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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1 K. M. MANN, F. R. HANSON and P. W. O'CONNELL, Fedn Proc. Fedn Am. Socs exp. Biol. *14*, 251 (1955).
2 A WINDAUS and J. BRUNKEN, Justus Liebigs Annln Chem. *460*, 225 (1928).
3 J. H. FORD and B. E. LEACH, J. Am. chem. Soc. *70*, 1223 (1948).
4 A. BUTENANDT, P. KARLSON and W. ZILLIG, Hoppe-Seyler's Z. physiol. Chem. *288*, 279 (1951).
5 H. K. ADAM, I. M. CAMPBELL and N. J. MCCORKINDALE, Nature *216*, 397 (1967).
6 T. KOSUGE and H. KAMIYA, Nature *188*, 1112 (1960).