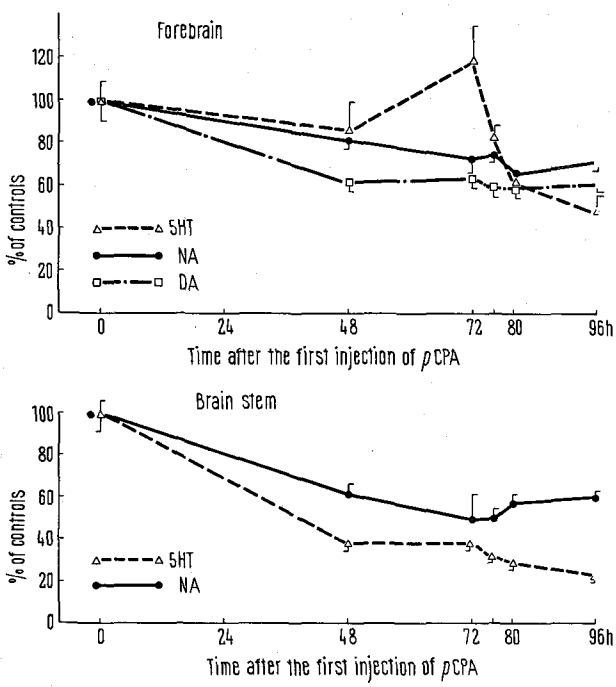
*p*CPA also induced a long-lasting decrease of catecholamines. Both NE and DA were reduced to 60–70% in the forebrain as well as NE in the brain stem (50–60%). The effect on NE seemed to be more pronounced in the brain stem (−50%) than in the forebrain (−34%).



Effect of *p*CPA on endogenous amines in brain stem and forebrain of the cat. The values are expressed in percent of controls and represent means with S.E.M. of 4 double experiments. Control values (μg/g) were 0.35 ± 0.03, 0.25 ± 0.01 and 0.53 ± 0.05 for 5HT, NA and DA in forebrain, 0.80 ± 0.06 and 0.46 ± 0.03 for 5HT and NA in brain stem, respectively.

Discussion. The present results confirm previous findings[1,2]. Thus, *p*CPA markedly reduces the 5HT content of the brain. In addition, these experiments show that the time course of 5HT depletion by *p*CPA is different in the forebrain and in the brain stem. This may suggest different effects of the drug on the synthesis of 5HT in regions which mainly contain nerve cell bodies and short axons (brain stem) from areas containing mainly terminals (forebrain). The slight transient rise of 5HT in the forebrain which precedes the depletion of the amine, is in agreement with previous findings in cat midbrain and diencephalon after a single dose of the drug[2]. However, its mechanism is as yet unclear.

*p*CPA markedly decreases the cerebral endogenous catecholamines in the cat as well as in the rat[6]. The mechanism of this depletion, which seems to be similar for brain stem and forebrain, remains to be elucidated. A direct effect of the drug on tyrosine hydroxylase[5] cannot be excluded. However, this action of *p*CPA might also be mediated by a neuronal mechanism.

In conclusion, these findings indicate that under the experimental conditions described, the depleting action of *p*CPA in cat brain is not selective for 5HT. Accordingly, if the drug is used as a tool for studying the role of 5HT in brain functions, a concomitant effect on catecholamines should be taken into account.

Zusammenfassung. *p*-Chlorphenylalanin, ein Inhibitor der Serotoninbiosynthese, senkte bei Katzen den Serotoningehalt des Gehirns nicht selektiv. Es wurde gleichzeitig eine signifikante und langanhaltende Erniedrigung des Noradrenalin- und Dopamingehalts gefunden.

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Axonal Growth from the Primitive Sympathetic Elements of Human Fetal Adrenal Medulla

The cytoarchitecture of human fetal adrenal medulla serves suitable conditions for isolating tather pure groups of primitive sympathetic cells. According to COUPLAND[1], the adrenomedullary primitive sympathetic elements showed delayed patterns of differentiation and persisted through the first postnatal years. The groups of primitive sympathetic developing AM cells formed tightly packed round to ovoid large cell islands surrounded by a thin connective tissue capsule. Electron microscopy of these cell groups revealed that sympathetic neurones were differentiating in the central region of the clusters while the peripheral zone facing to the cortical elements or medullary venous sinusoids differentiated to catecholamine storing medullary cells (HERVONEN[2]). The purpose of the present work was to develope an organ-culture method which could provide constant conditions for studying different humoral agents possibly exerting an effect on the differentiation of human fetal primitive sympathetic cells.

The adrenal glands of 3–4-month-old human fetuses were removed immediately after the disconnection of the fetomaternal nutritive contact and the medullary region was homogenized by gentle pipetting. The clusters of primitive sympathetic cells were identified under a stereomicroscope and placed on petri dishes for cultivation. The cultivation was performed in Hepes buffered Medium 199. A detailed description of the method will be published elsewhere (HERVONEN and RECHARDT[3]).

A strong fibroblast reaction and rapid monolayer formation by the cells of fetal cortex were the dominating features during the first 2 weeks. After this stage the dedifferentiation and/or degeneration of adrenocortical cells markedly reduced their number. The collections of primitive sympathetic cells were found to be mostly free from surrounding cortical cells. Axons grew rectangularly from the surface of the groups in all directions initially. During the 4th week the growth of the nerve fibres was most marked and complex anastomosing networks of fibres from neighbouring collection of primitive sympathetic elements were observed (Figure). The cells of Schwann were identified along the course of the fibres. The parallell

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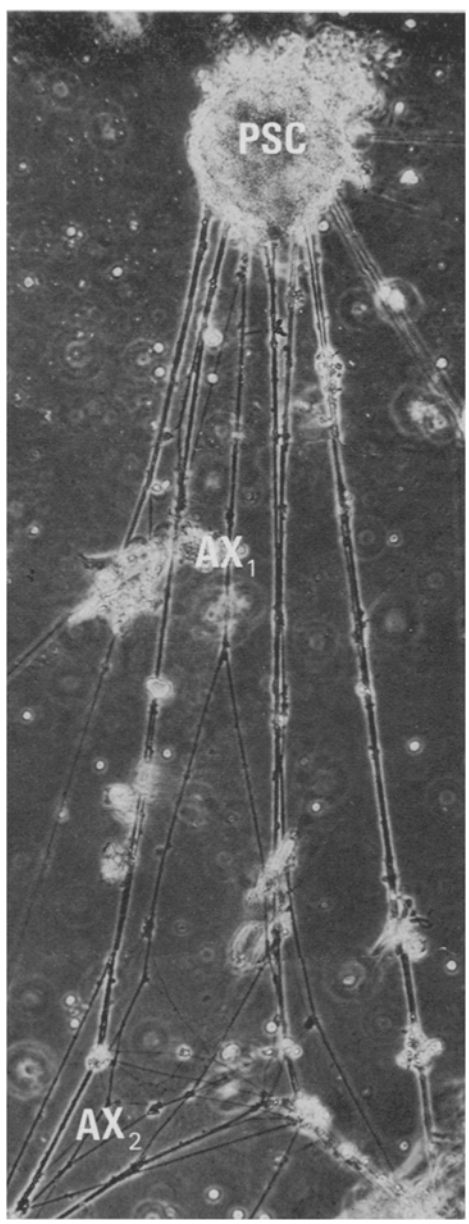
[2] A. HERVONEN, Acta physiol. scand. suppl. *368* (1971).
[3] H. HERVONEN and L. RECHARDT, in preparation.

axons had connecting branches to each other. The fibres terminated usually by a typical multipled division into 2 branches. The distal parts of the fibres had a marked tendency to form anastomosing networks. The length of the axons was calculated to be often over 2.5–10 mm in 4-week-old cultures. The diameter of the collections of primitive sympathetic cells was usually 0.1–1 mm. Tendency of central necrosis was not found in groups of this size.

Extensive neural growth was found in cultures of primitive sympathetic elements of adrenal medulla of midterm human fetuses. At this age both very primitive sympathetic cells and differentiated sympathetic neuroblasts were identified in these groups (Hervonen[2]). The present findings confirmed these electron microscopic observations by revealing an extensive and rapid axonal growth from the primitive sympathetic cell groups in cultures at the age of 3 weeks. Because these groups contain cells with neuronal characteristics, it is highly probable that these cells form a postanglionic nervous supply for some as yet unknown component of the adrenals. The axonal growth was obviously stimulated by some change in the micromilieu during the 3rd week of cultivation. This increased growth might be due to the dedifferentiation and/or degeneration of cortical cells which produce several progesterone metabolites at the age of 3–5 months (Villee et al.[4], Dizfalusy[5], Bloch[6]). The steroidogenic activity of human fetal adrenocortical cells was demonstrated also in tissue culture by Milner and Villee[7], who also stated that the cortical cells retained the ability to synthesize steroids throughout the culture periods. The human fetal adrenocortical cells were maintained up to 18 days, which is in accordance with the present observations on the degeneration of these cells. The glucocorticoids, especially hydrocortisone presumably exerted a diverse effect on rat developing primitive sympathetic cells. Hydrocortisone treatment promotes differentiation of new cells exhibiting positive Schmorl reaction after dichromate fixation (Lempinen[8]). These cells are catecholamine-storing as has been demonstrated using formaldehyde-induced fluorescence method, and possibly identical with the small intensely fluorescent cells normally present in sympathetic ganglia (Eränkö and Eränkö[9]). It has been reported independently by several workers that hydrocortisone is the regulating principle in the synthesis of adrenaline from noradrenaline (Eränkö, Lempinen, Räisänen[10], Coupland and McDougal[11], Wurtman and Axelrod[12]). Hence, a lot of evidence was available for the effect of hydrocortisone on the cytodifferentation of primitive sympathetic cells and, furthermore, these hormones directed the differentation towards catecholamine storing cells (Hervonen[2]).



Small, round cluster of primitive sympathetic elements (PSC) from the adrenal medulla of 14-week-old human fetus after 4 weeks culturing. Several axonal processes (AX_1) grow out from the cellgroup forming anastomosing network with processes from neighbouring implant of primitive sympathetic cells (AX_2). ×50,.

The present results suggest that the presence of actively functioning cortical cells in the culture might inhibite the neuronal growth, which was activated only after the disappearance of the influence of cortical elements, as suggested previously by Lempinen[8] and Hervonen[2]. Studies on the effects of different steroids on the differentiation and growth of sympathetic nerve cells are in progress.

Zusammenfassung. Die primitiven sympathetischen Zellen aus fetalem Nebennierenmark des Menschen wurden in Gewebekultur studiert. Der neuronale Zellcharakter konnte durch bedeutendes Axonwachstum bestätigt werden.

A. Hervonen, H. Hervonen and L. Rechardt

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