observed in dog and rat heart membranes. Assuming one phosphorylation site for cAMP-PK on each subunit of phospholamban a quantity of 6.7 nmoles phospholamban subunit per g wet heart weight can be calculated for dog myocardial tissue. Calculated values for the phosphoprotein in chick, frog, and carp hearts are one to two orders of magnitude lower. Small quantities of phospholamban in frog and fish hearts correspond to the sparse reticular systems in these hearts[8,9]. A low phospholamban content in chick myocardium is, however, in contrast with the extensive network of sarcotubules present in chick heart cells[10] and with the high Ca^{2+}-transport activity of sarcoplasmic reticulum fragments in crude chick membranes (table). The low level of ^{32}P-phospholamban in chick heart membranes, as compared to dog heart membranes thus suggests possible variations among species in the relationship between the phosphoprotein and sarcoplasmic reticulum Ca^{2+}-ATPase. In dog heart membranes a one-to-one stoichiometry has been established for the two proteins[11]. The quantity of Ca^{2+}-ATPase in crude chick heart membranes has not been estimated. It appears, however, that Ca^{2+}-ATPases of mammalian and avian hearts exhibit similar specific activities[12,13]. If this holds true also for dog and chick heart enzymes the results shown in the table imply that chick heart sarcoplasmic reticulum contains far fewer phospholamban molecules than Ca^{2+}-ATPase molecules. Evidence for independent changes in maximal phospholamban phosphorylation and sarcoplasmic reticulum Ca^{2+}-transport during cardiac muscle ontogenesis has been presented in Will et al.[4].

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Quantitative immunofluorescence of tyrosine hydroxylase in the adrenal medulla of spontaneously hypertensive rats[1]

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Summary. The amount of tyrosine hydroxylase protein in the adrenal medulla, which was estimated by a quantitative immunofluorescence method, was higher in spontaneously hypertensive rats than in normotensive control Wistar-Kyoto rats at 4 and 16 weeks of age before and after the development of hypertension.
Key words. Quantitative immunofluorescence; tyrosine hydroxylase; adrenal medulla; SHR.

Tyrosine hydroxylase (TH) activity was reported to be increased both in young spontaneously hypertensive rats (SHR)[2] (at 4 weeks of age) and in adult SHR (at 16 weeks of age) as compared to the normotensive control rats[3,4]. However, lower TH activity was also reported in the adrenal glands of young SHR[5]. Since enzyme protein contains an active form and an inactive form[6,7], we have tried to estimate the amount of TH protein in the chromaffin cells of the adrenal medulla of SHR by a quantitative immunofluorescence method. Cytofluorimetric quantitations of proteins using FITC-labeled antibodies[8] have been applied for this purpose. This cytofluorimetric quantitation has also been introduced for studying axonal transport of immunofluorescence materials[9]. A trial to quantitate TH based on the immunofluorescence intensity was made by taking photographs and measuring the density in the caudate nucleus of the rat[10]. In this paper, we intend to quantitate immunofluorescence intensity of TH in a single cell of the adrenal medulla in situ.

Materials and methods. Six SHR and six control Wistar-Kyoto rats (WKY) raised in our laboratory were used at 4 weeks or 16 weeks of age. 48 adrenal glands from 24 rats were obtained with perfusion (30 ml/min) of saline for 1 min and Zamboni's fixative[11] for 7 min, and were postfixed with the same fixative for additional 17 h. After washing with phosphate buffer containing 10% sucrose, 10 µm frozen sections were cut and put on a gelatin coated slide, and an immunofluorescence reaction was performed using antiserum against bovine adrenal TH. TH was purified homogeneously as judged by SDS-gel electrophoresis. No dopamine-β-hydroxylase or phenylethanolamine-N-methyltransferase activity was detected in the TH preparation. Antibodies raised in rabbits in our laboratory against the TH were examined by the immunodiffusion test of Ouchterlony[12], and the anti-TH gave a single precipitin line of identity when tested against the purified TH[13].

Immunostaining was performed as follows: Anti-TH antiserum (1:100–1:4000 in dilution with phosphate buffer saline (PBS) containing 0.3% Triton X-100) was applied on the specimens and incubated for 2 h in a moistened chamber at room temperature. After rinsing with PBS containing 1% Triton X-100, specimens were dried and fluorescein-labeled antiserum against rabbit IgG (Miles) was applied (1:250, diluted with PBS containing 0.3% Triton X-100). Following incubation at room temperature

Comparison of average fluorescence of 45–75 cells of the adrenal medulla of SHR and WKY at 4 weeks and 16 weeks of age. The measurement head delimited a square of 6.6 × 7.3 µm for a single cell but excluding the nucleus. The sensitivity is 10 times higher than figure 2

	Immunofluorescence of tyrosine hydroxylase Arbitrary units (means ± SE)	
Age (weeks)	4	16
WKY	245.8 ± 15.6 (45)	312.2 ± 18.5 (75)
SHR	311.7 ± 21.8* (45)	352.4 ± 17.3** (75)

Numbers of cells in parentheses. Difference from controls, *$p < 0.01$, **$p < 0.05$.

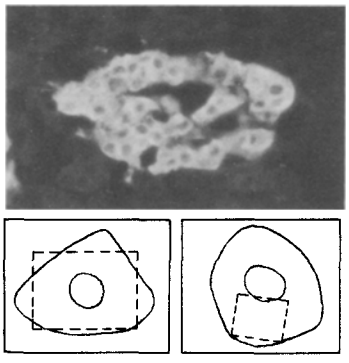

Figure 1. An immunofluorescence micrograph of the rat adrenal gland stained with anti-tyrosine hydroxylase antiserum. ×230. A large square of 11 × 15 μm is shown by a dotted line for dilution test of figure 2, and a small square of 6.6 × 7.3 μm by a dotted line for average fluorescence of the table.

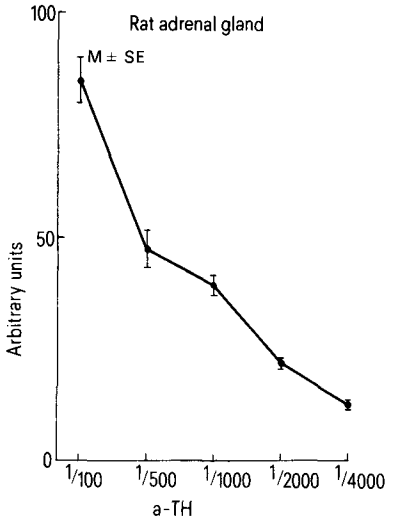

Figure 2. Change in fluorescence of the cells in the adrenal medulla of SHR exposed to dilutions of an anti-tyrosine hydroxylase antiserum. The measurement head delimited a square of 11 × 15 μm for a whole single cell including a nucleus. The sensitivity is 10 times lower than in the table.

for 30 min in a moistened chamber, specimens were rinsed as above. After air drying, glycerin/phosphate buffer was used for mounting.

A Leitz Dialux 20 fluorescence microscope with a MPV compact microscope photometer was used on the original section slide to give read-outs by printing. The standard light source for microscopic fluorimetry in incident light illumination was a XBO 75 W high-pressure Xenon lamp in a lamp housing 100 Z. All measurements were made with a single diagram setting in the measurement head delimiting a square of 11 × 15 μm including the nucleus (figs 1 and 2), or 6.6 × 7.3 μm excluding the nucleus (table) in the object plane for a single cell. Since the immunofluorescence faded slowly during excitation, the time of fluorescence measurements was made constant for 2.5 sec. and no more than one cell was measured per field.

Results. Figure 1 shows an example of a fluorescence micrograph of the rat adrenal gland stained with anti-TH antiserum. For the dilution test, the measurement head delimited a square of 11 × 15 μm for a whole single cell including a nucleus. The sensitivity used for measurement was 10 times lower than the table. For the comparison of average fluorescence of the adrenal medulla of SHR and WKY, the measurement head delimited a square of 6.6 × 7.3 μm for a single cell but excluding a nucleus. The sensitivity was 10 times higher than figure 2. Figure 2 shows the resulting plots of fluorescence brightness versus dilution of TH antiserum. Reduction in fluorescence was almost linear in the dilution range of 1:100–1:4000. At higher dilutions fluorescence levels approached base-line. In the experiments, measurements were made at a 1:500 dilution of TH antiserum only. Readings on single cells excluding nuclei were 100–800 against background values of 10–15. TH-immunoreactive fluorescence intensity in the cells of the adrenal medulla was compared between SHR and WKY at 4 weeks and 16 weeks of age. As shown in the table, TH-immunoreactive fluorescence intensity is higher in the cells of the adrenal medulla of SHR than in the cells of WKY both at 4 weeks of age and at 16 weeks of age. This result agrees with our previous reports[3,4] indicating increased TH activity in the adrenals of SHR, and suggests that the amount of TH protein in the adrenal medulla is increased in SHR.

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The egg-lipid composition of the 'living fossil' reptile tuatara *(Sphenodon punctatus)*

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Summary. The lipid composition of two tuatara eggs was examined. The eggs contained triacylglycerol (80%) and phospholipid (12%) as their major lipid fractions. Fatty acid analyses of the individual lipid classes indicated the presence of essential fatty acids, linoleic and arachidonic acids. The quantity of such acids in the egg yolk lipids would suggest they are factors for survival as illustrated in other species.

Key words. Tuatara; *Sphenodon punctatus;* egg composition; egg yolk lipids.