Alterations of Tryptophan Metabolism Induced by Sleep Deprivation

In our investigations of metabolic changes induced by sleep deprivation (SD) we focussed our attention on investigations of some tryptophan metabolites in view of the possible relations with psychic disorders occasionally in the course of SD[1].

Method. The results submitted were obtained in 15 healthy volunteers aged 20–30 years in the course of 120 h vigilance. During 2 control periods and the experimental period proper, the experimental subjects were on a weighed diet. They were maintained in a vigilant condition by means of direct psychic and physical stimulation. Pharmacological stimulants including caffeine were eliminated.

We investigated 3 main metabolic pathways of tryptophan. As an indicator of the metabolism of the serotonin branch we selected the excretion of 5-hydroxyindolacetic acid (5-HIAA)[2]. Abnormalities in the metabolism of the tryptamine pathway were assessed from variations in indolylacetic acid (IAA)[3]. From the kynurenine branch we assessed the kynurenine, xanthurenic acid and anthranilic acid excretion[4]. All selected parameters were expressed in absolute values of the total excretion/24 h and calculated in relation to 100 mg creatinine in order to eliminate the influence of glomerular filtration on changes in the parameters investigated. As statistically significant changes we considered only where we recorded significant deviations by both methods of evaluation.

Results and discussion. As apparent from the Table, SD leads to a considerable disturbance of the tryptophan metabolism. Above all between the first and second day of SD the excretion of 5-HIAA rises, in absolute values as well as those referred to creatinine. The mechanism of induction of this change can be explained in our opinion by 2 facts. First of all by LUBY's finding of a drop of the ATP level during the first stage of SD[1]. Because serotonin is bound in blood cells by means of ATP, its reduction must lead also to a decline of the binding capacity for serotonin. This means that the latter is released and bound to a smaller extent. Therefore a greater proportion of

[1] E. D. LUBY, C. E. FROHMAN, J. L. GRISELL, J. E. LENZO and J. S. GOTTLIEB, Psychosom. Med. *22*, 182 (1960).

[2] S. UDENFRIEND, E. TITUS and H. WEISSBACH, J. biol. Chem. *216*, 499 (7955).

[3] H. WEISSBACH, W. KING, A. SJOERDSMA and S. UDENFRIEND, J. biol. Chem. *234*, 81 (1959).

[4] S. L. THOMPSETT, Clinica chim. Acta *4*, 411 (1959).

	Days	Sleep deprivation					Second control period		
		1	2	3	4	5	1	2	3
5-HIAA/24 h	Δ%	+ 64.81[c]	+ 71.18[d]	+ 17.43	− 36.81	− 3.81	+ 7.68	+ 0.99	− 11.71
	S.D.	59.10	44.22	64.44	33.10	49.19	49.91	40.40	35.85
	No.	10	10	10	10	10	10	10	10
5-HIAA/100 mg creatinine	Δ%	+ 91.73	+ 57.64[a]	+ 54.76	− 21.81	+ 5.39	− 0.50	+ 13.08	+ 36.44
	S.D.	132.80	76.12	155.98	41.81	54.40	51.05	72.48	71.05
	No.	10	10	10	10	10	10	10	10
IAA/24 h	Δ%	− 17.93	+ 11.61	− 5.15	− 7.51	+ 20.32	− 18.18	+ 9.23	− 28.16[a]
	S.D.	27.33	31.35	48.18	53.75	55.42	28.40	13.92	36.98
	No.	10	10	10	10	10	10	10	10
IAA/100 mg creatinine	Δ%	+ 5.84	+ 18.69	+ 19.48	+ 34.05	+ 32.97	+ 5.28	+ 36.28	+ 0.29
	S.D.	45.90	27.65	33.88	47.94	54.14	38.67	39.04	41.08
	No.	10	10	10	10	10	10	10	10
Kynurenine/24 h	Δ%	+ 2.46	− 8.23	+ 8.97	− 1.28	− 3.23	− 18.39	− 8.27	+ 4.00
	S.D.	55.81	55.36	66.54	52.97	42.94	33.89	14.88	17.31
	No.	12	12	12	11	15	15	15	15
Kynureinine/100 mg creatinine	Δ%	+ 20.99	+ 20.35	+ 24.75	+ 21.39	− 16.85	− 7.77	− 5.22	+ 14.26
	S.D.	48.02	42.39	43.13	41.75	33.97	36.56	29.87	34.04
	No.	12	12	12	11	15	15	15	15
Xanthorenic acid/24 h	Δ%	+ 22.43	+ 38.48[a]	+ 30.53[d]	+ 27.60	+ 34.77[b]	+ 7.31	− 11.88	− 16.44
	S.D.	56.40	65.51	30.91	60.41	51.55	54.59	58.47	58.93
	No.	15	15	15	14	15	15	15	15
Xanthurenic acid/100 mg creatinine	Δ%	+ 7.49	+ 19.86	+ 17.60	+ 16.07	+ 25.17[a]	+ 6.01	− 28.72[b]	− 11.83
	S.D.	41.89	63.29	60.85	48.97	44.05	39.52	38.16	49.20
	No.	15	15	15	14	15	15	15	15
Anthranilic acid/24 h	Δ%	+ 2.94	− 17.40	− 13.48[a]	− 18.03[b]	− 38.79[d]	− 32.03[d]	− 1.85	− 13.11
	S.D.	45.33	32.57	27.36	38.56	40.32	26.02	56.24	53.61
	No.	15	15	15	14	15	15	15	15
Anthranilic acid/100 mg creatinine	Δ%	− 13.23	− 7.47	− 14.08	− 9.39	− 33.86[b]	− 17.85[b]	− 10.35	− 5.40
	S.D.	30.83	34.36	31.67	35.40	44.32	28.94	50.16	40.93
	No.	15	15	15	14	15	15	15	15

No., number of observations; the statistically significant change of both values is defined by a line; [a] $P < 0.05$, [b] $P < 0.02$, [c] $P < 0.01$, [d] $P < 0.001$.

serotonin is deaminated and the excretion of 5-HIAA rises. Moreover the temporary increase of 5-HIAA can be also considered a stress manifestation of SD, as suggested by the temporal agreement of changes in 17-OH steroid urinary excretion with a significant rise of roughly 73% between the first and second day of SD and the similar, though mirror image course of eosinophil levels in the morning[5].

The excretion of further tryptophan metabolites, i.e. IAA and kynurenine, did not change significantly in the course of the experiment. The absence of changes in IAA excretion permits the conclusion that the dietary supply of tryptophan in our subjects was balanced and that changes in the dietary supply of this amino acid did not participate in changes in the excretion of the metabolites investigated. The same applies to the kynurenine excretion. Despite this our finding was to a certain extent surprising as it did not comply with our assumption that the stressing effect of SD will stimulate the production of the adaptive enzyme-tryptophan pyrolase.

On the other hand, between the second and fifth day of SD the xanthurenic acid excretion rises and this increase is significant on the fifth day, similarly as the decline of the anthranilic acid excretion found on the same and subsequent day, i.e. the first day of the second control period. This finding can be explained only by a relative vitamin B_6 deficiency, pyridoxine being essential for the formation of anthranilic acid.

Zusammenfassung. Es wird gezeigt, dass Schlafentzug die Ausscheidung der 5-Hydroxyindolessigsäure und der Xanthurensäure vermehrt und diejenige der Antranilsäure herabsetzt.

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[5] E. KUHN, T. BRAUN, V. BRODAN, J. VÁLEK and M. VOJTĚCHOVSKY, *Metabolic Changes During Sleep Deprivation in Man* (Symp. on Fatigue of the Psychiatric Section of Czechosl. Med. Soc. J.E.P., Jasná pod Chopkom, 6 May 1965).

Decreased Restitution of Creatine Phosphate in White and Red Skeletal Muscles During Aging

The skeletal muscle system shows characteristic age changes. Mass and force of muscles decrease. However, no alterations of contraction metabolism are known during aging. It was supposed that aging of muscles may be the result of disturbed restitution[1].

In the following experiments we studied in rats of different ages, the rebuilding of creatine phosphate (KP) after complete rest for constant periods of time.

Wistar-rats from our institutes 'old age' colony were used. They were 1–37 months old and in good health. They were kept at 22 °C, with 8 h of light and a completely constant diet of food tablets ad libitum.

Creatine was estimated by the method of EGGLETON[2] and ENNOR and STOCKEN[3]; creatine phosphate after ENNOR[4].

The animals were lively and very active; only the 33- to 37-month-old ones were 'slow'. They were injected i.p. with 25 mg/kg nembutal (0.5 ml 5% solution/kg body weight). After a few minutes they were in narcosis, no spontaneous movements, only quiet breathing was seen. Care was taken that the body temperature should remain normal.

After 45 min the animals were transferred to a cold room (2–3 °C), the thorax was opened and the heart cut. This quick bleeding to death leads to no asphyxiation cramps, which may interfere with the quantity of KP rebuilt in the muscles during the period of rest.

Dissection followed immediately. Frozen CO_2 in ethanol was poured over the muscles. Since it is known that the functional activity is different in white (quick) and red (slow, tonic) muscles, all estimations were made in both types. In the hind legs of rats we used as white muscles: M. rectus femoris and M. gluteus maximus peripheral, and as red muscles M. piriformis, M. vastus intermedius and M. gluteus maximus centralis.

Muscles of both sides were united and weighed and transferred to frozen CO_2 in ethanol and solubilized in a Virtis homogenizer in 9 ml distilled water. Then 1 ml concentrated trichloracetic acid (TCA) was added and again homogenized for 1 min in the cold.

The homogenates were centrifuged at 3000 *g* and the precipitate washed with 5% TCA twice and centrifuged. The solutions were united and neutralized with NaOH to pH 7.0–7.3 and brought to equal volumes.

Creatine and creatine phosphoric acid values are expressed as mg/g wet weight of muscle.

Results are given for white (Table I) and red (Table II) muscles. The values are in 4 groups of different ages. Group I is 1–1½ months old (body weight 100–250 g), still in growth and the skeletal muscles in the process of upbuilding. Group II are adult animals after puberty, 6–9 months old, about 450–500 g body weight. Group III are 16–17 months old. (In female rats ovulation ceases between 12–14 months.) Group IV are old animals of 25–37 months of age. The age of 40 months is reached very rarely. Their weight generally declines.

Observations on the young developing group will be discussed later.

Total creatine is in all age groups fairly equal: 4.34 to 4.94 mg/g in the white, and 3.67–4.15 mg/g in the red muscle. The comparison of the white and red muscles of the same animals in both Tables show that in each animal the creatine content is reduced in the red muscles. There is no diminuition of total creatine content during aging in the muscles.

KP content is, by contrast, very different at different ages after 45 min of rest of these muscles. In the 6–37

[1] F. VERZÁR, *Lectures of Experimental Gerontology* (Thomas, Springfield 1963), p. 68.

[2] P. EGGLETON, S. R. ELSDEN and N. GOUGH, Biochem. J. *37*, 526 (1943).

[3] A. H. ENNOR and L. A. STOCKEN, Biochem. J. *42*, 557 (1948).

[4] A. H. ENNOR, in *Methods in Enzymology* (Ed. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1957), vol. 3.