was washed several times with 30% of NaCl solution until the acetone was completely removed. β-Carotene was separated from lycopene by column chromatography on aluminum oxide (basic, activity grade I, Woelm-Eschwege, FRG). β-Carotene and lycopene were eluted separately from the column using the following solvents:

β-carotene: n-hexane-acetone = 49:1 (v/v);
lycopene: n-hexane-acetone-ethanol = 95:4:2 (v/v/v).

The eluted fractions of pigments were evaporated to dryness at room temperature under a stream of nitrogen and redissolved in appropriate solvents for spectral measurement on a Specol 10 spectrophotometer[12]. β-Carotene and lycopene were identified by their visible spectra. Furthermore, β-carotene from tomato tissues was identified by rechromatography and spectral comparison with authentic β-carotene isolated from carrots. The quantities of lycopene and β-carotene were calculated from published extinction data[12].

Results and discussion. Mature green tomatoes, treated with methyl jasmonate, developed a yellow color at the stage of full ripeness in the areas treated, to a depth of 2 mm (fig.). Untreated areas or areas treated only with lanolin paste were red. Comparative carotenoid analysis of treated and control tissues showed high differences in lycopene and β-carotene contents. Treated tissue had about 3 times more β-carotene and only trace amounts of lycopene in comparison to the control (table). The presence of the small amount of lycopene in methyl jasmonate treated sample tissues was probably caused by difficulties in separating treated tissues from untreated tissues. Thus, the lack of red color in tissue treated with methyl jasmonate was caused by the lack of lycopene accumulation and the increased content of β-carotene.

The main carotenoids in ripe tomatoes are lycopene, β-carotene and in small amounts, phytoene, phytofluene, ζ-carotene, δ-carotene and sometimes traces of neurosporene[13-15]. It is possible that methyl jasmonate inhibits the conversion of neurosporene to lycopene and stimulates the conversion of neurosporene to β-zeacarotene → γ-carotene → β-carotene[16].

Raymundo et al.[13] and Jen[15] observed that β-carotene was synthesized first and quickly reached a plateau before mass lycopene accumulation in normal red and red lutescent tomatoes. The authors suggest that 2 pathways of carotenoid biosynthesis exist in ripening tomatoes.

According to our knowledge this is the 1st report on the action of methyl jasmonate on carotenoid metabolism in plants. Detailed study of carotenoid biosynthesis at different stages of the ripening of tomatoes after JA-Me treatment are in progress.

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Distribution and number of fluorescent granular perithelial cells in coronal sections of rats cerebrum

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Summary. The fluorescent granular perithelial cells (F.G.P.) of rats aged 1 week to 2 years were observed under a light microscope to investigate intracellular granules and localization. This study showed that a marked proliferation of F.G.P. occurs within 3 weeks after birth and the total number remains constant for 2 years. The F.G.P. are mainly distributed in the gray matter, and are scarce in the white matter. The number and distribution of F.G.P. seems to reflect a difference of vascularization and function in different cerebral regions.

During aging of animals and humans, neurolipopigments are deposited in neurons, glia and perivascular cells. Recently, the lipopigments in perivascular cells of the central nervous system have attracted the attention of anatomists and neuropathologists[1-3]. The perivascular cells described in their reports seem not to correspond to pericytes, but to the fluorescent granular perithelial cells (F.G.P.) of the present authors, judging from the profiles in the figures.

As reported previously[4-6], the F.G.P. were mainly localized in bifurcations of small cerebral blood vessels and involved specific round granules which were rich in acid phosphatase and stained with the PAS (periodic acid Schiff) technic. The F.G.P. are concerned with removing waste products in cerebral tissue and regulating the uptake of blood components. However, developmental sequence, number and distribution of F.G.P. remained unknown, because the discrimination of F.G.P. was not easy under a light microscope.

In this study, the authors identified the F.G.P. by referring to location, shape and stainability of intracellular granules by the PAS technic, and carried out a quantitative study on F.G.P., observing developmental and regional differences.

Material and methods. 12 Wistar rats at 1, 2, 3 and 6 weeks,

Total and numerical density of F.G.P at each stage

No. of F.G.P	Developmental stage 1-week	2-week	3-week	6-week	4-month Wistar	SHR-SP	2-year Wistar (normal)	Wistar (fat rich)
Total mean value	25.4	78.2	185.2	310.0	342.3	345.1	357.6	349.2
Standard deviation	4.5	12.0	24.0	45.2	49.3	38.0	37.6	40.4
Number per unit area/mm^2	0.5	1.1	2.7	4.4	4.8	4.7	4.0	4.2

4 months and 2 years after birth were employed for this study. In order to investigate species differences and the effect of food on F.G.P., 2 hypertensive SHR-SP rats (4-month-old) and 2 old Wistar rats (2-year-old) fed with a fat-rich chow (Oriental, Tokyo) for 3 months were also used.

The rat brains were removed into a cold physiological saline, just after decapitation under ether anesthesia, and cut frontally through the thalamus with a blade, under a binocular microscope. The specimens were fixed with neutral 10% formalin buffered with 0.1 M phosphate for 3 days, and then refixed with acetic Zenker-formalin for 4 days. They were then embedded in paraffin, and 4 µm sections cut with a microtome. The sections were deparaffinized and stained with PAS-iron hematoxylin. After staining, 50–100 sections were observed in each animal under a light microscope, and 4 sections showing a similar shape and size of fornix, lateral ventricles, mamillothalamic tract and hippocampus were selected from them. Counting was carried out using these 4 sections in each animal, and the schemes for distribution of F.G.P. were drawn from one of the sample specimens.

Results and discussions. The F.G.P. of younglings (1, 2, 3 and 6 weeks after birth) were slender in shape and contained several PAS positive granules, while those of old rats looked voluminous and included a lot of densely stained granules and vacuoles. The general view of F.G.P. from 2-week-old younglings is depicted in figure 1. In old rats fed with a fat-rich chow, a few F.G.P. possessed a pyknotic nucleus and were filled with large empty vacuoles. They were assumed to be degenerating F.G.P. The F.G.P. of SHR-SP rats looked relatively small in size and had numerous fine granules. The deformity of the granules might be related to a metabolic disorder in the vascular walls of SHR-SP[7]. The table shows mean values for the number of F.G.P. seen in coronal sections of each group, and the numerical density of F.G.P. per unit area (1 mm^2). Figures 2 and 5 are schematic drawings indicating a distribution of F.G.P. in the samples.

From these figures, the following is noticeable: At the first postnatal week, the F.G.P. appeared in the upper part of the thalamus, a cerebral cortex and an amygdaloid nucleus. The total number of F.G.P. in a coronal section was about 25 (fig. 2). At the 2nd week, the F.G.P. proliferated specifically in tahalmus – especially along the thalamostriate vessel – and in the cerebral cortex. They became slender in form and totaled about 78 (fig. 3). At the 3rd and 6th week, the F.G.P. were well developed in all parts of the gray matter, and the F.G.P. amounted to about 185 at the 3rd week and to 310 at the 6th week.

At 4 months, the F.G.P. increased gradually and reached about 340 in number (fig. 4). After this, the number of

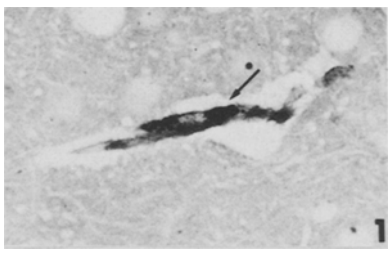
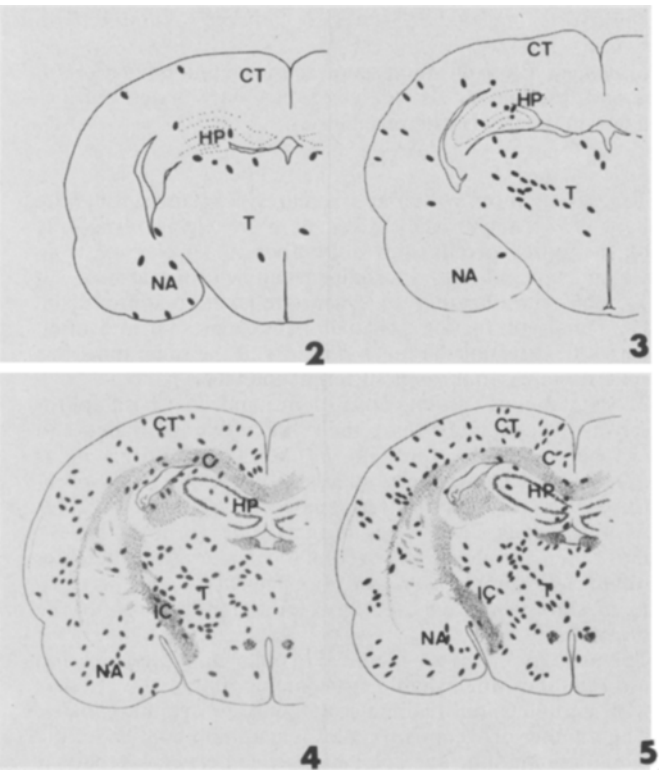


Figure 1. Microphotograph showing the general features of F.G.P. stained with PAS and iron-hematoxylin. The F.G.P. contains several round granules and is situated close to a small vessel. 2-week-old rat. × 870.



Figures 2–5. Number and distribution of F.G.P. in coronal sections of rat cerebrum at each developmental stage.
Fig. 2. 1-week-old rat. Fig. 3. 2-week-old rat. Fig. 4. 4-month-old rat. Fig. 5. 2-year-old rat.
C, corpus callosum; CT, cerebral cortex; HP, hippocampus; IC, internal capsule; NA, amygdaloid nucleus; T, thalamus.

F.G.P. fluctuated within a narrow range until 2 years old (fig. 5). That is, the F.G.P. appeared first just after birth, and a rapid proliferation of them occurred at an early stage of life; they then remained constant in number through out life. The finding called to the author's minds the formation of the vascular network in cerebral tissue. According to Bär[8], and to Mato and Ookawara's[9] observations, the pial vessels elongated and penetrated into the cerebral cortex, and branched frequently for 3 weeks after birth. Further, it is also known that myelination of nerve fibers takes place at the maximal rate in the cerebral cortex of rats in the 3rd postnatal week.

Recently, the authors found a primitive form of F.G.P. adjacent to venules running in the vicinity of the cerebral surface, in rats just after birth. They contained a lot of fibrous bundles and immature inclusion bodies. It is postulated that the primitive F.G.P. are able to migrate along vascular walls by their own motility (unpublished data).

In SHR-SP rats, as reported elsewhere[7], a small number of degenerating F.G.P. were recognized under the electron microscope. However, as shown in the table, the total of F.G.P. in SHR-SP did not deviate much from the mean value for contraol Wistar rats. In other words, the number of F.G.P. kept a constant level not depending on species, age or food, and was unaffected by some pathological conditions; a loss of F.G.P. seemed to be compensated by a temporal proliferation of F.G.P. Further, these schemes also demonstrated regional differences in the numbers F.G.P. The numerical density of F.G.P. was high in cerebral cortex and thalamus, moderate in hippocampus and amygdaloid nucleus, and low in the corpus callosum and internal capsule. Such regional differences in F.G.P. might reflect the differences in vasculature, function and metabolism between the cerebral regions. The production of metabolic wastes was estimated to be more vigorous in gray matter than in white matter. It has not yet been determined whether the number of F.G.P. is enough for the segregation and digestion of waste products in the central nervous system or not.

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Electron microscopical studies of gonads in primary and secondary males of protogynous hermaphroditic fish *Coris julis* L. (Labridae, Teleostei)[1]

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Summary. Electron microscopical examinations of *Coris julis* (L.) showed that Leydig cells are definitely present in the gonads of primary, as well as of secondary males. During sex change the Leydig cells develop from the remnants of the ovary in the newly organized testes.

The sex of most vertebrates remains constant throughout their life (gonochoristic). However in the teleosts, especially of the order perciformes a number of species exists in which 2 types of hermaphroditism can be distinguished.

1. True (simultaneous or synchronous) hermaphroditism. Here male or female gonadal tissue is present and often eggs and spermatozoa reach maturity at the same time. Sex cells are released through different channels[3-5].

2. Spontaneous (protogynous or protandrous) hermaphroditism. The animals start their life either as males and change to females (protandrous hermaphroditism) or as females and change into males (protogynous hermaphroditism)[6-13]. In some protogynous species 2 different kinds of males occur. The first are born as males (primary males), the other develop into secondary males from females (diandrie; example, *Coris julis* L.). The 2 types of *Coris julis* male differ from each other in color and in the shape of the dorsal fins[14-20].

The primary males are identical in color to the females and are thus indistinguishable from them. 70% of the animals with smooth color are females and only 30% primary males. The number of secondary males is smaller, about 8% of the whole population. The color-difference between secondary males on one side and primary males and females on the other side is a result of sex-inversion from female to secondary male. This process is correlated to some extent with gonadal change from females to males.

Chromosomal studies have shown that there are 2 types of female in *Coris julis*. One is relatively rare and remains female throughout its life. The other undergoes sex change, as an adult, into a secondary male with the characteristic color and fin pattern[20,21].

In our previous investigations, somatic cells of *Coris julis* gonads of 1 primary and 1 secondary male were found to be H-Y antigen positive. Two females were H-Y antigen negative. From these results it seems that H-Y antigen negative somatic cells of the female gonads become H-Y antigen positive during sex inversion[22].

The sex-reversal normally starts from a relatively large ovary, continues by ovarian degeneration and ends with formation of a testis. In most females, clusters of male germinal cells are located peripherally within the ovary. The testicular tissue develops from the region where these clusters are located, after degeneration of the ovaries[14,17,19]. Reinboth in his light microscopical studies could not find any Leydig cells in testes of primary and secondary males[15].