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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[14] H. KÄSER, unpublished data.
[15] K. TANIGUCHI, Y. KAKIMOTO and M. D. ARMSTRONG, J. Lab. clin. Med. *64*, 469 (1964).
[16] H. KASER, Schweiz. med. Wschr. *96*, 258 (1966).

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.

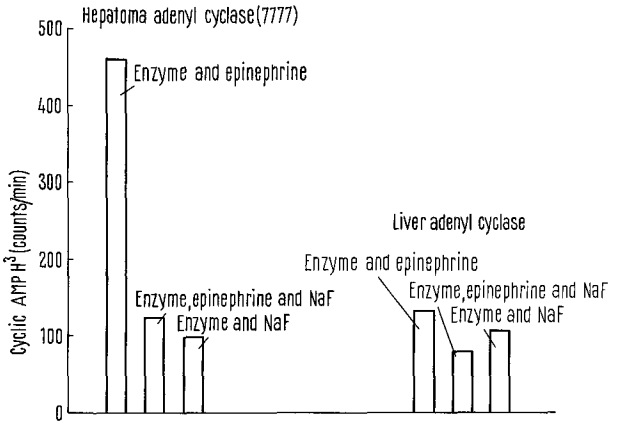
As a model system upon which to test the hypothesized difference between tumor and normal tissue, we have chosen Morris hepatomas types 7777 (52 generation), 7794A (21st generation), and 9618A (4th generation), versus normal liver. Morris hepatomas have elevated levels of adenyl cyclase relative to normal 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

[1] D. HEPP, Adv. Cancer Res. *10*, 93 (1967).
[2] M. GORLICH, E. HEISE and M. J. BINDER, Natn. Cancer Inst. *35*, 413 (1965).
[3] H. HERMAN, A. C. MARCHOK and E. F. BARIL, Natn. Cancer Inst. Monogr. *26*, 303 (1967).
[4] C. L. MARKERT, Cancer Res. *28*, 1908 (1968).
[5] D. M. PRESCOTT, Cancer Res. *28*, 1815 (1968).
[6] H. D. BROWN, S. K. CHATTOPADHYAY, H. P. MORRIS and S. N. PENNINGTON, Cancer Res., in press.

substrate (15.00 mg Na_2ATP, 3.54 mg $MgSO_4$, 2.08 mg NaF, 6.50 mg caffeine, in a 0.05*M Tris* buffer, pH 7.2)[7]. The reaction was allowed to proceed at 37 °C for 15 min, at which time it was stopped by placing the tube containing the reaction mixture into boiling water for 3 min and then into an ice-bath for 10 min. In certain of the experiments, 3 μC of ^3H-Na_2ATP was added to the reaction mixture, and the amount of cyclic AMP formed measured by radio-isotopic method[8]. This procedure involves the removal of the denatured protein material by centrifugation followed by partial separation of the reactants and products on a short Dowex-50 column. After this the fraction containing the partially separated cyclic AMP is treated with barium hydroxide and zinc sulfate which further separates the cyclic AMP by coprecipitating the contaminating materials. After coprecipitation and centrifugation, an aliquot of the supernatant activity is measured by liquid scintillation counting. The amount of cyclic AMP formed is given in terms of counts of tritiated cyclic AMP/min/mg.

Results and discussion. The stimulatory effect of epinephrine ($4.4 \times 10^{-5} M$) reported earlier[9] was not observed when NaF was present in the incubation mixture of enzyme from either normal or hepatoma tissue. We assume, prima facie, that NaF can stimulate adenyl cyclase maximally and that epinephrine has no further stimulatory effect. However, epinephrine does markedly further stimulate activity of hepatoma-derived enzymes in the absence of NaF. Several interpretations of this observation are possible. One thesis is that a structural abnormality of the enzyme molecule occurs in the tumors studied and that is related to the increase in the activity of the enzyme and the difference in response in epinephrine in the presence and absence of NaF. However, the possibility also exists that the variation in response to epinephrine is related to the state of disruption of the enzyme-membrane complex and that the variation in the disruption of this complex is a function of whether or not one has normal tissue or hepatoma tissue[10].



Bar graph relating tissue source and experimental conditions to amount of cyclic AMP formed.

Zusammenfassung. Die in vitro Stimulation der Adenyl-Cyclase durch Epinephrin zur Bildung der zyklischen AMP ist im Hepatomgewebe signifikant höher als in der normalen Rattenleber. Sodiumfluorid hemmt in beiden Fällen die Stimulation.

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[7] E. W. SUTHERLAND, T. W. RALL and T. J. MENON, Biol. Chem. *237*, 1220 (1962).
[8] G. KRISHNA, B. WEISAL and B. BRODIE, J. Pharmac. exp. Ther. 163, 379 (1968).
[9] G. A. ROBINSON, R. W. BUTCHER and E. W. SUTHERLAND, Ann. N.Y. Acad. Sci. *139*, 703 (1967).
[10] This work was supported by the U.S. Public Health Service.

Effect of Proteins on the Reversibility of the Thermal Inactivation of *Bacillus subtilis* α-Amylase

Since the precipitation which occurs upon heat denaturation of protein is probably due to noncovalent inter- and intrachain associations, and since such interactions are believed to be dissociated by urea or guanidine-HCl, attempts were made to regain activity from heat-denatured precipitated enzyme by dissolving it in either guanidine-HCl or urea and then removing the latter by dilution. This was successful with *Escherichia coli* galactosidase[1] and luciferase[2]. It is known that bacterial α-amylase can recover activity after treatment with high concentration of urea[3].

Experiments with urea or guadidine treatment were carried out. Crystalline amylase was dissolved in 8*M* urea or 6*M* guanidine and kept for 6 min at room temperature. The protein solution was then diluted 1000-fold with buffer, as below, and the recovery of activity was very close to 100%. To compare with the dilution experiment, the same sample was dialysed against successive dilutions of denaturing agents; in both cases, recovery was lower than after diluting sample. It is known that amylase once inactivated by urea is not reactivated by dialysis[4]. It was therefore thought interesting to test whether that enzyme, first denatured by heat, could recover its active configuration after being exposed to guanidine-HCl or urea.

Crystalline α-amylase (EC 3.2.1.1) from *Bacillus subtilis* (Sigma Type IIA) was dissolved in phosphate buffer 0.15*M* pH 6.8 with 0.05*M* NaCl and 0.05*M* NaF.

[1] D. PERRIN and J. MONOD, Biochem. biophys. Res. Commun. *12*, 425 (1963).
[2] J. FRIEDLAND and J. W. HASTINGS, Biochemistry *6*, 2893 (1967).
[3] T. TAKAGI and T. ISEMURA, J. Biochem., Tokyo *52*, 314 (1962).
[4] B. HAGIHARA, T. NAKAYAMA, H. MATSUBARA and K. OKUNUKI, J. Biochem., Tokyo *43*, 469 (1956).