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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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

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Table I. White muscles

Group	No.	Age (months)	Sex	Total creatine		Creatinphosphate (KP)		KP % of total creatine
				mg creatine/ g muscle	Mean	mg KP/ g muscle	Mean	
I	1	1.6	♀	4.61	4.45 ± 0.19	2.64	1.9 ± 0.86	26.5
	2	1.6	♀	4.61		1.76		
	3	1.3	♀	4.38		0.67		
	4	1.3	♀	4.21		2.52		
II	5	7.6	♀	4.59	4.94 ± 0.34	3.34	3.98 ± 0.66	50.1
	6	6.0	♀	4.71		4.57		
	7	6.0	♂	5.41		4.46		
	8	6.3	♀	5.16		3.89		
	9	9.3	♀	4.81		3.65		
III	10	16.6	♀	4.39	4.34 ± 0.11	2.64	2.78 ± 0.50	39.9
	11	16.6	♀	4.15		2.21		
	12	16.6	♀	4.41		3.57		
	13	17	♀	4.32		2.58		
	14	17	♂	4.43		2.88		
IV	15	25	♀	4.96	4.54 ± 0.32	0.66	1.29 ± 0.36	17.6
	16	25	♀	3.41		1.24		
	17	33	♂	4.96		1.49		
	18	33	♀	4.73		1.34		
	19	37	♂	4.62		1.72		

Table II. Red muscles

Group	No.	Age (months)	Sex	Total creatine		Creatinphosphate (KP)		KP % of total creatine
				mg creatine/ g muscle	Mean	mg KP/ g muscle	Mean	
I	1	1.6	♀	4.23	3.89 ± 0.37	0.79	0.34 ± 0.31	5.4
	2	1.6	♀	4.18		0.28		
	3	1.3	♀	3.68		0.22		
	4	1.3	♀	3.48		0.22		
II	5	7.6	♀	3.93	4.15 ± 0.37	0.54	1.58 ± 0.69	23.6
	6	6.0	♀	4.57		2.18		
	7	6.0	♂	4.52		2.26		
	8	6.3	♀	3.79		1.45		
	9	9.3	♀	3.93		1.49		
III	10	16.6	♀	3.78	3.67 ± 0.15	1.25	1.34 ± 0.39	
	11	16.6	♀	3.63		0.77		
	12	16.6	♀	3.74		1.84		22.6
	13	17	♀	3.92		1.39		
	14	17	♂	3.36		1.47		
IV	15	25	♀	4.06	3.99 ± 0.92	0.35	0.81 ± 0.42	12.5
	16	25	♀	2.67		0.67		
	17	33	♂	4.56		0.89		
	18	33	♀	4.66		1.34		
	19	no red preparation						

months old groups it diminishes with age in white as well as in red muscles. At all ages in the single animals as in the mean values, the white muscles contain considerably more KP than the red (slow, tonic) muscles.

If one calculates the percentage of all creatine which is phosphorylated, the difference becomes even more obvious. (For this calculation, to the 'total creatine' we related the creatine of KP. The percentage of phosphorylation was taken from this sum.) Between 6–37 months in white muscles KP decreases from 50.1–17.6% and in red muscles from 23.6–12.5%. Supposing that finally, in a long enough rest-period, all creatine would become KP the result means that with age the velocity of rephosphorylation slows down in these muscles.

Special attention has to be given to the values of the young animals of group I in which the muscles are in the state of building up. Their total creatine has already reached values similar to older animals. But their KP is

strongly reduced. One may suppose that while muscle structure is already built up, the rebuilding of KP has still not reached its final stage in muscle metabolism.

The problem of whether aging influences the restitution metabolism in muscle is answered by these experiments. They show that in the upgrown animal between 6–37 months there is a gradual decrease of creatine rephosphorylation in muscles. This is generally smaller (or rather slower) in red than in white muscles; but in both the decrease with aging is obvious.

The rephosphorylation of KP is the leading metabolic process of restitution after muscle work. KP transfers P to ADP to produce ATP. After breakdown of ATP to ADP again KP is phosphorylated. This basic restitution process is diminished during aging in muscles. It will be the problem of further work to explain the details of this mechanism.

Zusammenfassung. Die Fähigkeit des Skelettmuskels, in der Ruhe Kreatin-Phosphat (KP) zu restituieren, wird an der Ratte, gesondert an weisser und roter Muskulatur, untersucht. Das Gesamt-Kreatin ist in beiden Muskelarten zwischen jungen, erwachsenen (6–9 Monate) und alten Tieren (25–37 Monate) nicht verschieden. Dagegen ist die Restitution von KP nach gleich langer Ruheperiode bei jungen erwachsenen grösser als bei alten Tieren; in weissen Muskeln 50,1 gegenüber 17,6%, in roten Muskeln 23,6 gegenüber 12,5%.

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Acetylation of Cycloheximide by *Cunninghamella blakesleeana*

Addition of the antifungal antibiotic cycloheximide to the transformation of deoxycorticosterone by *Cunninghamella blakesleeana* H-334 has been reported to increase the yield of 11β-hydroxydeoxycorticosterone and decrease 14α-hydroxylation[1]. While studying the effect of cycloheximide on the 11β-hydroxylation of 6α-fluoro 21-hydroxy-16α,17α-isopropylidenedioxypregn-4-en-3,20-dione (F.H.S. acetonide) by *C. blakesleeana* (Lendner, A.T.C.C. 8688b) we detected the presence of a new UV-absorbing material by thin-layer chromatography (TLC). The absorbing material was produced in the absence of F.H.S. acetonide but not in the absence of cycloheximide. We describe here the isolation and identification of cycloheximide acetate as a microbiological transformation product of cycloheximide. Other products isolated from the transformation experiments were ergosterol peroxide, leucine-proline anhydride, and thymine. Cycloheximide inhibited the hydroxylation of F.H.S. acetonide by *C. blakesleeana* and its own transformation was inhibited by the sterol.

C. blakesleeana was grown in a nutrient solution containing cornsteep (solids, 0.3%), peptone (2%), dextrose (5%), and minor elements solution (0.1%), adjusted to pH 4.5 before sterilization. 500 ml flasks containing 125 ml nutrient solution were inoculated with *C. blakesleeana* (1.2×10^5 spores per flask) and incubated on a rotary shaker at 25 °C. After 48 h cycloheximide (31.25 mg) in acetone (2.5 ml) was added to each flask (cycloheximide, net 250 μg/ml). After a further 72 h the contents of 24 flasks (cycloheximide total 750 mg) were combined, adjusted to pH 7, and then extracted with ethyl acetate. The extract (3.1 g) was chromatographed on silica (80 g) with elution by increasing proportions of ethyl acetate in light petroleum (b.p. 60–80°) and then increasing proportions of methanol in ethyl acetate. The eluates were examined by TLC on silica gel G.F. plates developed in ethyl acetate. The plates were viewed in UV-light and then sprayed with chromic acid. Appropriate fractions were combined.

The early fractions contained fatty materials. A fraction (71 mg), Rf 0.58 (fluorescent in UV-light, 350 nm), eluted partly by ethyl acetate–light petroleum (3:1) and partly by ethyl acetate, gave ergosterol peroxide, m.p. and mixed m.p. 176°, from light petroleum (b.p. 60–80°), (lit. m.p. 178°)[2] identified initially by its mass spectrum, M^+ 428 ($C_{28}H_{44}O_3$). The main fraction (810 mg), Rf 0.57 (UV-absorbing, 254 nm), eluted partly with ethyl acetate and partly with methanol–ethyl acetate (1:19), gave cycloheximide acetate (650 mg), m.p. and mixed m.p. 147°, from ethyl acetate–light petroleum (b.p. 60–80°), $[\alpha]_D^{25}$ + 21.2° (*c*, 2.2 in methanol) (lit. m.p. 148–9°, $[\alpha]_D^{25}$ + 22°)[3]. (Found: C, 63.24; H, 7.81. $C_{17}H_{25}NO_5$ requires C, 63.14; H, 7.79%). A minor fraction (10 mg), Rf 0.17 (chromic acid spray), eluted by methanol–ethyl acetate (1:1), gave a solid M^+ 210 identified as leucine-proline anhydride, m.p. 161° from ethyl acetate (lit. m.p. 159°)[4] not depressed by L-leucine-L-proline anhydride. In a subsequent experiment which confirmed the formation of cycloheximide acetate, a fraction (20 mg) Rf 0.14 (UV-absorbing, 254 nm) eluted with ethyl acetate proved to be thymine, M^+ 126 ($C_5H_6N_2O_2$), m.p. and mixed m.p. 320° from methanol.

Cycloheximide is not converted (by ester exchange) to its acetate by heating under reflux with ethyl acetate for 2 h or by passing a solution in methanol–ethyl acetate (1:1) down a column of silica.

Ergosterol peroxide has been reported to be a metabolite of other fungi but may be an artefact[5]. Leucine-proline anhydride is a constituent of peptone[6].

Zusammenfassung. Das Cycloheximid wird von *Cunninghamella blakesleeana* in sein Acetat verwandelt. Ferner wurden Ergosterin-Peroxid, das Anhydrid von Leucin-Prolin, sowie Thymin aus diesem Kulturansatz isoliert.

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Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield (Cheshire, England), 13 May 1968.

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