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THE EFFECTS OF INFUSIONS OF SYNTHETIC ADRENOCORTICOTROPHIN IN THE CONSCIOUS CALF

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SUMMARY

1. A technique is described by which the whole of the effluent blood from the right adrenal gland can be collected as required from conscious, unrestrained calves. The technique may be used to measure adrenal blood flow gravimetrically and to compute the output of adrenal hormones under various conditions in the normal calf.

2. In a group of seven calves mean cortisol output from the right adrenal gland was found to vary between 20 and 40 ng.kg^{-1} min^{-1} and corticosterone between 6 and 18 ng.kg^{-1} min^{-1} during a 2 hr period, 24 hr after surgery.

3. Intravenous infusions of synthetic adrenocorticotrophin (5 ng.kg^{-1} min^{-1}) produced a significant increase in the output of both cortisol and corticosterone within 5 min. The output of both hormones rose to maximal values within 10–20 min and mean values of approximately 300 ng.kg^{-1} min^{-1} (cortisol) and 120 ng.kg^{-1} min^{-1} (corticosterone) were maintained thereafter for the duration of the infusion (120 min). The output of both steroids fell to values comparable with those observed initially within 45–60 min after the infusion was discontinued.

4. These changes in glucocorticoid output in response to adrenocorticotrophin produced a significant rise in the concentration of both cortisol and corticosterone in peripheral plasma. It is noteworthy that the rise in the mean corticosterone concentration in the peripheral plasma was substantially less than that which might be expected from relating the rise in mean plasma cortisol concentration to cortisol output.

5. The results of control experiments have eliminated the possibility that the sampling procedure might itself increase steroid output or peripheral plasma concentration. Comparison of results from calves of widely disparate ages (8–38 days) provided no evidence that either the resting

output of cortisol or corticosterone or the response to adrenocorticotrophin changes with age within the range examined.

6. Infusion of adrenocorticotrophin (5 ng.kg^{-1} min^{-1}) also stimulated an abrupt rise in adrenal blood flow; mean resting flow (210 ± 23 μl.kg^{-1}) increased by approximately 30% within 5 min and attained peak values (355–365 μl.kg^{-1} min^{-1}) between 10 and 30 min. Thereafter, adrenal blood flow steadily decreased and then fell rapidly to within the resting range when the infusion was terminated. No significant changes in heart rate or aortic blood pressure occurred during these infusions.

7. The results are discussed in relation to those obtained in other species and under differing conditions by other workers.

INTRODUCTION

Quantitative evaluation of the function of the adrenal gland necessitates the use of a technique whereby the whole of the effluent blood from the gland can be collected as and when required. This can be achieved relatively simply in acute experiments using anaesthetized animals; such methods have been in common use for many years (Dreyer, 1899; Balfour, 1953; Bush, 1953; Holzbauer & Vogt, 1957). However, both anaesthesia and surgery cause a pronounced increase in adrenal steroid production (Blair-West, Coghlan, Denton, Goding, Munro, Wintour & Wright, 1962; Domanski, Barcikowski & Skubiszewski, 1968) and the results of such experiments cannot therefore provide useful information about the variations in adrenal cortical function which occur within the normal physiological range.

Techniques involving the use of animals with transplanted adrenal glands (McDonald & Reich, 1959; Espiner, Jensen & Hart, 1972) suffer from the dual disadvantage that the innervation to the gland is necessarily destroyed and a new vascular supply must be established. The latter point may be of particular importance, in view of the changes in adrenal blood flow which have been found to occur in response to adrenocorticotrophin (Balfour, 1953; Frank, Frank, Korman, Macchi & Hechter, 1955; Urquhart, 1965; Urquhart & Li, 1968*a*, *b*). Suzuki, Yamashita & Mitamura (1958) modified a technique, originally pioneered by Sataké, Sugawara & Watanabé (1927), which enabled them to collect periodic samples of adrenal effluent blood, with the gland *in situ*, from conscious dogs. It seems likely that the sampling procedure caused some discomfort to the animals and no measurements of adrenal blood flow were made; the comparable technique developed by Hume & Nelson (1954) in the dog is susceptible to similar criticisms.

The most satisfactory techniques, in terms of minimizing disturbance to

the animal, appear to be those in which a vascular 'by-pass' has been constructed. Two groups of workers have employed such methods (Blair-West *et al.* 1962; Domanski *et al.* 1968) but these have necessitated long extra-corporeal circuits from the adrenal vein to the jugular vein or the cava cranialis. In such circumstances it is difficult to maintain a normal blood flow through the long 'loop' since the pressure differences are small. The present paper describes a technique which employs a short vascular by-pass to permit the collection of effluent blood samples from the right adrenal gland of conscious unrestrained calves. Adrenal venous pressures were maintained within the normal range and evidence is presented to show that the responses are not distorted by unphysiological stresses. The technique has been used to investigate the effects of intravenous infusions of synthetic adrenocorticotrophin on adrenal steroid output and blood flow and the results are discussed with reference to previous work in this and other species.

METHODS

Pedigree Jersey calves were obtained from local farms shortly after birth and used at ages ranging from 8 to 38 days (24–39 kg body weight). The animals were kept in individual pens in the laboratory animal house and maintained on a diet of milk (6–8 pints/day). Food was withheld for at least 6 hr prior to surgery. Daily records were kept of the weight and rectal temperature of each animal and particular care was taken to avoid using animals that were not fully fit.

Surgical procedure

Anaesthesia was induced with ethyl chloride and ether and maintained with halothane (Fluothane; I.C.I. Ltd) in oxygen, administered by means of a standard closed circuit system attached to an endotracheal tube. A braunula cannula was placed in the right jugular vein for subsequent I.V. infusions or injections and a narrow-bore polyethylene catheter was inserted into the right saphenous artery so that the tip lay in the abdominal aorta. This catheter was used later to monitor aortic blood pressure and heart rate and for collection of arterial blood samples.

A paravertebral incision was made in the right flank, immediately over the kidney, extending from just behind the last rib to a point 1–2 in. antero-ventral to the iliac crest. Perirenal fat was carefully stripped from the kidney and ligated as were the renal vessels and ureter. Care was taken to preserve the peritoneum intact and to ensure that the maximum length of renal vein was retained for ease of cannulation. The kidney was then removed, together with the renal lymph node. The renal vein was separated from the surrounding fascia by blunt dissection and a path cleared for the jaws of the clamp, on either side of the vena cava, from the renal vein posteriorly to a point level with the cranial pole of the right adrenal gland and anterior to the adrenal vein. Considerable care was required to accomplish this without damaging any of the small arteries which supply the adrenal gland. Such damage could invariably be recognized by the presence of small infarcts in the cortex of the gland at post-mortem examination.

The animal was then heparinized (1 mg/kg) and the renal vein cannulated. The cannula which was used for this purpose consisted of a siliconed glass tube with a bell-shaped end, firmly attached to siliconed polyethylene tubing (Portex N.T.5) and

filled with heparinized saline. The adrenal clamp was specially modified for the purpose in the laboratory workshop from a Potts porta-caval clamp and served to divert adrenal effluent blood back along the posterior vena cava from the right adrenal to the renal vein below the cannula (Fig. 1). A rubber sleeved, T-shaped projection, soldered to a photographic cable release (shutter control), provided a convenient means of occluding the renal vein immediately behind the jaws of the clamp and thereby collecting samples of adrenal venous effluent blood, through the renal vein cannula (Fig. 1). The jaws of the clamp were inserted into position along the vena cava in such a way that the tips lay anterior to the adrenal vein and the posterior bars gripped the renal vein centrally at its junction with the vena cava. A proportion of animals was found to possess a valve-like fold of intima at the posterior margin of the junction between renal vein and vena cava, which tended to obstruct the flow of blood at this point. In such animals it was usually possible to excise this fold of intima with fine scissors, inserted down the renal vein while the cannula was temporarily removed.

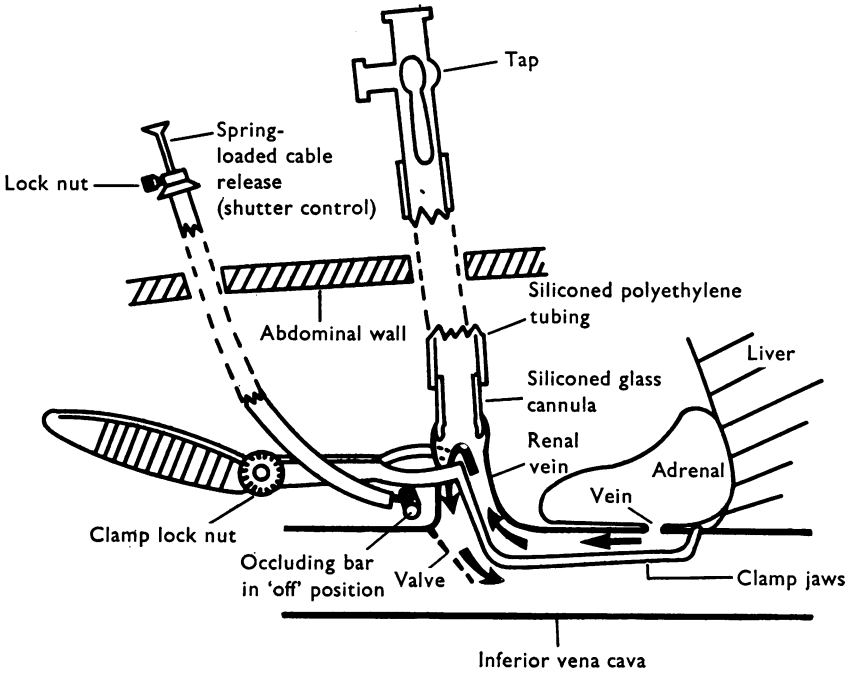

Fig. 1. Diagram to illustrate the positioning of the 'adrenal clamp' in relation to the adrenal gland and renal vein.

After the clamp had been inserted, the lock nut was secured and the assembly tested. It could be assumed that it had been placed in the correct position if the following criteria were satisfied.

(1) Well oxygenated blood flowed freely from the renal vein cannula when the photographic shutter control was depressed.

(2) The rate of adrenal effluent blood flow exceeded 4·0 ml./min.

(3) Flow persisted when the renal vein catheter was raised to a height of 20 cm.

(4) The blood then flowed freely back into the posterior vena cava when the shutter control was released.

When both clamp and cannula had been positioned correctly, absorbable gelatin sponge (Sterispon No. 1; Allen & Hanburys Ltd) soaked in thrombin was placed over the adrenal gland and the operation site was desensitized with 2% lignocaine (Xylocaine; Astra Chemicals Ltd). Rubber sleeves of appropriate dimensions were placed over the handle of the clamp and the protruding lock-nut thread and any small blood vessels which might cause local haemorrhage were ligated. The muscle edges were then firmly apposed by means of a continuous suture in such a way that the tube from the renal vein cannula emerged from the anterior end of the incision and the shutter control posteriorly. The assembly was tested a second time and the skin incision was then closed with Michel clips. Finally, the tube from the renal vein cannula and the arterial catheter were led into a small nylon wash-bag, sutured to the right flank, immediately over the incision, for protection.

Post-operative care

Each animal was given penicillin (600,000 i.u.) and dihydrostreptomycin (500,000 i.u.) by I.M. injection (Distavone; Dista Products Ltd) immediately after operation. The calves invariably recovered rapidly when anaesthesia was discontinued; they were normally able to stand within 10–20 min and drank 3 pts milk from a bucket 10 min later. They were kept in individual pens which permitted free movement and they showed little sign of discomfort. A solution of heparin (Heparin B.P.; Boots Co. Ltd, 153 u./mg) in sterile saline (32 mg/100 ml. 0·9% (w/v) NaCl) was infused continuously through the renal vein cannula at a rate of 0·10–0·25 ml./min in order to prevent blood clots forming at the point at which the glass cannula was tied into the renal vein. In several animals the anticoagulant Arvin (Arvin, Twyford Laboratories Ltd) was also administered by I.V. infusion at a dose of 1 u/kg.hr. No evidence was found to suggest that Arvin affected the response of the adrenal gland to adrenocorticotrophin in any way.

Experimental procedure

The experiments were carried out on the day after surgery; in all cases experimental infusions were begun between 11.00–13.00 hr to take into account any possible spontaneous diurnal fluctuations in steroid output. Heart rate and aortic blood pressure were monitored continuously, by means of a Devices L 221 pressure transducer connected to a Devices M19 or M2 recorder, and rectal temperature was recorded at intervals throughout the day. Adrenal venous pressure was determined at intervals and in most calves was invariably less than 10 mmHg. However, considerably higher pressures were recorded in certain animals, in which there was some resistance to the flow of blood back into the vena cava, when the snare was released. Post-mortem examination of such animals revealed partial obstruction of the return channel to the vena cava due to blood clots or remnants of a valve. Such obstructions were associated with severe haemorrhage in the adrenal medulla, presumably occasioned by persistently high venous pressures, and such animals have not been included in the present study.

Synthetic adrenocorticotrophin (tetracosactrin 100 i.u./mg; Synacthen; Ciba) was dissolved in a volume of sterile physiological saline, calculated to provide a dose of 5 $ng.kg^{-1}$ min^{-1} when infused into the right jugular vein at a rate of 1 ml./min for 120 min. Samples of arterial and of right adrenal venous effluent blood were collected at intervals before, during and after the infusion, with minimum disturbance to the animal and the haematocrit was determined on each occasion. When obtaining

adrenal venous blood samples, the snare was applied and the heparin-saline occupying the dead space was completely displaced before collection of the sample into a weighed heparinized tube. The end of the cannula was held at the level of the adrenal to ensure a constant and physiologically normal adrenal venous pressure and the period of collection was timed with a stop-watch. Blood flow was then determined gravimetrically. Arterial plasma glucose concentration was monitored throughout each experiment using the Beckman Glucose Analyzer.

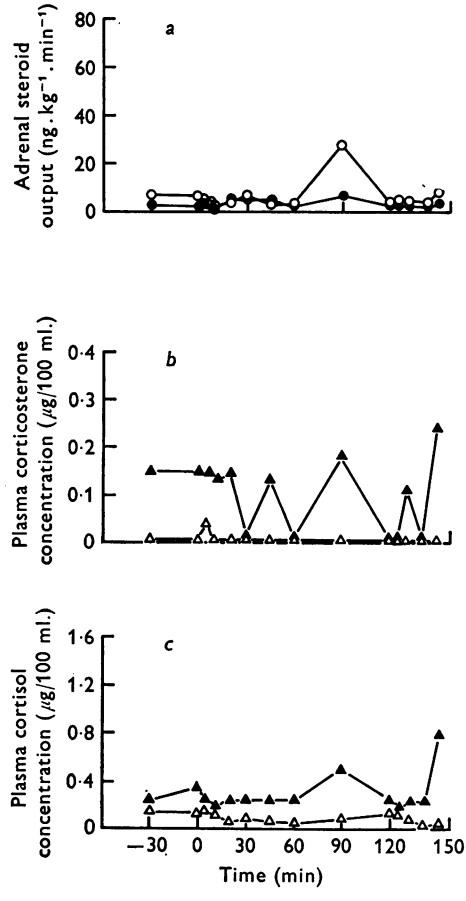

Fig. 2. Control variations in glucocorticoid output and plasma concentration in response to I.V. infusions of saline (1·0 ml.kg^{-1} min^{-1}) between 0 and 2 hr.

a: changes in glucocorticoid output from the right adrenal gland in an animal with an indwelling adrenal clamp. ○: cortisol. ●: corticosterone.

b: changes in the concentration of corticosterone in the arterial plasma of control calves given infusions of saline. △: adrenal clamp previously inserted. ▲: no previous operation.

c: changes in the concentration of cortisol in the arterial plasma of control calves given infusions of saline. △: adrenal clamp previously inserted. ▲: no previous operation.

Control procedures

The possibility that either the preparatory surgery, or the procedure involved in collection of blood samples, might cause sufficient 'stress' to alter the output of steroids from the adrenal gland was eliminated by control experiments in which saline was infused alone at the same rate as that employed experimentally. In these control experiments the variations in peripheral plasma glucocorticoid concentration, in response to infusions of physiological saline for 120 min in normal animals, were compared with those in animals with implanted adrenal clamps. Fig. 2 shows the results from one such typical comparison. The steroid output in the animal which had been subjected to the usual surgical procedure did not rise significantly during the period of the saline infusion and the arterial plasma steroid concentrations did not rise above those of the unoperated control animal (Fig. 2, note expanded scale).

At the end of each experiment a lethal dose of sodium pentobarbitone was administered by I.V. injection and the incision was reopened in order to examine the position of the clamp and adrenal vein cannula *in situ*. The adrenal gland, clamp and enclosed vena cava were then excised, together with the cannulated renal vein, and inspected to ensure that no blood clots had formed which might restrict the adrenal venous channel. Both adrenal glands were then weighed, sliced longitudinally and inspected for the presence of macroscopic infarcts before being fixed in Susa for subsequent histological examination. There was no significant difference between the mean weights of the two glands in this group of animals (right adrenal $2{\cdot}44 \pm 0{\cdot}21$ g, S.E. of mean; left adrenal $2{\cdot}18 \pm 0{\cdot}18$; $n = 7$) ($P > 0{\cdot}2$). Histological examination with the light microscope revealed no signs of damage to the right adrenal cortex and no structural difference between the two glands was observed in any of these animals.

Steroid analyses

Arterial and adrenal venous blood samples were collected into heparinized tubes and centrifuged immediately at $+4°$ C. Plasma was stored at $-20°$ C before analysis. Cortisol and corticosterone were measured by competitive protein-binding after preliminary separation on Sephadex LH 20 (Malinowska, Hardy & Nathanielsz, 1972).

Statistical analyses of the results were by the methods of Snedecor & Cochran (1967).

RESULTS

Changes in cortisol and corticosterone output from the right adrenal gland in response to infusions of Synacthen in individual calves 8–38 days after birth

Samples of right adrenal effluent blood were collected at 30 min intervals for 2–3 hr before each infusion. The output of cortisol was found to vary within the range 3–99 $ng.kg^{-1} min^{-1}$ during this period (Fig. 3*b*) and these variations were reflected by changes in the concentration of cortisol in the peripheral plasma. Output estimated from the first of these resting samples was usually quite low but generally rose during the next 60 min and fell steadily thereafter. The output of cortisol had fallen to below 45 $ng.kg^{-1} min^{-1}$ immediately prior to infusion in all seven animals and the concentration in the peripheral plasma was below 0·5 μg/100 ml. in all

but one of these at this time. The changes in corticosterone output and peripheral concentration during this period were much less pronounced and the absolute values were also lower.

Intravenous infusions of synthetic adrenocorticotrophin (Synacthen) at a dose of 5 ng.kg^{-1} min^{-1} produced an abrupt rise in the output of both steroids. This rise was detectable in most animals within 2½ min and maximal rates of release were achieved within 10–20 min (Fig. 3). Comparable values were maintained thereafter for the duration of the infusion

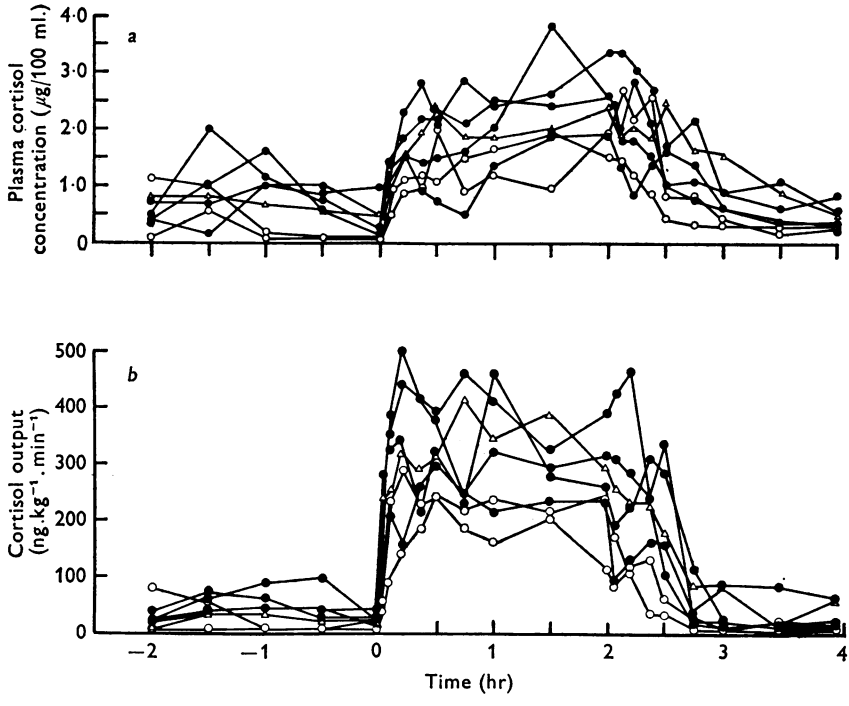

Fig. 3. Changes in plasma cortisol concentration (*a*) and cortisol output from the right adrenal gland (*b*) in response to I.V. infusions of Synacthen (5 ng.kg^{-1} min^{-1}) in conscious calves. ○: 35–38 days. ●: 18–26 days. △: 8 days.

but wide variations were found to occur in the outputs of both corticosterone and cortisol of individual animals. The size of the responses was found to be similar in animals of widely different ages and no evidence was obtained to suggest that the capacity of the adrenal cortex to secrete either steroid, in response to Synacthen, increases with age during the period 8–38 days. In fact, during infusion, the outputs of the two oldest calves were close to the lower end of the range for the whole group, although the difference was not statistically significant (Fig. 3). When the

infusion was discontinued, at 120 min, the outputs of both steroids returned to resting values within the next 45–60 min and these low levels were maintained thereafter until the end of the experiment (240 min).

The increase in the output of cortisol from the right adrenal gland, induced in response to Synacthen, was accompanied by a corresponding increase in the concentration of this steroid in peripheral arterial plasma (Fig. 3*a*). Both adrenal cortices may be assumed to have contributed to the change in peripheral concentration, which must therefore be related to actual changes in output approximately double those measured. The peripheral cortisol concentrations of these animals increased steadily to a plateau, which was then maintained until the infusion was discontinued, after which they returned to normal, but rather more gradually than the values for the cortisol outputs.

The changes in adrenal corticosterone output and peripheral plasma corticosterone concentrations evoked by Synacthen were essentially similar to those described for cortisol, although the absolute values were considerably less.

Comparison of the changes in mean steroid output with changes in mean plasma steroid concentration

Since the results of individual experiments indicated that there is no substantial change in the response of the adrenal cortex to Synacthen during this period of development, the results from all seven animals have been pooled for direct comparison of the mean values.

The mean outputs of both steroids from the right adrenal gland were falling before the infusion and the values were found to be 21 ± 5 and 6 ± 1 ng.kg^{-1}min^{-1} at time = 0 for cortisol and corticosterone respectively (Fig. 4*a*). Synacthen was found to produce a substantial increase in the output of both steroids within $2\frac{1}{2}$ min (cortisol: 152 ± 62 ng.kg^{-1} min^{-1}; corticosterone: 64 ± 23 ng.kg^{-1} min^{-1}) and peak values were achieved within 10–20 min. Thereafter, there was a gradual decline in the output of both steroids during the remainder of the infusion (Fig. 4*a*). In contrast, the mean concentrations of the two steroids in peripheral arterial plasma rose more slowly to a maximum, which was then maintained until the infusion was discontinued (Fig. 4*b*).

Comparison of the rise in the mean arterial plasma cortisol concentration produced by the increase in cortisol output during infusion, with the rise in mean arterial plasma corticosterone concentration in relation to the output of this steroid from the gland, shows a substantial discrepancy. This is best illustrated by comparing the ratio of the outputs of the two steroids with the ratio of their mean peripheral concentrations during the infusion (Fig. 4*c*). Comparison of the two ratios shows that, whereas

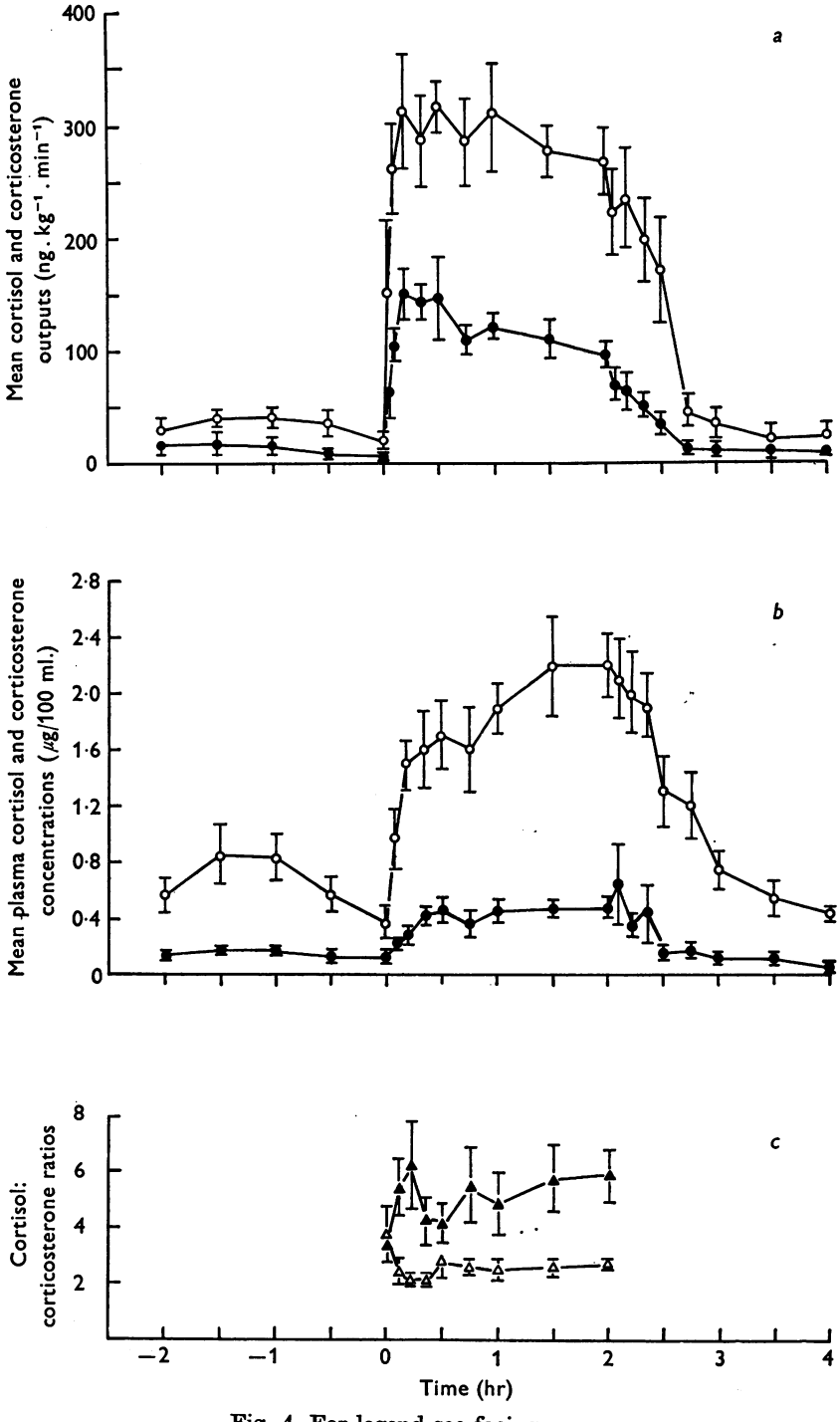

Fig. 4. For legend see facing page.

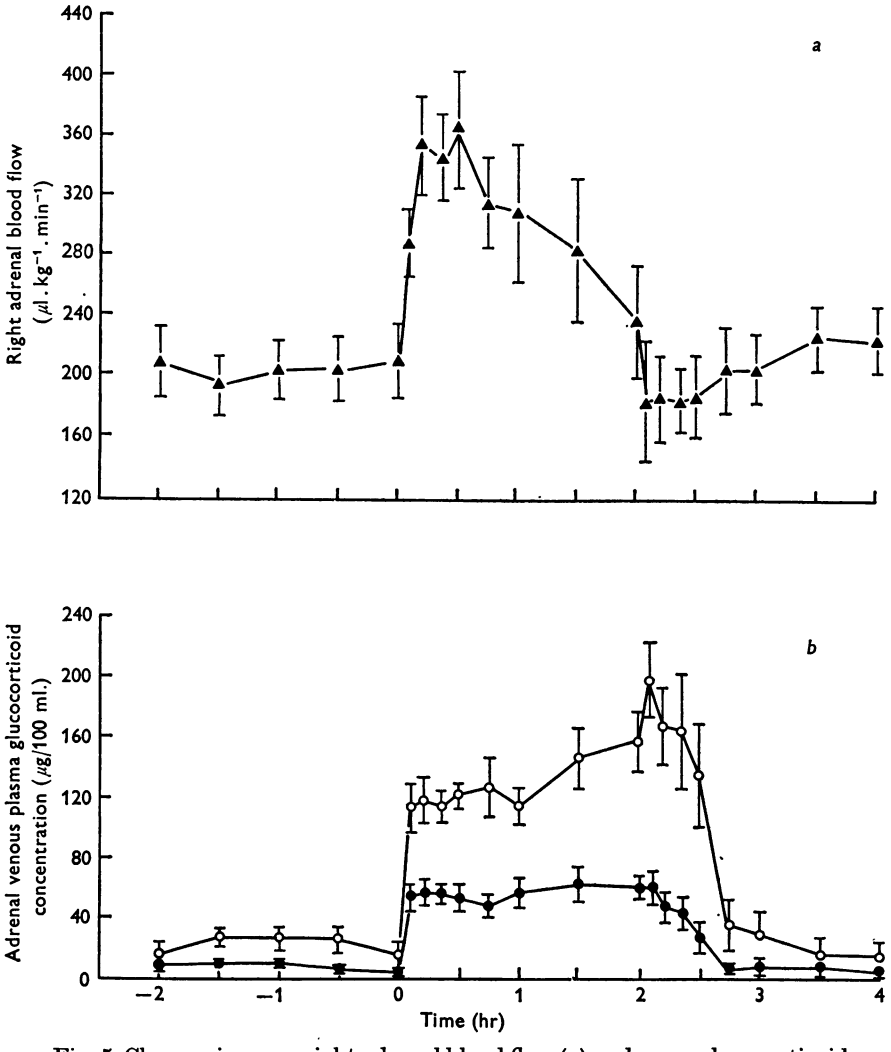

Fig. 5. Changes in mean right adrenal blood flow (*a*) and mean glucocorticoid concentration in the right adrenal effluent blood (*b*) in response to intravenous infusions of Synacthen (5 ng.kg^{-1} min^{-1}) in conscious calves (n = 7). ●: cortisol. ○: corticosterone. Vertical bars show S.E. of each mean value.

Fig. 4. Changes in the mean plasma cortisol and corticosterone outputs from the right adrenal gland (*a*), mean plasma concentrations (*b*) and the ratios of the two steroids (*c*) in response to intravenous infusions of Synacthen (5 ng.kg^{-1} min^{-1}) in conscious calves (n = 7). ○: cortisol. ●: corticosterone. △: ratio of cortisol: corticosterone outputs. ▲: ratio of cortisol: corticosterone plasma concentrations. Vertical bars show S.E. of each mean value.

Synacthen stimulates a proportionately greater increase in corticosterone output; the rise in the concentration of this steroid in the peripheral plasma is proportionately less than that of cortisol. (It proved impossible to calculate mean ratios for either output or peripheral concentration before or after infusion as several of the corticosterone values were below the sensitivity of the assay.)

The output of adrenal steroids discussed above represents the product of the adrenal venous steroid concentration and plasma flow through the gland, both of which were estimated independently.

TABLE 1. Changes in mean adrenal blood flow associated with I.V. infusion of Synacthen (5 ng.kg^{-1} min^{-1}) in the conscious calf (n = 7). Infusion period 0–120 min. Values represent mean ± S.E. of mean

Time (min)	Flow (ml. min^{-1})	Flow (μl. kg body wt.$^{-1}$ min^{-1})	Flow (ml. 100 g gland^{-1} min^{-1})
−120	6·6 ± 0·76	208 ± 23	280 ± 40
−90	6·0 ± 0·67	191 ± 20	260 ± 36
−60	6·5 ± 0·77	202 ± 21	270 ± 36
−30	6·4 ± 0·79	202 ± 21	270 ± 40
0	6·7 ± 0·94	210 ± 23	280 ± 45
5	8·8 ± 0·37	288 ± 21	370 ± 27
10	10·9 ± 0·61	355 ± 33	460 ± 35
20	10·7 ± 0·70	344 ± 29	450 ± 33
30	11·3 ± 0·82	365 ± 38	470 ± 38
45	9·8 ± 0·71	316 ± 28	420 ± 37
60	9·4 ± 0·92	310 ± 45	400 ± 48
90	8·7 ± 1·1	285 ± 48	370 ± 53
120	7·3 ± 0·54	239 ± 34	310 ± 41
125	5·7 ± 1·2	184 ± 39	240 ± 47
130	5·9 ± 0·77	189 ± 24	240 ± 23
140	5·8 ± 0·80	184 ± 22	240 ± 25
150	6·0 ± 0·89	189 ± 25	250 ± 32
165	6·6 ± 1·1	205 ± 28	270 ± 49
180	6·6 ± 0·93	206 ± 23	280 ± 45
210	7·2 ± 0·83	229 ± 20	310 ± 42
240	7·1 ± 0·91	225 ± 24	290 ± 32

It will be seen from Fig. 5*a* that the infusion of Synacthen into a peripheral vein resulted in a rapid increase in right adrenal blood flow. Before infusion the mean resting blood flow was approximately 200 μl.kg^{-1} min^{-1} (270 ml./100 g adrenal^{-1} min^{-1}; see also Table 1), but within 5 min of beginning the Synacthen infusion the flow had increased by about 30% and subsequently reached a transient peak of approximately 350 μl.kg^{-1} min^{-1} by 10–30 min. Thereafter, blood flow declined progressively and fell abruptly when the infusion was discontinued. It was concluded that the increase in adrenal blood flow during Synacthen infusion was due to

vasodilatation within the gland itself since these infusions produced no significant changes in either heart rate or mean aortic pressure.

The variations in the plasma concentrations of cortisol and corticosterone in adrenal venous blood during these experiments are shown in Fig. 5*b*. Onset of infusion resulted in an immediate increase in the venous concentration of both steroids. However, whereas the cortisol concentration of the adrenal venous effluent showed a progressive increase throughout the infusion period and a further brief increase immediately after the infusion, the corticosterone concentration was maintained at a plateau level during the infusion. Relatively high concentrations of the two steroids persisted for 20–30 min after the infusion was discontinued, even though the outputs were falling rapidly during this period. This apparent anomaly can be accounted for by the rapid fall in the blood flow and suggests that adrenal vasodilatation during Synacthen infusions is independent of local glucocorticoid concentrations.

Analysis of present results clearly shows that I.V. infusions of Synacthen produced an increase in the output of both cortisol and corticosterone by the adrenal cortex together with local changes in the gland which resulted in an increase in the blood flow through it. At low steroid outputs (< 70–80 ng.kg^{-1} min^{-1} corticosterone: < 225 ng.kg^{-1} min^{-1} cortisol) the mean blood flow was unrelated to mean output. Above these 'threshold' outputs, blood flow increased linearly with increase in steroid output. These results show that the increase in mean steroid output in response to I.V. infusions of Synacthen at 5 ng.kg^{-1} min^{-1} was associated with a proportional increase in mean adrenal blood flow.

DISCUSSION

Before any detailed discussion of the present results can be undertaken it is necessary to examine the validity of the technique and in particular the justification for the belief that it allows assessment of *normal* adrenal function. The preparatory surgery and anaesthesia presumably constitute a considerable stress and, assuming that the hypothalamic-pituitary-adrenal axis is functional in the young calf, would be expected to stimulate secretion of cortisol and corticosterone. The output of both steroids from the right adrenal gland during and immediately after operation was found to be high (cortisol 216 ± 46 ng.kg^{-1} min^{-1}, corticosterone 114 ± 25 ng.kg^{-1} min^{-1}, $n = 7$). However, this adrenal cortical response to surgical trauma was relatively short-lived and the steroid output had declined to extremely low values by the time each experiment was started, 18–22 hr post-operation (Fig. 4). These low steroid outputs, together with the values obtained at this time for peripheral adrenocortical steroid concentrations (comparable

TABLE 2. Comparison of resting adrenal steroid output in the dog, sheep and calf

Species	Technique for obtaining adrenal venous blood	Method of steroid assay	Published values for resting steroid output		Resting steroid output expressed as (ng.kg^{-1} min^{-1})		Reference	Remarks
			Cortisol	Corticosterone	Cortisol	Corticosterone		
Dog	Acute cannulation lumbo-adrenal vein	Paper chromatography/ colorimetry	18·5±7·1 μg.kg^{-1} min^{-1}	13·0±3·9 μg.kg^{-1} min^{-1}	18,500±7100	13,100±3,900	Holzbauer & Vogt (1961)	Chloralose anaesthesia. Continuous collection of adrenal venous blood. Homologous blood or dextran infused to maintain blood volume. Plasma steroid concentrations only noted here
Dog	Collection from lumbo-adrenal cannula (conscious)	Colorimetry	0·0–2·0 μg/min		0·0–100		Hume & Nelson (1954)	During sampling snare applied to adrenal vein/caval junction (dogs assumed to be 20 kg)
Dog	Collection from lumbo-adrenal cannula (conscious)	Paper chromatography/ fluorimetry	389 μg.hr^{-1}	253 gland^{-1}	308	200	Hechter, Macchi Korman, Frank & Frank (1955)	Continuous flow from vein. Samples taken after recovery from ether anaesthesia. Output quoted is mean of 17 dogs but wide variation noted (weight taken as 21 kg)
Dog	Collection from lumbo-adrenal cannula (conscious)	Double-isotope dilution derivative	—	< 1·46 μg.min^{-1}	—	73	Davis, Carpenter, Ayers & Bahn (1960)	Technique as Hume & Nelson (1954) (dogs taken to weigh 20 kg)
Dog	Collection from lumbo-adrenal cannula (conscious)	Colorimetry	0·09±0·023 μg.kg^{-1} min^{-1}		90±23		Suzuki, Higashi, Hirose, Ikeda & Tamura (1972)	Adrenal vein/caval junction occluded by snare during sampling. Prior denervation T14-L3. Most recent of a series of papers using this technique

TABLE 2 (*cont.*)

Sheep	Neck transplant (other adrenal removed)	Chromatography	0·04–0·6 mg.hr^{-1}	0·00–0·02 mg.hr^{-1}	17–250	0–8	McDonald & Reich (1959)	Sheep assumed to weigh 40 kg
Sheep	Bypass renal vein–jugular vein	Double-isotope dilution derivative	92–455 μg.hr^{-1}	5–32 μg.hr^{-1}	38–189	2–13	Blair-West *et al.* (1962)	1–3 days post operation
			742–1342 μg.hr^{-1}	57–181 μg.hr^{-1}	309–559	24–75		Taken during operation: cyclopropane anaesthesia
	Neck transplant (other adrenal removed)		161±20 μg.hr^{-1}	10·0±0·9 μg.hr^{-1}	67±8	4·2±0·4		Trained sheep, neck transplant (all sheep assumed to weigh 40 kg)
Sheep	Acute cannulation (renal vein)	Paper chromatography/ fluorimetry	16–55 μg.min^{-1}		400		Harrison, McDonald & Patterson (1964)	Nembutal anaesthesia (all sheep assumed to weigh 40 kg)
	Neck transplant (other adrenal removed)		10–32 μg.min^{-1}		250–800			
Sheep	Bypass renal vein–cava cranialis	Not stated	9–17 μg.min^{-1}	—	225–425	—	Domanski *et al.* (1968)	Samples collected after recovery from surgery (all sheep assumed to weigh 40 kg)
Sheep	Neck transplant (other adrenal removed)	Fluorimetry	9·7–22 μg.min^{-1}	—	242–550	—	Espiner *et al.* (1972)	—
Sheep	Neck transplant (other adrenal removed)	Competitive protein-binding	0·49–3·5 μg.min^{-1}	0·005–0·102 μg.min^{-1}	12·2–87·5	0·12–2·5	Clements & Newsome (1973)	(Sheep assumed to weigh 40 kg)
Calf	Acute cannulation	Chromatography	*ca.* 1 mg.hr^{-1} adrenal^{-1}	0·7 mg.hr^{-1} adrenal^{-1}	555	388	Balfour (1962)	Nembutal anaesthesia. Values refer to calves 3–4 weeks old (calves assumed to weigh 30 kg) Jersey calves
Calf	'Adrenal clamp'	Competitive protein-binding	20–40 ng.kg^{-1} min^{-1}	6–18 ng.kg^{-1} min^{-1}	20–40	6–18	Present paper	Jersey calves

with those obtained from unoperated calves) constitute one reason for supposing that the animals had effectively recovered from the surgical and anaesthetic procedures and were not suffering significant discomfort. Further evidence to support this contention is provided by the stable and normal levels of glucose in the blood, blood pressure and heart rate, together with the general condition and behaviour of the animals. It remains therefore to establish that the experimental procedures did not *per se* interfere with adrenal cortical behaviour. Our evidence to support this assumption depends upon the results of control experiments in which saline alone was infused intravenously for 2 hr either into normal calves, or calves with an adrenal clamp implanted; two such are illustrated in Fig. 2. These show that the sampling procedure did not alter significantly the output of steroids from the adrenal gland or the amounts present in peripheral blood. It therefore appears that the 'adrenal clamp' technique provides a valid index of normal adrenal function in the conscious calf and, since a normal adrenal venous pressure is preserved, allows more precise measurement of adrenal blood flow than has hitherto been possible. Furthermore, the use of competitive protein binding for steroid estimation has permitted sensitive and specific measurement of plasma steroid concentrations.

Comparison of these results with previous studies of adrenal cortical function is difficult for three reasons; first the extreme sensitivity of the gland to stress and the consequential problem of assessing the degree of stress imparted to the animal by different experimental techniques; second, species differences in the behaviour and responses of the gland, and finally the problems associated with estimation of low concentrations of specific steroid hormones in plasma. Table 2 embodies a representative selection of data obtained by other workers who have attempted to measure the same indices of adrenal function in the dog, sheep and calf.

Examination of these data confirms that, as would be expected from the well-documented increase in peripheral adrenal glucocorticoid concentrations during stress, the adrenal output of these steroids is also increased under these circumstances.

The values for the resting output of steroids from the adrenal gland reported in the present paper are the lowest yet published. This may be attributed in part to the relative lack of stress associated with this method for collection of blood samples, as discussed previously, together with the high specificity of the competitive protein-binding assay used, since it is well known that this method consistently produces lower steroid concentrations than the less specific techniques previously used such as fluorimetry (see, for example, Malinowska, Hardy & Nathanielsz, 1972; Malinowska & Nathanielsz, 1974). However, it is also possible that the

physiological output of these steroids in the calf is genuinely lower than that in the adult dog and sheep.

The results of the current study show that arterial plasma cortisol concentration is usually less than 1·0 μg/100 ml. and corticosterone less than 0·4 μg/100 ml. in the conscious calf at rest. The value for cortisol is comparable with values for venous plasma obtained using the same analytical method by Nathanielsz, Comline, Silver & Paisey (1972) for calves of this age, and is also in general agreement with several other recent reports of peripheral cortisol concentrations in the calf some days after birth (Eberhardt & Patt, 1971; Purohit & Estergreen, 1971; Dvorak, 1971).

In both the calf and the lamb the concentration of cortisol in the peripheral plasma is high immediately after birth and falls progressively during the first week of post-natal life (Khan, Dickson & Meyers, 1970; Eberhardt & Patt, 1971; Nathanielsz *et al.* 1972): values up to 12 μg/100 ml. have been reported in the calf. There is abundant evidence in the sheep that these high cortisol concentrations in the new-born are a consequence of dramatic changes in foetal adrenal cortical function at the end of gestation associated with the initiation of parturition (Liggins, 1969). It seems probable that similar mechanisms are also operative in the bovine. However, it is clear from the present results that perinatal adrenal hyperfunction has abated within a few days of birth in this species as there were no obvious variations with age, either in the resting output of the gland, or in the responses to synthetic adrenocorticotrophin between 8 and 30 days of age.

It was of particular interest to define the response to adrenocorticotrophin using this new technique, for it is well known that adrenocorticotrophin is the major factor in the normal control of glucocorticoid synthesis and release from the adrenal gland and also causes an increase in adrenal blood flow. The present results represent the first quantitative analysis of both the glucocorticoid output and the blood-flow responses *in vivo* with measurements at frequent intervals before, during and after infusion of adrenocorticotrophin. Intravenous infusion of synthetic corticotrophin at a rate of 5 ng.kg^{-1} min^{-1} (0·005 i.u. kg^{-1} min^{-1}) resulted in a prompt increase in the output of both cortisol and corticosterone, together with an increase in blood flow.

The steroidogenic action of adrenocorticotrophin has been the subject of much investigation using *in vitro* techniques and considerable information has been obtained about the mode of action of the hormone within the adrenal cortex. Much is also known about the effect of adrenocorticotrophin administration *in vivo* on peripheral plasma glucocorticoid concentrations. Extremely little is known, however, about the response of the adrenal cortex itself *in vivo* to corticotrophin administration, since such

investigation requires collection of adrenal venous blood from conscious, unstressed animals. Previous reports of direct measurements of glucocorticoid output in response to adrenocorticotrophin in conscious animals have been largely concerned with the adult sheep. McDonald & Reich (1959) defined the threshold dose of adrenocorticotrophin necessary to increase cortisol output from a transplanted adrenal when infused into the jugular vein at 1·2 i.u./hr (0·005 i.u. kg^{-1} min^{-1}). This suggests that the adult sheep adrenal is less sensitive to adrenocorticotrophin than that of the calf, particularly since the transplanted left adrenal had hypertrophied as a result of previous right adrenalectomy in these experiments. Furthermore, it is difficult to understand why the cortisol output rose so slowly, only attaining a peak output some 2 hr after the infusion began. Beaven, Espiner & Hart (1964) also investigated the response of the transplanted sheep gland to adrenocorticotrophin but in this study the animals had been pre-treated with dexamethasone and the adrenocorticotrophin was administered as a single intravenous injection which negates direct comparison between this and the present work. It is, however, noteworthy that the adrenal response to adrenocorticotrophin was apparent about 3 min after injection, which is in good agreement with the latency found in the current experiments. Close intra-arterial injection did not appreciably reduce this latency.

There have been no previous systematic studies of the possible differential effect of adrenocorticotrophin on cortisol and corticosterone output *in vivo*, together with the correlation between output from the adrenal and the peripheral arterial venous concentrations of each steroid. Fig. 4*c* demonstrates the changes in output ratio of cortisol and corticosterone and the simultaneous variation of the steroid ratio in arterial plasma during Synacthen infusion. It can be seen that there is an overall decrease in the cortisol:corticosterone output ratio which implies that during infusion there is a relative increase in the output of corticosterone, even though the *absolute* output of cortisol is greater (Fig. 4*a*). Conversely, however, the cortisol:corticosterone ratio in arterial plasma increases during infusion, suggesting a greater peripheral utilization of corticosterone. It is intended to extend these observations in the future, since quantitative correlations between adrenal steroid output and the peripheral concentration of these hormones are likely to provide a valuable index of changes in peripheral utilization during conditions such as hypoglycaemia and cold exposure.

The finding that adrenal blood flow increases in response to adrenocorticotrophin is in accord with observations of numerous other workers since the phenomenon was first described by Balfour (1953). However, the 'adrenal clamp' technique ought to provide a more accurate estimate than other methods of measuring normal adrenal blood flow in the conscious

animal for a variety of reasons. Adrenal venous pressure approximates to the normal value since the adrenal vein itself represents the narrowest point in a relatively short artificial shunt (Fig. 1) and care was taken to see that the outflow tube was held at the height of the gland during the sampling procedure: adrenal venous pressures during sampling were found to fall within the range 2–8 mmHg. Previous estimates of adrenal blood flow in conscious animals have been made in transplanted glands in the sheep (McDonald & Reich, 1959; Beavan *et al.* 1964; Espiner *et al.* 1972), or in sheep with lengthy extravascular by-passes (Blair-West *et al.* 1962; Domanski *et al.* 1968). In the former method, section of the splanchnic innervation and prior removal of the contralateral gland, together with radical changes in blood supply associated with revascularization in a carotid-jugular loop, may well combine to alter the normal blood flow characteristics. In the case of long extravascular by-passes the resistance to flow is high when samples are not being taken and during the sampling procedure a siphon effect is apparently necessary to facilitate collection of blood.

Table 3 summarizes the results obtained in conscious sheep and two previous studies of adrenal blood flow in the calf. Although the absolute resting values in the sheep may not be reliable, it is noteworthy that adrenal blood flow during anaesthesia was higher than that after recovery (Blair-West *et al.* 1962; Domanski *et al.* 1968). This effect was demonstrated dramatically in the calf by Balfour (1953) and was also evident during the operative procedures in the present work; it seems likely that these high values are due to the release of endogenous adrenocorticotrophin.

Comparison of the changes in adrenal blood flow in response to exogenous adrenocorticotrophin in different preparations shows that the transplanted sheep adrenal is relatively insensitive (Table 3) and that comparatively large doses of adrenocorticotrophin are required to elicit an increase in blood flow through the sheep adrenal *in situ* (Blair-West *et al.* 1962). In contrast, the adrenal gland of the conscious calf responds rapidly to intravenous infusions of extremely small quantities of synthetic adrenocorticotrophin. This increase in blood flow occurs in association with increased steroid output and there appears to be a distinct threshold output for each steroid (225–250 $ng.kg^{-1}\ min^{-1}$, cortisol; 70–80 $ng.kg^{-1}\ min^{-1}$, corticosterone) above which blood flow first begins to increase beyond basal levels. The changes in adrenal blood flow which occurred during the present experiments could not be related to changes in either heart rate or aortic blood pressure and it is therefore concluded that they were due to vasodilatation within the adrenal gland itself.

The steroid concentrations in arterial plasma during infusions of synthetic adrenocorticotrophin were within the range encountered in normal animals. Since, in the former case, an increase in adrenal blood flow was

TABLE 3. Comparison of adrenal blood flow in the sheep and calf

Species	Technique for obtaining adrenal venous blood	Resting blood flow (ml. min^{-1})	Effect of ACTH	Reference	Remarks
Sheep	Neck transplant	*ca.* 2·5–9·0	No effect	McDonald & Reich (1959)	Siphon effect during collection: outflow tube 18 in. below adrenal
Sheep	Neck transplant	*ca.* 5	> 1000 mu.: increase to *ca.* 7 ml. 20–50 mu.: slight effect 5 mu.: no effect	Beavan *et al.* (1964)	Dexamethasone treated. Siphon effect: outflow tube 24–30 in. below adrenal. ACTH injections, jugular vein
Sheep	Neck transplant	5–15	Slight increase during intra-arterial infusion of high doses (16·6 mu. min^{-1})	Espiner *et al.* (1972)	As above
Sheep	By-pass renal vein–jugular vein	4·7–16·6 (7 sheep)[1] 3–6 (7 sheep)[2]	Increase e.g. 4·7–10·9 ml. min^{-1} 12 i.u. (I.V.)	Blair-West *et al.* (1962)	Collection from T-piece in by-pass tube held 10 cm below adrenal. [1]During operation (cyclopropane/O_2): [2]24 hr after operation.
Sheep	By-pass renal vein–cava cranialis	10·3–12·0 (3 sheep)[1] 5·9–6·8 (3 sheep)[2]	—	Domanski *et al.* (1968)	Collection from by-pass tubing held an unspecified distance below adrenal. [1]During operation (N_2O/O_2): [2]4 days after operation
Calf	Acute cannulation	40[1] 10–20[2] < 5[3]	Increased flow in calves 10–40 days old. Little effect in calves < 8 days old	Balfour (1953)	[1]During anaesthesia (ether/chloralose): [2]Decerebrate animal: [3]Some hours after operation in [1] and [2] above
Calf	Acute cannulation	8·5 ± 3·1	—	Whipp, Weber, Usenik & Good (1967)	Pentobarbitone anaesthesia. Very large calves (*ca.* 100 kg). All animals routinely given 10 i.u. ACTH.hr^{-1}
Calf	'Adrenal clamp'	6–7	See Fig. 6 and Table 1	Present paper	—

observed, it seems likely that fluctuations in adrenal blood flow may occur under physiological conditions. Although the present experiments provide no direct evidence that sufficient endogenous corticotrophin is released from the pituitary to promote adrenal vasodilatation in response to any physiological stimulus, the results of preliminary work (S. R. Bloom, A. V. Edwards, R. N. Hardy, K. W. Malinowska & M. Silver, unpublished work) indicate that this may occur during hypoglycaemia.

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