J. Physiol. (1960), **152**, *pp.* 14–29
With 5 *text-figures*
Printed in Great Britain

THE OUTPUT OF ADRENALINE AND NORADRENALINE FROM THE ADRENAL MEDULLA OF THE CALF

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(*Received* 1 *September* 1959)

The adrenal medulla contains both adrenaline and noradrenaline but their proportions vary considerably in different species: from 99–100% adrenaline in the rabbit to 20–30 % in the whale (for references see von Euler, 1956). In the cat and dog, stimulation of the glands leads to the liberation of adrenaline and noradrenaline in approximately the same proportions as in the medulla (Lund, 1951; Outschoorn, 1952; Vogt, 1952; Rapela, 1956; Butterworth & Mann, 1957), but it is not certain whether a similar parallel between output and content occurs in other species.

In contrast with the wide variation in the relative and absolute pressor amine content of adult adrenal glands, foetal adrenal medullary tissue contains primarily noradrenaline (Shepherd & West, 1951; West, Shepherd, Hunter & MacGregor, 1953). There is a gradual increase in the percentage of adrenaline with age, but the adult stage may not be reached until some months or even years after birth (Holton, 1951; Shepherd & West, 1951; Hökfelt, 1952; West *et al.* 1953). It seemed of interest to study the output of pressor amines from the adrenal glands of young animals of various ages, and to determine whether the change in the proportion of adrenaline in the glands was accompanied by any similar changes in output. Preliminary experiments on the lamb (Comline & Silver, 1958) showed that, in this species, the major increase in the proportion of adrenaline in the adrenal glands occurred *in utero*. During the latter part of pre-natal life there was an increase in the proportion of adrenaline released during splanchnic nerve stimulation, which paralleled the rise in the percentage of this amine in the glands. The present work is concerned with similar experiments on the post-natal development of adrenal medullary function in the calf, a preliminary account of which has already been published (Silver, 1954).

METHODS

Male Jersey calves from 3 hr to 220 days, and adult cows of the same breed aged 2–3 years, were used in these experiments. Food was withheld from the young animals on a milk diet on the morning of the experiment; from weaned animals, solid food, but not water, was withdrawn for 36–48 hr before the experiment.

Anaesthesia was induced in the calves by ethyl chloride and ether and thereafter maintained by intravenous chloralose (50 mg/kg). The adult cows were anaesthetized by the intravenous injection of Na pentobarbitone (10 mg/kg).

The left adrenal gland was prepared for the collection of adrenal effluent blood. It was exposed by a flank incision, and in the young calf its dissection was facilitated by removal of the last rib. In all animals the left kidney was removed, and after cannulation of the renal vein, blood from the adrenal gland was diverted backwards into the cannula by means of a Blaylock clamp placed across the junction of the renal vein with vena cava, just medial to the entrance of the very short adrenal vein. Coagulation of the blood was prevented by intravenous heparin (1000 i.u./kg.). The adrenal effluent blood was returned to the general circulation through an external circuit of polythene tubing connected via a drop chamber to the femoral vein. The left splanchnic nerve was exposed and cut as close as possible to its point of emergence through the diaphragm, thereby leaving 3–4 cm of the peripheral end free for stimulation. In all experiments blood pressure was recorded from the carotid artery to check the efficacy of splanchnic nerve stimulation.

Adrenal effluent blood. Samples of about 20 ml. were collected through a side arm inserted as close to the renal-vein cannula as possible. The sampling time varied from a half to 1 min in the cows and from ¾ to 2 min in the calves, depending on the rate of blood flow, and there was no evidence that pressor amine output changed during this time; in any one animal the sampling period was kept constant. A resting sample of adrenal blood was taken at the beginning of each experiment about 30 min after the left splanchnic nerve had been cut; subsequent samples were taken during periods of stimulation starting 15–30 sec after the onset to allow for the dead space. When the splanchnic nerve was stimulated continuously for 40 min, blood samples were collected at intervals during this period. Siliconed glassware was used throughout, and after collection the blood was immediately centrifuged on ice.

Stimulation of the peripheral end of the left splanchnic nerve with platinum electrodes was carried out either from a Palmer induction coil (frequency *ca.*/80 sec) or by condenser discharges at either, 5, 10 or 30/sec; the stimulus strength was always adjusted to give a maximal increase in blood pressure on splanchnic nerve stimulation. No special precautions were taken to prevent polarization of the electrodes or deterioration of the nerve, but neither of these factors appeared to be affecting the response, since the frequent removal of the electrodes from the nerve for a few seconds (ensuring depolarization) and changing their position along the length of the nerve did not cause any increase in the blood-pressure response.

Acetylcholine (1–3 mg) was given by close arterial injection into the anterior mesenteric artery. The coeliac artery was tied previously and the aorta occluded caudal to the left adrenal gland during the period of injection. Administration of atropine sulphate (0·2 mg/kg i.v.) before each acetylcholine test prevented all cardiovascular effects of any acetylcholine reaching the general circulation. The dose of acetylcholine varied with the age of the animal: 1 mg induced a maximal response in calves under 1 week of age, 3 mg was given to calves of 1–3 months.

Adrenal glands. The adrenaline and noradrenaline content of the adrenal glands of calves of different ages was determined from the right gland, which was removed at the end of each experiment. In those animals subjected to prolonged splanchnic nerve stimulation both glands were removed immediately after the end of stimulation. No glands were used for pressor amine estimations after acetylcholine injections.

Adrenaline and noradrenaline were separated chromatographically before estimation, by the method of Crawford & Outschoorn (1951) as modified by Vogt (1952). 5–7 ml. samples of plasma were used for extraction, and after the final evaporation of the eluates from the chromatograms the amines were taken up in 1 ml. NaCl 0·9 % (w/v). The adrenal glands were ground with sand and 0·1 N-HCl, 10 ml. acid/g tissue; 1 ml. samples of the homogenate were used for extraction. In the majority of experiments the final eluates from plasma and adrenal tissue were assayed biologically, either by the blood-pressure response of the anaesthetized rat given atropine and hexamethonium to increase the sensitivity (Outschoorn, 1952), or by the inhibition of carbachol-induced contractions of the isolated rat uterus (Gaddum & Lembeck, 1949). In general four-point assays were carried out on each sample. L-Adrenaline-HCl or bitartrate and L-noradrenaline bitartrate were used for the preparation of standard solutions and the quantities are expressed as weights of base. No systematic search for other pressor amines or their derivatives was made, but several chromatograms of plasma and adrenal tissue were analysed by assaying the eluates from paper strips taken at levels other than those corresponding to adrenaline and noradrenaline; no pressor activity was found in these samples.

Recently the fluorimetric method of von Euler & Floding (1955) has also been employed for catechol estimation, after chromatographic separation and elution of the amines. When compared with biological assay in a series of analyses of adrenaline and noradrenaline in plasma, with twelve random samples of each, there was no consistent difference between the results obtained by the two methods of estimation. Mean figures for each series were (μg/ml.): noradrenaline 1·16 (assay and fluorimetry), mean difference 0·005 ± 0·06; adrenaline, 1·30 (assay) and 1·31 (fluorimetry), mean difference 0·01 ± 0·03. From this series of analyses the fluorimetric method appears to be unbiased; a detailed statistical investigation into the comparative reliability of fluorimetry and biological assay for the estimation of pressor amines will be presented elsewhere.

RESULTS

Adrenaline and noradrenaline in the adrenal glands

Preliminary experiments showed that the adrenaline and noradrenaline concentrations of the right and left unstimulated adrenal glands of the calf were indistinguishable. The right adrenal, which remained intact during the course of these experiments, was therefore used to examine the changes in content and proportions of the two amines at different ages. It was assumed that the right gland would be unaffected by stimulation of the left splanchnic nerve, and that the slight depletion due to endogenous splanchnic discharge during the course of the experiment (Elmes & Jefferson, 1942) would be small compared with the high amine concentration in the medulla.

The results given in Table 1 show that there is an increase in the concentration of adrenaline and a fall in that of noradrenaline during the 2nd week of life with a consequent increase in the proportion of adrenaline during this period. However, when the results are expressed as total amounts of amines per adrenal gland (Fig. 1), it is seen that the noradrenaline content remains constant throughout the period investigated, whereas the adrenaline content rises from the second week onwards. The discrepancy between the figures for pressor amine concentration (Table 1) and those for total glandular content (Fig. 1), can presumably be ascribed to the more rapid growth of the adrenal cortex relative to the medulla in the growing calf.

TABLE 1. Change in adrenaline and noradrenaline concentration in the adrenal glands with age

Age	Number of animals	Mean adrenal weight (g)	Mean pressor amine concentration (mg/g adrenal tissue)		Mean % adrenaline ± S.E.	
			Noradrenaline	Adrenaline		
Calf (weeks)						
Birth–1	10	1·6	0·86	0·91	51 ± 1·7	P < 0·01
2–3	6	1·8	0·53	1·11	67 ± 2·4	
4–5	3	1·9	0·65	1·54	71	
11	2	2·4	0·67	1·5	69	
15–18	4	3·7	0·5	1·49	75	
Cow (2–3 yr)						
Non-pregnant	4	9·9	0·42	1·22	75	
Pregnant	3	12·2	0·29	1·07	79	

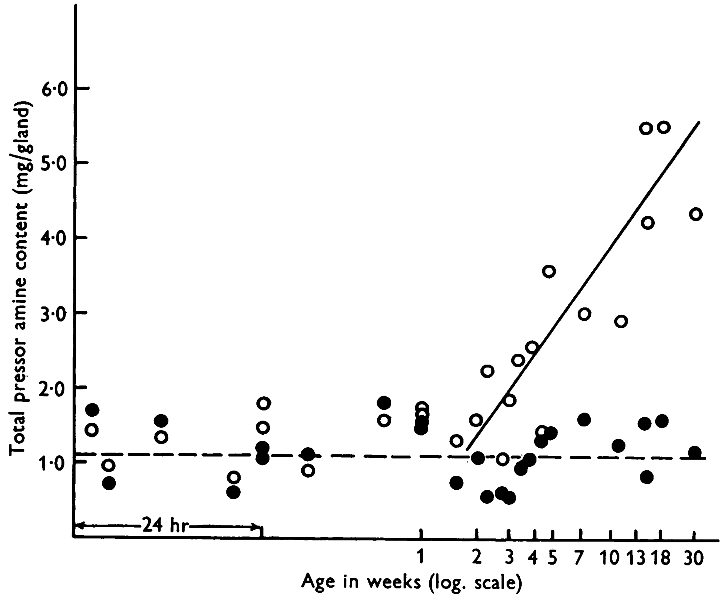

Fig. 1. The change in the total amount of adrenaline and noradrenaline in the adrenal glands of the calf with age; logarithmic time scale. ●, mg noradrenaline/gland; ○, mg adrenaline/gland; ———, regression line fitted by the method of least squares from data from animals of over 10 days of age; dependent variable, glandular content of adrenaline (regression coefficient = 3·59 ± 0·59, $P < 0\cdot001$).

Adrenaline and noradrenaline in the adrenal effluent blood

Resting level of secretion from acutely denervated gland

This is summarized in Table 2. The amounts secreted were always small and the proportion of adrenaline to noradrenaline in the effluent blood corresponded approximately to that found in the gland, the percentage of adrenaline increasing after the first week of life. When expressed as μg/kg body weight/min the total resting output in the older calves was comparable with that found by Dunér (1953) and Rapela (1956) using the acutely denervated adrenal glands of the cat and dog, respectively.

TABLE 2. Resting level of secretion from calf adrenal medulla: splanchnic nerve cut ½ hr previously

		Mean rate of secretion of pressor amines into adrenal effluent blood				
Age (weeks)	Number of animals	Noradrenaline (μg/min)	Adrenaline (μg/min)	Total output (μg/kg/min)	Mean % adrenaline ± s.e.	
Birth–1	8	0·65	0·55	0·047	44 ± 6·1	0·05 > P > 0·02
2–5	8	0·24	0·45	0·027	66 ± 7·5	
8–30	8	0·23	0·75	0·017	75 ± 7·5	P > 0·1

TABLE 3. Rate of output of pressor amines from the adrenal medulla during ½–2 min stimulation of the peripheral end of the splanchnic nerve

		Mean rate of pressor amine output				Mean % adrenaline	
		(μg/min ± s.e.)		(μg/kg body wt./min)			
Age	No. of animals	Noradrenaline	Adrenaline	Noradrenaline	Adrenaline	Effluent blood	Right gland
Calf							
1st 24 hr	5	1·2 ± 0·25	3·2 ± 0·80	0·07	0·15	73	51
½–1 week	13	7·0 ± 1·3	5·0 ± 1·0	0·27	0·20	42	51
2–3 weeks	8	15 ± 3·6	8·1 ± 1·2	0·67	0·39	36	66
4–5 weeks	7	17 ± 2·2	11 ± 0·54	0·60	0·43	41	71
7 weeks	4	18 ± 5·8	13 ± 2·0	0·63	0·45	42	—
11 weeks	3	30	29	0·66	0·61	49	69
15–18 weeks	5	32 ± 8·8	39 ± 8·5	0·42	0·55	54	75
30 weeks	4	31 ± 7·3	39 ± 3·9	0·30	0·48	56	—
Cow 2–3 yr							
Non-pregnant	3	23	60	0·055	0·14	72	75
Pregnant	5	17 ± 4·7	45 ± 9·0	0·045	0·13	73	79

Level of secretion during splanchnic nerve stimulation. Short period of stimulation (½–2 min)

Induction coil (ca. 80/sec). The mean rates of pressor amine secretion from the adrenal medulla during splanchnic nerve stimulation in calves of different ages and in the adult are given in Table 3. The rate of output of both amines increased with age, but this rise in the level of adrenal medullary secretion was not directly related to general body growth, since the major increases in pressor amine output occurred during the first few weeks of life, at a time when changes in body weight are negligible. Hence an even larger initial increase in adrenaline and noradrenaline output is found when the data are calculated as μg/kg body weight/min (Table 3).

Noradrenaline output was higher than that of adrenaline in calves from $\frac{1}{2}$ to 7 weeks of life, while after 15–18 weeks the same type of stimulus released larger amounts of adrenaline. Comparison of individual observations showed that both these differences were statistically significant ($P = 0{\cdot}01$).

In the adult the rate of noradrenaline secretion was considerably lower, and that of adrenaline higher, than in calves of 15–30 weeks. The differences between pregnant and non-pregnant animals (Table 3) were negligible when the data were calculated as μg/kg body wt./min.

The contrast between adrenal medullary secretion in the calf and in the adult is emphasized when the figures for percentage adrenaline in the effluent blood are compared with those for the glands (Table 3). In the adult the two values are almost equal, while in all calves tested after 24 hr from birth the percentage of adrenaline in the effluent was much lower than that in the gland. This very striking difference between the proportions of the two amines released and their proportions in the gland seemed to be due almost entirely to the unexpected increase in the rate of noradrenaline output with age, which was difficult to reconcile with the constant total amount of this amine in the glands throughout the period investigated (Fig. 1). Further experiments were therefore carried out to study the factors concerned in the high noradrenaline output in the calf, and to determine whether such a level of secretion could be maintained for long periods of stimulation.

Comparison between stimulation at ca. 80/*sec and at lower rates.* The rate of stimulation employed in the preceding experiments was *ca.* 80/sec, and since the probable maximum rate of endogenous splanchnic nerve discharge is considerably lower than this (Celander, 1954; Rapela, 1956), the secretions obtained with three low frequencies of stimulation (5, 10 and 30/sec) were also studied. The different frequencies of stimulation were applied in random order; the rest period between successive tests was 15–20 min. Four calves of 3–4 weeks old were used for this series since it was at this age that the divergence between glandular content and output was greatest.

The proportions of the two amines released by nervous stimulation were not affected by the rate at which the splanchnic nerve was made to discharge (Fig. 2). However, the rate of both adrenaline and noradrenaline output rose with frequency of stimulation to a maximum at 30 stimuli/sec. Rapela (1956) also found that in the adult dog stimulation at 40/sec gave a bigger response than either 10/sec or 160/sec, but he reports a significant increase in the percentage of noradrenaline.

Comparison between the effect of acetylcholine and nerve stimulation. The mean levels of adrenaline and noradrenaline secreted in response to acetylcholine in four calves aged 3–4 weeks were compared with the results obtained with different rates of nerve stimulation. It will be seen from Fig. 2 that acetylcholine resulted in the output of a very much larger amount of noradrenaline than any frequency of nerve stimulation. The proportion of adrenaline in the effluent blood after acetylcholine was only 22 %, compared with 40–44 % during splanchnic stimulation and 70 % in the glands.

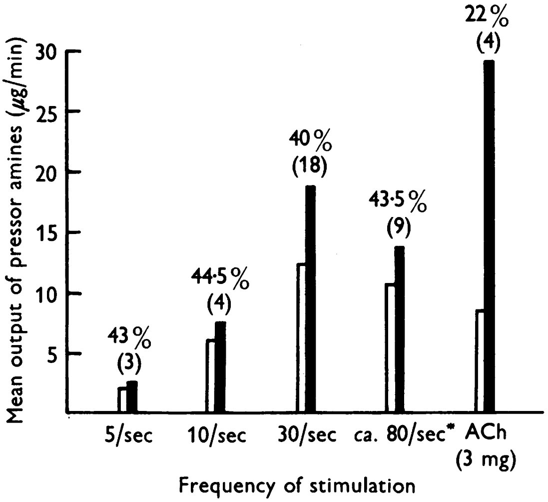

Fig. 2 Mean output of pressor amines from the adrenal glands of calves aged 3–4 weeks, obtained during nerve stimulation with different frequencies, and after the intra-arterial injection of acetylcholine: □ adrenaline; ■, noradrenaline; mean % adrenaline given above each histogram; number of observations in parenthesis. * Induction coil.

Prolonged stimulation (induction coil)

Continuous stimulation. The course of adrenal medullary secretion during 40 min continuous splanchnic nerve stimulation was followed in calves of different ages and in three adults. The mean values for adrenaline and noradrenaline output in each age-group are plotted against duration of stimulation in Fig. 3: the changes in the proportion of adrenaline in the effluent blood are shown in the upper part of each graph. In the first few days of life (Fig. 3*a*) the initial output of both amines was very low and showed no marked changes during the period of prolonged stimulation. In all other groups of calves and in the adult animals the levels of both adrenaline and noradrenaline decreased with continuous splanchnic stimulation; the higher the initial rate of secretion, the greater was the fall in rate of output during the 40 min period. But whereas in the adult the output of both amines declined at the same rate, in the calves the output of noradrenaline fell more quickly. This fall was greatest in the first 10 min of stimulation and after 40 min the output had dropped to one third of the initial level. In contrast, the adrenaline secretion changed little during the first 25 min of stimulation, but fell more quickly during the last 15 min (Fig. 3*b*–*e*). These changes are reflected in the proportion of adrenaline secreted during the stimulation period.

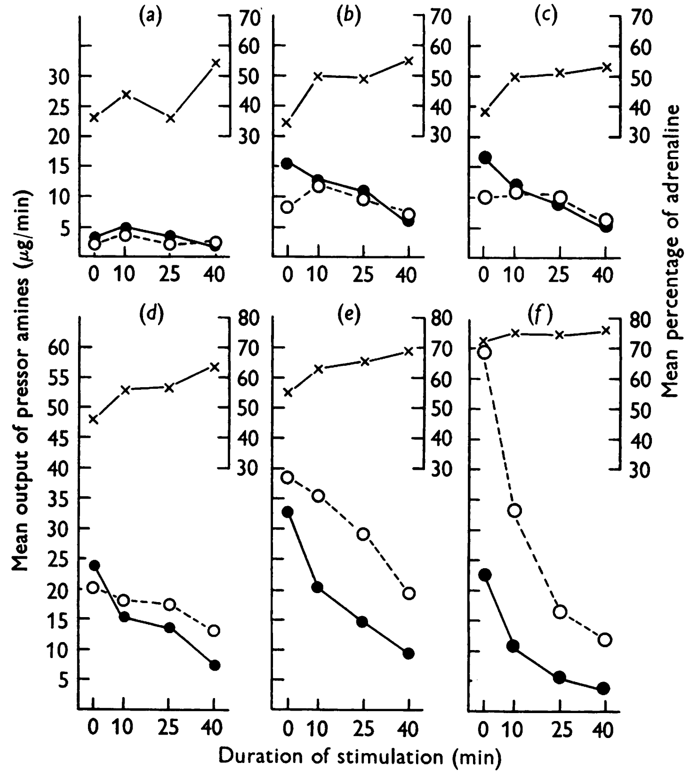

Fig. 3. Mean output of pressor amines from the bovine adrenal gland during prolonged splanchnic stimulation (induction coil). ●, noradrenaline; ○, adrenaline; ×, % adrenaline. Calves (*a*) under 1 week (9 animals); (*b*) 2–3 weeks (6 animals); (*c*) 4–5 weeks (5 animals); (*d*) 7–11 weeks (6 animals); (*e*) 15–30 weeks (9 animals). Cows (*f*) 2–3 years (3 animals).

In the adult the proportion of adrenaline in the effluent blood remained constant at 73–75 %, which is close to the corresponding values for adrenal glands. In the young animals the proportion of adrenaline rose by 13–20 %, the major increases in all groups occurring during the first 10 min of stimulation (Fig. 3*b*–*e*). Therefore, although the percentage of adrenaline in the blood initially was about 20–30 % lower than that in the glands, the final values after 40 min stimulation were only 5–10 % lower.

Intermittent stimulation. A similar change in the proportions of the two amines in the adrenal effluent blood was found when an interval of 15–20 min occurred between brief periods of stimulation. The mean rates of adrenaline and noradrenaline secretion and the corresponding figures for the percentage of adrenaline, shown in Fig. 4, are similar to those obtained during continuous stimulation in calves of comparable age (Fig. 3*b*, *c*). The rise in the percentage of adrenaline which occurred with each successive stimulation was due primarily to a fall in noradrenaline output while the rate of adrenaline secretion, after a slight initial increase, remained relatively constant.

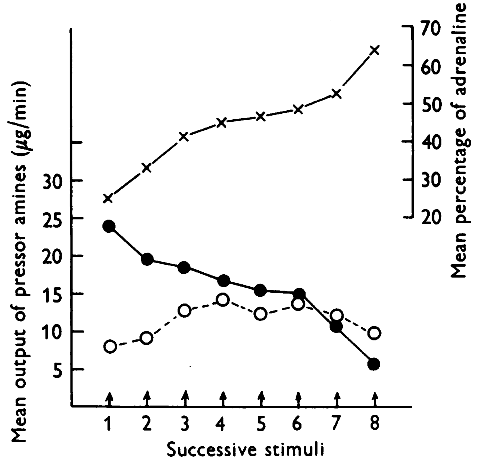

Fig. 4. The mean output of pressor amines from the adrenal glands of calves aged 3–4 weeks, during intermittent stimulation of the splanchnic nerve. Duration of stimulation ¾–2 min applied at 15–20 min intervals; rate of stimulation = 30 and *ca.* 80/sec, applied in random order. Each point represents a mean value for three or more observations. Symbols as for Fig. 3.

The amount of noradrenaline in the adrenal glands of the calves used in the preceding experiments was low compared with the adrenaline concentration (Fig. 1), and therefore it was important to determine whether lack of noradrenaline could be responsible for its rapid decline in output during prolonged stimulation. However, when the content of the left adrenal glands was investigated it was found that considerable amounts of both amines were still left after 40 min continuous stimulation; the mean level of noradrenaline was 0·98 mg/gland, while the adrenaline levels varied with age from 1·12 to 3·76 mg/gland.

Effect of acetylcholine given before and after a period of prolonged splanchnic nerve stimulation

The results given in the preceding section showed that the pressor amines in the adrenal medulla were by no means exhausted by 40 min continuous stimulation of the splanchnic nerve, yet the output of both amines fell during this period. It was possible that not all the amines in the medulla were immediately available for release and that the decline in output reflected this. Alternatively a progressive failure in transmission would explain the changes in output. In the latter event direct excitation of the medulla with acetylcholine at the end of a period of prolonged nerve stimulation should restore the original secretory levels. Experiments were accordingly performed to test this possibility.

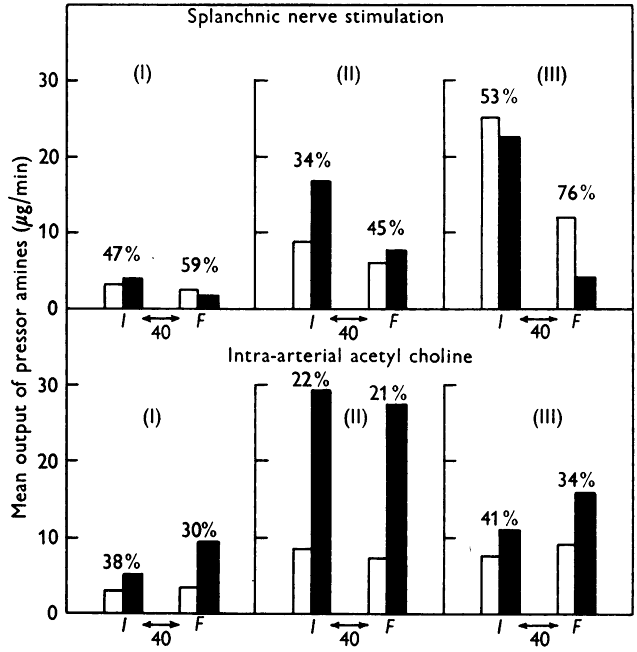

Fig. 5. Comparison between the effects of splanchnic nerve stimulation (above) and of intra-arterial injection of 1–3 mg acetylcholine (below), before and after a 40 min period of continuous nerve stimulation (←40→). *I*, mean initial output and *F*, final output of pressor amines; □, adrenaline; ■, noradrenaline. Mean % of adrenaline is given above each histogram: for further details see text.

The left adrenal gland was again subjected to 40 min continuous stimulation and the output of adrenaline and noradrenaline obtained at the beginning and end of this period was compared with the response elicited by the intra-arterial injection of acetylcholine given before and immediately after the stimulation period. Three groups of three calves, aged (i) under 1 week, (ii) 3–4 weeks, and (iii) 8–12 weeks, were used in these experiments. The mean levels of secretion obtained in each group are given in Fig. 5.

The results with prolonged splanchnic stimulation, shown in the upper part of Fig. 5, are typical of those obtained in the previous experiments, with noradrenaline output falling to a greater extent than that of adrenaline. Acetylcholine, on the other hand, induced the secretion of large amounts of noradrenaline both before and after prolonged splanchnic stimulation. The high proportion of noradrenaline released initially by acetylcholine in all three groups confirmed the results obtained previously with 3–4 weeks old calves (see Fig. 2). However, in the present experiments, involving animals of widely differing ages, the absolute levels of noradrenaline secreted varied considerably; in groups (i) and (ii) acetylcholine was a far more effective stimulus for noradrenaline release than nerve stimulation, while in the oldest group of calves the response to acetylcholine was small compared with the effects of splanchnic discharge. Despite these variations in absolute levels, the percentage of adrenaline in the effluent blood after acetylcholine was considerably lower in all groups than that obtained with nerve stimulation. At the end of the 40 min period of continuous stimulation acetylcholine, in contrast to splanchnic nerve stimulation, released as much adrenaline and noradrenaline as at the beginning. These results indicate that in glands previously subjected to prolonged splanchnic discharge both adrenaline and noradrenaline were available for release and suggest that the fall in output obtained with continuous splanchnic stimulation was due to a gradual failure in transmission.

DISCUSSION

An increase in the percentage of adrenaline in the adrenal glands with age has been found in the calf, confirming previous reports on this and other species by Holton (1951), Shepherd & West (1951) and Hökfelt (1952). The increase, from 51 % at birth to 77 % in the adult, occurred early in post-natal life, and was brought about by a rise in the total amount of adrenaline in the glands while the noradrenaline content remained unchanged. The adrenal glands were not removed until the end of the experiment, and hence any losses due to endogenous splanchnic discharge during 2–3 hr anaesthesia could not be gauged. However, all studies on the pressor amine concentration of adrenal glands necessarily involve either anaesthesia or rapid killing before the glands can be removed, and the sympathetic discharge accompanying these procedures may well induce large adrenal medullary losses.

The development of adrenal medullary function in the calf, as judged by the response to splanchnic nerve stimulation, lagged behind the changes in content. Thus, immediately after birth the medulla was comparatively unresponsive to stimulation despite the presence of apparently adequate amounts of both amines in the glands. Nervous stimulation elicited a response which was only 2–3 times the resting level of secretion, whereas a week later a twentyfold increase in output was obtained under similar conditions without any changes in glandular content.

In the older animals the proportion of noradrenaline in the adrenal effluent blood during stimulation was higher than that in the glands. This was a constant finding in calves from 1 to 30 weeks, and although the increase in glandular adrenaline with age was to some extent paralleled by a corresponding rise in adrenaline output, this relationship was obscured by the anomalous production of noradrenaline. In lambs no phase analogous to this was found either pre- or post-natally (Comline & Silver, 1958); the major increase in the percentage of adrenaline in the glands occurred in the foetus, but, in contrast to the calf, this change was closely paralleled by a rise in the proportion of adrenaline secreted on splanchnic stimulation.

In the few species in which adults have been studied a slightly greater proportion of noradrenaline is liberated on stimulation than is found in the gland, although some of the early work on the cat is at variance with this (see von Euler, 1956). In the cow this difference was only 4–6 % and was negligible compared with the discrepancy of 20–30 % in the calf. Nevertheless, the fact that a high proportion of noradrenaline has been found in the effluent blood of other species suggests that the phenomenon in the calf portrays a developmental phase which persists, to a limited extent, in adult life.

More detailed analysis of the response to splanchnic stimulation in calves showed that the characteristically high output of noradrenaline was obtained only during the initial periods of stimulation; the response rapidly waned with repeated or prolonged stimulation, while the amount of adrenaline released often increased and only fell during the last 15 min of prolonged stimulation. In the adult a fall in output of both amines occurred during continuous stimulation, but the percentage of adrenaline in the effluent blood remained unchanged. This finding in the cow agrees with the observations of Outschoorn (1952) on the adult cat.

The fall in pressor amine output with prolonged splanchnic stimulation could be attributed either to a progressive failure of transmission at the splanchnic nerve endings, or to a decrease in the concentration of available amines in the medulla, or to a combination of both these factors. The results with acetylcholine showed that there was no lack of releasable material at the end of the 40 min period of stimulation in the calf. In fact acetylcholine was a particularly effective stimulus, and it released an even greater proportion of noradrenaline than did splanchnic stimulation. This finding was not in accord with previous work on adult cats, in which both acetylcholine (Outschoorn, 1952; Butterworth & Mann, 1957) and KCl (Vogt,

1952) had the same effect as nerve stimulation, and released approximately the same proportion of adrenaline into the effluent blood as that found in the glands. In the calf, on the other hand, with only 30 % noradrenaline in the glands as much as 80 % noradrenaline was released after acetylcholine, but only 50–60 % during nerve stimulation. These results suggested that the injected acetylcholine was not exciting the same cells as the acetylcholine released from the splanchnic nerve endings; for if the sites of action had been the same, then the proportions of the two amines released should have remained constant. The discrepancy between the effects of splanchnic stimulation and intra-arterial injections of acetylcholine can be explained, at least in part, if it is assumed that some of the noradrenaline-releasing cells in the medulla of immature animals are not directly excitable by nerve stimulation. These cells might, however, show a phenomenon akin to the sensitivity of denervation i.e. extreme sensitivity to acetylcholine until the tissue became fully innervated.

Histo-chemical evidence for separate adrenaline and noradrenaline secreting cells in the medulla has been provided by the work of Hillarp & Hökfelt (1954), and Eränkö (1955*a*, *b*), but there is as yet no direct histological evidence for their separate innervation, although the separate nervous control of adrenaline and noradrenaline release has been shown by hypothalamic stimulation (Redgate & Gelhorn, 1953; Folkow & von Euler, 1954). The rate of development of innervation in the immature adrenal gland is not known, although it may well be very slow; in human sympathetic ganglia development is not complete even at seven years after birth (Gairns & Garven, 1959).

The above hypothesis would go far to explain many of the results obtained in young calves, such as the preferential release of noradrenaline by acetylcholine, the discrepancy between content and initial output during nerve stimulation, and the smaller response to acetylcholine at 2–3 months of age, at a time when the effects of splanchnic stimulation have become more pronounced. If it is assumed that during nerve stimulation the initial output of the transmitter is sufficiently high to excite many of the noradrenaline cells which are not directly innervated, the decline in the rate of noradrenaline secretion on continuous stimulation would be explained by a gradual decrease in the amount of transmitter liberated by each nerve impulse. However, certain anomalies remain; it is not easy to understand why transmission should fail so readily with successive stimulations at 15–20 min intervals, for the output of noradrenaline drops even under these circumstances. It is possible that in the young animal with a cut splanchnic nerve only a limited amount of transmitter is available, and that at relatively high rates of stimulation the output rapidly falls to a level which is too low to excite the majority of non-innervated cells. The hypothesis also offers no explanation for the results with calves under one week of age. In these animals the small secretory response elicited by nerve stimulation suggests that innervation may be incomplete at this stage, yet there was no evidence for the increased sensitivity to acetylcholine which would be expected under these circumstances. There is also the problem of the low output of adrenaline compared with its relatively high concentration in the medulla. The apparent inability to release much of this amine either in response to nerve stimulation or to acetylcholine suggests that much of the adrenaline extractable from the glands may not be immediately available for release.

SUMMARY

1. The pressor amine content and secretory capacity of the adrenal gland were determined in calves aged from 3 hr to 220 days, and in pregnant and non-pregnant cows 2–3 yr old.

2. In the calves the percentage of adrenaline in the glands rose with age from 51 to 75 %, while 77 % was found in the adult. The total adrenaline per gland rose sharply from about the tenth day of life, while the noradrenaline content remained constant during the period investigated.

3. In the first fews days of life the output of pressor amines during stimulation of the peripheral end of the splanchnic nerve was only 2–3 times the resting level of secretion.

4. In all older calves a brief period of splanchnic stimulation resulted in the liberation of large amounts of both amines, which increased with age. The proportion of noradrenaline secreted was invariably higher than that in the glands and was unaffected by altering the frequency of stimulation.

5. Prolonged splanchnic nerve stimulation led to a rapid fall in the output of noradrenaline, while the decline in adrenaline output was less pronounced and slower in onset. This resulted in an increase in the percentage adrenaline in the effluent blood during the stimulation period. Essentially the same results were obtained when the nerve was excited intermittently.

6. The percentage of adrenaline secreted in response to splanchnic nerve stimulation in the adult was only about 5 % below that in the glands. The output of both amines fell with prolonged stimulation, but the proportions remained unchanged.

7. In the calf a higher proportion of noradrenaline was released by the intra-arterial injection of acetylcholine than by nerve stimulation. The response to acetylcholine at the end of 40 min splanchnic stimulation

was as high as that at the beginning of the experiment, which suggested that transmission failure rather than lack of available amines was primarily responsible for the fall in output during prolonged stimulation.

8. The anomalies in the development of adrenal medullary function in the calf are discussed, and a hypothesis is advanced which could explain many of the discrepancies between output of adrenaline and noradrenaline during nerve stimulation and acetylcholine injection, and the glandular concentration of these amines.

I should like to thank Dr R. S. Comline for advice and criticism, Dr D. A. Titchen for help with the cows, and G. Baker, M. Smith and P. Hughes for technical assistance. This work was begun during the tenure of a personal research grant from the Agricultural Research Council, by whom part of the expenses were defrayed.

REFERENCES

BUTTERWORTH, K. R. & MANN, M. (1957). The release of adrenaline and noradrenaline from the adrenal gland of the cat by acetylcholine. *Brit. J. Pharmacol.* **12**, 422–426.

CELANDER, O. (1954). The range of control exercised by the sympathico-adrenal system. *Acta physiol. scand.* **32**, Suppl. 116.

COMLINE, R. S. & SILVER, M. (1958). Response of the adrenal medulla of the sheep foetus to asphyxia. *Nature, Lond.*, **181**, 283–284.

CRAWFORD, T. B. B. & OUTSCHOORN, A. S. (1951). The quantitative separation of adrenaline and noradrenaline in biological fluids and tissue extracts. *Brit. J. Pharmacol.* **6**, 8–19.

DUNÉR, H. (1953). The influence of the blood glucose level on the secretion of adrenaline and noradrenaline from the suprarenal. *Acta physiol. scand.* **28**, Suppl. 102.

ELMES, P. C. & JEFFERSON, A. A. (1942). The effect of anaesthesia on the adrenaline content of the suprarenal glands. *J. Physiol.* **101**, 355–361.

ERÄNKÖ, O. (1955*a*). Distribution of fluorescing islets, adrenaline and noradrenaline in the adrenal medulla of the hamster. *Acta endocr., Copenhagen*, **18**, 174–179.

ERÄNKÖ, O. (1955*b*). Distribution of fluorescing islets, adrenaline and noradrenaline in the adrenal medulla of the cat. *Acta endocr., Copenhagen*, **18**, 180–188.

FOLKOW, B. & VON EULER, U. S. (1954). Selective activation of noradrenaline and adrenaline producing cells in the cat's adrenal gland by hypothalamic stimulation. *Circulation Res.* **2**, 191–195.

GADDUM, J. H. & LEMBECK, F. (1949). The assay of substances from the adrenal medulla. *Brit. J. Pharmacol.* **4**, 401–408.

GAIRNS, F. W. & GARVEN, H. S. D. (1959). Sympathetic ganglion cells in infants and young children. *J. Physiol.* **148**, 44–45*P*.

HILLARP, N. A. & HÖKFELT, B. (1954). Evidence of adrenaline and noradrenaline in separate adrenal medullary cells. *Acta physiol. scand.* **30**, 55–68.

HÖKFELT, B. (1952). Noradrenaline and adrenaline in mammalian tissues. *Acta physiol. scand.* **25**, Suppl. 92

HOLTON, P. (1951). High concentration of noradrenaline in calves' suprarenals. *Nature, Lond.*, **167**, 858–859.

LUND, A. (1951). Release of adrenaline and noradrenaline from the suprarenal gland. *Acta pharm. tox., Kbh.*, **7**, 309–320.

OUTSCHOORN, A. S. (1952). The hormones of the adrenal medulla and their release. *Brit. J. Pharmacol.* **7**, 605–615.

RAPELA, C. E. (1956). Differential secretion of adrenaline and noradrenaline. *Acta physiol. latinoamer.* **6**, 1–14.

REDGATE, E. S. & GELHORN, E. (1953). Nature of sympathetico-adrenal discharge under conditions of excitation of central autonomic structures. *Amer. J. Physiol.* **174**, 475–480.

SHEPHERD, D. M. & WEST, G. B. (1951). Noradrenaline and the suprarenal medulla. *Brit. J. Pharmacol.* **6**, 665–674.

SILVER, M. (1954). The output of adrenaline and noradrenaline from the adrenal glands of the calf. *J. Physiol.* **125**, 45–46*P*.

VOGT, M. (1952). The secretion of the denervated adrenal medulla of the cat. *Brit. J. Pharmacol.* **7**, 325–330.

VON EULER, U. S. (1956). *Noradrenaline.* Springfield, Illinois: Thomas.

VON EULER, U. S. & FLODING, I. (1955). A fluorimetric micro-method for differential estimation of adrenaline and noradrenaline. *Acta physiol. scand.* **33**, Suppl. 118, 45–56.

WEST, G. B., SHEPHERD, D. M., HUNTER, R. B. & MACGREGOR, A. R. (1953). The function of the organs of Zuckerkandl. *Clin. Sci.* **12**, 317–325.