Hematological Effects of Lathyrism[1]

In connection with other experiments on lathyrism we noticed a high hemoglobin level in the blood. Since the synthesis of collagen (especially of hydroxyproline) is decreased and the relative concentrations of serum proteins reversed[2], a hematological study on the cell maturation and hemoglobin synthesis seemed warranted with reference to the unknown way of action of the toxic agent (β-aminopropionitrile). In the literature we found only a passing note on the polycythemia in lathyrism[3].

The rats were kept for 2 months on a diet containing 56·0% of sweet pea[4]. Hemoglobin was determined with the photometer at 5500 Å after dilution 1:200 with 0·1% sodium carbonate. The white and red cells were counted by standard methods in Buerker's chamber. The bone marrow was studied from smears (stained with May-Gruenwald-Giemsa-solution) obtained from the thighbones. Usually one thousand (in few samples 500) cells were counted.

From the marrow following comparison was obtained.

Table II

	Lathyrus-group	Control-group
	%	%
No. of animals	4	7
Proerythroblasts	1·0	–
Erythroblasts	12·5	7·0
Normoblasts	45·0	42·7
Erythropoesis: total	*58·5*	*49·7*
Myeloblasts	–	0·3
Promyclocytes	0·4	1·8
Myelocytes	14·4	15·6
Matured neutrophils	12·6	15·1
Eosinophils	4·1	7·3
Lymphocytes	6·9	7·1

To conclude, β-aminopropionitrile seems not to affect the division or maturation of the cells nor the synthesis of hemoglobin. This is in agreement with the histological findings on the connective tissue. These symptoms seem to be due to a chronic nitrile intoxication and not directly connected with the characteristic symptoms of lathyrism, which depend on an available amino group[5].

[1] This work forms a part of a program which has been aided by Sigrid Juselius' Stiftelse. The help is gratefully acknowledged.

[2] Unpublished experiments.

[3] B. J. Geiger, H. Steenbock, and H. T. Parsan, J. Nutr. *6*, 427 (1933).

[4] The animals and diet are described in L. Kalliomäki, Maija Yli-Pohja, and E. Kulonen, Exper. *13*, 495, footnote 2 (1957).

[5] T. E. Bachhuber, J. J. Lalich, D. M. Angevine, E. D. Schilling, and F. M. Strong, Proc. Soc. exp. Biol. Med. *89*, 294 (1955).

If the β-aminopropionitrile would compete with β-alanine, symptoms of pantothenic acid deficiency, including anemia, were to be expected[6].

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Zusammenfassung

Bei experimentellem Lathyrismus wurde eine deutliche Vermehrung von Erythrozytenproliferation und Hämoglobinsynthese beobachtet. Hämoleukozyten und Myelopoese im Knochenmark wurden nicht beeinflusst.

[6] Anemia is found in human lathyrism, which seems to be a different condition, C. Gopalan, Trans. R. Soc. trop. Med. Hyg. *44*, 333 (1950).

5-Hydroxytryptamine in Mammalian Thyroid Gland[1]

Thyroid glands of rats, sheep, rabbits, and dogs were examined for 5-hydroxytryptamine (5-HT). Considering the great quantities of 5-HT present in gastrointestinal mucosa[2], it is not surprising that high concentrations of 5-HT were found in mammalian thyroid glands, which phylogenetically are derived from the phyrangeal epithelium. Surprisingly, however, there are great differences between the 5-HT content of the thyroids of different species.

The tissues were extracted with 90% v/v acetone; otherwise the procedure suggested by Amin *et al.*[3] was followed. The glands were taken from rats and rabbits killed by a blow on the head and from dogs bled to death under ether anesthesia. The sheep thyroids were obtained from the slaughterhouse, where they were immediately put on dry ice. In most cases the cut tissues were left in acetone for a few days at −15°C, and the rest of the extraction was done just before the assay. 5-HT was assayed biologically by using the isolated heart of *Venus mercenaria* in natural sea water with 6 mg/l of benzoquinonium chloride (generously supplied by Sterling Winthrop Research Institute).

[1] This work was supported by a grant from the U.S. Public Health Service (H-2205).

[2] V. Erspamer, Pharmacol. Rev. *6*, 425 (1954).

[3] A. H. Amin, T. B. B. Crawford, and J. H. Gaddum, J. Physiol. *126*, 596 (1954).

Table I

	Hemoglobin g%		Erythrocytes mill./mm³		Mean hemoglobin	Leukocytes in mm³
	Mean	Range	Mean	Range	Mean	Mean
Lathyrus-group (8)	14·5	(13·1 – 16·4)	7·44	(6·60 – 8·01)	19·5	8690
Control-group (8)	12·9	(12·4 – 14·1)	6·25	(5·73 – 6·77)	20·7	8860
P of chance occurrence of the difference	< 0·01		< 0·001		> 0·10	–

The differentiation of the leukocytes was similar in both groups.

The test conditions were described previously[4]. 5-HT was used as creatinine sulfate (generously supplied by Abbott Laboratories); the doses refer to the base.

5-Hydroxytryptamine in Mammalian Thyroid Glands

									Mean µg/g*
Rat	2·5	2·6	2·4	3·3	5·0	5·0	3·9	4·6	3·7
Sheep	3·5	5·7	7·5	3·8	2·4				4·6
Dog	0·025	0·018	0·038	0·02					0·025
Rabbit	0·29	0·09							0·19

* Wet weight.

The 5-HT equivalents are presented in the Table. The concentrations found in the rat and sheep thyroids were more than hundred times those found in the dog thyroids. Rabbit thyroids contained more than those of dogs. In order to ascertain the specificity of these findings, the following studies were carried out.

(1) Ascending paper chromatograms were run using the rat and sheep thyroid extracts in the upper organic phase of *n*-butanol/glacial acetic acid/water (4:1:5). When pieces of the paper strips were eluated and tested on *Venus* heart, the active material was found to travel like 5-HT. When paper chromatograms of sheep thyroid extract were treated according to JEPSON and STEVENS[5], a fluorescing spot was found at the same site as the spot of the reference 5-HT.

(2) Parallel assays were done with rat and sheep thyroid extracts on the atropinised estrus rat uterus[3] and *Venus* heart. Sheep extracts gave about one third lower results on the uterus. The reason for this is supposed to be the presence of catechol amines, which relax the uterus. In one sheep thyroid gland 0·2 µg/g of norepinephrine and 0·03 µg/g of epinephrine were found when analysed according to v. EULER[6]. The rat extracts, on the other hand, were about 30% more active on the uterus than on the heart. LSD treatment did not completely abolish the response of the uterus to rat thyroid extracts, although it blocked the effect of 5-HT. There was little, if any, sign of this LSD-resistant activity in sheep and dog thyroid extracts. The low amount of 5-HT in the dog thyroid gland was also confirmed on the uterus. The amounts of norepinephrine and epinephrine in one dog thyroid were 0·3 and 0·04 µg/g respectively.

(3) That the activity measured on *Venus* heart was due to 5-HT is strengthened by the similar time course of the response. After washing, the effect of bufotenine, and, to a much lesser degree, the effect of N-methyl-5-HT, lasts longer than the effect of 5-HT. The molluscan heart is resistant to most substances likely to have an effect on smooth muscle[7].

(4) The rat thyroid extract did not lose its activity on the *Venus* heart when boiled for 10 min in 1 *N* HCl, while the same treatment in 1 *N* NaOH destroyed about 80%.

(5) Reserpine (5 mg/kg intraperitoneally) was not able, within 4 h, to decrease significantly the *Venus* heart activity of rat thyroid glands; however, neither was their a clear-cut 5-HT depletion in the duodenum of these rats.

For comparison some salivary glands were extracted and their activity tested on the *Venus* heart. In the rat rather high values were found (in µg/g, parotid: 0·68, 0·44; submaxillary: 0·19, 0·31; sublingual: 0·61, 1·0; extraorbital lacrimal: 0·34, 0·26). In the dog, the values were again lower (parotid: 0·03, 0·005; submaxillary; 0·02, 0·004); and in the rabbit, they were between those of the rat and the dog (parotid: 0·16; submaxillary: 0·13).

Conclusions: High 5-HT values were found in rat and sheep thyroid glands and very low values in dog thyroids. In the rat thyroid, there appears to be an unidentified uterus-stimulating substance which is soluble in 90 % acetone. On rat uterus, it is atropine-resistant. It is not histamine, because the tissues used for analysis are resistant to this amine. GARVEN[8] tested two acetone extracts of rabbit thyroid on the rat uterus and found one of them to produce an LSD-resistant response. The smooth muscle-stimulating substance of KOEPF and MEZEN[9], which is present in rat salivary glands, also was found to contract the rat uterus. It is not known where 5-HT is localized in the mammalian thyroid gland. The mast cells are a possible site, since they are known to contain this amine[10]; certain cells of the enterochromaffin type are another possible location. It is improbable that 5-HT has anything to do with the specific thyroid function.

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Zusammenfassung

Schilddrüsen von Ratten und Schafen enthalten relativ grosse Mengen von 5-Hydroxytryptamin. In der Thyreoidea von Hunden wird dieser Stoff nur in einer hundertmal kleineren Konzentration vorgefunden. Weiter wird gezeigt, dass in der Rattenthyreoidea eine nicht identifizierte, acetonlösliche Substanz vorhanden ist, welche Uteruskontraktionen auszulösen vermag.

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Fat Metabolism in Experimental Obesities VIII. Blood Total Lipids and Ketones in Four Kinds of Obese Mice*

To our knowledge, no systematic study has been conducted of blood lipids and ketone levels in various

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