single-stranded DNA indicates much weaker binding of histones by the phosphate groups of RNA. The existence of histone-RNA complexes in cell nuclei is still uncertain; the in vitro dissociation of these complexes by low ionic strength (0.15 *M* NaCl and higher) and by DNA suggests that such complexes based on electrostatic bonding, may not exist under in vivo conditions. In addition, the failure of RNA to prevent the histone inhibition of the in vitro synthesis of RNA is probably due to the dissociation of histone-RNA complexes by the template DNA[12].

Zusammenfassung. Eine Bestimmungsmethode für die Interaktion zwischen Histon und Nukleinsäuren (RNS oder DNA) wurde beschrieben. Beide Nukleinsäuren komplexieren mit Histon; RNS-Histonkomplexe sind schwach und dissozieren in Gegenwart von DNS. Damit wird die Hemmung der RNS-Synthese in vitro mit Histon, die unabhängig von der Gegenwart der RNS im Reaktionsgemisch ist, erklärt.

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3-Methoxytyramine, a Catecholamine Catabolite regularly present in Human Urine[1]

In 1958 AXELROD, SENOH and WITKOP[2] could demonstrate by paperchromatography that rats given i.p. injections of dopamine excrete 3-methoxytyramine in the urine. This observation suggested that dopamine can serve as substrate for the catecholamine-*O*-methyltransferase not only in vitro as shown previously by AXELROD[3], but also in vivo. Since subsequently 3-methoxytyramine was found by fluorimetric methods to be present in brain homogenates of various mammals[4–6], no doubt remained that part of the dopamine formed in the organism is *O*-methylated under physiological conditions.

In view of the fact that the methoxyderivatives of norepinephrine and epinephrine, i.e. normetanephrine and metanephrine, are always excreted in certain amounts in the urine of normal individuals, we wondered whether this was not true also for 3-methoxytyramine, which, up to now, was rarely assessed and not always found in the urine[7–11]. Assuming that chromatographic techniques might not be sensitive enough and that the procedure of CARLSSON and WALDECK[13] for the determination of 3-methoxytyramine in tissue homogenates was inappropriate for our purposes, we developed a fluorimetric method[12] for quantitative measurements of 3-methoxytyramine in urine and used this procedure for the estimation of 3-methoxytyramine excretion in healthy individuals.

Materials and method. To 20 ml of a 24 h urine specimen 1 ml of 0.2 *M* EDTA (ethylene diamine tetraacetate · $2H_2O$) solution, 0.25 ml of a 2% ascorbic acid solution and 1 ml of 0.1 *M* phosphate buffer pH 6.5 (Sörensen) are added. After titration to pH 6.5 the urine is passed at 25 °C through a thermostatized column with an inner diameter of 4 mm containing Dowex AG 50 W × 8, 200–400 mesh, in Na^+-form, up to a height of 35 mm. This column adsorbs the catecholamines and their methoxyderivatives and, after rinsing with a mixture consisting of 10 ml of phosphate buffer, 1 ml of ascorbic acid solution and 40 ml water, norepinephrine and epinephrine can be eluted with 7 ml 1 *N* HCl (flow-rate 7–9 drops/min). If thereafter 25 ml of 5 *N* HCl are used for elution at the same flow-rate, a fraction containing dopamine and 3-methoxytyramine is obtained. To 5 ml of this fraction 3 ml of 0.5 *M* citrate-boric acid buffer pH 6.5 and 3 ml of 10 *N* NaOH are added. By heating this alkaline solution to 50 °C during 20 min dopamine but not 3-methoxytyramine will be destroyed quantitatively. The same is then adjusted to pH 6.5 with HCl (= solution for oxidation). 3 ml from this solution are then oxidized according to the scheme given in Table I, the 3-methoxytyramine being converted thereby into a fluorescent indole. Another sample of the aforementioned solution for oxidation is used as blank and processed as shown in Table I.

If the intensity of fluorescence is determined as described above in a sample of plain urine ($F_1 - B_1$) as well as in a second sample of the same urine to which a known amount of 3-methoxytyramine (Q) was added ($F_2 - B_2$), the quantity of 3-methoxytyramine initially present can be calculated according to

$$\text{3-methoxytyramine } (\mu g/24\text{ h}) = \frac{(F_1 - B_1)}{(F_2 - B_2) - (F_1 - B_1)} \cdot Q \cdot \frac{V}{20}$$

where: F_1 = fluorescence units of the urine to be analyzed; B_1 = fluorescence units of the corresponding blank; F_2 = fluorescence units of the same urine to which 3-methoxytyramine was added; B_2 = fluorescence units of the corresponding blank; Q = amount of 3-methoxytyramine added (μg); V = 24 h urine volume (ml).

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Results and discussion. Assessing the urinary excretion of free 3-methoxytyramine in normal individuals of different ages by the method described, we found the results as shown in Table II. These results demonstrate that in children the excretion of free 3-methoxytyramine expressed in µg/24 h is lower than in adults, whereas the reverse is true if the excretion is expressed in µg/mg creatinine.

Since free 3-methoxytyramine was present in all urines examined, even in those of individuals on a vegetable-free diet, one can assume that part of this endogenously formed metabolite is excreted by the kidneys, as normetanephrine and metanephrine, the methoxyderivatives of norepinephrine and epinephrine. It is of interest to note that the free 3-methoxytyramine excretion in normal adults is 3 times higher than that of free metanephrine (mean: ~30 µg/24 h) and 4 times higher than that of free normetanephrine (mean: ~20 µg/24 h), but considerably less than that of the corresponding phenolic acids, i.e. homovanillic acid (mean: 8 mg/24 h) and vanilmandelic acid (mean: 4.5 mg/24 h)[14,15].

In view of the fact that quantitative determinations of the catecholamines and of their catabolites in the urine have become increasingly important for diagnostic as well as other purposes[16], one might assume that the analysis of the 3-methoxytyramine excretion will create additional investigative possibilities.

Table I. Oxidation scheme of 3-methoxytyramine

	Sample (ml)	Blank (ml)
Solution for oxidation	3.0	3.0
Saturated NaCl solution	0.5	0.5
5 *N* NaOH	–	0.45
0.02 *N* iodine solution	0.2	–
	Wait 4 min	
Alkaline sulphite solution[13]	0.5	–
	Wait 5 min	
5 *N* HCl	1.0	–
	Keep at 80 °C for 30 min	
2 *M* Na_2SO_3 solution	–	0.05
5 *N* HCl	–	1.0
0.02 *N* iodine solution	–	0.2

Read fluorescence of sample (F) and of blank (B) at 330/385 nm.

Table II. Urinary excretion of free 3-methoxytyramine in normal individuals

Individuals examined (age in years)	Number	Urinary 3-methoxytyramine µg/24 h	µg/mg creatinine
Children (2–13)	14	Mean: 37.0 Range: 12.7–72.0	Mean: 0.10 Range: 0.04–0.18
Adults (25–102)	14	Mean: 88.4 Range: 30.3–175.0	Mean: 0.067 Range: 0.02–0.13

Zusammenfassung. Es wird eine säulenchromatographisch-fluorimetrische Methode zur Bestimmung des freien 3-Methoxytyramins beschrieben. Mit ihrer Hilfe konnte festgestellt werden, dass sich dieses Dopaminabbauprodukt stets in bestimmter Menge im Urin gesunder Menschen nachweisen lässt.

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Effect of Sodium Fluoride on the Epinephrine Response of Liver and Hepatoma Adenyl Cyclase

Some tumors appear to be exempt from the regulatory control found in normal tissue with the result that unrestricted cell proliferation occurs. In attempts to define the control points within tumor cells that vary as compared with normal cells, a variety of parameters have been studied. The functions which have been considered include respiration[1] and metabolism[2], cell growth[3], cell differentiation[4] and division[5]. We have investigated the enzyme adenyl cyclase, known to be the mediator of the hormonal effect of epinephrine and thought therefore to function at a control point. The product of the adenyl-cyclase reaction (3'-5' cyclic AMP) regulates the formation of the phosphorylated products of glycolysis. Adenyl cyclase, therefore, interfaces hormonal effects and cellular metabolism.

As a model system upon which to test the hypothesized difference between tumor and normal tissue, we have chosen Morris hepatomas types 7777 (52 generation), 7794A (21st generation), and 9618A (4th generation), versus normal liver. Morris hepatomas have elevated levels of adenyl cyclase relative to normal liver, and the amount of increased adenyl cyclase activity correlates with the growth rate of the hepatoma (i.e., the shorter the doubling time for the particular hepatoma, the higher is its adenyl cyclase content)[6].

Materials and methods. Adenyl cyclase activity from transplanted hepatoma and normal liver of Buffalo rats was determined in a 20,000 *g* fraction. The reaction mixture contained 0.5 ml of the enzyme and 4.0 ml of the

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