estimated that the profile shapes in glass tubes above the 40 μm diameter was nearly parabolic at all $\overline{U}$ (average tube velocity/diameter) above $\overline{U} = 6\ sec^{-1}$. On the other hand, the crossed-beam Doppler method employed in the present study has the advantage that the beat signal is obtained only in the clearly defined small volume (10 μm in the cross-section diameter and 15 μm in the depth) in which the 2 incident beams are crossed. The results obtained by using that method is expected to be little affected by the process of averaging the whole profile. The present measurements were performed in the venule in vivo and the estimated $\overline{U}$ is 9 sec^{-1} nearly equivalent to the critical value of 6 sec^{-1} below which the flow profile changes according to BAKER and WAYLAND. Thus, the velocity profile in the venule seems to deviate slightly from the Newtonian flow. In the frog venule, a pulsatile blood flow was also often observed and the periodic changes of flow velocity were weaker than those in mammalian mesentric venule[7]. The present measurements were made in a venule where the pulsatile changes of flow velocity was almost undetectable.

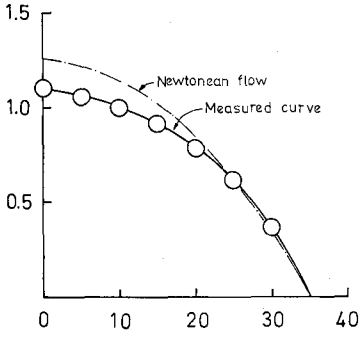

Fig. 3. A profile of the blood flow velocity in the venule. Peak velocity, obtained from Figure 2, on the abscissa against the distance of the probing point from the centre of the flow. The broken line indicates a parabolic flow for the flow rate estimated from the flow profile experimentally obtained.

Finally it must be mentioned that the response and reproducibility of the present method were ascertained through measurements of the capillary blood flow on a repetition of exposing the frog web to normoxic (20% O_2), hypoxic (0.5% O_2) and hypercapnic (80% CO_2) gas mixtures. The broken line in Figure 1 indicates a vinyl bag covering the frog and a part of microscope, into which the above gas mixtures were blown. The output signals of the microscope showed a clear decrease of the blood flow velocity by exposing the web for 6 min to both the hypoxic and hypercapnic gases. Once the gas in the bag was replaced with normoxic gas, the signals showed a smooth recovery of the blood flow velocity to the initial level.

Summary. The flow velocity profile in the venule of frog web was measured by using a laser Doppler microscope of a crossed-beam which permitted a measurement of flow velocity in a clearly defined small volume. The flow velocity profile in the venule seems to deviate slightly from the Newtonian parabola.

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Noradrenaline Synthesis in Human Fetal Heart[1]

The adrenergic system is important for the regulation of the fetal circulation[2], for the maintenance of the cardiac function under conditions of asphyxia[3], and for the adaptation of the fetal cardiovascular system to extrauterine life[2]. Noradrenaline-containing nerve terminals have been visualized by the histochemical method of Falck and Hillarp in human fetal hearts from the 11–12th gestational weeks the innervation starting in the atria and reaching the ventricles a few weeks later[4]. Hitherto, it has been uncertain whether the noradrenaline in the human fetal heart is of extra-cardiac origin extracted from the circulating blood or is synthesized within the adrenergic components of the heart itself. This study investigated the capacity of the human fetal heart to synthesize noradrenaline.

Material and methods. 6 fetal hearts from human fetuses (crown-heel length 11–23 cm) were obtained from therapeutic interruptions of pregnancy by laparotomy. The hearts were dissected and placed in a modified Krebs-Ringer solution containing glucose 3.3 m*M* and low molecular weight dextran 2.5% (w/v), aerated with 95% O_2 and 5% CO_2 giving the solution a pH of 7.35 ± 0.06. A coronary perfusion via aorta was started within a few min after the interruption with the Krebs-Ringer solution, and the hearts were placed in a thermostated (37 ± 0.2 °C) jacketed glass bath. After an equilibration time of 15 min, the perfusion was changed to the following solution: To 200–400 ml of the Krebs-Ringer solution was added 10-20 μCi L-tyrosine-C^{14} (243 mCi/m*M*) and 3.7 mg nonradioactive L-tyrosine (in experiment D, 18.1 instead of 3.7 mg L-tyrosine was used). Shortly before

Table I. Data of the human fetal hearts

Case	Gestational age (weeks)	Fetal crownheel length (cm)	Rate of perfusion (ml/min)	Heart activity during perfusion
A	13	11	2.7	normal
B	14	11	1.2	slow
C	16	16	2.3	normal
D	19	20	4.4	normal
E	20	22	6.3	normal
F	23	23	4.0	normal

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Table II. Radioactivity of noradrenaline in perfused human fetal hearts and calculated values for formation rates of noradrenaline (dopamine is not separated, see Methods)

Case	Tyrosine in perfusate specific activity (cpm/nmol)	Noradrenaline cpm/g of tissue of I atrium II ventricle III mediastinum		Perfusion time (h)	Rate of noradrenaline formation (μg/g of tissue/h)	
A	2166	I	1949	1.25	I	0.122
		II	2528		II	0.158
		III	2125		III	0.133
B	2166	I	8566	2.0	I	0.334
		II	2394		II	0.093
		III	5127		III	0.200
C	1085	I	1590	2.5	I	0.099
		II	708		II	0.044
		III	1758		III	0.110
D	444	II	2039	1.5	II	0.517
		III	414		III	0.105
E	2166	I + II	1461	1.0	I + II	0.114
		III	893		III	0.070
F	2122	I	2191	1.7	I	0.109
		II	1391		II	0.065
		III	595		III	0.028

the perfusion began, 10 μ*M* ascorbic acid was added to the solution to prevent oxydation of the catecholamine. The perfusion pressure was kept constant during the experiment (30–35 mm Hg), the perfusion with tyrosine lasted 60–150 min with a rate ranging from 1.2 to 6.25 ml/min in the various hearts. The perfusion fluid was collected and not recycled. During the perfusion, 5 of the hearts showed vigorous spontaneous contractions; in one heart (No. B), the beating was slow and weak (Table 1).

Preliminary studies in 2 hearts showed that, after 1 h of perfusion, the noradrenaline content was 20–30% lower than in 11 non-perfused hearts. No correction for this decrease was made at the calculation of the rate of noradrenaline formation.

After the perfusion, the heart was divided into atria, ventricles, and extracardiac tissue at the base of the heart including the most proximal parts of the great vessels (here denoted 'mediastinum'). The various tissue parts were weighed, frozen, kept at −70 °C, and analyzed within 6 days.

The specimens were homogenized in ice-cooled perchloric acid. After centrifugation (30,000 × *g*, 10 °C, 10 min) the catecholamines were absorbed from the clear supernatant to alumina and eluated with 0.05 *N* HCl. A small aliquot was taken for fluorometric determination of catecholamines according to Anton and Sayre[5]. Pilot studies revealed that, with the methods used, practically no adrenaline and only traces of dopamine could be found in the human fetal heart; consequently, only the content of noradrenaline was measured in the eluate. Spector et al.[6] demonstrated that, in the perfused guinea-pig heart, apart from noradrenaline, small amounts of dopamine were produced. Therefore 15 μg noradrenaline and 15 μg dopamine were added to the remainder of the eluate and the mixture was absorbed to a Dowex-50 (H^+) column. Noradrenaline and dopamine were eluated separately according to the method of Bertler et al.[7], and the aliquots were dissolved in Bray's solution[8] for scintillation counting. In 3 experiments, the radioactivity of the dopamine fraction was less than 8% of that in the noradrenaline eluate; therefore the separation of dopamine from noradrenaline was omitted in the following experiments, where the results obtained of the formation rate of noradrenaline thus include that of dopamine. The rate of noradrenaline formation was calculated according to the equation given by Spector et al.[6].

For further identification of noradrenaline, in 2 perfusion experiments the major part of the eluated material from the alumina columns was lyophilized and chromatographed (Whatman No. 1, descending, *n*-butanol saturated with 1 *N* HCl) after addition of 15 μg dopamine and 15 μg noradrenaline. After chromatography, the catecholamines were localized on the chromatogram with ethylendiamine. Radioactivity was only registered in the noradrenaline area.

Results. Table II summarizes the results of 6 experiments. The mean rate of formation in the atria, ventricle, and mediastinum was 0.166, 0.175, and 0.108 μg noradrenaline/g tissue/h, respectively. There was no consistent difference of formation rate between the parts of the heart investigated. Within the period of fetal life studied (13–23 week of gestation), the formation rate was not correlated to the gestational age. In one experiment (No. B), the contractile activity of the heart was slow and weak, most probably owing to hypoxia before the start of perfusion; the rates of noradrenaline synthesis in this heart did not exceed the range of values in the whole material of hearts.

Discussion. The estimated rate of noradrenaline synthesis in human fetal hearts accords well with the noradrenaline synthesis rate estimated in intact mammalian heart (0.11–0.17 μg/g/h)[9,10]. The rate of synthesis

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of noradrenaline has been proved to be dependent on the concentration of the perfused precursor[11]. The concentrations used in the present experiments ($0.5-1 \times 10^{-4} M$ tyrosine) were very close to tke levels that give a maximum rate of synthesis in the guinea-pig heart[11]. Despite the network of adrenergic nerve fibres in the atria being denser than in the ventricles[4], there was no corresponding difference in the formation rate of noradrenaline between those regions of the fetal heart.

A small part (less than 8%) of the radioactive catecholamine recovered in the heart after the perfusion was, in some hearts, identified as dopamine. In several species of laboratory animals, dopamine in the heart seems to be localized to 'small intensely fluorescent cells'[12]. Such cells have been found also in the human fetal heart from the 10th week of gestation in the atria and around the base of the heart[4]. These cells are not confined to the ganglia, and it is interesting that high doses of nicotine have been found to produce a propranolol-blockable excitation of human fetal atria at an early stage where field stimulation had no effect, suggesting a non-neuronal store of catecholamine[13].

The ability to form noradrenaline early in the development of the human fetal heart seems of biological significance by contributing to the integrity of the intrinsic catecholamine stores in the intact fetus. Furthermore, the capacity here demonstrated of the isolated fetal heart to form noradrenaline from tyrosine seems to be a factor of importance for the feasibility of experimental work on isolated hearts. Noradrenaline is known to be released locally by electrical stimulation[14] and by reduced oxygen tension[15], and part of the cardiac noradrenaline content is washed out by perfusion in amounts dependent on the perfusion rate[11]. Noradrenaline lost by degradation by MAO and COMT and/or washed out by the perfusion might be replaced in the in vitro heart preparation not only by the various uptake mechanisms[16] but also by the formation of new catecholamines from a dietary precursor. In fact, inhibitors of tyrosine hydroxylase, which turn off endogenous noradrenaline synthesis, decrease the levels of endogenous noradrenaline in adrenergically innervated tissue[17]. Both fetal and adult hearts in isolated preparations have been extensively used in physiological work. As the presence of noradrenaline contributes to the cardiac ability to withstand asphyxia[3], the intact capacity to form the neurotransmitter from available precursors might be essential for the heart to remain in a functional state during longtime studies of isolated hearts, especially under conditions that promote the release of endogenous noradrenaline.

Summary. The capacity of noradrenaline synthesis was investigated in 6 isolated human fetal hearts (13–23 gestational week). The mean rate of transforming perfused labelled tyrosine to noradrenaline in atrial, ventricular, and mediastinal tissue was 0.175, 0.168, and 0.108 μg/g tissue/h, respectively.

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Nonsympathetic Dilator Innervation of Cat Cerebral Arteries

In 1928 FORBES and WOLFF[1] reported that stimulation of the vagus nerves in the neck caused pial artery dilation in the cat. This observation was confirmed by COBB and FINESINGER[2] and CHOROBSKI and PENFIELD[3], who also provided anatomical and physiological evidence that cerebral blood vessels receive a dilator innervation. Subsequently, although interest has been focussed mainly on sympathetic vasoconstrictor innervation, based on the orientation of agranular vesicles in surviving nerve terminals in sympathetically denervated tissue[4–6] and positive histochemical staining for acetylcholinesterase[5, 7], several authors have suggested that cerebral blood vessels are richly innervated by cholinergic neurons.

Several in vivo studies support the idea that cholinergic nerves may be involved in cerebral vasodilation. This conclusion is based on observations that intravascular acetylcholine dilates pial arteries or increases blood flow[1, 8], that pial artery dilation follows the local application of carbachol[9, 10], and that autoregulatory cerebral vasodilation following a decrease in systemic arterial blood pressure was blocked by atropine[11]. Not all observations, however, support such a conclusion; neurogenic vasodilation of dog cerebral arteries following electrical stimulation of the trigeminal nerve and medulla was not atropine sensitive[12] and no significant change in cerebral blood flow resulted when the petrosal nerve was stimulated[13]. It is also well known from studies at other sites that an atropine-sensitive response to parasympathomimetic drugs is by no means an indication of cholinergic innervation.

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