bis 10^{-2} Torr durch Zertrümmerung der Ampulle mittels Magneten in die Apparatur verdampft.
Stickstoff (reinst), Luft und Stickstoffmonoxid wurden vor dem Einströmen in die Reaktions- bzw. Aliquotierungsgefässe sorgfältig mit KOH und P_2O_5-Bimsstein getrocknet. Alle Schliffverbindungen und Vakuumhähne wurden mit einem Präparat der Firma Halocarbon Product Corporation (USA), «Halocarbon 25-5 S Grease», eingefettet. Zur Druck- bzw. Vakuummessung kamen je nach dem zu messenden Bereich Pirani-, McLeod- oder Huygens-Manometer bzw. ein einfaches, mit Quecksilber gefülltes U-Rohr zum Einsatz. Vor jeder Umsetzung erfolgte ein Ausheizen der Reaktions- bzw. Aliquotierungsgefässe bei 10^{-1} bis 10^{-2} Torr, um an den Glaswänden adsorptiv gebundenes Wasser weitgehend zu entfernen.
Analytik. Nach entsprechenden Reaktionszeiten wurden Diäthylnitrosamin, unumgesetztes Stickstoffdioxid sowie das als Aerosol anfallende Diäthylammoniumnitrat durch intensives Schütteln des geschlossenen Reaktionskolbens mit 0,1 N Kalilauge in die wässerige Phase übergeführt. Unumgesetztes Stickstoffdioxid wurde – nach Absorption in 0,1 N KOH, Diazotierung mit Sulfanilsäure und Kupplung mit N-(1-Naphthyl)-äthylendiamin – als Azofarbstoff bei 540 nm bestimmt.
Im NO_2-Konzentrationsbereich von 20 bis 250 ppm wurde unter den beschriebenen experimentellen Bedingungen der Umwandlungsgrad von Stickstoffdioxid zu Nitrit bzw. zum Azofarbstoff untersucht. In diesem Bereich wurde ein konzentrationsunabhängiger Wert von 0,64 («Saltzman-Faktor») gefunden.
Nitrat wurde nach einem von Sawicki und Scaringelli beschriebenen[18] und von uns modifizierten Verfahren[19] analysiert, wobei die zuvor ermittelten Nitrit- und Nitrosaminkonzentrationen berücksichtigt wurden. Diäthylnitrosamin wurde nach Wasserdampfdestillation und katalytischer Spaltung im wässerigen, alkalischen Milieu ebenfalls spektralphotometrisch bestimmt[19]. Die Identität des gebildeten Diäthylnitrosamins wurde dünnschichtchromatographisch[20] durch Vergleich mit authentischen Referenzproben bestätigt. Diäthylammoniumnitrat wurde spektralphotometrisch[19] (Nitrat) und durch Nitrosierung[21] (Diäthylamin) mit nachfolgender Dünnschichtchromatographie bzw. katalytischer Spaltung charakterisiert.

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Correlation between serum dopamine-β-hydroxylase activity and dopamine-β-hydroxylase and tyrosine hydroxylase activities in central and peripheral adrenergic neurons and adrenal glands

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Summary. Serum dopamine-β-hydroxylase activity in spontaneously hypertensive rats and Wistar-Kyoto rats had a positive correlation with dopamine-β-hydroxylase and tyrosine hydroxylase activities in mesenteric vessels, vas deferens, and adrenal glands at 14–16 weeks of age, a negative correlation with dopamine-β-hydroxylase activity in locus coeruleus at 3 weeks and 14–16 weeks of age, and a positive correlation with tyrosine hydroxylase activity only at 3 weeks of age, but not at 14–16 weeks of age.

Since dopamine-β-hydroxylase (DBH) is secreted into blood from the nerve endings of the sympathetic nerves together with noradrenaline[1], it is speculated that serum DBH activity can be an index of the peripheral sympathetic nerve activity. However, serum DBH activity appears to be an insensitive index of acute changes of the sympathetic nerve activity as compared with the noradrenaline level in blood[2]. One question is whether or not serum DBH can express the changes of sympathetic nerve activity during a long-term period such as during the onset of hypertension.
We have examined the relation between the serum DBH activity, which is mainly derived from the peripheral sympathetic nerve terminals, and the DBH and tyrosine hydroxylase (TH) activities in the peripheral sympathetically innervated tissues, adrenal glands, and in the catecholaminergic regions of brain with spontaneously hypertensive (SH) rats and control Wistar-Kyoto rats during the development of hypertension, in order to evaluate the significance of serum DBH activity as an index of peripheral and central noradrenergic neuronal activity.

The SH rats[3] and Wistar-Kyoto rats examined were at 3, 14 and 16 weeks of age. They were kindly supplied by Drs Okamoto and Yamori (Kyoto University, Kyoto) and raised in our laboratory under the same conditions. The mean values of DBH and TH activities in tissues of rats at 3 weeks[4] and 14 weeks[5] were reported previously. Rats were decapitated, and mesenteric vessels, vas deferens, adrenal glands and brain were quickly removed, weighed, frozen on dry ice and stored at –80 °C. 'Mesenteric vessels' consist of superior, inferior and coeliac mesenteric arteries and veins plus connective tissues after removing fat from the mesentry. Blood samples were

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Correlation of serum dopamine-β-hydroxylase (DBH) activity with dopamine-β-hydroxylase and tyrosine hydroxylase (TH) activities in peripheral tissues and brain of Wistar-Kyoto rats and spontaneously hypertensive rats

	Age (weeks)	Number of rats	Coefficient of correlation	p
Serum DBH vs mesenteric vessel DBH	14 and 16	39	0.7321	< 0.01
Serum DBH vs vas deferens DBH	14 and 16	33	0.4077	< 0.02
Serum DBH vs adrenal DBH	14 and 16	28	0.5730	< 0.01
Serum DBH vs	3	8	−0.8940	< 0.01
locus coeruleus DBH	14 and 16	25	−0.7123	< 0.01
Serum DBH vs mesenteric vessel TH	14 and 16	31	0.3460	< 0.1
Serum DBH vs vas deferens TH	14 and 16	33	0.4301	< 0.05
Serum DBH vs adrenal TH	14 and 16	33	0.3793	< 0.05
Serum DBH vs	3	8	0.7806	< 0.05
hypothalamus TH	14 and 16	25	−0.3726	NS

Serum DBH: nmoles/min/ml of serum. DBH and TH in mesenteric vessels, vas deferens and adrenal glands: nmoles/min/g of tissue. DBH and TH in locus coeruleus and hypothalamus: nmoles/min/mg of protein.

obtained by exsanguination and were put into a test-tube kept in ice, and the serum was removed. The regions of noradrenergic neurons (locus coeruleus and hypothalamus) were dissected out under a microscope from frozen sections of the brain[6].

DBH activity was determined based on formation of octopamine from tyramine by dual-wavelength spectrophotometry[7], as described before[5]. TH activity was assayed by measuring the [^{14}C]dopa formed from L-[U-^{14}C] tyrosine[8,9], as described before[5]. 6-Methyltetrahydropterin or L-erythro-tetrahydrobiopterin was used as a cofactor for the assay of TH in the peripheral or central tissues, respectively. The results are shown in the table. A significant positive correlation was observed between the DBH activity in serum and that in mesenteric vessels, vas deferens and adrenal glands of adult rats at 14 and 16 weeks of age. A significant inverse correlation was seen between serum DBH activity and the activity in the locus coeruleus area of the brain of young (3 weeks of age) and adult rats (table and figure 1). Less marked but still significant correlations were observed between serum DBH activity and TH activity of mesenteric vessels, vas deferens and adrenal glands of adult rats. Serum DBH activity had a significant positive correlation with TH activity in the hypothalamus aera of the brain, but only in young rats, and not in adult rats (table and figure 2). There has been much discussion whether or not serum DBH activity can be an index of peripheral sympathetic nerve activity. The present results suggest that serum DBH activity has a positive correlation with DBH and TH activities in mesenteric vessels, vas deferens and adrenal glands. However, since increased levels of DBH activity in serum and mesenteric vessels of young SHR disappear during development of SHR[4,5], serum DBH may reflect mainly the DBH activity in the peripheral sympathetic nerve terminals in the blood vessels. The noradrenergic neuron in the brain stem is believed to play a role in depression of peripheral sympathetic nerves through an α-receptor to decrease the blood pressure[10], whereas that in the hypothalamus may play a role in activation of peripheral sympathetic nerves through a β-receptor to increase the blood pressure[11]. The negative correlation between DBH activity in serum and that in locus coeruleus and a positive correlation between DBH

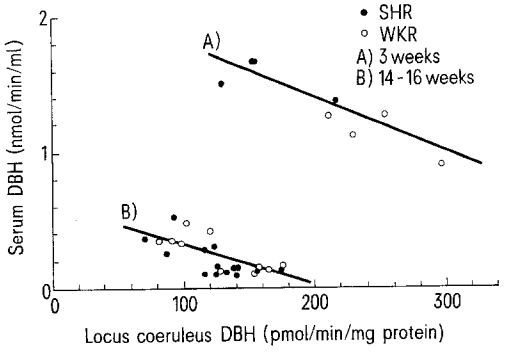

Fig. 1. Correlation between serum dopamine-β-hydroxylase (DBH) activity and DBH activity of locus coeruleus in Wistar-Kyoto rats (WKR) and spontaneously hypertensive rats (SHR) at 3 weeks (*A*) and 14–16 weeks (*B*) of age. *A* Coefficient of correlation, −0.8940, $p<0.01$; equation of regression line, $y = -0.004\ x + 2.205$. *B* Coefficient of correlation, −0.7123, $p<0.01$; equation of regression line, $y = -0.003\ x + 0.610$.

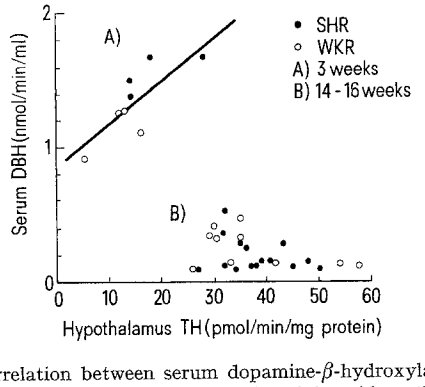

Fig. 2. Correlation between serum dopamine-β-hydroxylase (DBH) activity and tyrosine hydroxylase (TH) activity of hypothalamus in Wistar-Kyoto rats (WKR) and spontaneously hypertensive rats (SHR) at 3 weeks (*A*) and 14–16 weeks (*B*) of age. *A* Coefficient of correlation, 0.7806, $p<0.05$; equation of regression line, $y = 0.033\ x + 0.846$. *B* No significant correlation.

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activity in serum and TH activity in hypothalamus supports this view. Lamprecht et al.[12] also reported that, after 4 weeks of immobilization stress to rats, there was a significant increase in the activity of hypothalamus TH and in the activity of serum DBH. Interesting phenomenon in the present study is that TH activity in hypothalamus has a positive correlation with serum DBH activity only in young rats, but not in adult rats.

It may be concluded from the present results that serum DBH activity can be an index of peripheral sympathetic neurons during a long-term period and may be indirectly regulated by the central noradrenergic neurons through changes of peripheral sympathetic nerves, especially in blood vessels. Human serum DBH activity is determined by genetic factors, and therefore a great variation exists in men[1]. However, the present results indicate that a follow-up study of serum DBH activity with an individual patient may reveal the changes in the peripheral and central noradrenergic activity and may give an useful information as a diagnostic index in diseases such as essential hypertension in which the implication of the sympathetic nerves are suspected.

Linkages between chromophore and apoprotein in the biliverdin-protein of the scales of big blue parrotfish, Scarus gibbus Rüppell[1]

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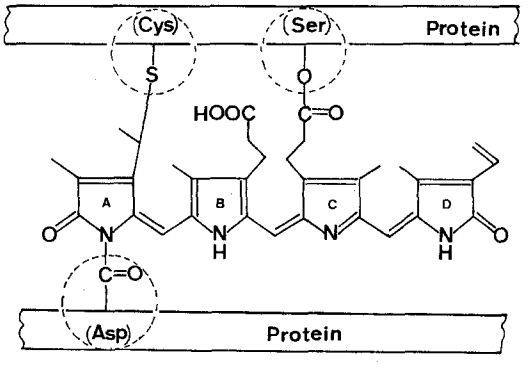
Summary. On the basis of the amino acid composition of the chromophore peptides, it can be assumed that Asp, Ser and $^1/_2$ Cys are located near the biliverdin chromophore. Experiments for splitting of the chromophore lead us to expect a thioether bond in the linkages between biliverdin and apoprotein.

Recently, Abolinš et al.[3,4] demonstrated that a blue chromoprotein isolated from the fins of Crenilabrus pavo C.V. was a biliverdin-protein. Subsequently it was shown by the present authors that the scale pigments of several species[5] of parrotfish, including S. gibbus and the skin pigment of the sculpin, Pseudoblennius percoides Günther[6], were also biliverdin-proteins, the fact suggesting a fairly wide occurrence of such chromoproteins in the epidermis of blue-coloured fishes. This investigation attempts to characterize the mode of linkages and the amino acids involved in the biliverdin-protein of S. gibbus[7].

The thin film in which the blue pigment is found was stripped off the scales, cut into small pieces and crushed with sea sand in a mortar. The pigment was extracted with 0.9% NaCl and fractionated with $(NH_4)_2SO_4$ between 0.2 and 0.4 saturation at pH 7.0 to collect the blue chromoprotein. Further purification by starch block electrophoresis[8] in 0.04 M phosphate buffer pH 6.8 afforded an electrophoretically homogeneous biliverdin-protein.

In order to find out which amino acid residues are involved in the linkage with biliverdin, the following procedure was applied. Approximately 50 mg of the lyophilized biliverdin-protein (P_0), of which amino acid composition was analyzed beforehand[9], were digested with 1 mg of pepsin[10] at pH 2.0 and 37 °C for 16 h. The digest obtained was submitted to Sephadex G-100 gel filtration (column 2.6 × 32 cm) with 0.1 N CH_3COOH as eluent. Fractions of 5 ml were collected and assayed for absorbance at 280 and 650 nm. The elution pattern gave 1 green and 8 colourless peaks. The green fractions were collected and lyophilized to obtain the biliverdin-peptide mixture (P_1), a part of which was subsequently analyzed for amino acid composition. P_1 was then digested with thermolysin[11] (enzyme to substrate ratio, 1:25) at pH 6.8 and 45 °C for 2 h. The digest was placed on a Sephadex G-10 column (1.6 × 70 cm) and eluted with 0.5 N CH_3COOH. The elution pattern gave one green and 5 colourless peaks. Green-coloured biliverdin-peptide mixture (P_2) was collected as above. A small portion of P_2 was assayed for amino acid composition. The remaining P_2 was further hydrolyzed with 2 N HCl at 100 °C for 2 h. The hydrolysate was subjected to paper chromatography with n-BuOH/CH_3COOH/water (4 : 1 : 5, by vol.) as solvent, resulting in the appearance of a green spot of biliverdin-



Presumed linkages between chromophore and apoprotein.

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10 A 3-times crystallized preparation of Worthington Biochemical Corp.
11 A crystalline preparation of Seikagaku Kogyo Co., Ltd.