In the presence of scavenger, the initial rate of acid formation is zero. As the efficiency of the radical trap falls off – due to the incorporation of monomer into the growing polymer radicals – the acidity of the system correspondingly increases. The initial zero rate of acid production emphasizes however that such an increase in acidity is due to reaction (2) and not to any significant contribution of reaction (1) to the production of acid.

It is apparent that the mechanism of FRONAEUS and OSTMAN[2] does not account for these experimental findings which strongly support the view that reaction (4) is the first step of the decomposition sequence[11].

Zusammenfassung. Säurebildung als Zerfallsprodukt des Peroxodisulfations wurde in Gegenwart eines Radikalsammler mittels pH-stat-Technik untersucht: Die Säurebildung ist zu Beginn null. Dieses Ergebnis stützt die Theorie, wonach $S_2O_8^{2-} \rightarrow 2SO\cdot_4^-$ die Anfangsstufe der Zerfallsfolge ist.

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Retardation of Protein Synthesis in Rat Tumours on Inhibiting Histamine Formation

The discovery of high histidine decarboxylase activity in foetal rat tissues was the starting point for a new field of study: the association between high histamine forming capacity (HFC) and rapid growth[1]. High HFC has been found in various normal and malignant rapidly growing tissues. In healing rat skin wounds collagen formation and tensile strength of the wound could be reduced or enhanced by respectively inhibiting or elevating tissue HFC. Histidine decarboxylase (EC 4.1.1.22) could be specifically and strongly inhibited by α-methyl histidine in vitro and in vivo[2]. The relevant literature has been reviewed by KAHLSON and ROSENGREN[3].

In the rat foetus histidine decarboxylase activity of the liver exceeds that of the young or adult by about 1000 times. Under the influence of α-methyl histidine the rate of protein synthesis, as measured by the incorporation of ^{14}C-leucine, has been shown to be substantially diminished in foetal liver but not in that of the young[4]. The retarded leucine incorporation could not be restored by adding histamine to foetal liver tissue, an instance, among others already known, where extracellular histamine lacks the function of 'nascent histamine', a term coined for the kind of intracellular histamine believed to be associated with rapid growth.

The present study extends the earlier investigation[4] to include rapidly growing malignant tissues, the Walker 256 mammary carcinosarcoma and the Rous virus sarcoma. These rat tumours form histamine at relatively high rates, and the enzyme concerned, histidine decarboxylase, is specifically inhibited by α-methyl histidine[5,6]. The methods employed in the present report are the same as in the previous study[4]. In some experiments, besides α-methyl histidine, the compound 4-bromo-3-hydroxy benzyloxyamine (NSD-1055) was used. This compound inhibits histidine decarboxlase strongly in vitro[7–9].

Both tumours were transplanted subcutaneously to female Sprague-Dawley rats. The Walker 256 tumour was allowed to grow for 5–7 days and the Rous sarcoma for 10–13 days. In each experiment pooled tissue from 2 or 3 animals was used. The tumours were rapidly excised and freed of necrotic parts before mincing. For HFC determinations[2], minced tissue samples of about 200 mg were incubated for 90 min with or without inhibitor. To determine the rate of protein synthesis[4], about 100 mg of minced tumour tissue was preincubated for 20 min at 37 °C with or without inhibitor, 0.05 μmoles of 1-^{14}C-L-leucine (25 mCi/mmole) was added and the samples were incubated for 90 min. Incorporation rates of leucine were estimated in 2 fractions, referred to as soluble and insoluble protein fractions, obtained by the centrifugation of the homogenized samples at 30,000 *g* for 30 min. Results are detailed in Tables I and II.

The rate of histamine formation was higher in the Rous virus sarcoma than in the Walker 256 tumour. In the former tumour 2.5 m*M* DL-α-methyl histidine depressed histamine formation by about 80% (Table I). In the latter tumour (Walker 256), however, inhibition by α-methyl histidine was inconsistent, and in 2 cases it had no effect. The inhibitor NSD-1055 was employed in the Rous sarcoma only, in which 0.5 m*M* concentration strongly depressed histamine formation (Table I).

The rate of leucine incorporation was about the same in the 2 protein fractions in both tumours (Table II). This result differs from the situation in the rat liver in which the incorporation rate into the insoluble protein fraction was only 66% of that in the soluble fraction[4]. In the absence of inhibitor, the rate of incorporation was nearly the same in both tumours. In the Walker 256 tumour, leucine incorporation under the influence of α-methyl histidine (2.5 m*M*) was about 80% of the control values in both protein fractions, and in 2 instances there was no effect (Table II). It would thus appear that this tumour to a minor extent, or not at all, depends on nascent histamine for its growth. In the Rous tumour, by contrast, either of the inhibitors at the concentration indicated, depressed leucine incorporation into both protein frac-

[1] G. KAHLSON, Lancet *1*, 67 (1960).
[2] G. KAHLSON, E. ROSENGREN and R. THUNBERG, J. Physiol. *169*, 467 (1963).
[3] G. KAHLSON and E. ROSENGREN, Physiol. Rev. *48*, 155 (1968).
[4] B. GRAHN, R. HUGHES, G. KAHLSON and E. ROSENGREN, J. Physiol. *200*, 677 (1969).
[5] M. JOHNSTON, Experientia *23*, 152 (1967).
[6] C. G. AHLSTRÖM, M. JOHNSTON and G. KAHLSON, Life Sci. *5*, 1633 (1966).
[7] R. J. LEVINE, T. L. SATO and A. SJOERDSMA, Biochem. Pharmac. *14*, 139 (1965).
[8] M. JOHNSTON and G. KAHLSON, Br. J. Pharmac. Chemother. *30*, 274 (1967).
[9] L. ELLENBOGEN, E. MARKLEY and R. J. TAYLOR JR., Biochem. Pharmac. *18*, 683 (1969).

tions by about 50%. The effect of α-methyl histidine on histamine formation and protein synthesis is in accordance with the earlier observations in rat foetal liver[4].

The interpretation of the experiments involving α-methyl histidine as inhibitor needs some comments. The enzymes catalyzing the amino-acid activation and amino-acyl-t-RNA synthesis are highly specific for the natural amino-acids and will be inhibited by amino-acid analogues. However, a number of such analogues exist which are readily incorporated instead of the natural amino-acids[10]. It is unknown whether α-methyl histidine can similarly be incorporated into the peptide chain. Since leucine incorporation into the 5-day-old rat liver was not affected by the histidine decarboxylase inhibitor[4], it appears unlikely that this analogue per se would interfere with protein synthesis.

We have not yet investigated whether α-methyl histidine or NSD-1055 would retard tumour growth in vivo. For this purpose the concentration of inhibitor, judged from our in vitro experiments, must remain rather high in the neoplasm, which seems difficult to attain, as the first compound undergoes rapid destruction[2] and the second is toxic[7,8].

The process of rapid tissue growth is not always associated with high histidine decarboxylase activity. Several exceptions have been recorded. RAINA and his colleagues[11,12] found increased ornithine decarboxylase activity in the regenerating rat liver following partial hepatectomy and increased formation of spermidine in Ehrlish ascites tumour cells in mice and in the developing chick embryo. RUSSEL and SNYDER[13] have recently demonstrated a high formation rate of diamines in certain artificial tumours, some of which formed putrescine and others histamine. An inverse relationship between the activities of ornithine decarboxylase and histidine decarboxylase was noted. Confirming RAINA[11] and JÄNNE[12], they found high levels of ornithine decarboxylase in the regenerating rat liver after partial hepatectomy, and in the chick embryo, whereas the foetal rat liver displayed high histidine decarboxylase activity. It has not been investigated whether inhibition of ornithine decarboxylase would retard growth and protein synthesis in any of these tissues.

On the assumption that in some rapidly growing tissues, putrescine and spermidine play the role of 'nascent histamine', we suggest that in the light of new information, the original hypothesis[1] be broadened to state that high rates of intracellular diamine formation is associated with and presumably essential to certain types of rapid growth[14].

Table I. Formation of ^{14}C-histamine from 2-ring-^{14}C-L-histidine by rat tumour tissue in 90 min

Walker 256 mammary carcinoma		
No inhibitor	Inhibitor DL-α-Methyl histidine 2.5 m*M*	
161	55	
54	69	
74	20	
205	210	
134	28	
Rous virus sarcoma		
No inhibitor	Inhibitor DL-α-Methyl histidine 2.5 m*M*	NSD-1055 0.5 m*M*
304	55	
167	47	
154	27	
202	29	
286	33	17
408		12
227	53	20

The figures are ng histamine formed per g wet tissue and are the means of duplicate determinations on samples of minced tissue. Molarities are final concentrations.

Table II. Incorporation of 1-^{14}C-L-leucine into rat tumour tissue in 90 min

Walker 256 mammary carcinoma			
Soluble fraction		Insoluble fraction	
No inhibitor	Inhibitor DL-α-Methyl histidine 2.5 m*M*	No inhibitor	Inhibitor DL-α-Methyl histidine 2.5 m*M*
7.2 (3)	5.6 (2)	8.1 (3)	6.3 (2)
7.1 (2)	5.5 (3)	9.1 (2)	9.6 (3)
16.1 (5)	15.1 (3)	28.5 (5)	22.1 (4)
8.2 (3)	5.6 (3)	12.1 (3)	7.1 (3)
10.4 (3)	9.0 (3)	14.6 (3)	11.9 (3)
19.3 (4)	16.1 (4)	24.7 (4)	20.4 (4)

Rous virus sarcoma					
Soluble fraction			Insoluble fraction		
No inhibitor	Inhibitor DL-α-Methyl histidine 2.5 m*M*	NSD-1055 0.5 m*M*	No inhibitor	Inhibitor DL-α-Methyl histidine 2.5 m*M*	NSD-1055 0.5 m*M*
10.7 (3)	4.7 (3)		7.0 (3)	3.3 (3)	
13.8 (4)	8.1 (4)		11.9 (4)	7.9 (4)	
10.1 (5)	4.4 (5)		14.0 (5)	6.3 (5)	
8.7 (2)	4.4 (2)	4.8 (2)	10.0 (2)	5.7 (2)	6.3 (2)
9.6 (2)		5.6 (4)	11.4 (2)		6.7 (4)
7.5 (2)	4.1 (2)	4.3 (4)	9.7 (2)	5.7 (2)	5.1 (4)

The figures are mean cpm × 10^3 per mg protein in samples of minced tissue. The number of determinations in each experiment is given in parenthesis. Molarities are final concentrations.

Zusammenfassung. Untersuchung der Proteinsynthese bei Virustumoren. Anhand der Inkorporierungsgeschwindigkeit von ^{14}C-Leucin wurde festgestellt, dass die Hemmung der Proteinsynthese im Rous-Sarkom höher ist als im Walker-Karzinom.

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[10] H. R. MAHLER and E. H. CORDES, *Biological Chemistry*, 4th edn. (Harper International Editions, New York 1968), p. 796.
[11] A. RAINA, Acta physiol. scand. *60*, Suppl. 218 (1963).
[12] J. JÄNNE, Acta physiol. scand. Suppl. 300 (1967).
[13] D. RUSSEL and S. H. SNYDER, Proc. natn. Acad. Sci. USA *60*, 1420 (1968).
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