Tab. I. Aktivität und Hitzedenaturierung verschiedener Enzyme in der Dorschmuskulatur

Enzym	Enzymquelle	Messtemperatur °C	Aktivität gesamt µMol/h/g Frischgewicht	Aktivität spezifisch µMol/h/mg Protein	Temperatur, bei der nach 1stündiger Einwirkung 50%ige Denaturierung erfolgt
Kathepsin, Rohextrakt	Dorschmuskel	37	2.9(Tyrosin)	0,17	44
Kathepsin, teilgereinigt	Dorschmuskel	37	—	5,3	52
Glycylglycindipeptidase	Dorschmuskel	40	420	158	42
Glycylglycindipeptidase	Rindermuskel	40	185	35	46
Milchsäuredehydrogenase	Dorschmuskel	25	20 100	750	52
Aldolase	Dorschmuskel	25	1 250	43	41
Triosephosphatdehydrogenase	Dorschmuskel	25	4 600	180	51
Enolase	Dorschmuskel	25	3 080	128	45
Pyruvatkinase	Dorschmuskel	25	3 410	141	51
Isocitricodehydrogenase	Dorschmuskel	25	202	8,4	30

Tab. II. Q_{10}-Werte von zwei Enzymen aus Dorschmuskulatur

Messbereich in °C, der zur Berechnung benutzt wurde	Glycylglycin-dipeptidase	Kathepsin, teilgereinigt
von 37 bis 27	1,66	2,03
32 22	1,84	
27 17	1,97	1,86
22 12	2,13	
17 7	2,17	2,13
12 2	2,67	
7 – 3		2,41
–3 –13		2,45

et al.[7], Aldolase, Triosephosphatdehydrogenase und Pyruvatkinase nach BÜCHER *et al.*[8], Enolase nach BÜCHER[9], Proteinbestimmung nach LOWRY *et al.*[10].

Ergebnisse. Die in Tabelle I enthaltenen Versuche zeigen, dass der Bereich 50%iger Denaturierung in sehr weiten Grenzen, zwischen 30 und 52°C, schwankt. Die von PARTMANN untersuchte Adenosintriphosphatase fällt mit etwa 38°C in den unteren Bereich. Gesetzmässigkeiten bezüglich der Lebensweise als Kaltblüter lassen sich aus diesen Daten daher nicht ableiten. Proteinchemische Konsequenzen hinsichtlich besonders geringer thermischer Stabilität (etwa im Gegensatz zu den hochgradig hitzeresistenten Enzymproteinen thermophiler Bakterien) können erst erörtert werden, wenn die betreffenden Enzyme in gereinigter Form vorliegen. Reinigungseffekte durch Erhitzung haben wir beobachtet bei Milchsäuredehydrogenase (2fach), Enolase (2,2fach), Pyruvatkinase (2,8fach) und Triosephosphatdehydrogenase (3,5fach), jedoch nicht weiter verfolgt. Von den verschiedenen Erklärungsmöglichkeiten, warum gereinigtes Kathepsin (SIEBERT und v. MALORTIE[2]) eine grössere Hitzeresistenz als das im Rohextrakt vorliegende Enzym aufweist, wird keine durch die bisher vorliegenden experimentellen Erfahrungen gestützt.

Wie zu erwarten, sind die thermodynamischen Qualitäten der Kaltblüterenzyme gleich denen der Warmblüterenzyme. Dies geht aus Tabelle II hervor, in der für Glycylglycin-dipeptidase und für Kathepsin aus Dorschmuskulatur die Temperaturkoeffizienten Q_{10} zusammengestellt sind. Der im Messbereich 37–27°C aus der Reihe fallende Wert für Kathepsin, der einen im Verhältnis zu geringen Umsatz bei 37°C anzeigt, dürfte auf bereits beginnende thermische Denaturierung zurückzuführen sein, obwohl erfahrungsgemäss bei den hier untersuchten Enzymen Substratgegenwart vor der Hitzedenaturierung in gewissem Umfang zu schützen vermag.

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R. v. MALORTIE und E. ADLOFF

Physiologisch-chemisches Institut der Universität Mainz (Deutschland), 14. Juli 1960.

Summary

The temperature, which leads to 50% reduction of catalytic activity by heat denaturation, has been determined for 8 different enzymes from cod muscle, as being in the range between 30 and 52°C. Therefore, there are no indications of a generally different heat resistance of enzymes from cold-blooded animals as compared with those from warm-blooded animals. The same conclusion is derived from calculations of Q_{10}-values, measured between + 37 and – 13°C for cathepsin and glycylglycine dipeptidase.

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Release of 5-Hydroxytryptamine and Histamine from Neoplastic Mast Cells by Alkaline Tissue Extracts[1]

The pharmacological release of biogenic amines from a number of tissues by reserpine and related compounds is now a well-documented phenomenon[2,3]. In the intact anesthetized dog, without pharmacological intervention, 5-hydroxytryptamine (5-HT, serotonin) has been shown to be released from the gastro-intestinal tract, but the natural stimulus responsible for this release is not known[4].

[1] Aided in part by grant B-940 from National Institute of Neurological Diseases and Blindness, and in part by the James Hudson Brown Memorial Fund of Yale Medical School.

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In this connection, the observations of Toh[5,6] that alkaline extracts of certain mammalian tissues, under *in vitro* conditions, will liberate both 5-HT and histamine from platelets (dog), are of some interest.

In using suspensions of neoplastic mast cells (mouse) instead of platelets as a source of 5-HT and histamine we have confirmed and extended the observations of Toh, in a preliminary attempt at characterizing the active principle(s).

Extracts of various tissues of the rat were prepared by a slight modification of the method of Toh[5] so that approximately 0·2 ml of extract was derived from each 50 mg of tissue. The neoplastic mast cells, harvested from the ascitic fluid of mice inoculated 7 to 10 days earlier, were centrifuged, washed and suspended in either Hank's solution[7] or a calcium-free fluid originally described by Tullis[8] and modified by Toh[5]. The cold suspension of cells (usually 1 ml containing 10×10^6 cells) and a small volume of extract (usually 0·1 ml) were mixed and incubated at 37°C for 40 min. At the end of that time the cells were separated by centrifugation, and both cells and supernatant medium were analyzed separately for their content of 5-HT (and, occasionally, of histamine). The 5-HT was extracted by means of 95% acetone as described by Amin, Crawford, and Gaddum[9] and assayed on the heart of *Mercenaria* (*Venus*) *mercenaria*. Histamine was extracted with 1 N HCl; and the centrifuged, neutralized extract was assayed on the isolated ileum of the guinea pig.

Untreated suspensions of cells, with pH adjusted appropriately, served as controls in each experiment. These always showed a small release of 5-HT (10%) at the end of the incubation procedure; and, therefore, all results are expressed as % total release minus % release found in control tubes.

A number of tissues of the rat were extracted and the extracts tested for their 5-HT releasing activity. Composite results of 5 experiments are shown in Table I.

It is clear that extracts of brain had the greatest activity. Extracts of lung, kidney, duodenum and spleen were also active; those from heart were barely active; and extracts of blood and skin were inactive. These findings agree generally with those of Toh[5,6] and of Archer[10]. Toh, however, laid special emphasis upon activity of kidney extracts, without having observed the equal or greater activity of extracts of brain.

Varying the number of neoplastic mast cells in the test suspensions from 8 to 23×10^6 cells per ml of suspending medium had no consistent effect on the % of 5-HT released by a given concentration of brain extract; however, the amount of 5-HT released varied with the amount of extract (over the range: 5 to 56 mg, i. e., the tissue equivalent, diluted to 0·2 ml with suspending medium). Results of two representative experiments are summarized in Table II.

In experiment 2, the brain extract used was one found to have relatively low activity (maximum release; 52%); however, it is evident that the extract from as little as 5 mg of brain can cause a substantial release of 5-HT. These data would indicate that an excess of extract was used in all other experiments.

The extent of release of histamine studied under identical conditions was similar to that of 5-HT. For instance, a potent brain extract which caused the release of 86% 5-HT liberated 95% of the histamine in the cells.

The release of 5-HT by extracts of brain was dependent upon temperature, but not upon pH (in the range, pH 5·0 to pH 8·0), and it increased directly with the duration of incubation. Furthermore, the active principles in the

Tab. I. 5-HT Releasing Activity of Alkaline Extracts of Various Tissues of the Rat

Tissue	Equivalent Weight Tested (mg)	5-HT Content (γ)			5-HT Release (%)
		Supernatant	Cells	Total	
Control	—	0.1	2.4	2.5	4
Brain	68	4.8	0.4	5.2	92
Lung	74	4.4	1.0	5.4	81
Kidney	59	1.0	0.3	1.3	76
Duodenum	64	0.8	0.3	1.1	73
Spleen	60	2.6	1.3	3.9	67
Heart	50	0.8	1.6	2.4	33
Blood	50	0.3	2.0	2.3	13
Skin	50	0.2	4.8	5.0	3

Tab. II. Relation of Concentration of Alkaline Extracts of Rat's Brain to Release of 5-HT from Mast Cells

Experiment	Tissue Equivalent of Extract (mg)	5-HT Content (γ)			5-HT Release (%)
		Supernatant	Cells	Total	
1	14	2.8	0.6	3.4	84
	28	2.8	0.3	3.1	92
	56	3.2	0.2	3.4	94
2	5	0.7	1.6	2.3	29
	10	0.8	1.5	2.3	33
	15	0.5	1.4	1.9	38
	20	1.1	1.2	2.3	48
	25	1.0	1.3	2.3	42
	50	1.2	1.1	2.3	52

Tab. III. Influence of Suspending Medium on Amine-Releasing Potency of Alkaline Extracts of Rat's Brain

Suspending Medium	% Release of 5-HT	% Rel. of Histamine	% Cells «Non-Viable»[a]	
			Treated	Control
Isotonic Salt	20	—	20	18
Tullis-Toh's	86	95	18	5
Ringer-Locke's	50	—	22	7
Locke-Lewis'	2	—	4	4
Hanks'	10	12	2	4

[a] As indicated by microscopic examination.

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[14] This work was done in partial fulfillment of the requirements for the degree of Doctor of Medicine by L. T. P.

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extracts were stable to heat, acid and alkali, and could not be separated completely from protein by these and other precipitants, or by dialysis.

To test the hypothesis that the amine-releasing activity of the extracts might be mediated by damage to the membrane of the neoplastic mast cell, experiments were carried out in a variety of media ranging from simple isotonic salt solution to Hanks' solution (a complex salt solution containing glucose, used as the basal salt medium in the culture of neoplastic mast cells *in vitro*[11]. Table III summarizes the results of these experiments.

In those solutions containing glucose, Hanks' and Locke-Lewis', the potency of the brain extract was markedly inhibited, as was destruction of the cells. In fact, the degree of amine-release which occurred in Tullis-Toh's or Ringer-Locke's solutions could be accounted for in part by destruction of the cells. By contrast, the entire release of 5-HT produced in isotonic salt solution was directly related to cell destruction. Further experiments have served to show that the most of the antagonism to amine-release seen in Hanks' solution is attributable to the presence of glucose in this medium. Such an effect of glucose might be expected if the extracts had a non-specific damaging action on the cell membrane which could be opposed by increased metabolic activity.

The activity of these tissue extracts may be compared most closely to the action of certain monoamines and surface-active agents (e. g., saponin)[12,13]. The extracts caused some apparent damage to the cells, but lysis of the cells was not sufficient to explain the activity observed. These extracts probably did exert a surface action, but it is unlikely that purely toxic, surface-active principles would have had all the characteristics of temperature dependence, variation in tissue source, relatively low lytic activity and «dosage»-effect relationships which were observed. It is generally concluded that degradation products (probably polypeptides or simpler amines) derived from injured tissues can cause the release of 5-HT and histamine from certain storage sites.

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Zusammenfassung

Alkalische Extrakte diverser Rattengewebe setzen 5-Hydroxytryptamin (Serotonin) und Histamin aus Suspensionen neoplastischer Mastzellen der Maus frei. Gehirn- und Nierenextrakte zeigen die grösste Aktivität. Zugabe von Glukose in die Nährlösung verhindert das Freiwerden der Amine. Eine vorläufige Charakterisierung des aktiven Materials ist unternommen worden.

Histamine et métaux cancérigènes

Un travail précédent a établi la fixation de l'histamine par les substances cancérigènes organiques de structure différente[1,2]. Il a paru intéressant d'élargir le champ et d'observer le comportement de l'amine avec les métaux actifs (argent, nickel, cobalt, chrome, glucinium).

En effet, lorsque histamine est au contact de divers sels de ces métaux, une base complexe prend naissance en phase aqueuse. Dans certains cas le complexe apparaît sous une forme cristallisée qui entraîne une étude minéralogique, dans d'autres cas le complexe est hydrosoluble et la méthode des variations continues de Job s'applique à l'absorption lumineuse dans l'ultra-violet[3].

L'argent s'est révélé cancérigène à divers auteurs qui implantèrent des fragments métalliques dans la paroi abdominale de rats. Les tumeurs se sont développées *in situ*[4]. L'étude physico-chimique comporte le fluorure, le nitrate, le sulfate, l'acétate et le butyrate d'Argent. Le complexe $[Ag\ Hi_2]^+$ est caractérisé quelle que soit la nature de l'anion. La constante de stabilité $K = 3 \cdot 10^{-10}$ à 20°C (Fig. 1).

Le nickel a fait l'objet d'études biologiques très poussées. Les applications de métal divisé produisent le cancer au lieu même du dépôt chez les rats et les lapins avec un certain caractère de spécificité quand le véhicule utilisé pour la poudre métallique s'est révélé strictement inactif. De plus, les cancers des voies respiratoires se sont développés nombreux, provoqués par la poussière de nickel dans l'industrie du métal[5,6]. La complexion du nickel par l'histamine a déjà été entrevue dans une étude électrochimique des solutions diluées par divers auteurs (voir réf. [7]). L'étude présente permet de caractériser les complexes $[Ni\ Hi_2]^{++}$ et $[Ni\ Hi_3]^{++}$ en solution et à l'état cristallisé[7].

Le cobalt métallique divisé, administré par injections intramusculaires dans la cuisse de rats, provoque l'apparition de tumeurs *in situ*. Les injections intra-fémorales pratiquées chez le lapin produisent des tumeurs *in situ* et au loin[8]. Les sels cobalteux forment avec histamine le complexe soluble $[Co\ Hi_2]^{++}$ déjà mis en évidence par un important travail électrochimique (voir réf. [7]). La méthode de Job confirme et complète cette étude. L'examen spectral s'étend au nitrate, au chlorure et au sulfate cobalteux.

Les particules de chrome implantés dans le fémur ou introduites dans le poumon de divers animaux (lapins, chiens) font naître des tumeurs au lieu du dépôt et à distance. La fréquence des cancers observés chez les ouvriers de l'industrie vient à l'appui de l'expérimentation[9,10]. L'étude physico-chimique donne les résultats suivant: 1° Si l'on met au contact de solutions d'histamine ($M/100$) des solutions de Cl_3Cr de même titre, on observe dans les mélanges de Job une précipitation lorsque le milieu devient alcalin. L'étude spectrale des mélanges restés clairs, donne après 10 jours de contact, une courbe où la complexion de Cr par histamine apparaît déjà (Fig. 2). 2° Dans les mélanges faits à force ionique constante $\mu = 1$, le pH reste compris entre 2,75 et 4,60 zône ou Cr^{+++} existe soluble. On caractérise le complexe $[Cr\ Hi_2]^{+++}$ la constante de stabilité $k = 5 \cdot 10^{-6}$ (Fig. 3).

Le glucinium est hautement cancérigène[11]. De nombreux cas de cancérisation chez les ouvriers en contact avec les poussières ou les vapeurs de métal ont été relevés[12–14]. Le glucinium introduit dans la peau de jeunes verrats, administré par voie respiratoire, injecté par voie sous-cutanée ou intraveineuse à des souris, à des rats et à des lapins, est retrouvé *in situ* et même trois mois après

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