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RESPONSES OF THE PITUITARY-ADRENAL SYSTEM OF THE PIG TO ENVIRONMENTAL CHANGES AND DRUGS

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1 The reactivity of the pituitary-adrenal axis of the young pig was tested for its suitability as a sensitive index for any discomfort that might be experienced under certain conditions of intensive husbandry.

2 In a thermoneutral environment, most undisturbed piglets showed only slight variations in the plasma concentrations of adrenocorticotrophic hormone (ACTH) and corticosteroids.

3 Stimuli such as exposure to ambient temperatures of +40°C or −5°C were required to cause large rises in the plasma concentrations of ACTH and corticosteroids.

4 Apparently milder stimuli, such as change of environment, slight frustration or changes in ambient temperatures between +5°C and +30°C only rarely caused a significant rise in plasma corticosteroids. Thus changes in plasma corticosteroid concentrations are not a sensitive index for the reaction of a piglet to its environment.

5 Increases in plasma ACTH concentrations occurred faster than those of the corticosteroids, were larger when expressed as a percentage of the basal values and occurred following relatively small disturbances such as omission of the reward in an operant behaviour test when corticosteroid changes were often not detectable. Thus rises in plasma ACTH might be a useful indication that a given situation is disturbing to a pig. The reaction of plasma ACTH concentrations to chronic irritations as they might occur in intensive husbandry remains to be investigated.

6 Azaperone (2 mg/kg i.m.), a drug which is used as a sedative in pigs, caused a rise of about 50% in plasma corticosteroid concentrations. It did not diminish the large steroid output seen when the animals were exposed to high and low ambient temperatures.

Introduction

The activity of the pituitary-adrenal system can be altered by changes in the environment. The response of the system could therefore be a useful index for the degree of discomfort which a domestic animal might experience under some conditions of intensive husbandry. Because of the differences in the magnitude of the responses of this endocrine system known to exist between species and even between breeds, it is essential to test its reactivity for any given group of animals. Only then is it possible to draw correct conclusions regarding the degree of disturbance an animal has experienced, from the presence or absence of alterations in plasma adrenocorticotrophic hormone (ACTH) or corticosteroid concentrations.

On previous occasions (Holzbauer & Newport, 1967; 1969) the adrenal corticosteroid secretion of the young pig was studied in experiments in which adrenal venous blood was collected under anaesthesia from eviscerated animals. Cortisol and corticosterone were found to be secreted at ratios varying between 1:1 and 4:1. In addition, considerable quantities of Reichstein's Compound S, androstane derivatives, pregnenolone, progesterone, 11β-hydroxyprogesterone and 16α-hydroxyprogesterone were present in the blood. The rates at which glucocorticoids were secreted by the piglets were similar to those found in puppies of the same age under similar conditions. Thus it appears that the degree of the response of the pituitary-adrenal system to severe abdominal surgery is the same in the two species.

On the other hand Baldwin & Stephens (1971) reported that in the conscious pig, significant rises of the corticosteroid concentrations in the peripheral plasma could only be elicited by quite severe stimuli, whereas smaller disturbances which are known to raise plasma corticoids in rats considerably, were without effect in the pig. It was felt that a more detailed investigation into the degree of disturbance

required to elicit a significant response of the pituitary-adrenal system in this species was indicated. Experiments were therefore carried out on conscious piglets with a venous catheter implanted in a jugular vein for the withdrawal of blood. Firstly the time course and extent of the response of plasma corticosteroids to injected ACTH were studied. Secondly, plasma ACTH and steroid concentrations were measured in piglets exposed to different environmental temperatures, subjected to mild frustration or injected with drugs which cause the release of ACTH. Furthermore the effect of azaperone, a drug that causes sedation in the pig, on plasma corticosteroid concentrations has been tested.

Methods

Animals

Pigs: 6 to 10 week old females (Large White breed, free of enzootic pneumonia) weighing between 15 and 20 kg were used. They were fed with commercial pig food (4.5% of body weight per day). Rats: male Wistar rats of 200–250 g body weight were used. Dog: a mature female was trained to stay quietly in a Pavlov stand. On the day of an experiment, a sterile catheter was temporarily inserted into a saphenous vein under local anaesthesia.

Surgery

Surgery on pigs was carried out under halothane (ICI) anaesthesia and sterile conditions. A polyethylene catheter was inserted into one or both jugular veins through an incision in the neck and advanced into the right atrium. The other end of the catheter was led under the skin to the back of the neck from where it emerged. The end was plugged with a stainless steel stiletto, rolled up to form a loop and kept in position with adhesive plaster. The cannula was regularly flushed with a sterile 0.9% w/v NaCl solution (saline) containing a trace of heparin. Each pig was fitted with a harness to aid handling.

Some animals had a thermode implanted into the anterior region of the hypothalamus. It consisted of a disc shaped tank, 5 mm in diameter, with an entrance and exit tube which led up to the surface of the head. A thermistor bead was fixed to the surface of the thermode and its connection was also led to the surface of the skull. The whole assembly was implanted into the brain using a series of radiographs to guide it into place. The surface temperature of the thermode could be varied by perfusion with either hot or cold fluid. Cooling the surface of the thermode to 10°C reduced the brain temperature up to 5 mm away by about 2°C. Further details of the method are described in Baldwin & Ingram (1967a) and Ingram & Legge (1971).

Experimental procedures

Before the first experiment was carried out, each piglet had on several occasions been placed in a climatic chamber (air conditioned room, 3 m × 2 m × 2 m) where it was loosely tied by its harness to a stall so that it was able to lie down or stand up but was unable to turn round completely. On the day of an experiment the animals were taken in a large cage-trolley from the pig holding house to the laboratory. Half an hour later one or two blood samples were taken while the animals were still in the trolley. Then they were placed in the climatic chamber. All blood samples were taken via the indwelling catheter with plastic syringes, transferred to plastic, heparin-coated tubes, cooled, centrifuged and the plasma taken off for hormone assays.

Rectal temperature was measured with a thermistor probe inserted 10 cm into the rectum. The rectal temperature was registered outside the chamber.

Operant conditioning

Some pigs were taught to perform an operant response in the form of switching on a battery of infra red lamps (Baldwin & Ingram, 1967b). The animals were placed in a cold room (+10°C) in a cage which was equipped with a switch panel. When the panel was pushed with the snout (response), infra red bulbs were switched on for 5 s (reinforcement). After several sessions of an hour or more the pigs made regular responses for reinforcements of heat at an environmental temperature of +10°C.

Chemical procedures

Plasma steroids were estimated by a protein-binding assay using dog plasma for the preparation of corticosterone-binding globulin. Bound steroids were separated from free steroids on 2 cm columns of Sephadex G25 fine (Basset & Hinks, 1969). The same standard curves were obtained for cortisol alone, or for cortisol and corticosterone as a 1:1 mixture. The steroid content of each plasma sample was estimated six times (duplicates at 3 dilutions each differing by a factor of 2) and the mean value calculated. As the number of pigs available was limited, a detailed analysis of variability between animals was not often possible. In most cases the assessment as to whether a given stimulus caused a significant change in plasma corticosteroid concentrations had to be made from results on small groups of animals and the accuracy of the estimate obtained for each individual plasma sample was of great importance. For this reason there

are included in Figures 2, 3 and 5 to 11 the standard errors of the mean for the estimate of the plasma corticosteroid concentration in each individual plasma sample as derived from six assays. An exception is Figure 1 in which each dot represents the mean for the values obtained over 5 days and the vertical lines the s.e. of these means indicating the day-to-day variations in the same pig.

ACTH was measured either by a histochemical bioassay (Chayen, Loveridge & Daly, 1972) which is usually referred to as the 'Red-ox' assay, or by a radioimmunoassay (Rees, Cook, Kendall, Allen, Kramer, Radcliffe & Knight, 1972). Antidiuretic hormone (ADH) was measured by bioassay on water-loaded rats as described by Forsling (1974). Some of the ADH values discussed in the present paper have been published previously (Forsling, Ingram & Stanier, 1976).

Clearance rate of cortisol from the blood stream

[^{3}H]-cortisol (2 μCi/pig) was injected through one indwelling catheter in a volume of 3 ml saline and washed in with 5 ml saline. Blood samples were then taken from the second indwelling catheter in the contralateral jugular vein. The steroids were extracted either from whole blood or from plasma and blood cells separately (Holzbauer & Newport, 1969). The cortisol was separated from its metabolites by chromatographing the purified extracts on paper in the E_2B system of Eberlein & Bongiovanni (1955). The paper region corresponding to cortisol was cut out and transferred to counting vials, 10 ml of Unisolve (Koch-Light) was added and the radioactivity counted in a liquid scintillation spectrometer.

Drugs and hormones

The following were used: ACTH: Cortrosyn (Tetracosactryn, Organon) synthetic β1-24 corticotrophin; Synacthen: (Tetracosactryn, Ciba) synthetic β1-24 corticotrophin; histamine acid phosphate (BDH); nicotine tartrate (BDH) and [^{3}H]-cortisol, (Amersham; in benzene:ethanol 1:1).

Results

'Spontaneous' variations in plasma steroid and ACTH concentrations

The normal physiological variations in the plasma concentrations of corticosteroids occurring in the course of the day were investigated in 10 pigs. The animals were fed at 08 h 45 min and then brought to the laboratory and kept in a cage-trolley outside the climatic chamber until 09 h 30 min when the first blood sample was taken. The pig was then transferred to the stall in the temperature-controlled room at 20°C. This temperature is a little below the thermally neutral zone for the pig (about 25°C). Thereafter a number of blood samples were taken at intervals. Food was provided at 16 h 00 min, their normal feeding time. Trained pigs accepted these movements quietly and usually lay down and fell asleep in the stall.

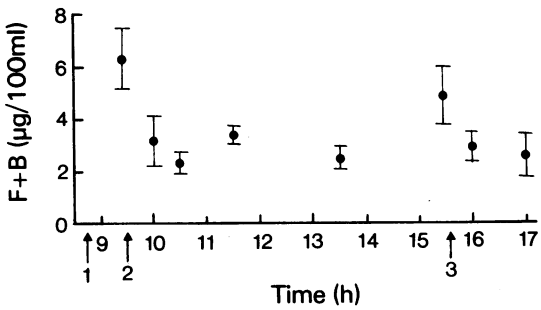

Figure 1 Plasma corticosteroid concentrations (F + B) of a pig measured on 5 consecutive days at the same hours of day. Mean values of the observations are given; vertical lines show s.e. mean. Arrows on abscissa scale: (1) pig moved from holding house to laboratory; (2) pig moved into temperature-controlled room (20°C); (3) pig fed.

In Figure 1 the mean values for plasma corticosteroid concentrations as estimated in one pig on five consecutive days are shown. On each day the concentrations were higher in the sample taken immediately before the pig entered the temperature-controlled room than half an hour later. An increase occurred in the afternoon followed by a fall after feeding. In a second pig which was also studied over five consecutive days the steroid concentrations showed much larger variations. On 4 days sudden large rises occurred which reached values of 9 μg/100 ml although the pig was not obviously excited. This pig ate the food offered in the afternoon on only one occasion and developed severe diarrhoea during the week following this experiment.

In another eight pigs the steroid concentrations in plasma were measured for only one day between 09 h 30 min and 17 h 00 min. In five animals absolute values and the fluctuations observed were similar to those illustrated in Figure 1. The initial high value just before entering the temperature-controlled room and the higher values at 15 h 30 min were not always present. In the three remaining pigs the plasma corticosteroid concentrations at 09 h 30 min were in the region of 4.5 μg/100 ml, but increases up to 15 μg/100 ml occurred at some time or other during the day even though the pigs displayed no signs of discomfort.

The only abnormalities in these pigs were that one developed diarrhoea during the following week and another one had a leucocythaemia.

In Table 1, plasma corticosteroid and ACTH concentrations are listed as they were estimated in 12 different pigs on 23 different days. ACTH was estimated by the bioassay method (B) or by a radioimmunoassay (RI). All blood samples were taken before the start of any experimental procedures and should therefore be regarded as 'basic control values'. It can be seen that in individual pigs the day to day variations were small for both types of hormones with the exception of pig 'K'. This pig had low steroid and ACTH concentrations at the first occasion when the blood sample was taken in the morning. Five days later a sample was taken in the early afternoon and the values for ACTH and steroids were several times higher. A comparison between steroid concentrations in control samples taken before 12 h 00 min or after 14 h 00 min was carried out on 84 samples from 13 pigs. The mean value for the corticosteroid content (μg/100 ml plasma) of the morning samples was 3.50 ± 1.53 (s.e. mean, $n = 58$), of the afternoon samples 3.91 ± 2.24 ($n = 26$).

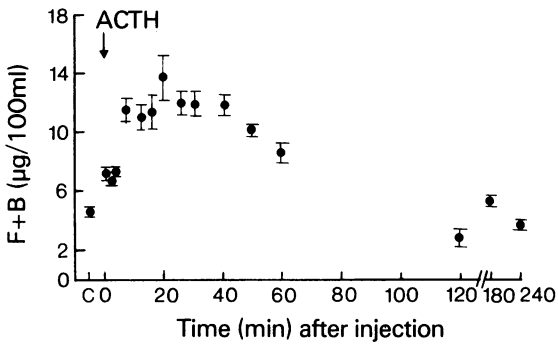

Figure 2 Plasma corticosteroid concentrations (F + B, mean values of 6 assays on the same sample; vertical lines show s.e. mean) in a pig injected intravenously (arrow) with ACTH (1.0 i.u/kg body weight). C: control before injection.

Table 1 Plasma corticosteroid and ACTH concentrations of 12 undisturbed piglets at 18 to 20°C

		Plasma concentrations		
Pig	*Exp. No.*	*F + B (μg/100 ml)*	*ACTH (pg/ml)*	*Time (h)*
A	1	—	57 B	09.25
		—	54 B	15.30
A	2	—	22 B	15.00
B	1	—	48 B	09.46
C	1	—	47 B	09.20
D	1	—	36 B	09.30
E	1	1.20	37 B	09.20
		1.16	42 B	09.23
F	1	3.03	34 B	10.00
F	2	2.16	43 B	09.25
F	3	1.80	43 B	12.21
F	4	2.19	317 B	10.00
		3.96		12.00
		2.46	72 B	14.30
		3.30	76 B	16.20
F	5	1.47	37 B	10.00
M	1	5.66	76 B	14.32
M	2	2.30	76 B	14.22
G	1	7.23	333 RI	10.00
G	2	5.16	222 RI	10.00
G	3	5.88	224 RI	09.40
H	1	1.56	<81 RI	09.55
I	1	3.68	<80 RI	10.15
I	2	3.84	<92 RI	11.25
I	3	4.28	<80 RI	09.45
J	1	2.2	<80 RI	13.30
		4.6	155 RI	15.30
		2.9	102 RI	17.00
K	1	1.59	<80 RI	09.05
K	2	10.80	282 RI	14.30

B: Bioassay ('Red-ox' method, Chayen *et al.*, 1972); RI: radioimmunoassay (Rees *et al.*, 1971); F + B: cortisol plus corticosterone.

Effect of exogenous ACTH on plasma corticosteroid concentrations

The time course of the response of the adrenal cortex of a pig to a single intravenous injection of 1 iu ACTH per kg body weight is shown in Figure 2. The plasma corticosteroid concentration was doubled within the first 2 min after the injection and near maximal values were reached after 8 minutes. The high concentrations were maintained for about 40 min; after one hour the values were still similar to those seen 1–5 min after the injection, after 2 h control values were reached.

When this dose of ACTH was given as an intravenous infusion over a period of one hour (17 mu min^{-1} kg^{-1} body wt.) the plasma corticosteroid concentrations increased within the first 5 min of the infusion from 3.2 to 7.4 μg/100 ml. This was followed by a more gradual rise to reach 10.6 μg/100 ml by the end of the infusion; 10 min later the value was 13.3 μg. After 1 h the concentrations were similar to those before infusion.

In another set of experiments the response of plasma corticosteroid concentrations to intravenous injections of increasing amounts of ACTH was studied in a pig and, for interspecies comparison, also in a dog. After collecting two control blood samples, ACTH was injected intravenously at 30 min intervals

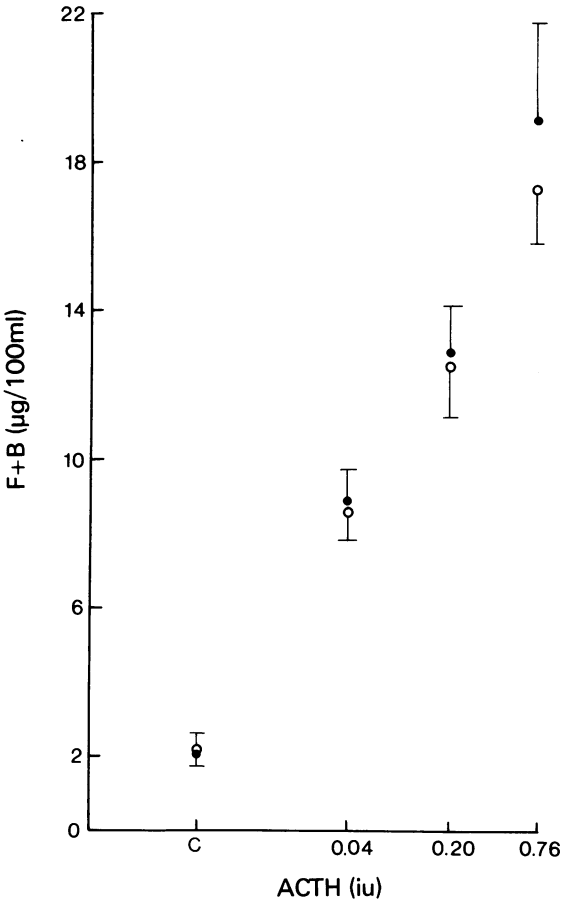

Figure 3 Dose-response relation between the logarithm of the dose of ACTH (tetracosactrin, Organon) injected intravenously and the plasma corticosteroid concentration 29 min after the injection (F + B, mean values of 6 assays on the same sample, vertical lines show s.e. mean) in a pig (●) and a dog (○). C: control before ACTH.

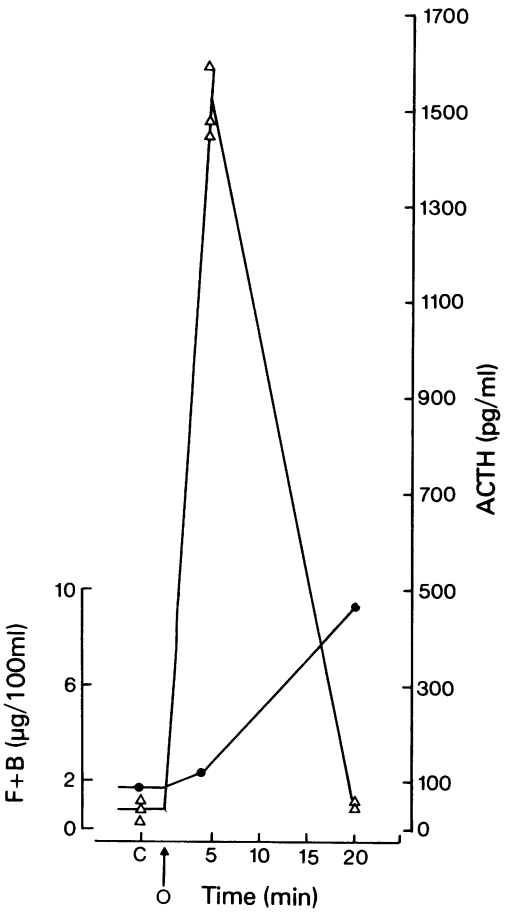

Figure 4. The effect of histamine (2 or 3 mg/pig, injected intravenously at arrow) on plasma ACTH (bioassay, △, right ordinate scale) and corticosteroid (F + B, ●, left ordinate scale) concentrations. C: control before injection.

in doses increasing by a factor of 5. Blood samples were taken 10 and 29 min after each injection. Figure 3 shows the straight line relation between the logarithm of the amount of ACTH injected and the plasma corticosteroid concentrations 29 min after each injection in a pig and a dog. The slope of the dose-response curve was similar in the two species. A five-fold increase of the dose of ACTH led approximately to a 50% rise in the plasma corticosteroid concentration.

In two other pigs larger quantities of ACTH were injected in doses increasing by a factor of 2 to establish the plateau of the response. The plasma corticosteroid concentration of one pig rose from 5.7 µg/100 ml to 11.8 µg, 30 min after the injection of 1.25 iu ACTH per kg body weight; there was a further rise to 13.4 µg in response to 2.5 iu ACTH per kg and to 14.8 µg in response to 5 iu ACTH per kg. After 1 h the value was still 13.6 µg, after 2.5 h it was back to 5.3 µg. In another pig the plasma steroid concentration rose from 4.8 µg/100 ml to 11.00 µg, 30 min after the injection of 1.25 iu ACTH per kg. Increasing the dose of ACTH to 2.5 and 5.0 iu per kg did not cause any further rises. These experiments demonstrate the individual variations in the extent to which adrenal steroid secretion can be stimulated by exogenously administered ACTH. They also showed that the plasma corticosteroid concentrations after the largest doses of exogenous ACTH were not

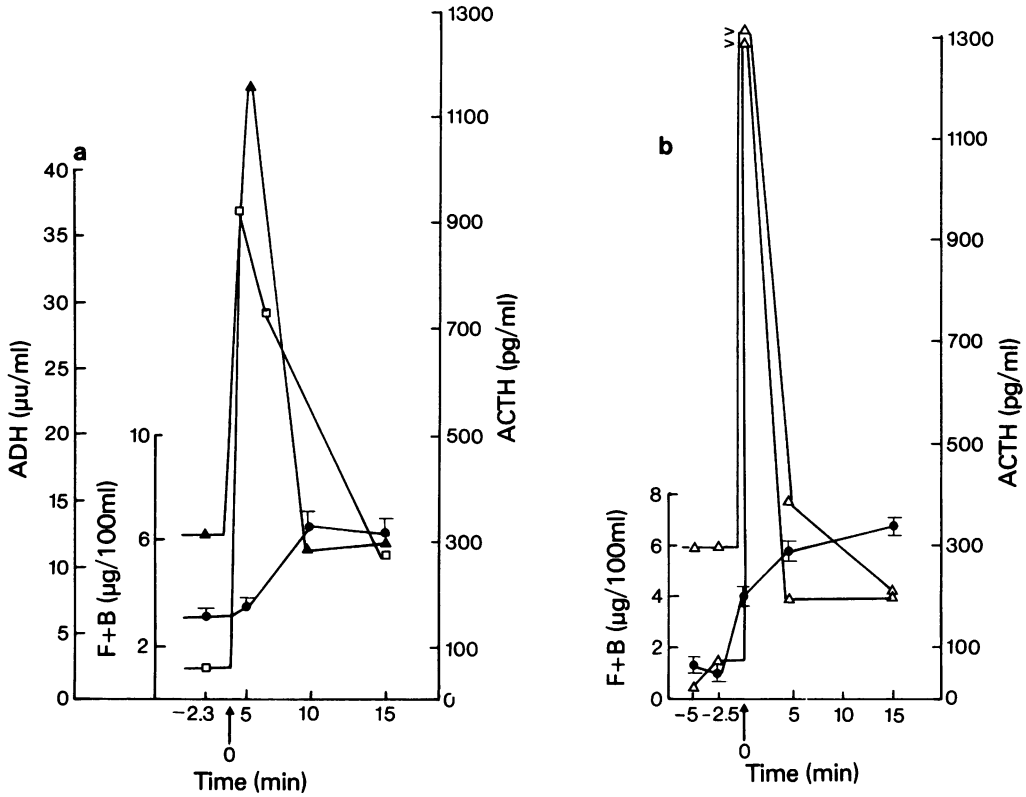

Figure 5 The effect of nicotine (4 mg/animal) injected intravenously at arrows on plasma hormone concentrations. (a) Antidiuretic hormone (ADH) (□); ACTH, radioimmunoassay (▲). (a & b) Corticosteroids (F + B, mean values of 6 assays on the same sample; vertical lines show s.e. mean) (●); (b) ACTH, bioassay (△).

higher than those found on some occasions in apparently undisturbed pigs.

Effect of drugs which release ACTH on plasma concentrations of ACTH and corticosteroids

The effect of a slow intravenous injection of histamine (2 or 3 mg/pig over 1 min) which lead to strong salivation for 1–2 min, was examined in three pigs. Two minutes after the end of the injection the plasma ACTH concentration was increased more than 30-fold (see Figure 4) whereas the steroid concentration was hardly changed. After 20 min the ACTH (bioassay) concentration was again back to control values, however that of the corticosteroids was increased about five-fold. Figure 5a shows the response of a piglet to the intravenous injection of 4 mg nicotine base which also caused transient salivation. As after histamine, plasma ACTH (radioimmunoassay) rapidly increased and was back to normal 4 min later. The corticosteroids again showed a delayed response. In this piglet, plasma samples were also analysed for antidiuretic activity (ADH). As with ACTH there was an immediate rise in the plasma ADH concentrations; 2 min later the activity had fallen by 20% and 15 min later by 70% of the maximum value. Figure 5b shows plasma ACTH (bioassay) and corticosteroid concentrations found in another two piglets after the intravenous injection of 4 mg nicotine base. There was again a rapid, steep but transient rise in plasma ACTH and a slower but longer lasting rise in plasma steroids. The time course of the increase in plasma corticosteroid concentrations after nicotine and histamine was reminiscent of that seen after a single dose of ACTH.

Effect of abdominal surgery on plasma ACTH and corticosteroid concentrations

The reaction of the pituitary-adrenal system of the

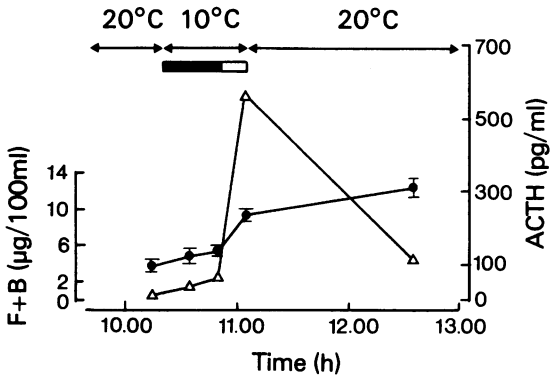

Figure 6 The effect of 'frustration' on plasma ACTH (bioassay, △, right ordinate scale) and corticosteroid (F + B, ●, left ordinate scale, mean values of 6 assays on the same sample; vertical lines show s.e. mean) concentrations. The pig was trained to respond to a cool environment (+10°C) by switching on infrared lights (dark bar). Switching off the infrared lights permanently (light bar) led to frustration. Figures between arrows: ambient temperature. The first sample was taken in the laboratory 15 min after the pig had been moved from the holding house, samples 2, 3 and 4 in the temperature-controlled room, sample 5, 90 min after the pig had been brought back to the holding house.

pig to abdominal surgery under halothane anaesthesia, involving midline incision and handling of the intestine was examined in two pigs. In the first animal the control samples taken before anaesthesia had corticosteroid concentrations of 1.2 and 1.1 µg/100 ml and the values for ACTH (bioassay) were 37 pg/ml and 42 pg/ml. Two further blood samples were taken 20 and 30 min after surgery had begun and steroid values of 4.6 µg and 5.2 µg were found. The corresponding values for ACTH were 75 pg/ml and 51 pg/ml. In the second pig the plasma concentration of ACTH 10 min after the onset of the operation was three times higher than in the control sample and the steroid concentration was doubled. To judge from the time course of the ACTH reaction to stimuli seen in other experiments (see Figures 4, 5, 8 & 9) it seems likely that the plasma ACTH concentrations were higher soon after the start of surgery.

Effects of 'frustration' on ACTH and corticosteroids

These experiments were carried out in order to test whether a disturbance elicited by interfering with an operant response situation causes the release of ACTH. Pigs were trained to press a button to switch on a battery of infra red bulbs in an environment of +10°C. After the pigs had learned this task they were first allowed to obtain heat and then they were 'frustrated' by interrupting the connection between the switch and the heater so that although the response was made, no reward was obtained. Sometimes these 'frustrated' animals became very restless, rubbed their noses on the cage floor and began to eat their faeces, on other occasions they simply retreated into a corner of the cage and lay down. Figure 6 shows the changes of plasma ACTH (bioassay) and corticosteroid concentrations of one pig in this situation. During the period when pushing the light switch was rewarded with heat (black bar) the hormone concentrations hardly changed. However, 14 min after the infra red bulbs had been permanently switched off (open bar) the plasma ACTH concentration was increased nearly ten-fold and that of the corticosteroids was nearly doubled. Ninety minutes after the pig had been returned to an environmental temperature of 20°C plasma ACTH was back to the control values whereas the corticosteroid concentrations showed a further rise. Similar observations were made in three experiments on another two piglets. In the first one the plasma ACTH concentration was 34 pg/ml at the end of the 15 min period at +10°C during which the pig was able to switch on the heating bulbs and 89 pg/ml (bioassay) at the end of the 'period of frustration'. The corresponding values for corticosteroids were 3.0 and 3.4 µg/100 ml. Two days later the same pig showed in a similar experiment ACTH values of 43 and 170 pg/ml and corticosteroid values of 2.2 and 3.4 µg/100 ml. These two experiments and the experiment shown in Figure 6 were carried out in the morning. The experiment was done in the afternoon in another pig in which the initial corticosteroid concentration was high, 10.8 µg/100 ml. It increased further to 20.8 µg/100 ml during the period of 'frustration'. The ACTH values (radioimmunoassay) for this pig were also high. In spite of this, frustration caused a rise from 282 to 827 pg/ml. Simply exposing pigs to an environment of +10°C for periods up to 4 h did not cause significant rises in plasma corticosteroids although some shivering occurred.

The effect of low environmental temperatures on the plasma concentration of ACTH and corticosteroids

In these experiments piglets were exposed to temperatures between −5 and −7°C. The results of one such study are shown in Figure 7. Forty-five minutes after the pig was introduced to the cold the plasma corticosteroid concentration was doubled and the ACTH concentration (radioimmunoassay) had increased five-fold. After 2 h the corticosteroid concentration was three times, the ACTH concentration ten times the control value and stayed so for the next hour. The pig was then returned to an ambient temperature of

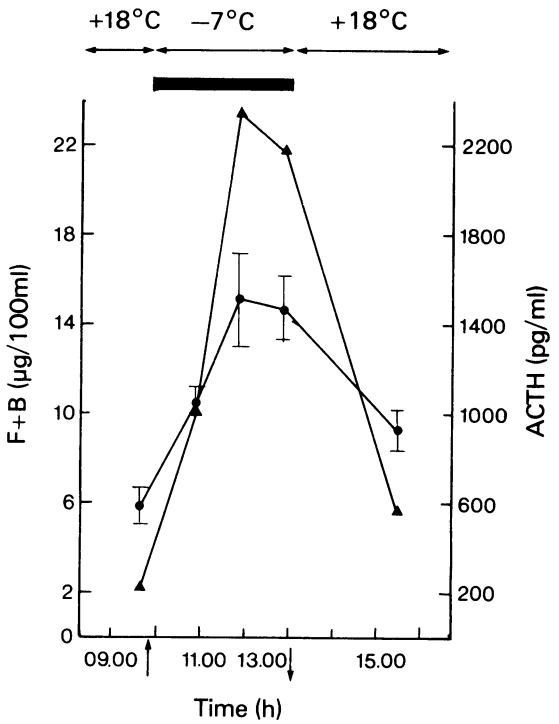

Figure 7 Effect of a cold environment (−7°C) on plasma ACTH (radioimmunoassay, ▲, right ordinate scale) and corticosteroid (F + B, ●, left ordinate scale, mean values of 6 assays on the same sample, vertical lines show s.e. mean) concentrations. Figures between arrows on top of graph: ambient temperature. Arrows on abscissa scale: (1) pig introduced to the cold environment; (2) return to ambient temperature of 18°C.

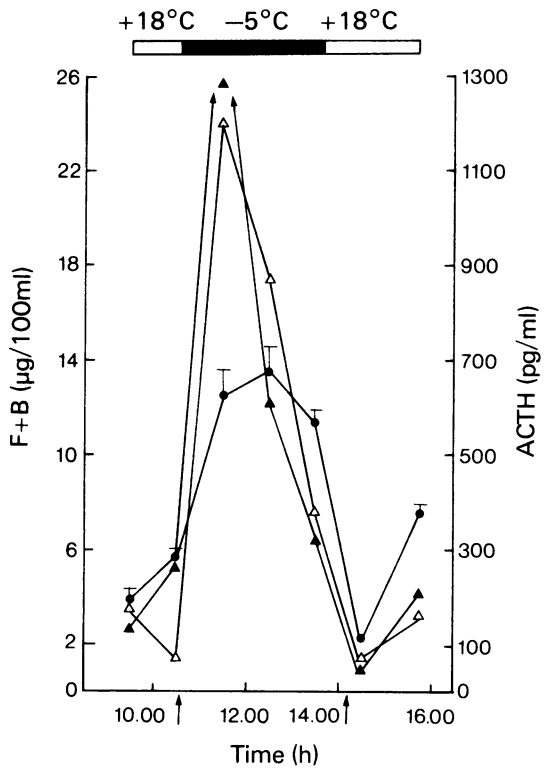

Figure 8 Effect of a cold environment (−5°C) on plasma ACTH (bioassay: △; radioimmunoassay: ▲, right ordinate scale) and corticosteroid concentrations (F + B, ●, left ordinate scale, mean values of 6 assays on the same sample; vertical lines show s.e. mean). Arrows on abscissa scale: (1) pig introduced to the cold environment; (2) return to ambient temperature of 18°C.

20°C and 165 min later the hormone concentrations had fallen to near control values. In this experiment the peaks in the ACTH and corticosteroid secretions occurred apparently at the same time and both hormone concentrations remained elevated in the cold. In another pig, ACTH was increased more than 15-fold after the pig had been exposed to −7°C for 45 min but returned to values below the sensitivity of the radioimmunoassay after 135 min in the cold. By contrast the plasma corticosteroid concentration showed the highest value at the end of the 3 h cold exposure (4 times the control value) and remained at the same high level for 2 h after the pig had been returned to 20°C. Figure 8 shows the results of yet another experiment in which a pig was exposed to −5°C. In this case the plasma ACTH concentrations were measured by the radioimmunoassay as well as by the bioassay. The ACTH concentrations at the end of the first hour in the cold were very high, one hour later they had decreased and after 2 h the values were even lower. One hour after the pig had been returned to a room at 18°C the ACTH values were similar to those of the controls. There was a fair agreement between the values obtained by the two assay methods. In contrast to ACTH, plasma corticosteroids stayed high until the end of the cold exposure and returned to normal after 1 h at 18°C. Pigs kept at temperatures below −5°C had increased rectal temperatures (40°C to 41°C) and were often shivering and restless.

To allow a comparison of the time course of the response of the pituitary-adrenal system of the pig to a cold stimulus with that of another species experiments were carried out on a group of male rats

(200–250 g) which were exposed for different periods to an environment of −5°C. Their plasma ACTH concentrations (bioassay) increased from 30 pg/ml to 1700 pg/ml within the first 2.5 min of cold exposure. The highest value (2000 pg/ml) was found at 10 min, at 30 min the ACTH concentrations had fallen to 1000 pg/ml, after 1 h in the cold it was 800 pg. These ACTH concentrations are very similar to those seen in the pig. The plasma corticosterone concentrations in the rats exposed to a cold environment increased gradually from 15 μg/100 ml (control value) to a maximum of 50 μg/100 ml after one hour. Thus in the rat, ACTH secretion was already maximally stimulated within the first minutes in a cold environment and fell rapidly during further exposure. In contrast the response of the adrenal cortex to this burst of ACTH was gradual and continued to rise when plasma ACTH was already considerably decreased.

Effect of high environmental temperatures on plasma concentrations of ACTH, ADH and corticosteroids

In these experiments six pigs were exposed to environmental temperatures above 40°C, conditions under which the pig can no longer maintain its normal body temperature. The rectal temperature was therefore carefully watched and when it reached 43°C the pigs were taken out of the hot room. Figure 9 illustrates the results obtained from one of these experiments. The first changes in hormone concentration occurred when the rectal temperature was about 41°C. The highest values were reached after 2 h when the rectal temperature was 43°C. On return to a thermal neutral environment both ACTH and corticosteroid values fell. The same time course was seen in another two experiments in which plasma concentrations of ACTH and corticosteroids also reached maxima only at the end of the heat exposure. In a fourth experiment the maximal ACTH value occurred after 45 min when the rectal temperature was 41.5°C. After 80 min, when the rectal temperature was 42.8°C, the ACTH concentration had fallen by 30%.

In two other piglets figures were also obtained for the plasma concentration of ADH (Forsling *et al.*, 1976). Both the plasma concentrations of ADH and corticosteroids increased on exposure to heat (Figure 10); the ADH returned to the control value sooner than the plasma corticosteroids.

The effect of azaperone on plasma corticosteroids

Azaperone (4'-fluoro-4-[4-(2-pyridil)-1-piperazinyl]-butyrophenone, Stresnil, Suicalm) is a drug that was reported to act as a potent sedative in pigs and to possess a high antishock activity in this species (see e.g. Marsboom & Symoens, 1968). Consequently this

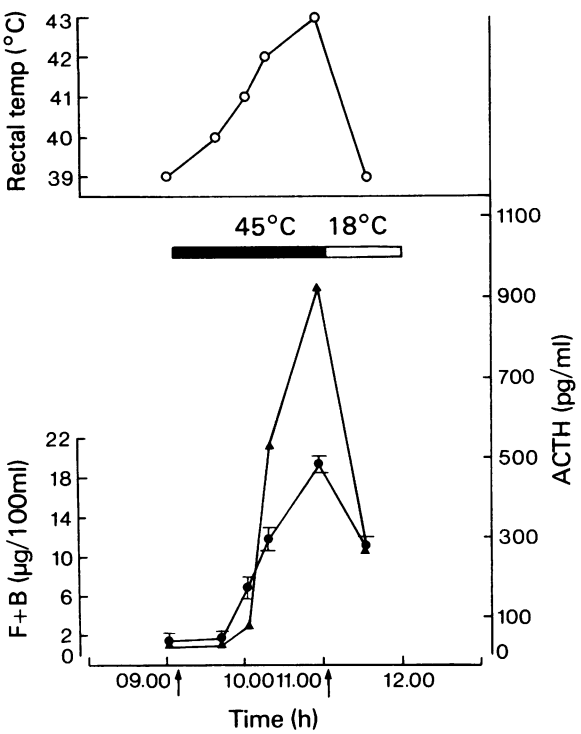

Figure 9 Effect of a hot environment (+45°C) on plasma ACTH (radioimmunoassay, ▲, right ordinate scale) and corticosteroid concentrations (F + B, ●, left ordinate scale, mean values of 6 assays on the same sample; vertical lines show s.e. mean). Rectal temperature: (○). Arrows on abscissa scale: (1) pig introduced to hot environment; (2) return to ambient temperature of 18°C.

drug was tested for its ability to prevent the increase in the plasmacorticosteroid concentrations which is caused by changes in the environmental temperature. The following experiments were carried out: 30 min after a piglet (about 8 weeks old) had been transported from the holding shed to the laboratory, 2 control blood samples were taken with an interval of 10 minutes. Azaperone (2 mg/kg) was then injected into the musculature of a hindquarter. Control animals received an intramuscular injection of an equal volume of saline. A third blood sample was taken 15 min after the injection. The pig was then transferred into the climatic chamber kept either at +45°C or at −5°C. In one instance it was 26°C (pig 3400, 13th of May, Table 2).

The injection of azaperone was usually followed by ataxia which started within 5 min and lasted for at

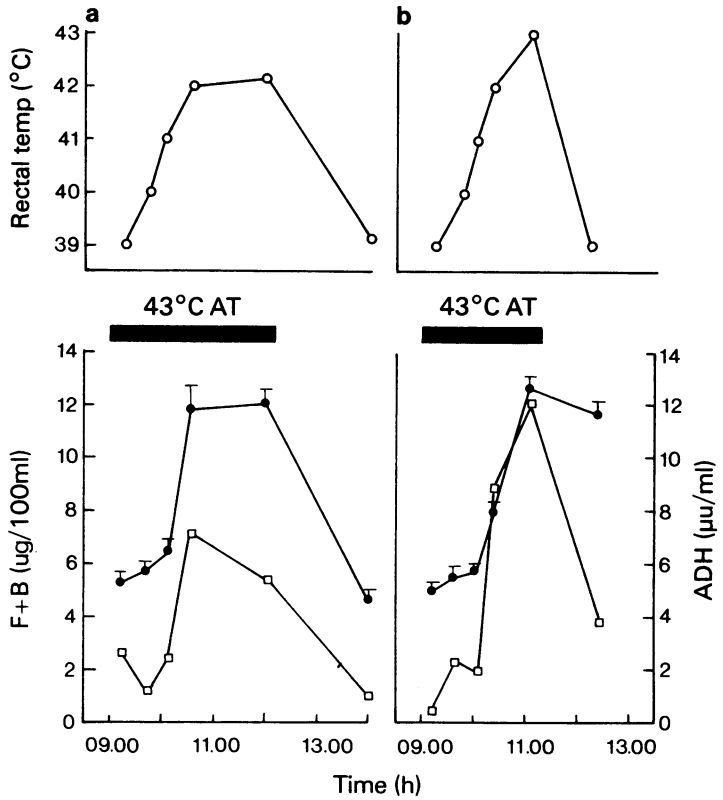

Figure 10 Effect of a hot environment (+43°C) on plasma concentrations of corticosteroids (F + B, ●, left ordinate scale, mean values of 6 estimations on the same sample; vertical lines show s.e. mean) and antidiuretic hormone (ADH, □, right ordinate scale) in 2 pigs (a and b). Period of heat exposure indicated by black bar. Rectal temperature: (○). AT: ambient temperature.

least 2 hours. Some pigs went through a short excitatory phase (1 to 2 min) during which they tried to jump out of the cage and vocalized. While exposed to the high or low environmental temperatures the behaviour varied from pig to pig and was also different in the same pig on different occasions. Pigs injected with saline were usually quiet before being put into the climatic chamber but during exposure to heat they became restless when their body temperature began to rise and panting set in. This restlessness could either be diminished by azaperone (e.g. pig 3396, May 24th) or exaggerated (e.g. pig 3400, May 14th, second experiment with azaperone). Pigs 6643 and 6645 belonged to an especially aggressive litter. In both animals azaperone had a calming effect. Another pig (not in Table 2) was, for the first 15 min after the injection of azaperone, very agitated although severely ataxic. When transferred into the climatic chamber it lay down and remained quiet for 30 min until its rectal temperature reached 41.9°C. Then it became agitated and broke loose from the stall.

The plasma corticosteroid concentrations measured in these piglets are listed in Table 2. After an intramuscular injection of saline, plasma corticosteroids did not alter. In contrast, the injection of azaperone caused a mean rise of 52% ($P < 0.001$). These increases occurred whether the pigs went through an excitatory phase after the injection or not. Furthermore, the maximal values for the corticosteroid concentrations reached during hot or cold exposure were equal in the pigs injected with azaperone and in the saline injected controls. Thus azaperone does not block ACTH and corticosteroid secretion in hostile environmental conditions.

Effect of changing the temperature of the hypothalamus on plasma corticosteroid and ADH concentrations

The effect of warming to 43°C a thermode implanted

into the preoptic area of the hypothalamus on plasma corticosteroid concentrations was examined in six pigs at environmental temperatures of 20, 30 and 35°C. In no instance was there any unequivocal change in the plasma concentrations of corticosteroids.

The thermodes were also cooled to a temperature of 10°C while the pig was in an ambient temperature of 10 or 20°C but again there was no unequivocal change in the concentration of plasma corticosteroids.

Lastly in two pigs the surface temperature of the thermode was cooled to 10°C while the pigs were exposed to an ambient temperature of 45°C and their rectal temperature allowed to increase to 43°C. In these pigs the plasma concentration of ADH was measured in addition to the corticosteroid concentration. As can be seen in Figure 11, when the thermode was cooled the rise in corticosteroids in an ambient temperature of 43°C still occurred, but the rise in ADH which is normally seen on exposure to heat (Forsling *et al.*, 1976) (compare Figure 10) did not occur.

Clearance rate of [^3H]-cortisol from the blood stream

The rate at which [^{3}H]-cortisol (3 μCi) injected intravenously disappeared from the blood stream was studied at ambient temperatures of +20°C, +5°C and +35°C. The reason for choosing these temperatures was the possibility that the absence of significant alterations in plasma corticosteroid concentrations at +5°C and +35°C might have been due to an increase

Table 2 Plasma corticosteroid concentrations (F + B, μg/100 ml)

Pig No. and date	*Control* S_1	S_2	*15 min after i.m. injection of:*	*Maximal F + B conc. reached during exposure to heat (45°C)*	*cold (−5°C)*	*Behaviour*
Controls:			*Saline*			
3400 10/5	1.74	4.02	3.42	7.80	—	Calm throughout.
3400 12/5	4.40	4.37	4.37	8.40	—	Calm when injec. In hot room very restless.
3396 21/5	5.84	4.97	5.10	8.30	—	Restless when injec. In hot room very restless.
3396 26/5	5.62	6.09	5.79	—	8.5	Calm throughout.
6645 14/6	5.25	3.39	5.13	14.1	—	Very agitated throughout experiment.
	—	—	—			
Mean ± s.e.	4.57 ± 0.75	4.57 ± 0.46	4.76 ± 0.40 +4%*			
Experimental:			*Azaperone*			
3400 6/5	4.68	5.07	6.68	9.15	—	Atax. after injec. In hot room calm.
3400 13/5	3.17	3.47	5.46	—	—	Atax. after injec. Room at 26°C; tremor, calm.
3400 14/5	5.10	5.20	8.0	9.50	—	Atax. but agitated after injec. In hot room first calm, after 30 min very agitated, 30 min later calm.
3400 27/5	5.28	4.52	5.05	—	7.3	Atax. after injec. In cold room calm.
3396 24/5	5.00	5.01	7.3	7.50	—	Short period of violent agitation after injec., ataxia. In hot room calm.
6645 15/6	4.4	4.74	7.34	11.3	—	Aggressive before but calm after injec., ataxia. In hot room calm.
6643 18/6	1.98	2.49	6.48	—	6.9	Aggressive before but calm after injec., ataxia. In cold room first very agitated, later calm.
Mean ± s.e.	4.23 ± 0.46	4.36 ± 0.38	6.62 ± 0.40 +52%* ($P < 0.001$)			

* Difference from S_2.

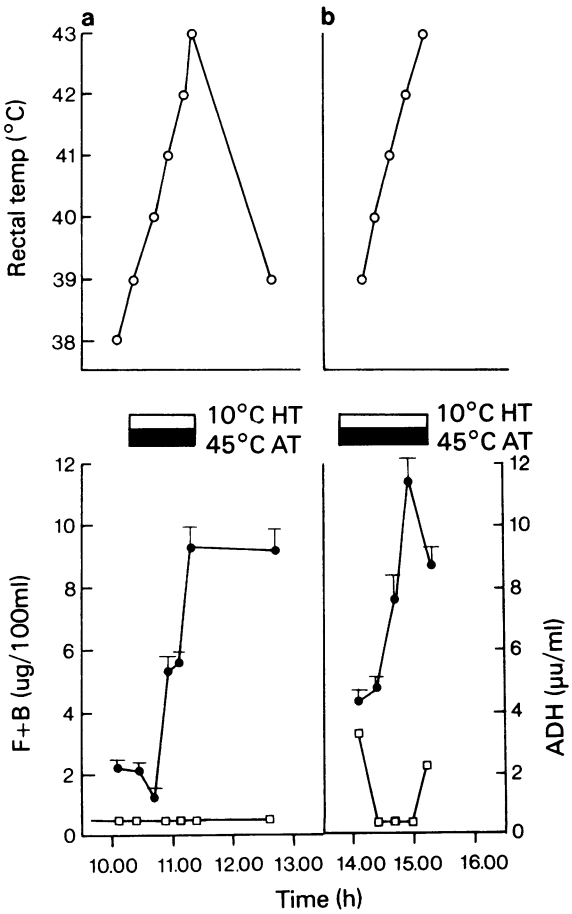

Figure 11 Plasma concentrations of corticosteroids (F + B, ●, left ordinate scale, mean values of 6 estimations on the same sample; vertical lines show s.e. mean) and antidiuretic hormone (ADH, □, right ordinate scale) in a pig at an ambient temperature (AT) of 45°C (black bar). At the same time a thermode implanted in the preoptic region of the hypothalamus was cooled (open bar). Rectal temperature: (○); HT: hypothalamic thermode temperature.

in the rate at which corticosteroids were used thus masking any increases in adrenocortical secretion. In 9 experiments carried out on 2 pigs a mean metabolic clearance rate of 4.1 ± 0.43 l blood/min was found. No significant difference between the metabolic clearance rates at the three ambient temperatures was present.

On four occasions the blood samples taken after the injection of [^{3}H]-cortisol were centrifuged and the [^{3}H]-cortisol present in the plasma and cell fraction measured separately. As previously seen in other species (Holzbauer, 1972), a considerable portion of the radioactive cortisol present in the pig's blood was associated with the cell fraction. In 20 samples taken 2 to 10 min after the injection, 34% (±1.4, s.e. mean) of the total [^{3}H]-cortisol present in the blood was cell bound. In 11 samples taken 30 to 90 min after the injection the cell bound fractions were even larger (43% ± 3.14).

Discussion

In the present work the sensitivity of the pituitary-adrenal system of the domestic pig and the consistency of its response to disturbances in the environment of the animal has been tested.

Corticosteroid concentrations in blood samples taken under standard environmental conditions at the beginning of an experiment showed but small variations on different days in the same pig or in different pigs. When several consecutive samples were collected in the course of one day without changing the experimental conditions occasionally a high value was observed without apparent reason.

The plasma ACTH concentrations in initial blood samples taken under 'resting' conditions were often below the sensitivity of the radioimmunoassay. When the more sensitive bioassay (Red-ox assay) was used, ACTH could be detected in all samples. The values were relatively consistent on different days in the same animal and also varied little in different animals. On those occasions on which ACTH could be detected by the radioimmunoassay, relatively high estimates were obtained. This may be due to the fact that sometimes antibodies are present in the assay mixture which react with fragments of the ACTH molecule that are no longer biologically active (see e.g. Nicholson, Liddle & Puett, 1976).

Mild stimuli, to which other species like the laboratory rat are known to respond with significant rises in their plasma corticosteroid concentrations (for example decreased (+10°C) or raised (+35°C) ambient temperatures or moving the animals into different locations) did not cause regularly significant increases in plasma corticosteroids in the piglets.

Depriving pigs of the reward in an operant response situation was disturbing enough to cause consistently a rise in their plasma ACTH concentrations (2–10 fold) within 15 minutes. Reliable estimates of these rises could only be obtained with the sensitive bioassay method which allowed the small quantities of ACTH present in pig's plasma under resting conditions to be measured. In the same experiments small rises in plasma corticosteroid concentrations were sometimes, but not always, observed.

Larger changes in ambient temperature (above +40°C or below −5°C) always caused considerable

stimulation of ACTH and corticosteroid secretion, the relative changes in ACTH concentrations being usually much larger than those in the corticosteroids.

The response of the pituitary-adrenal system of the young pig to an intravenous injection of histamine or nicotine was similar to that known to occur in other species. A large but very short-lasting rise in plasma ACTH was gradually followed by a smaller rise in plasma corticosteroids which was of longer duration. When pigs were injected with insulin a similar time course in the response of plasma ACTH and corticosteroids was observed (Donald, Salisbury-Murphy & Nabarroo, 1968).

The rise in plasma corticosteroids after injecting azaperone was unexpected. The dose of azaperone used has been reported to cause a fall in systemic blood pressure by about 30% after 15 min (Clarke, 1969) which may have been sufficient to stimulate the pituitary-adrenal axis. Exposure of injected pigs to heat or cold led to a further rise in plasma steroids even in those pigs which appeared calmer than on the occasions when they had undergone the same experience without the drug.

Some experiments were also carried out on pigs in which the preoptic region of the hypothalamus was cooled or heated. In contrast to observations on the dog (Chowers, Hammel, Stromme & McCann, 1964; Chowers, Hammel, Eisenman, Abrams & McCann, 1966) in the pig these procedures did not cause consistent changes in plasma corticosteroid concentration. Neither did cooling the hypothalamus prevent the rise in plasma corticosteroid concentrations elicited by high environmental temperature whereas the rise in plasma ADH concentrations occurring in the heat could be prevented (Forsling *et al.*, 1976). A dissociation of ADH and ACTH release has also been described on previous occasions in the rat when a rise of plasma ADH, but not of ACTH, was caused by osmotic stimuli (Nichols & Guillemin, 1959).

For practical purposes we have to conclude from the results presented that changes in plasma corticosteroid concentrations in the young pig are not consistent enough to be used as a measure for the mild disturbances an animal might experience under conditions of modern intensive husbandry.

Changes in plasma ACTH concentrations could be detected after smaller irritations, e.g. depriving a pig of a reward in an operant response experiment. However these changes can only be reliably measured with a sensitive and specific bioassay method.

Our thanks are due to Professor J. R. Hodges and Drs Julia Buckingham and Sandra Vellucci (Department of Pharmacology, Royal Free Hospital School of Medicine, University of London) for their help with the ACTH assays by the 'Red-ox' method and to Dr Lesley Rees (The Royal Hospital of St. Bartholomew, London) for the radioimmunoassays of ACTH. We also thank Mr K. Legge for his help in the animal work and for drawing the illustrations and Mrs Urma Godden for her assistance in the experiments on the half life time in blood of injected [^{3}H]-cortisol.

References

BALDWIN, B.A. & INGRAM, D.L. (1967a). The effect of heating and cooling the hypothalamus on behavioural thermoregulation in the pig. *J. Physiol.*, **191**, 375–392.

BALDWIN, B.A. & INGRAM, D.L. (1967b). Behavioural thermoregulation in pigs. *Physiology and Behavior*, **2**, 15–21.

BALDWIN, B.A. & STEPHENS, D.B. (1971). The effects of conditioned behaviour and environmental factors on plasma corticosteroid levels in pigs. *Physiology and Behavior*, **10**, 267–274.

BASSET, J.M. & HINKS, N.T. (1969). Micro-determination of corticosteroids in ovine peripheral plasma: Effects of veinipuncture, corticotrophin, insulin and glucose. *J. Endocr.*, **44**, 387–403.

CHAYEN, J., LOVERIDGE, N. & DALY, J.R. (1972). A sensitive bioassay for adrenocorticotrophic hormone in human plasma. *Clin. Endocr.*, **1**, 219–233.

CHOWERS, I., HAMMEL, H.T., EISENMAN, J., ABRAMS, R.M. & McCANN, S.M. (1966). Comparison of effect of environmental and preoptic heating and pyrogen on plasma cortisol. *Am. J. Physiol.*, **210**, 606–610.

CHOWERS, I., HAMMEL, H.T., STROMME, S.B. & McCANN, S.M. (1964). Comparison of effect of environmental and preoptic cooling on plasma cortisol levels. *Am. J. Physiol.*, **207**, 577–582.

CLARKE, K.W. (1969). Effect of azaperone on the blood pressure and pulmonary ventilation in pigs. *Vet. Rec.*, **85**, 649–651.

DONALD, R.A., SALISBURY-MURPHY, S. & NABARROO, J.D.N. (1968). The plasma corticotrophin response to insulin hypoglycaemia, lysine-vasopressin and metyrapone in pigs. *J. Endocr.*, **41**, 509–518.

EBERLEIN, W.R. & BONGIOVANNI, A.M. (1955). New solvent systems for the resolution of corticosteroids by paper chromatography. *Archs Biochem. Biophys.*, **59**, 90–96.

FORSLING, M.L. (1974). Extraction of neurohypophysial hormones for bio-assay. *J. Physiol.*, **241**, 3–5P.

FORSLING, M.L., INGRAM, D.L. & STANIER, M.W. (1976). Effects of various ambient temperatures and of heating and cooling the hypothalamus and cervical spinal cord on antidiuretic hormone secretion and urinary osmolality in pigs. *J. Physiol.*, **257**, 673–686.

HOLZBAUER, M. (1972). The association of steroids with blood cells *in vivo*. *J. Steroid Biochem.*, **3**, 579–592.

HOLZBAUER, M. & NEWPORT, H.M. (1967). Evidence for the presence of 16α-hydroxypregn-4-one-3,20-dione in adrenal venous blood of young pigs. *J. Physiol.*, **191**, 691–697.

HOLZBAUER, M. & NEWPORT, H.M. (1969). Adrenal se-

cretion rates and adrenal tissue concentrations of pregnenolone, progesterone, 11βOH-androstenedione and some other steroids in young pigs and dogs. *J. Physiol.*, **200**, 821–848.

INGRAM, D.L. & LEGGE, K.F. (1971). The influence of deep-body and skin temperature on peripheral blood flow in pigs. *J. Physiol.*, **215**, 693–707.

MARSBOOM, R. & SYMOENS, J. (1968). Experiences with azaperone, a sedative for pigs. *Tijdschr. Diergeneesk.*, **93**, 3.

NICHOLS, B.L. & GUILLEMIN, R. (1959). Endogenous and exogenous vasopressin on ACTH release. *Endocrinology*, **64**, 914–920.

NICHOLSON, W.E., LIDDLE, R.A. & PUETT, D. (1976). Corticotropin: plasma clearance, catabolism and biotransformations. *58th Annual Meeting American Endocrine Society*, Abstr. 5.

REES, L.H., COOK, D.M., KENDALL, J.W., ALLEN, C.F., KRAMER, L.H., RATCLIFFE, J.G. & KNIGHT, R.A. (1971). A radioimmunoassay for rat plasma ACTH. *Endocrinology*, **89**, 254–261.

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