Effects of four peptides (physalaemin, d-d-oxytocin, d-d-Arg-vasotocin and d-d-Arg-vasopressin), when treated by chymotrypsin or trypsin, on the excitability of 2 giant neurones are summarized in the table, in comparison with those of these peptides untreated. In these cases, we examined solutions of these peptides in concentrations of 2×10^{-4} kg/l.

Figure 1 demonstrates effects of these enzyme-treated peptides on TAN excitability. Physalaemin lost its excitatory effect on this neurone after trypsin treatment. Physalaemin, when treated by chymotrypsin, unexpectedly showed an inhibitory effect on the same neurone, opposite to that of untreated physalaemin. 3 peptides analogous to neurohypophyseal hormones (d-d-oxytocin, d-d-Arg-vasotocin and d-d-Arg-vasopressin) had no effect on the TAN, whether treated or not.

Figure 2 shows effects of these treated peptides on the PON. D-d-oxytocin and d-d-Arg-vasotocin continued to show their excitatory effect on the PON after chymotrypsin treatment for 6 h. However, d-d-Arg-vasotocin lost its effect after trypsin treatment. Physalaemin and d-d-Arg-vasopressin, whether treated or not, had no effect on the PON.

In spite of its remarkable excitatory effect of physalaemin on the TAN[2], this substance, when treated by trypsin, no longer showed its effect. As shown schematically in the table, trypsin must cleave the peptide bond of 'Phe-Lys' of physalaemin. Konishi and Otsuka[11] reported that several hypotensive peptides including physalaemin commonly affected the ventral root potential of the frog spinal cord, and assumed that a common C-terminal sequence (-Phe-X-Gly-Leu-Met-NH_2) of these peptides caused the depolarization of spinal motoneurones. We could not confirm their hypothesis with our experimental material, since a fragment of physalaemin, 'Phe-Tyr-Gly-Leu-Met-NH_2', which is considered to be produced by trypsin treatment, showed no effect on the TAN. We can say that a certain amino acid sequence of physalaemin, longer than the above-mentioned fragment, is necessary to produce the effect of untreated physalaemin on this neurone. After treatment with chymotrypsin, not only physalaemin's excitatory effect on the TAN disappeared, but also an inhibitory effect on the same neurone was apparent. We are convinced that some new peptide showing the inhibitory effect on the TAN was produced by the chymotrypsin treatment, although we cannot conclude whether inhibitory active sites of this new peptide are identical with those of untreated physalaemin in producing the slight inhibitory effect on the same neurone.

Previously we reported[3] that d-d-oxytocin and d-d-Arg-vasotocin, but not d-d-Arg-vasopressin, showed an excitatory effect on the PON. Trypsin treatment of d-d-Arg-vasotocin made its effect disappear. Since trypsin must cleave the peptide bond of 'Arg-Gly' of this substance, glycylamine (Gly-NH_2) in the C-terminal is indispensable in producing the effect. On the other hand, 'Ile' in the second position of the amino acid sequence of this substance is also indispensable to produce the effect, since d-d-Arg-vasopressin, having 'Phe' instead of 'Ile' of d-d-Arg-vasotocin, showed no effect on the same neurone. Therefore, it is assumed that almost complete amino acid sequences of d-d-oxytocin and d-d-Arg-vasotocin are necessary to produce the effect on the PON. After the chymotrypsin treatment for 6 h in the condition described above, the 2 peptides analogous to neurohypophyseal hormones continued to show their effect. The amino acid sequences of d-d-oxytocin, d-d-Arg-vasotocin and physalaemin necessary to produce effects on our experimental materials have to be ascertained in a further study.

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Development of noradrenaline uptake in the human foetal heart

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20 July 1976

Summary. The development of NA-uptake mechanisms in the human foetal heart start at the same time as the adrenergic terminals were visible. The highest ^{3}H-NA values in the human foetal heart were only 25–30% of those found in the mouse heart.

The adrenergic nervous system develops later in the human foetal heart than in many other peripheral tissues according to mainly morphological examinations[2–7]. Present knowledge of the functional development of the adrenergic nervous system in the human foetal heart is more limited. The adrenergic receptors are believed to respond at 9 weeks of gestation to adrenaline[8], but not before 13 weeks of gestation to field stimulation[9], and the metabolic inactivation mechanisms are believed to be of more significance than the uptake mechanisms in the second trimester of pregnancy[10].

In the present work the functional development of the adrenergic nervous system was examined by estimating the ^{3}H-noradrenaline (^{3}H-NA) uptake in the isolated atria and ventricles of human foetal heart as compared with the mouse atria and ventricles, and with the development of the adrenergic nerve fibres observed histochemically.

Material and methods. Foetal hearts were obtained from legal terminations of pregnancy performed by evacuation or by hysterotomy. In all of the 48 cases, the premedi-

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cation was the same: atropine, pethidine and promethazine. The evacuations were performed under paracervical local anaesthesia (lidocain 1%) and the hysterotomies under general anaesthesia using a nitrous oxide-oxygen mixture after intravenous barbiture induction. The foetuses ranged in size from 8 to 20 weeks of gestation according to the obstetric history and measurement of the foetal length from the crown of the head to the rump. The weights of the female Albino Swiss mice were 18–25 g. Both the right and the left atria from each heart were dissected free of the ventricles and each was kept in Krebs-Ringer solution, aerated with a gas mixture of 95% O_2 and 5% CO_2 and thermostatically controlled at 37.5 °C. l-^{3}H-NA, specific activity 10.3 Ci/mmol (Amersham Radiochemical Centre, England) was added to the organ bath in a concentration of 10^{-7} M. The incubation time was 30 min in the bath containing l-^{3}H-NA and thereafter 10 min in an activity-free Krebs-Ringer solution[11]. The ^{3}H-activity taken by tissues was estimated by liquid scintillation counting: the weighed and dried tissues were oxygenated by a sample oxidizer introduced by Kaartinen[12] and the quantity of tritiated water produced was determined by liquid scintillation counting. The recovery of this method is 99.0 ± 2.4% (mean ± S. D.)[10].

The metabolites of l-^{3}H-NA taken up by tissues were separated by paper chromatography using Whatman No. 1 paper and n-butanol: formic acid: water (70 : 12 : 15) as a solvent system, and the percentage of different metabolites was then estimated by liquid scintillation counting after oxygenation as was the total ^{3}H activity. (For details see Saarikoski[10]). The data in figure 1 show only the intact ^{3}H-NA. The formaldehyde-induced histochemical fluorescence of the adrenergic nerve fibres was examined in part of the atria using stretch preparations[11, 13]. The statistical analysis was done using Student's t-test.

Results. Figure 1 shows the uptake of l-^{3}H-NA by foetal heart tissue. The values are quite low at 8–13 weeks of gestation, after which the capacity for NA uptake in the heart atrium and ventricle showed a tendency to rise. The highest values were seen in the atria and ventricles of 16–20-week-old foetuses. These mean values in NA-concentration 10^{-7} M were very significantly higher than the

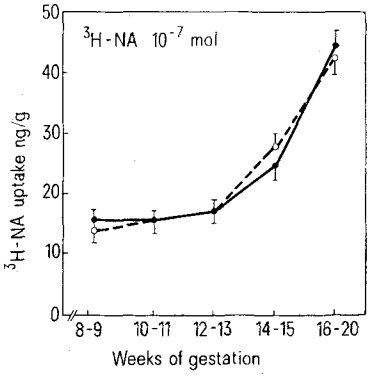

Fig. 1. Uptake of ^{3}H-NA in atria (○) and ventricles (●) of human foetuses. The incubation time was 30 min, followed by a 10 min reincubation in fresh buffer. Mean ± S. E. M. values for 8–10 determinations.

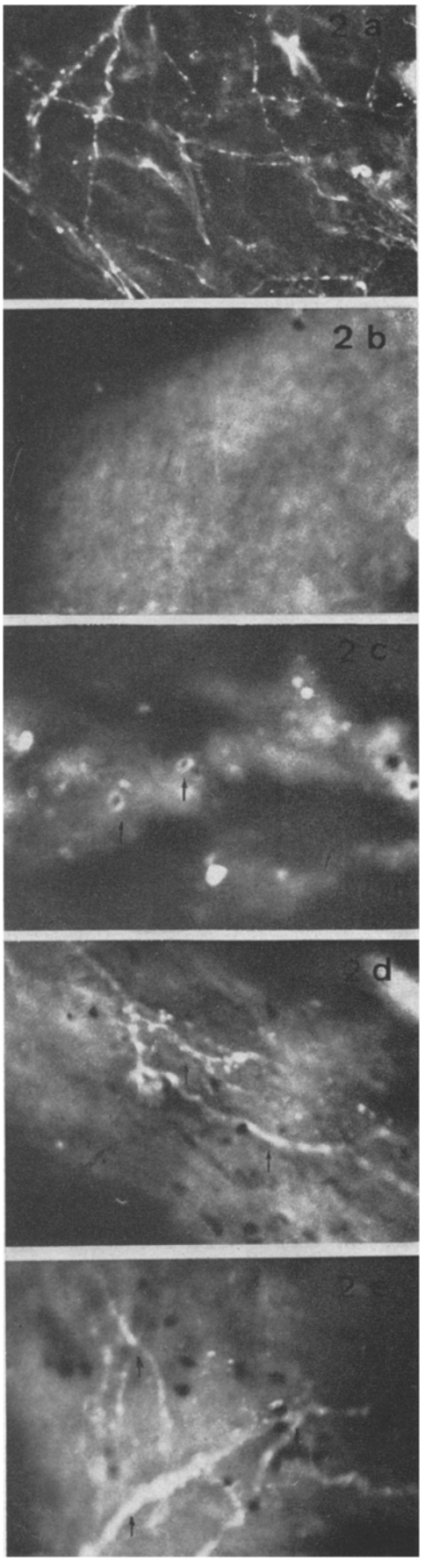

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Fig. 2. Histochemical demonstration of adrenergic nerves in stretch preparations of mouse atrium (2a), of human foetal atrium at 10 weeks of gestation (2b), at 12 weeks of gestation (2c), at 14–15 weeks of gestation (2d) and at 17–18 weeks of gestation (2e).

mean values at the stages 8–9, 10–11 or 12–13 weeks of gestation ($p<0.001$) and significantly higher than the values at 14–15 weeks of gestation ($p<0.01$–0.02). The ^{3}H-NA uptake values for the mouse atria and ventricles in NA-concentration 10^{-7} M were 186 ± 21 ng/g and 143 ± 15 ng/g (mean ± S. E. M. of 10 determinations). The highest mean values, 42 and 44 ng/g found in the human foetal heart, were only about one-quarter to one-third of those found in the mouse atria and ventricles.

Histochemistry. In the mouse atria (figure 2a), quite a thick net of adrenergic terminals was seen in contrast to the human foetal heart preparations (figures 2b–e). In some of the foetal atria at 12–13 weeks of gestation, a few terminals were found around the coronary vessels and in the hearts of the foetuses at 14–15 weeks of gestation and thereafter a few fluorescent adrenergic terminals could be seen in the myocardial tissue.

Metabolites. It was found (table) that the ^{3}H activity taken up into the mouse heart was non-metabolized ^{3}H-NA in a higher proportion than that in the foetal heart, in which the quantity of deaminated metabolites proved to be rather high, especially in the older foetuses.

^{3}H-NA and its metabolites in human foetal atria and mouse atria in vitro

	Foetal heart at 8–13 weeks	Foetal heart at 14–20 weeks	Mouse heart
NA	82.4±2.7	81.5±2.6	97.1±0.5
NMN	4.8±1.2	2.9±0.9	0.5±0.2
DHPG-DHMA	6.1±1.2	11.9±1.8	2.2±0.4
MHPG-VMA	3.8±0.7	2.1±0.3	0.2±0.0
F	3.0±0.6	1.6±1.0	0.1±0.0

NA = noradrenaline, NMN = normetanephrine, DHPG = 3,4-dihydroxyphenylglycol, DHMA = 3,4-dihydroxymandelic acid, MHPG = 3-methoxy-4-hydroxyphenylglycol, VMA = vanillylmandelic acid, F = the ^{3}H activity spreading outside of the metabolites on the paper. Mean ± S. E. M. of 8–10 determinations. Quantity of metabolites expressed as percentage of total activity.

Discussion. The present results show that the uptake of ^{3}H-NA into the human foetal heart tissues was rather low during the first half of gestation as compared with the uptake of ^{3}H-NA into the adult mouse heart under the same experimental conditions. Some gradual increase in the uptake was found during the first half of the foetal life, and the development of neural NA uptake mechanisms was found to start at the beginning of the second trimester of pregnancy. The histochemical findings were in good agreement with this: formaldehyde-induced fluorescence terminals were seen after 12–13 weeks in the coronary arteries and after 14–15 weeks of gestation in the heart muscle. The present findings are also in good agreement with the findings of Walker[9], who observed that the atria from human foetuses of less than 13 weeks of gestation did not respond to field stimulation. There was clearly a higher percentage of non-metabolized ^{3}H-NA in the activity taken by the mouse heart than in that taken by the foetal heart. This finding suggests that the metabolic inactivation mechanisms play a greater part in the immature human foetal adrenergic nervous function than in the mature mouse adrenergic nervous function.

The present results suggest that during the first trimester of human foetal life the neural mechanisms are of lesser importance, and the functional development of adrenergic nervous mechanisms starts at the same time as the morphological development of adrenergic terminals in the heart at the beginning of the second trimester of pregnancy.

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Acidic metabolite of prednisolone[1]

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Summary. The metabolic fate of the 17β-ketol side chain of (21-^{3}H) prednisolone was studied with an enzyme preparation from male golden hamster liver. The acidic metabolite of prednisolone was identified by mass spectrometry as 11β, 17α,20ξ-trihydroxy-3-oxo-1,4-pregnadien-21-oic acid. The enzyme showed substrate specificity, depending on the nature of substituent on the steroid nucleus.

Evidence has been presented to support the existence of acidic corticosteroid metabolites called 'polar compounds' or 'bicarbonate extractable materials' in human urine[2,3]. 2 classes of acidic cortisol metabolites characterized by having a glycolic or glyoxylic acid side chain in human urine have been identified; they have been shown to constitute 5–25% of the administered cortisol radioactivity[4]. The formation of uncharacterized acidic metabolites of progesterone and deoxycorticosterone by the mitochondrial fraction of rabbit liver was recently reported[5]. We have presented the purification of an enzyme from hamster liver which is responsible for the oxidation of corticosteroid to steroidal-20-ol-21-oic acid[6]. In this communication the isolation and characterization of an acidic metabolite of prednisolone are described.

Steroids were bought from Research Plus Laboratory, Inc., Denville, N. J. NaB^3H_4 was purchased from New England Nuclear Corp., Boston, Mass. 21-Tritiated prednisolone was synthesized from 21-dehydroprednisolone

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