

STATISTICAL TABLE IV

Values of 't' together with D.F. for statistical tests of significance for different types of 'intra-group' paired comparisons regarding adrenal venous 17-OHCS output in dogs (decortication and burn)

							D.F.	't'
Observation	No.	1	vs.	Observation	No.	2	9	15·900 *
,,	,,	2	vs.	,,	,,	3	9	− 9·705 *
,,	,,	2	vs.	,,	,,	4	9	− 9·267 *
,,	,,	2	vs.	,,	,,	6	9	− 8·514 *
,,	,,	4	vs.	,,	,,	5	9	− 9·730 *
,,	,,	6	vs.	,,	,,	7	9	− 9·366 *
,,	,,	6	vs.	,,	,,	8	9	− 9·097 *
,,	,,	6	vs.	,,	,,	9	9	− 7·725 *
,,	,,	6	vs.	,,	,,	10	9	− 5·641 *
,,	,,	10	vs.	,,	,,	11	9	−10·984 *

* Significant at 0·1% level or more stringent level.

TABLE III

Control experiments for the brain lesioned dogs and burn

Dog numbers		147	148	149	150	151	Mean	S.D.	D.F.
Observation numbers		Adrenal venous 17-OHCS output (gamma/minute)							
1	During adrenal vein cannulation	10·0	9·4	9·5	8·5	10·7	9·620	0·811	4
2	Forty-eight hours after cannulation	1·3	2·5	2·7	2·0	2·5	2·200	0·566	4
3	Thirty minutes post-operative	11·4	10·7	9·9	8·0	11·5	10·300	1·438	4
4	Sixty minutes post-operative	11·6	11·0	6·5	7·5	10·0	9·320	2·222	4
5	Three hours post-operative	10·5	7·1	7·2	8·4	10·6	8·760	1·713	4
6	Six hours post-operative	8·0	6·5	6·0	7·0	6·4	6·780	0·769	4
7	Twenty-four hours post-operative	5·2	3·6	3·5	2·9	3·8	3·800	0·852	4
8	Thirty minutes after burn	23·0	20·7	21·6	23·3	24·0	22·520	1·341	4
9	Sixty minutes after burn	21·9	18·1	18·5	17·8	22·1	19·680	2·134	4
10	Two hours after burn	21·0	15·4	18·0	19·0	17·5	18·180	2·052	4
11	Three hours after burn	18·2	16·4	17·2	18·6	18·7	17·820	0·981	4
12	Six hours after burn	18·7	13·5	14·5	14·7	16·2	15·520	2·023	4
13	ACTH (I.V.)	21·0	18·6	20·3	20·1	22·1	20·420	1·283	4

TABLE IV

Docortication and burn

Dog numbers		157	158	159	160	161	162	163	164	165	166	Mean	S.D.	D.F.
Observation numbers		Adrenal venous 17-OHCS output (gamma/minute)												
1	During adrenal vein cannulation	12·4	8·4	7·5	10·0	9·2	8·6	10·0	9·0	11·5	7·4	9·400	1·619	9
2	Forty-eight hours after cannulation	3·2	2·4	2·7	3·1	2·8	1·9	2·6	3·5	3·0	2·7	2·690	0·567	9
3	Thirty minutes after decortication	22·6	12·0	16·9	11·7	13·0	8·1	18·5	14·0	18·2	13·5	14·850	4·539	9
4	Sixty minutes after decortication	18·0	10·2	19·4	13·5	12·0	11·5	23·0	15·2	23·0	20·0	16·580	4·749	9
5	ACTH (I.V.)	27·1	18·5	23·1	27·2	20·0	19·5	32·1	28·0	34·0	26·5	25·600	5·264	9
6	24 hours after decortication	14·4	9·2	12·2	10·1	9·3	8·5	15·0	12·0	20·0	16·5	12·720	3·725	9
7	Thirty minutes after burn	30·1	19·0	20·0	25·0	22·1	16·7	34·0	29·0	28·6	27·5	25·200	5·579	9
8	Sixty minutes after burn	24·0	20·5	18·1	19·0	16·5	12·5	27·1	18·4	28·7	21·0	20·580	4·895	9
9	Two hours after burn	24·6	17·6	18·4	15·9	14·0	16·1	30·5	21·2	26·0	21·7	20·600	5·212	9
10	Three hours after burn	18·4	14·1	14·5	12·0	13·5	14·0	26·7	18·5	26·6	19·5	17·780	5·287	9
11	ACTH (I.V.)	30·0	23·0	20·5	22·0	24·7	26·6	35·2	28·0	32·1	25·0	26·710	4·656	9

When the results at 60 minutes after decortication were compared to those of the control brain operations (Statistical Table XI), the difference was significant at 1% level, the decortication group manifested with higher adrenocortical activity. At 24 hours the difference was significant at 0·1% level. By this time the corticosteroid output reached a basal level in the dogs with control brain operations, but the decorticated dogs had high 17-OHCS output. Regarding the responses after burn trauma there was practically no difference between the two groups.

III. *Animals with brain removal up to thalamo-hypothalamic level* (Table V and Statistical Tables V and VIII)

TABLE V

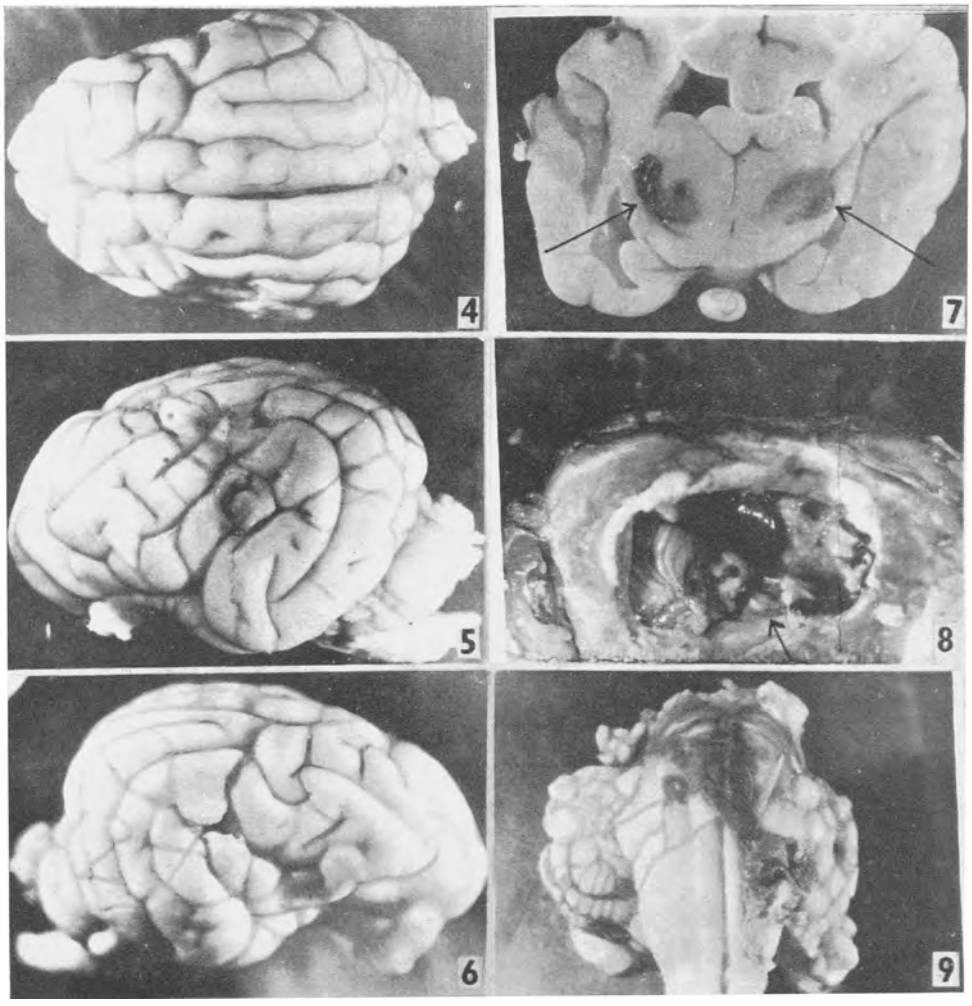
Brain romoval down to thalamo-hypothalamic level (posterior connections remaining intact) and burn

Dog numbers		167	168	169	170	171	Mean	S.D.	D.F.
Observation numbers		Adrenal venous 17-OHCS output (gamma/minute)							
1	During adrenal vein cannulation	11·2	13·0	7·0	10·4	7·4	9·800	2·557	4
2	Forty-eight hours after cannulation	3·1	2·7	1·9	2·6	2·0	2·460	0·503	4
3	Thirty minutes after the preparation	7·1	1·5	6·4	7·0	5·0	5·400	2·336	4
4	Sixty minutes after the preparation	11·5	1·2	6·0	10·5	6·4	7·120	4·106	4
5	Thirty minutes after burn	30·6	8·5	21·5	26·8	23·2	22·120	8·380	4
6	Sixty minutes after burn	25·0	13·4	17·8	20·2	18·0	18·880	4·218	4
7	Two hours after burn	20·4	12·7	19·0	14·5	14·1	16·140	3·355	4
8	Three hours after burn	17·0	13·0	22·0	18·2	21·3	18·300	3·622	4
9	ACTH (I.V.)	18·0	16·6	26·9	24·2	26·4	22·420	4·809	4

Basal 17-OHCS output was noted 48 hours after cannulation. Surgery for removal of the brain above the hypothalamic level did not show significantly high 17-OHCS output. However, when burn trauma was superimposed, increased 17-OHCS output was observed and the adrenal cortex was well responsive to exogenous ACTH.

Thirty minutes after control brain operation the corticosteroid output was significantly higher than in the group of dogs with lesions up to thalamo-hypothalamic level. At no other periods there was any significant difference.

In a few experiments when only bilateral thalamic lesions were produced, burn trauma to the right hind limb (on the same day) did not manifest with immediate increased 17-OHCS output (Figs. 4, 5, 6 and 7 of the same dog).



FIGS. 4–9. 4, dorsal view of the brain. 5, left lateral view of the same brain as of Fig. 4. 6, right lateral view of the same brain as of Fig. 4. 7, shows the bilateral thalamic lesions of the same brain. 8, shows the solitary pituitary and the intact hind brain within the calvarium of dog No. 172. 9, ventral view of the infratentorial structures of dog No. 172.

IV. *Animals with solitary pituitary and intact hind brain* (Table VI and Statistical Tables VI and X)

Forty-eight hours after cannulation the basal 17-OHCS output was noted. The solitary pituitary with intact infratentorial structures responded well to surgery at 30, 60 and 180 minutes. Superadded burn trauma manifested with high 17-OHCS output up to three hours and the adrenal cortex was well responsive to exogenous ACTH.

STATISTICAL TABLE V

Values of 't' together with D.F. for statistical tests of significance for different types of 'intra-group' paired comparisons regarding adrenal venous 17-OHCS output in dogs (brain removal down to thalamo-hypothalamic level—posterior connections remaining intact and burn)

							D.F.	't'
Observation	No.	1	vs.	Observation	No.	2	4	7·565 *
,,	,,	2	vs.	,,	,,	3	4	— 2·753
,,	,,	2	vs.	,,	,,	4	4	— 2·630
,,	,,	4	vs.	,,	,,	5	4	— 7·440 *
,,	,,	4	vs.	,,	,,	6	4	—19·216 †
,,	,,	4	vs.	,,	,,	7	4	— 5·767 *
,,	,,	4	vs.	,,	,,	8	4	— 5·526 *
,,	,,	8	vs.	,,	,,	9	4	— 4·741 *

* Significant at 1% level.

† Significant at 0·1% level or more stringent level.

STATISTICAL TABLE VI

Values of 't' together with D.F. for statistical tests of significance for different types of 'intra-group' paired comparisons regarding adrenal venous 17-OHCS output in dogs (solitary pituitary, hind brain and burn)

							D.F.	't'
Observation	No.	1	vs.	Observation	No.	2	7	17·089 †
,,	,,	2	vs.	,,	,,	3	7	— 3·528 *
,,	,,	2	vs.	,,	,,	4	7	— 3·683 *
,,	,,	2	vs.	,,	,,	5	7	— 6·810 *
,,	,,	5	vs.	,,	,,	6	7	—11·198 †
,,	,,	5	vs.	,,	,,	7	7	— 6·081 †
,,	,,	5	vs.	,,	,,	8	7	— 5·908 †
,,	,,	5	vs.	,,	,,	9	7	— 7·289 †
,,	,,	9	vs.	,,	,,	10	7	— 5·958 †

* Significant at 1% level.

† Significant at 0·1% level or more stringent level.

TABLE VI

Solitary pituitary, intact hind brain and the effect of burn

Dog numbers		172	173	174	175	176	177	178	179	Mean	S.D.	D.F.
Observation numbers		Adrenal venous 17-OHCS output (gamma/minute)										
1	During adrenal vein cannulation	6·9	9·1	10·2	11·0	10·5	12·0	7·6	10·8	9·763	1·759	7
2	Forty-eight hours after cannulation	1·4	1·7	2·6	2·7	3·2	3·0	2·0	3·4	2·500	0·727	7
3	Thirty minutes after the preparation	7·6	8·0	1·5	7·1	10·0	2·1	9·0	7·8	6·638	3·122	7
4	Sixty minutes after the preparation	7·5	10·0	2·2	9·3	11·5	1·9	6·8	8·4	7·200	3·495	7
5	Three hours after the preparation	7·9	12·0	6·4	9·5	8·6	5·4	9·1	8·0	8·363	2·003	7
6	Thirty minutes after burn	23·5	23·9	20·0	24·6	25·5	15·7	16·0	20·0	21·150	3·832	7
7	Sixty minutes after burn	18·0	18·4	18·5	17·3	26·4	15·2	12·4	14·6	17·600	4·157	7
8	Two hours after burn	16·1	17·0	13·5	18·6	25·0	12·9	12·5	20·5	17·013	4·292	7
9	Three hours after burn	15·0	14·4	15·4	18·1	20·0	10·8	14·5	14·3	15·688	2·791	7
10	ACTH (I.V.)	27·3	20·6	22·9	22·0	25·5	18·6	20·2	19·4	22·063	3·039	7

STATISTICAL TABLE VII

Values of 't' together with D.F. for statistical tests of significance for different types of 'intra-group' paired comparisons regarding adrenal venous 17-OHCS output in dogs (all brain including hind brain ablated—absolutely solitary pituitary and burn)

							D.F.	't'
Observation	No.	1	vs.	Observation	No.	2	4	9·115 ‡
,,	,,	2	vs.	,,	,,	3	4	— 1·666
,,	,,	2	vs.	,,	,,	4	4	— 1·668
,,	,,	4	vs.	,,	,,	5	4	— 4·316 *
,,	,,	4	vs.	,,	,,	5	4	—10·385 ‡
,,	,,	4	vs.	,,	,,	7	4	— 5·709 †
,,	,,	4	vs.	,,	,,	8	4	— 7·158 †
,,	,,	8	vs.	,,	,,	9	4	— 5·700 †

* Significant at 5% level.
† Significant at 1% level.
‡ Significant at 0·1% level or more stringent level.

STATISTICAL TABLE VIII

Showing D.F. and values of 't' and their significance for different types of 'intergroup' comparisons

Table I			vs.	Table V			D.F.	't'
Observation	No.	3	vs.	Observation	No.	3	8	4·853 *
,,	,,	4	vs.	,,	,,	4	8	1·382
,,	,,	5	vs.	,,	,,	5	8	0·190
,,	,,	6	vs.	,,	,,	6	8	0·559
,,	,,	7	vs.	,,	,,	7	8	1·496
,,	,,	8	vs.	,,	,,	8	8	—0·314

* Significant at 1% level.

STATISTICAL TABLE IX

Showing D.F. and values of 't' and their significance for different types of 'intergroup' comparisons

Table I			vs.	Table VII			D.F.	't'
Observation	No.	3	vs.	Observation	No.	3	8	1·224
,,	,,	4	vs.	,,	,,	4	8	0·095
,,	,,	5	vs.	,,	,,	5	8	1·902
,,	,,	6	vs.	,,	,,	6	8	— 0·482
,,	,,	7	vs.	,,	,,	7	8	0·073
,,	,,	8	vs.	,,	,,	8	8	0·294

Out of the eight dogs in this group two dogs (Dog numbers 174 and 177) showed low 17-OHCS output at 30 and 60 minutes after the brain operation.

Comparison of the results of this group with those of the control brain operation-group showed significant difference only at 30 minutes.

Photographs of the brain lesions after solitary pituitary experiments have been presented in figure numbers 8, 9, 10, 11 (dog number 172), 12, 13, 14 (dog number 176) and 15, 16, 17 (dog number 178).

Histologically the pituitary did not show any gross change and there was no evidence of any serious vascular jeopardization.

V. *Absolutely solitary pituitary with removal of the hind brain* (Table VII and Statistical Tables VII and X)

STATISTICAL TABLE X

Showing D.F. and values of 't' and their significance for different types of 'intergroup' comparisons

Table II			vs.	Table VI			D.F.	't'
Observation	No.	3	vs.	Observation	No.	3	11	2·841 *
,,	,,	4	vs.	,,	,,	4	11	1·612
,,	,,	5	vs.	,,	,,	5	11	− 0·314
,,	,,	6	vs.	,,	,,	6	11	0·226
,,	,,	7	vs.	,,	,,	7	11	1·372
,,	,,	8	vs.	,,	,,	8	11	0·861
,,	,,	9	vs.	,,	,,	9	11	0·462

* Significant at 5% level.

STATISTICAL TABLE XI

Showing D.F. and values of 't' and their significance for different types of 'intergroup' comparisons

Table III			vs.	Table IV			D.F.	't'
Observation	No.	3	vs.	Observation	No.	3	13	− 2·152
,,	,,	4	vs.	,,	,,	4	13	− 3·202 *
,,	,,	7	vs.	,,	,,	6	13	− 5·195 †
,,	,,	8	vs.	,,	,,	7	13	− 1·041
,,	,,	9	vs.	,,	,,	8	13	− 0·387
,,	,,	10	vs.	,,	,,	9	13	− 0·985
,,	,,	11	vs.	,,	,,	10	13	− 0·016

* Significant at 1% level.

† Significant at 0·1% level or more stringent level.

The high adrenal venous 17-OHCS output reached a basal level 48 hours after cannulation. The corticosteroid output at 30 and 60 minutes after absolute isolation of the pituitary was not significantly high in comparison to the value noted at 48 hours after cannulation and, moreover, in 2 dogs (dog numbers 180 and 183) there was practically no tendency for the corticosteroid output to rise. No rest period was allowed and in the post-burn period there was significantly high level of corticosteroid output. The adrenals were well responsive to exogenous ACTH.

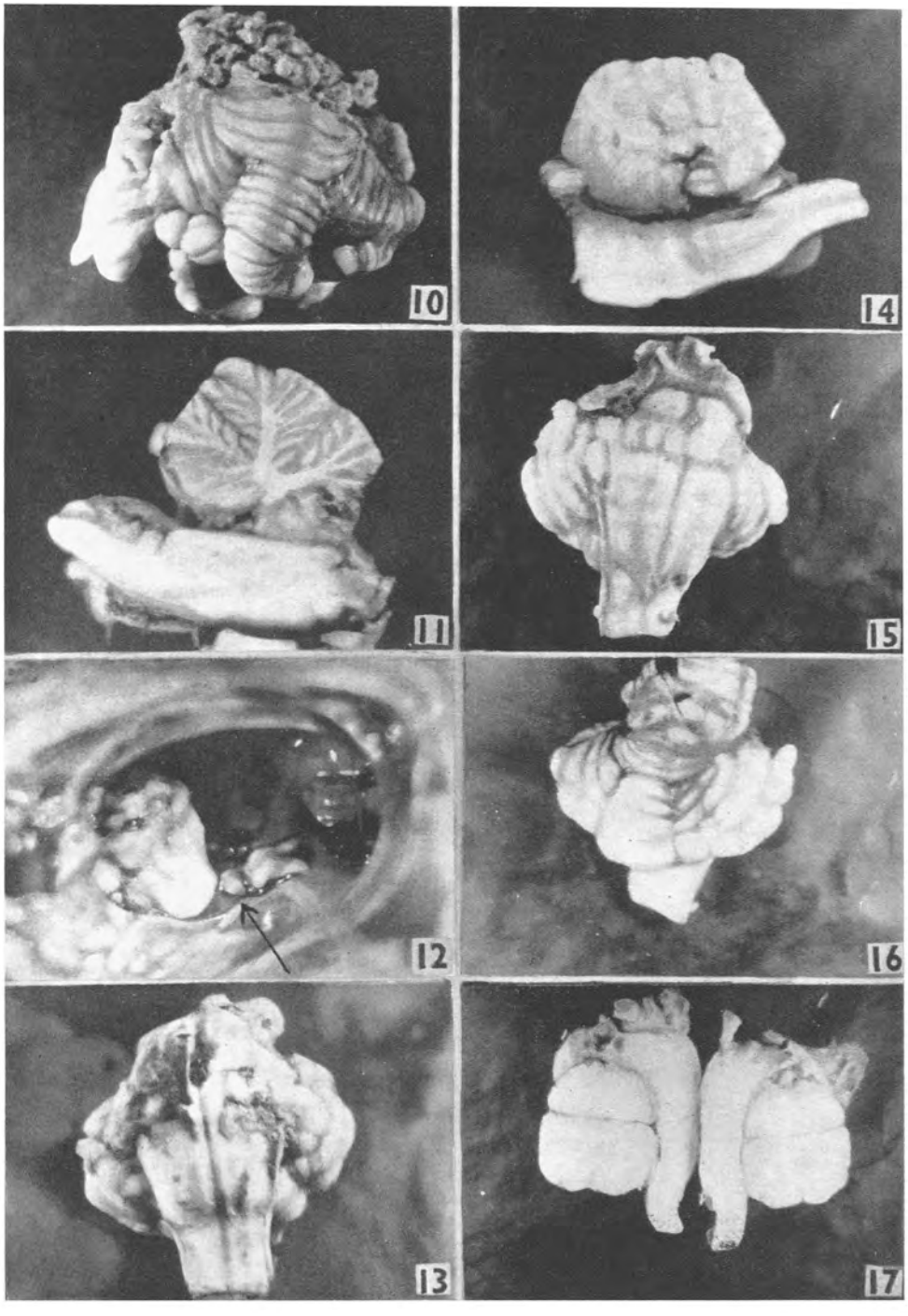
TABLE VII

All brain including hind brain ablated (absolutely solitary pituitary) and the effect of burn

Dog numbers		180	181	182	183	184	Mean	S.D.	D.F.
Observation numbers		Adrenal venous 17-OHCS output (gamma/minute)							
1	During adrenal vein cannulation	13·1	10·0	14·5	11·6	7·0	11·240	2·904	4
2	Forty-eight hours after cannulation	4·5	2·9	3·8	2·7	1·6	3·100	1·107	4
3	Thirty minutes after the preparation	0·9	12·5	10·6	2·5	13·1	7·920	5·780	4
4	Sixty minutes after the preparation	1·5	16·0	10·5	1·4	18·0	9·480	7·828	4
5	Thirty minutes after burn	4·2	20·5	19·0	3·9	24·4	14·400	9·652	4
6	Sixty minutes after burn	11·2	28·1	27·0	14·7	28·5	21·900	8·282	4
7	Two hours after burn	8·7	20·7	24·5	10·0	29·0	18·580	8·935	4
8	Three hours after burn	7·0	25·0	24·3	10·5	28·2	19·000	9·552	4
9	ACTH (I.V.)	12·1	29·6	26·2	14·0	30·6	22·500	8·805	4

From the scrutiny of Table VII it appears that dog numbers 180 and 183 show results different from those shown by dog numbers 181, 182 and 184 at every stage of experiment after the brain operation. In order to examine whether the subset consisting of dog numbers 180 and 183 differ from the subset consisting of dog numbers 181, 182 and 184, statistical tests have been applied. The values of Fisher's 't' for the comparisons of the two subsets are given on page 266.

FIGS. 10–17. 10, dorsal view of the infratentorial structures of dog No. 172. 11, midline sagittal section of the infratentorial structures of dog No. 172. 12, shows the solitary pituitary of dog No. 176. 13, ventral view of infratentorial structures of dog No. 176. 14, mid-sagittal section of the infratentorial structures of dog No. 176. 15, ventral view of infratentorial structures of dog No. 178. 16, dorsal view of infratentorial structures of dog No. 178. 17, mid-sagittal section of infratentorial structures of dog No. 178.


10
14
11
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12
16
13
17

Observation numbers	Mean and S.D. of dog Nos. 180 and 183	Mean and S.D. of dog Nos. 181, 182, 184
	Subset 1	Subset 2
3	1·70 (1·131)	12·07 (1·305)
4	1·45 (0·071)	14·83 (3·884)
5	4·05 (0·212)	21·30 (2·787)
6	12·95 (2·475)	27·87 (0·777)
7	9·35 (0·919)	24·43 (4·155)
8	8·75 (2·475)	25·83 (2·079)
9	13·05 (1·344)	28·80 (2·311)

Observation numbers	Subset 1 vs. Subset 2	
	D.F.	't'
3 vs. 3	3	9·086 †
4 vs. 4	3	4·630 *
5 vs. 5	3	8·289 †
6 vs. 6	3	10·453 †
7 vs. 7	3	4·907 *
8 vs. 8	3	8·432 †
9 vs. 9	3	8·472 †

Histologically the pituitaries did not show any gross change.

* Significant at 5% level.

† Significant at 1% level.

Discussion

In the control brain operation groups low 17-OHCS outputs are noted at 24 and 48 hours after such operations. The corticosteroid output is high during and after such operations. In all cases there is increased adrenocortical response after burns. ACTH response is good in these experiments.

The cerebral cortex of the dog has an inhibitory influence over the centrally located structures responsible for ACTH release, because 24 hours after

decortication the 17-OHCS output is significantly higher than that of the control brain operation-group. That the increased level at this period is not due to the stress of surgery only is proved by the low level of 17-OHCS output in the control brain operation-group. Burn trauma and ACTH manifested with significantly increased activity of the pituitary-adrenal axis. Roy (1962) mentioned about this cortical inhibition. Egdahl (1961*b*) discussed about the cerebral control inhibition of the pituitary-adrenal secretion. He used sciatic nerve stimulation in the anaesthetized decorticated animal as a form of stress. With this stress there was a significant adrenal cortical response. There was no further increase in corticosteroid output after exogenous ACTH. However, in the present investigation increased response to exogenous ACTH has been noted.

There is a species difference in the adrenocortical response after bilateral decortication. Matsuda *et al.* (1963) did not observe any cerebral cortical inhibition in the rat as in these animals there was no sustained rise in peripheral plasma corticosteroid level after removal of the cerebral cortex and subjacent brain.

Setekleiv *et al.* (1961) observed increase in plasma 17-hydroxycorticosteroids by cerebral cortical and amygdaloid stimulation in the cat. There was no significant increase on stimulation through 21 cortical electrodes on the lateral surface of the brain. The position of the electrodes were mainly in the frontal and parietal lobes including sensory-mother cortex and the orbital gyrus. The middle and the posterior cingulate areas were also negative regarding the response.

Adrenocortical response to burn trauma is more in dogs having the hypothalamus intact (brain removed up to thalamohypothalamic level).

In the solitary pituitary experiments with the infratentorial structures intact high 17-OHCS output has been observed and burn stimulates the same axis further. Egdahl (1960) thought that the hind brain factor could pass through the systematic circulation and act on the isolated pituitary. Roy (1960) observed similarly and thought that when the inhibitory influences from higher centres were removed, the pituitary could activate in a more pronounced way. Possibilities of infratentorial neurosecretion and histamine release at the injured site were also thought of. The pituitary gland is very labile in such conditions and any chemical alteration or *p*H change in blood will lead to increased ACTH discharge. Roy (1962) found that zinc can stimulate the infratentorial neurosecretion which could pass via the systemic circulation and act on the solitary pituitary. Moreover, in such a preparation, as the inhibitory influence of the neocortex and hippocampus was lost, the basal value of 17-OHCS output was increased.

Subsequently it has been observed by Wise *et al.* (1962) and Egdahl (1962) that the pituitary can respond even in the absence of the hind brain.

Removal of the total brain and spinal cord or removal of posterior pituitary or abdominal viscera does not change the response (Egdahl 1962). In the present investigation removal of the infratentorial structures cannot block the raised 17-OHCS output in response to burn trauma, proving thereby that the HBF is not important for the increased ACTH response after stress. When the infratentorial structures are removed, there is low 17-OHCS output up to 60 minutes after the preparation, but such dogs respond to burn trauma with increased 17-OHCS output. In a total brainless dog the pituitary has only systemic circulation and any fluctuation in the ACTH secretion is solely due to substances released in circulation. The neurosecretion and chemicals from the sympathetics may be an answer to this problem, but we have seen increased adrenocortical response in partially sympathectomized and splanchnicectomized dogs with total ablation of central nervous system structures after burns. Moreover, Egdahl (1962) has shown that posterior pituitary, kidneys or other abdominal organs are not responsible for the adrenocortical secretory status of these animals. Gann *et al.* (1961) thought of an ACTH releasing substance produced by the kidney in dogs with isolated pituitaries.

What we surgically perform in solitary pituitary experiments is seen in nature-made anencephaly. In the latter condition there is a fundamental defect in the formation of the brain and so there is hypoplasia of the secretory elements of the pituitary which is reflected over the ductless glands.

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