Histamine Formation in Rats Bearing the Walker Mammary Carcinosarcoma

During recent years certain observations have led to the belief that there is a connection between rapid tissue growth and histamine formation[1]. For example, embryonic tissue in the rat, mouse and man, rat bone marrow, wound and granulation tissue in rat and man, rat hepatoma and Walker rat mammary carcinosarcoma have been shown to be capable of forming histamine at high rates in vitro, and an elevated excretion of histamine in the urine of pregnant and hepatoma-bearing rats has been observed[1,2]. Furthermore, investigation of the Ehrlich ascites tumour of mice showed that there was a positive correlation between histamine forming capacity (HFC) and mitotic index[2].

The present report gives results of further experiments on the Walker mammary carcinosarcoma. HÅKANSSON[3] measured the HFC of this tumour in vitro and obtained values very much higher than those given by normal mammary tissue, but no in vivo measurements of urinary histamine excretion were performed. HALLENBECK and CODE[4], in their experiments on the Walker rat carcinosarcoma in Sprague-Dawley rats, found no great or consistent changes in the levels of urinary histamine during growth or after excision of the tumour. However, since a striking rise in histamine excretion was reported for hepatoma-bearing rats, a fuller investigation of the Walker tumour, employing the best of available methods, seemed desirable.

Materials and methods. The tumour was obtained by courtesy of Prof. A. HADDOW, Chester Beatty Institute, London, and was transplanted into adult female rats of a strain bred at the Institute of Physiology, Lund. A limited number of experiments were done using Sprague-Dawley rats. Under sterile conditions and using a trocar, small pieces of neoplastic tissue were deposited subcutaneously near the scapula. The tumours grew rapidly, reaching 30–40 g at about 14 days, by which time central necrosis had developed.

The urinary excretion of histamine, which has been shown to reflect the rate of whole-body endogenous formation of the substance[5], was followed in female rats before and after implantation of the tumour. The animals were kept in metabolism cages and fed a semisynthetic, histamine-free diet[6]. Urine was collected continuously in 24 h samples in flasks containing a few drops of concentrated hydrochloric acid, to prevent formation or destruction of histamine by bacteria. Free histamine was assayed on the atropinized guinea-pig ileum.

Excretion of ^{14}C-histamine after injection of ^{14}C-histidine was also investigated. 2 rats were injected s.c. with 50 μg ^{14}C-histidine before and after tumour implantation, and aliquots of the urines were assayed for ^{14}C-histamine for the 3 consecutive days after injection, using a method which has been described previously[7].

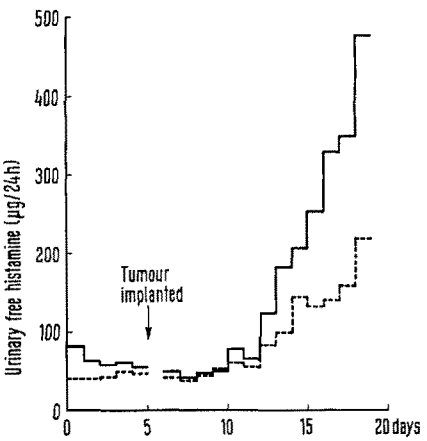
The rate of formation of histamine by the tumour tissue in vitro was measured by a method which has been described in detail previously[7]. Briefly, finely-cut tissue was incubated with ^{14}C-histidine and the ^{14}C-histamine formed was measured. The coenzyme pyridoxal-5-phosphate, 7.9 μg per sample, was added to the incubation mixture since it was found to enhance the enzyme activity. Results are expressed as ng ($=10^{-9}$ g) histamine formed/g tissue/3 h.

The well-known phenomenon of liver enlargement in the tumour-bearing host[8] was also noticed in this investigation, and it seemed of interest to discover whether the liver enlargement was another example of growth accompanied by high HFC. Using the same method as described above for the tumour tissue, the rate of histamine formation in vitro of liver tissue from tumour-bearing and control animals was measured. The effect of the enzyme inhibitors α-methylhistidine and α-methyl DOPA on tumour and liver tissue was investigated in vitro. Each was added separately to the usual incubation mixture and HFC determined as described above.

Results and discussion. Urinary free histamine excretion was found to increase as the tumours grew, in both Sprague-Dawley and Institute-strain rats. Increases of from 3–8 times the pre-implantation values were observed and 2 examples from individual rats are shown in the Figure. In both rats injected with ^{14}C-histidine the excretion of ^{14}C-histamine was higher during the course of tumour growth than it was in the control period before implantation (Table I).

Table I. Excretion of ^{14}C-histamine after injection of ^{14}C-histidine (Counts per min/Total daily urine volume)

		Before implantation	12–15 days after implantation
Sprague-Dawley rat	1st day	195	240
	2nd day	28	115
	3rd day	14	78
Institute strain rat	1st day	83	259
	2nd day	18	73
	3rd day	6	81



Urinary histamine excretion patterns in 2 female rats implanted at the arrow with the Walker carcinosarcoma.

[1] G. KAHLSON, Perspect. Biol. Med. *5*, 179 (1962).
[2] G. KAHLSON, E. ROSENGREN, and C. STEINHARDT, J. Physiol., Lond. *169*, 487 (1963).
[3] R. HÅKANSSON, Experientia *17*, 402 (1961).
[4] G. A. HALLENBECK and C. F. CODE, Proc. Soc. exp. Biol. Med. *110*, 649 (1962).
[5] B. GUSTAFSSON, G. KAHLSON, and E. ROSENGREN, Acta physiol. scand. *41*, 217 (1957).
[6] G. KAHLSON, E. ROSENGREN, and H. WESTLING, J. Physiol., Lond. *143*, 91 (1958).
[7] G. KAHLSON, E. ROSENGREN, and R. THUNBERG, J. Physiol., Lond. *169*, 467 (1963).
[8] F. N. GHADIALLY and E. W. PARRY, Cancer, N.Y. *18*, 485 (1965).

The in vitro results on tumour HFC (Table II) show that the tumour tissue has a considerable ability to form histamine but, as is generally observed for enzyme activities in transplanted tumours[9], the values fall within the extremes found in normal tissues (values for rat tissues, expressed as ng/g/3 h, as measured in this laboratory: gastric mucosa up to about 15,000, small intestine often no detectable activity). The rate of histamine formation in vitro in the liver tissue of tumour-bearing rats (Table II) was found to be very much higher than in non-tumour-bearing controls.

The effects of the compounds α-methylhistidine and α-methyl DOPA on the rate of histamine formation in tumour tissue and liver of the tumour-bearing host were different (Table III). Whereas the former compound produced 70–90% inhibition of enzyme activity, the latter had practically no inhibitory effect. The contrasting effects of these 2 substances have been used to distinguish histidine decarboxylase from what is referred to as 'non-specific' histidine decarboxylase, the former being strongly inhibited by α-methylhistidine but not by α-methyl DOPA, which latter compound strongly inhibits the 'non-specific' decarboxylase[7]. The results obtained in the present study are similar to those previously recorded for mouse foetal tissues[10] and Ehrlich ascites tumour[2], thus indicating that the enzyme involved is histidine decarboxylase.

Parabiosis experiments. The finding that the presence of a tumour in these rats seemed to cause an elevation in liver HFC prompted investigation of parabiotic rats, one partner tumour-bearing, to see whether a blood-borne agent(s) was responsible. Pairs of parabiotic rats were prepared, using female litter-mates aged 5–6 weeks. A large area of lateral skin extending from the ear to the tail was removed from each partner and the cut edges were joined by interrupted everting sutures. This procedure is known to result in a degree of common blood flow. 6 weeks after the operation, when risk of parabiosis intoxication was negligible, 1 partner from each of 2 pairs was implanted with the tumour as described above. After a further 2 weeks both pairs were killed and the HFC of their tissues determined. A control pair which had not been implanted was also investigated.

The results (Table IV) indicate a general elevation of HFC in many tissues, including the liver. The values for the non-tumour-bearing partners of pairs 2 and 3 fall between to those of their tumour-bearing counterparts and the control pair (pair 1). This is consistent with the involvement of a blood-borne agent(s) in the mechanism causing elevation of HFC. The effect is naturally greater in the tumour-bearing partner since the concentration of the agent(s) in the blood of the other rat will not be so high, possibly due to destruction or excretion. In the case of abdominal skin, there seems to be no eleva-

Table II. Rate of histamine formation in tumour and liver tissue

Tumour age, days	Tumour weight, g	Tumour HFC, ng/g/3 h	L/C[a]	Liver HFC, ng/g/3 h
14	40	278	0.074	290
14	35	416	0.054	437
14	34	821	0.071	582
14	32	884	0.063	296
14	38	1090	0.073	1200
17	60	770	0.081	613
17	49	1190	0.064	371
17	37	1890	0.065	886
25	69	900	0.043	219
Controls			0.038	31
			0.044	27
			0.039	22
			0.049	25
			0.032	18

[a] L/C is the ratio liver weight/carcass weight (weight of rat exsanguinated and minus the tumour).

Table III. Effect of α-methylhistidine and α-methyl DOPA (10^{-3} *M*) on the rate of histamine formation of tumour tissue and liver of the tumour-bearing host (ng histamine formed/g tissue/3 h)

Rat	Tumour			Liver		
	No addition	α-methyl-histidine	α-methyl DOPA	No addition	α-methyl-histidine	α-methyl DOPA
1	166	42	191	62	15	58
2	900	211	806	219	26	219
3	1550	381	1560	2530	354	2310
4	416	79	437	437	70	418

[9] J. P. GREENSTEIN, *Biochemistry of Cancer* (Academic Press, New York 1954), p. 362.

[10] E. ROSENGREN, Experientia *18*, 176 (1962).

Table IV. HFC of tissues of parabiotic rats (ng histamine formed/g tissue/3 h)

	Pair 1		Pair 2		Pair 3	
	− Tumour	− Tumour	− Tumour	+ Tumour	− Tumour	+ Tumour
Abdominal skin	61	43	49	989	58	464
Liver	58	44	95	305	278	523
Lung	963	838	1,932	3,374	3,337	7,460
Spleen	935	678	1,844	2,684	4,337	4,510
Gastric mucosa	15,050	18,500	7,439	2,149	16,140	21,660
Small intestine	–	–	–	–	–	4
Kidney	6	1	18	35	51	80
Tumour				398		412

– = not detectable.

tion of HFC in the non-tumour-bearing partners, and the figures for gastric mucosa are inconsistent. A similar general elevation of HFC has previously been noted in the tissues of mice dying from Ehrlich ascites tumour[2], but no indication as to the mechanism of the process was obtained.

It appears that the unusually high excretion of histamine in the urine of female rats bearing the Walker mammary carcinosarcoma may be explained by the ability of the tumour itself to form histamine and also by the increased capacity of the liver in this respect. From the results of the experiments on parabiotic rats it seems that the HFC of several tissues, including the liver, may become elevated and that a blood-borne factor is involved. The observations of high HFC in the tumour and enlarged liver tissue support the view that there is a connection between high rates of histamine formation and certain types of rapid tissue growth[11].

Zusammenfassung. Die Geschwindigkeit der Histaminbildung wurde bei Ratten von experimentellem Walker Carcinosarcom kontrolliert. Bei Parabiosen konnte gezeigt werden, dass der Faktor, welcher diese Histaminbildung auslöst, mit dem Blutstrom übertragen wird.

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Oscillations in the Pigeon's Pupil Servomechanism in Relation to Illumination

The cybernetic approach considers the pupil reflex to light as a self-regulated control device to regulate light impinging on the retina. Similarly to the human eye[1], the pupil of the chicken continuously undergoes small fluctuations in area even in steady illumination[2], comprehensible when considered as sustained oscillations in the pupil servosystem because of the time lag of the feedback path which completes the loop. This induced pupillary hippus may originate in, or be modified by, properties of the iris neuromuscular system (i) or central nervous system elements such as reflex centres of the brain stem (ii) or the retina (iii).

Comparison of the frequency spectrum of the pupillary unrest shows values up to 2 c/sec in the smooth muscle of the human iris[3] and up to 15 c/sec for the pigeon's iris consisting of striate muscles, which clearly indicates the effect of the iris neuromuscular system on the spontaneous oscillations of the pupil area. Similarly, the effect of the central nervous system can be inferred from the close correlation of unrest of one iris with the simultaneously recorded unrest of the contralateral iris in humans[4], and from the effect of brain cooling on the frequency of pupil oscillations in pigeons[5]. The present experiments, which are part of a study of the pupillary reflex to light in the pigeon[6], were undertaken in order to determine how far the pupil's unrest can be modified by changes at the loop input, i.e. at the retinal level.

Awake, unanaesthetized pigeons were used throughout the investigation. The head of the animal was fixed by holding the skull between the occiput and the beak. The pupil area was measured continuously by reflecting IR-light from the iris onto an IR-photocell shielded with a Kodak 87C filter to eliminate the effects of visible light of the conditioning beam. The output of the photocell was fed into the input of a d.c. amplifier of an oscilloscope (Tektronix type 502A) and recorded photographically on moving film. In order to correct the luminance readings into retinal illumination, it was necessary to measure the actual size of the pupil. This was done by taking photographs of the pigeon's pupil in IR-light. The experiment started after a dark period of 1 h by determining the threshold of the pupillary light reflex. The experiments were performed by exposing the eye to constant lights of increasingly higher luminances. Records were taken after the adapting light was presented for a period of not less than 10 min.

The Figure, showing records of a typical experiment, gives samples of the fluctuations of the pupillary area at different constant luminances of adaptive illumination. As can be seen from part A of the Figure there are, at low levels of illumination, only a few oscillations of larger amplitude. With increasing luminances of the conditioning light both the amplitude and the frequency of the sustained oscillations increase[7]. Quantitatively (Figure B) the frequency of the pupillary oscillations increases from low values (about 1 c/sec) in dim light (retinal illumination less than 10 Troland) to high values (about 15 c/sec) at high luminances of the conditioning light (1500 Troland). Thus the increase of frequency of oscillations of the pupillary diameter is clearly in the photopic range of luminances of the pigeon's pupillary response to light[6]. While it is commonly believed that the pupillary response to light is mainly governed by the retinal cones[8], this is certainly untrue in the dark-adapted state and for light stimuli evenly distributed over the entire retinal surface[9]. However, the present experiments indicate it to be true for the oscillatory changes of the

[1] L. STARK, F. W. CAMPBELL, and J. ATWOOD, Nature, Lond. *182*, 857 (1958).
[2] H. G. THIENEMANN, Zool. Jb. Allg. Zool. Physiol. *57*, 293 (1937).
[3] J. STEGEMANN, Pflügers Arch. ges. Physiol. *264*, 113 (1957).
[4] L. STARK, Proc. IRE *47*, 1925 (1959).
[5] Unpublished data of the laboratory. A full account will be published elsewhere.
[6] E. ALEXANDRIDIS, Pflügers Arch. ges. Physiol. *289*, R 63 (1966).
[7] A similar phenomenon has been described previously in the chicken[2]. However, for technical reasons neither the frequencies of oscillations nor the photometric quantities are comparable with the present results.
[8] H. HARMS, Albrecht v. Graefes Arch. Ophthal. *149*, 1 (1949).
[9] N. M. J. SCHWEITZER, Documenta ophth. *10*, 1 (1956).