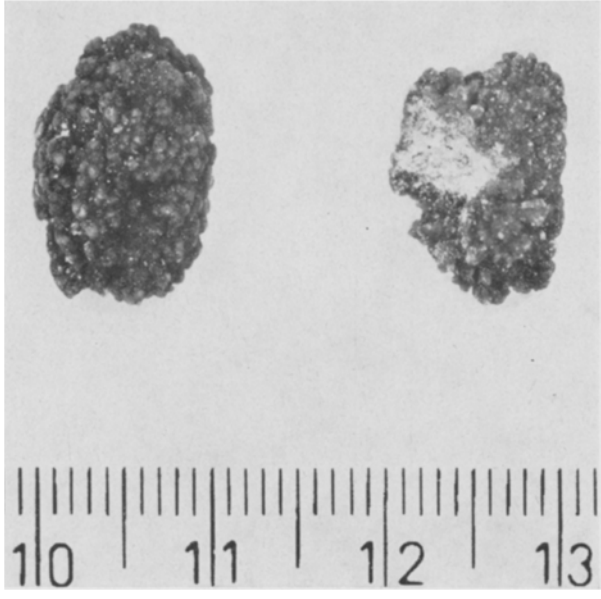
Cystinurie und L-Cystinsteinbildung beim Hund

Die Cystinurie, eine erbliche Stoffwechselanomalie, und die Cystinsteinbildung ist bei Menschen eine relativ seltene Krankheit. Eigene Untersuchungen von 1564 Nierensteinen (Stand 30. 8. 1972) mit Hilfe der IR-Spektrographie haben ergeben, dass insgesamt 24 Steine chemisch aus L-Cystine zusammengesetzt sind, d.h. die Häufigkeit der Cystinsteinbildung betrug bei unserem Kollektiv 1,53%. Obwohl diese Krankheit selten vorkommt, können Genese und Auflösung von Cystinsteinen als Modell für die Entwicklung einer Therapie des Steinleidens dienen, da die Vorgänge bei diesen Krankheitszuständen ähnlichen Prinzipien folgen. Am Beispiel der Cystinsteinauflösung lassen sich die chemischen und physikalischen Grundlagen der Steinauflösung besonders gut studieren, weil es sich bei den Cystinsteinen meistens um sehr reine Steinbildungen handelt[1].

Von Interesse wäre es deshalb, die experimentelle Erzeugung von Cystinsteinen bei Versuchstieren zu probieren, oder noch besser Tiere zu suchen bei denen eine Cystinurie bzw. eine Cystin-Urolithiasis vorliegt. Für diesen Zweck wäre die Zusammenarbeit mit frei praktizierenden Tierärzten oder mit veterinär-medizinischen Fakultäten, wo solche Fälle auftauchen, unerlässlich.



Der aus dem Rüden operativ gewonnene L-Cystinstein, links: äussere Sicht; rechts: Querschnitt. Die weissen Stellen entstanden bei der Probeentnahme (Kern, mit mittlere und äussere Schicht). Gewicht des Steins 1,6 g; Länge 16 mm; Breite 10 mm; Höhe 9 mm.

Methodik und Resultate. Bisher wurden von uns insgesamt 20 Steine, die von verschiedenen Tierarten stammen, infrarot-spektrographisch untersucht (15 Hunde, 2 Stuten, 1 Hammel, 1 Rind und 1 Maus). Die grösste Anzahl waren Steine von Hunden, die operativ entfernt wurden. Sie setzen sich vorwiegend aus Magnesium-Ammonium-Phosphat (bei infizierten Nieren) und Calcium-Oxalat zusammen.

Bei einem 3jährigen Rüden, Rasse Basset (23 kg Körpergewicht), wurde röntgenologisch ein Blasenstein festgestellt. Nach operativer Entfernung konnte infrarotspektrographisch nachgewiesen werden, dass der Stein chemisch aus L-Cystin besteht (Figur).

Der Cystinnachweis im Urin mit Cystinognost (Fa. Heyl, Berlin) bzw. Natrium-nitroprussid war ebenfalls positiv.

Post-operativ wurde als Prophylaktikum Thiola (α-Mercapto-propionyl-glycin) verabreicht, um die L-Cystin-Löslichkeit im Urin zu steigern, damit die Rezidivhäufigkeit reduziert wird.

Es ist anzunehmen, dass durch systematische Stoffwechseluntersuchungen bei Tieren, speziell bei Hunden vermutlich eine grössere Anzahl von Tieren, bei denen eine Cystinurie vorkommt, entdeckt werden könnte. Solche Tiere sind für das Studium verschiedener wissenschaftlicher Probleme, insbesondere für die Urolithiasis von grosser Bedeutung.

Summary. Cystinuria and L-Cystine lithiasis, is a rather rare hereditary metabolic disease in human beings. Due to the high purity of L-Cystine stones formed in the urinary tract, they can serve as ideal models for studies on the chemical dissolution of kidney stones in general. By systematic examinations, it is possible to find for these purposes, dogs in which a cystinuria is present.

G. Kallistratos[2], O. Fenner und U. Berg

Forschungsinstitut Borstel,
Institut für experimentelle Biologie und Medizin,
D–2061 Borstel BRD 23. Oktober 1972.

[1] G. Kallistratos and G. Malorny, Arzneimittel-Forsch. *22*, 1434 (1972).

[2] Herrn Prof. Forenbacher, Vet. Med. Fakultät der Universität Zagreb, danken wir für die Übersendung von Steinen, und Frau M. Stoffers für die technische Mitarbeit.

Distribution of Histamine in 7 Brain Regions in Different Species and Strains of Mammals

The central actions of histamine (HT)[1] and its catabolites[2], together with the non-homogeneous regional[3] and subcellular[4] localization of the amine in brain tissue, support the idea that this substance has a neurotransmitter function.

The total HT in the whole brain and in 7 brain regions of several species and strains has been determined after purification with Cellex P and subsequent spectrofluorometric determination. In addition, the HT content in the sediment and supernatant of homogenates of 7 brain regions of the rat in isotonic medium has also been determined.

[1] M. Monnier and A. M. Hatt, Experientia *25*, 1297 (1969).
[2] R. J. Marcus, W. D. Winters, E. Roberts and D. G. Simonsen, Neuropharmacology *10*, 203 (1971).
[3] J. P. Green, *Handbook of Neurochemistry* (Ed. A. Lajtha; Plemun Press, New York and London 1970), Vol. IV, p. 221.
[4] M. Kataoka and E. de Robertis, J. Pharmac. exp. Ther. *156*, 114 (1967).

Material and methods. The following animals were used: Swiss mouse males, 5 weeks old, weighing 18 ± 2 g; Black mouse (DBA) ('quaking mouse') males, 4 weeks old, weighing 16 ± 2 g; Sprague Dawley rat males, 6 weeks old, weighing 180 ± 10 g; Long Evans rat males, 4 weeks old, weighing 80 ± 10 g; Guinea-pig tricolour males, 6 weeks old, weighing 180 ± 10 g; New Zealand white rabbit males, 4 years old, weighing 6 ± 0.5 kg; Fauve de Bourgogne rabbit males, 6 months old, weighing 2.5 ± 0.5 kg.

The brains were removed and dissected at 2 °C[5]. HT was purified using 2 successive chromatographies over columns of Amberlite CG-50 and Cellex P and quantified spectrofluorometrically[6].

In another series of experiments carried out with rat brains, the tissue was homogenized in 0.32 *M* sucrose and centrifuged at $10^5 \times g$. HT was determined in the sediment and in the supernatant fractions[7].

Results and discussion. Though the metabolism of HT does not seem to be identical in all rodents,[8] the brain concentrations are very similar in all the animals studied, the only exception being the Fauve de Bourgogne rabbit. (Table I). The HT levels in the Fauve de Bourgogne rabbit seem to be age-conditioned rather than strain-conditioned. This idea is supported by the fact that the brain development in the rabbit is extremely slow, and also because the brain concentration of HT in the rat is closely related to the brain development[8–10].

With the exception of the New Zealand white rabbit, the relative concentrations of HT, in the different regions studied allows them to be classified in an order that hardly varies from one species to the other (Table II). Due to the great mass of tissue corresponding to the cortex, the HT concentration in the whole brain constitutes a reflection of the amine levels in this region. In the species

Table I. Concentrations of HT in the whole brain of different species and strains of mammals

Animals	HT (ng/g fresh tissue)
Sprague Dawley rat	41.5 ± 1.4 (40)
Long Evans rat	44.7 ± 2.8 (24)
Swiss mouse	47.9 ± 3.8 (20)
Black mouse (DBA) 'quaking mouse'	43.4 ± 2.8 (15)
Tricolour Guinea-pig	59.1 ± 1.6 (14)
New Zealand white rabbit	49.1 ± 4.4 (5)
Fauve de Bourgogne rabbit	112.7 ± 4.7 (12)

Each value represents the mean ± S.E.M. Number of determinations in brackets.

Table II. Distribution of the concentrations of HT in 7 brain regions of mammals of distinct species and strains

Animals	HT (ng/g fresh tissue)						
	Cerebellum	Striatum	Cortex	Hippocampus	Hypothalamus	Thalamus	Pons-medulla
Sprague Dawley rat	18.9 ± 1.5 (10)	56.0 ± 2.1 (10)	39.0 ± 0.9 (40)	39.8 ± 1.9 (10)	240.0 ± 13.0 (20)	157.5 ± 6.7 (20)	30.0 ± 1.5 (10)
Tricolour Guinea-pig	27.7 ± 2.2 (8)	57.1 ± 2.7 (6)	56.5 ± 2.8 (24)	56.4 ± 3.9 (12)	233.9 ± 16.7 (12)	86.1 ± 7.6 (12)	47.5 ± 3.0 (12)
New Zealand white rabbit	52.8 ± 2.5 (12)	74.4 ± 8.0 (12)	36.3 ± 2.2 (12)	41.3 ± 3.4 (12)	268.3 ± 29.3 (12)	49.1 ± 2.6 (12)	45.1 ± 2.5 (12)
Fauve de Bourgogne rabbit	76.7 ± 4.4 (12)	147.9 ± 7.5 (12)	88.6 ± 4.0 (12)	128.7 ± 6.0 (12)	366.3 ± 26.0 (12)	195.1 ± 14.3 (12)	123.7 ± 4.3 (12)

Only in the cases of the Sprague Dawlwy rat and the Tricolour Guinea-pig, homologous regions coming from more than 1 brain were grouped together. Rat: hypothalamus and thalamus, 2 brains; cerebellum, striatum hippocampus and pons-medulla, 4 brains. Guinea-pig: striatum, 4 brains; cerebellum, 3 brains; hippocampus, hypothalamus, thalamus and pons-medulla, 2 brains. Each value represents the mean ± S.E.M. Number of determinations in brackets.

[5] L. GLOWINSKI and L. L. IVERSEN, J. Neurochem. *13*, 665 (1966). Some technical details were slightly modified according to I. BLANCO, (see ref.[9]).

[6] J. R. BOISSIER, M. GUERNET, J. P. TILLEMENT, I. BLANCO and M. BLANCO, C.r. hebd. Séanc. Acad. Sci, Paris *268*, 1448 (1969). It should be noted that 0.4 *M* perchloric acid was used as deproteinizing agent instead of 74% (v/v) ethanol as described in the original technique.

[7] J. R. BOISSIER, M. GUERNET, J. P. TILLEMENT, I. BLANCO and M. BLANCO, Life Sci. *9*, part 2, 249 (1970).

[8] L. A. PEARCE and S. M. SCHANBERG, Sci. *116*, 1301 (1969).

[9] I. BLANCO, Thèse Doctoral, Faculté de Pharmacie de l'Université de Paris, (1970); J. P. TILLEMENT, M. GUERNET, H. DIEHL, I. BLANCO, M. BLANCO and J. R. BOISSIER, J. Pharmac., Paris *2*, 1 (1971).

[10] J. C. SCHWARTZ, C. LAMPART, C. ROSE, M. C. REHAULT, S. BISCHOFF and H. POLLARD, J. Neurochem. *18*, 1787 (1971).

Table III. Distribution of HT in the sediment and in the supernatant of whole brain and 7 brain regions of Sprague Dawley rats, homogenized in a isotonic medium and centrifuged at 100,000 × g during 1 h

Region	HT (ng/g fresh tissue) Sediment a)	Supernatant b)	a/b	HT (%) Sediment	Supernatant
Cerebellum	16.1 ± 3.1 (4)	13.2 ± 0.1 (4)	1.3	54.9	45.1
Pons-medulla	20.8 ± 0.9 (4)	15.0 ± 1.4 (4)	1.3	58.0	42.0
Hippocampus	22.6 ± 2.5 (4)	15.9 ± 1.9 (4)	1.4	58.7	41.3
Cortex	25.4 ± 1.1 (12)	13.9 ± 1.5 (12)	1.8	64.6	35.4
Striatum	54.7 ± 1.4 (4)	20.1 ± 8.0 (4)	2.7	73.8	26.2
Thalamus	128.3 ± 10.6 (6)	46.3 ± 4.5 (6)	2.8	73.5	26.5
Hypothalamus	200.3 ± 12.3 (6)	56.8 ± 5.1 (6)	3.5	77.9	22.1
Whole brain	31.2 ± 0.7 (10)	13.6 ± 0.6 (10)	2.3	69.6	30.4

Homologous regions coming from more than 1 brain were grouped together; cortex, 2 brains; hypothalamus and thalamus, 4 brains; cerebellum, striatum, hippocampus and pons-medulla, 6 brains. Each value represents the mean ± S.E.M. Number of determinations in brackets.

studied, the highest HT levels are registered in the hypothalamus, as has also been demonstrated in the monkey[11] and man[12]. The concentration of HT in the thalamus takes second place in importance except in the New Zealand white rabbit. The HT levels of the striatum and the hippocampus are low and similar to those found in the cortex. Cerebellum, cortex and pons-medulla are poor in HT.

The regional levels of HT in the rat are conditioned both by the histidine concentration[9] and the histidine decarboxylase activity in each region[13]. These 2 parameters are also age-dependent.[9,10]. The regional distribution of the amine in other species could probably be determined by the some factors. In the Fauve de Bourgogne rabbit the regional distribution of HT could correspond to a pre-stabilization level, as the animals used were too young.

After homogenization of the brain of the rat in an isotonic medium and centrifugation at $10^5 \times g$, 70% of the HT was found in the sediment (Table III). An identical distribution was found by SCHANBERG et al.[14] in the case of serotonin. In all areas, the HT content in the sediment is higher than in the supernatant. The ratio, HT concentration in the sediment/HT concentration in the supernatant makes it possible to differentiate between the 2 types of structures: a) cerebellum, pons-medulla, hippocampus and cortex in which the HT concentration in the sediment is less than twice that in the supernatant, b) striatum, thalamus and hipothalamus in which the HT concentration in the sediment is more than twice that in the supernatant.

In the cortex[4] and hipothalamus[15], HT, like other biogenic amines, is found expecially concentrated in the subfractions rich in nerve endings. The analysis of the results of Table III shows that the regions rich in nerve endings have a higher concentration of HT in the sediment, the fraction in which the cell organelles are found.

The presence of the enzymes histidinedecarboxylase[16] and N-imidazolemethyltransferase[15] in the sediment, together with the fact that the intraperitoneal administration of 100 mg/kg of L-histidine, causes a rise in the levels of HT exclusively in this fraction[7], support the interpretation that the HT dectected in the supernatant is synthesized by some of the structures found in the sediment.

Resumen. En las especies estudiadas el hipotálamo es la región mas rica en histamina (HT). En la rata, la mayor proporción de HT se localiza en el sedimento de los homogenizados en medio isotónico centrifugados a $10^5 \times g$; la proporción de HT en el sedimento varia ampliamente de una región a otra.

I. BLANCO, M. BLANCO, M. GRAU, J. M. PALACIOS, F. PICATOSTE and G. SCHERK

Instituto de Biologia Fundamental, Centro Coordinado C.S.I.C., Universidad Autónoma de Barcelona. San Antonio Ma Claret, 171, Barcelona-6 (Spain),
15 January 1973.

[11] I. A. MICHAELSON, P. Z. COFFMAN and D. F. VEDRAL, Biochem. Pharmac. *17*, 2435 (1968).
[12] T. L. PERRY, S. HANSENS and L. MCDOUGALL, J. Neurochem. *14*, 775 (1967).
[13] J. C. SCHWARTZ, C. LAMPART and C. ROSE, J. Neurochem. *17*, 1527 (1970).
[14] S. M. SCHANBERG and M. J. GIARMAN, Biochem. Pharmac. *11*, 187 (1963).
[15] M. J. KUHAR, K. M. TAYLOR and S. H. SNYDER, J. Neurochem. *18*, 1515 (1971).
[16] T. ITO and M. KAWAAI, Nipon. Univ. J. Med. *1*, 185 (1959).

Evidence for an Adipokinetic Mechanism in the Ventromedial Hypothalamus

Long-term regulation of feeding appears to be controlled by adipose tissue metabolism. Rats which have been artifically fattened eat little while the excess fat is being catabolized[1]. Conversely, chronic diabetics are vigorous eaters[2,3], possibly because their fat stores are almost totally depleted. In normal animals, the circadian cycle of lipogenesis and lipolysis is paralleled by high and low feeding rates, respectively[4]. The ventromedial hypothalamus (VMH) appears to mediate these behavioral responses: After VMH lesions, rats do not adjust food intake appropriately in response to either body nutrient repletion[5] or depletion[6]. These feeding adjustments may be mediated directly by local nutrient transactions within the medial hypothalamus, for this area of the