Tabelle II. Die Hemmung der ATPasen von WM, RM und HM durch Zusatz von KCl

pH	6,5		7,6	
Quotient der sA	TM–37 / TM–287	n	TM–37 / TM–287	n
WM	1,54 ± 0,16	6	1,76 ± 0,22	7
RM	1,31 ± 0,04	6	1,68 ± 0,17	9
HM	1,15 ± 0,04	6	1,45 ± 0,14	7

WM/RM $p < 0{,}01$ (+), $p = 0{,}43$ (—); WM/HM $p = 0{,}01$ (+), $p = 0{,}01$ (+); RM/HM $p = 0{,}02$ (+), $p = 0{,}05$ (±).

Angegeben sind die Quotienten ($\bar{x} \pm s_{\bar{x}}$) der Aktivitäten in zwei Ansätzen mit verschiedener KCl-Konzentration. Die statistischen Berechnungen erfolgten nach LINDER[20], p. 106ff. Signifikanz (+) wurde angenommen, wenn $p < 0{,}05$.

Tabelle III. Die Abhängigkeit der spezifischen Aktivität der ATPasen von WM, RM und HM vom pH bei zwei verschiedenen KCl-Konzentrationen

Puffer	TM–37	n	TM–287	n
WM	1,33 ± 0,04	6	1,52 ± 0,11	6
RM	1,39 ± 0,14	6	1,74 ± 0,09	6
HM	1,25 ± 0,16	6	1,58 ± 0,09	6

WM/RM $p > 0{,}05$ (—), $p < 0{,}01$ (+); WM/HM $p > 0{,}05$ (—), $p = 0{,}3$ (—); RM/HM $p > 0{,}05$ (—), $p = 0{,}02$ (+).

Angegeben sind die Quotienten pH 6,5:7,6 ($\bar{x} \pm s_{\bar{x}}$). Signifikanz (+) wurde angenommen, wenn $p < 0{,}05$. Statistische Berechnung nach LINDER[20].

Die spez. Aktivitäten aller 3 Myosine hängen in einem Milieu mit geringem KCl–Zusatz in gleicher Weise vom pH ab[19]. Bei höherem KCl–Zusatz ergeben sich signifikante Unterschiede. Die unterschiedliche Empfindlichkeit auf KCl hängt nicht vom Reinheitsgrad oder der Präparationsmethode ab[14]. SRETER et al.[7] konnten keine Inhibitoren oder Inaktivatoren von Myosin-ATPasen nachweisen. Wir glauben deshalb, dass nicht nur WM von HM und RM wesentlich verschieden ist, sondern dass auch zwischen RM und HM keine enzymatische Identität besteht.

Summary. The Ca^{2+} activated myosin-ATPase of white skeletal muscle of the rabbit has a much higher specific activity than the corresponding enzymes from red and cardiac muscle. The pH-dependence of the 3 myosin-ATPases is identical. However, they differ significantly in the extent of their inhibition by KCl. We conclude, therefore, that all 3 myosins are enzymatically dissimilar.

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Effects of Cholesterol-Derived Photoproducts on the Incorporation of ^{14}C-Acetate into Human Skin Lipids

Previous studies have demonstrated that either ultraviolet (UV) or sunlight reduces the level of freely extractable human skin cholesterol in vitro[1]. The fate of the cholesterol, reflected by these reduced levels, is not fully understood. However, the instability of cholesterol in the presence of air and light is well documented. The formation of cholesterol-derived photo-oxidation products under various experimental conditions have been reported[2–4]. Preliminary studies have shown that cholesterol-derived photo-oxidation products are formed in human skin upon exposure to UV-light (unpublished data).

Recently BLACK and RAUSCHKOLB[5] reported that broad spectrum light (simulated sunlight) inhibited the in vitro incorporation of 1-^{14}C-acetate into human skin sterols as well as other classes of lipids. The major site of this inhibition was shown to be activation of acetate in the formation of acetyl CoA, the precursor of lipogenesis[6]. As these phenomena, reduced cholesterol levels, formation of cholesterol-derived photo-oxidation products and inhibited lipogenesis, are the result of light irradiation upon human skin, the possibility exists that they are related. The present investigations were undertaken to determine the possible role of cholesterol-derived photo-oxidation products upon skin lipogenesis.

Materials and methods. Cholesterol-derived photo-oxidation products (UV-PP) were produced by UV-irradiation of cholesterol absorbed on thin layer plates. 2 mg of cholesterol containing a trace amount of cholesterol-4 ^{14}C (sp. act. 61.4 mC/mmole) in 1.0 ml of chloroform-methanol (2:1, v/v) was applied to silica gel coated thin-layer plates. The plates were irradiated for 40 min with a Burdick QA-450 N mercury arc lamp (1.43×10^5 ergs/sec/cm^2). After irradiation the plates were developed in 1, 2-dichloroethane. Polar UV-PP remaining at the origin of the chromatogram were scraped off and extracted 3 times with 5 ml of chloroform-methanol (2:1, v/v). The extracts were pooled and evaporated to dryness. The radioactivity of dry residue, representing UV-PP, was equivalent to 5% of the total radioactivity of the original cholesterol.

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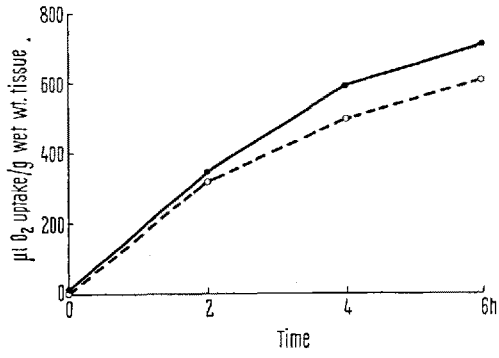
Effect of cholesterol photo-oxidation products on 1-^{14}C-acetate incorporation into human skin lipids[a]

Experimental	Total lipids	Polar[b] lipids	Fatty acids	Sterols	Triglycerides	Sterol esters
			(cpm/100 mg wet wt. tissue)			
Control	5175	3039	684	722	383	126
UV-PP	1824 (–64.9%)	1407 (–53.7%)	299 (–55.3%)	125 (–82.7%)	113 (–70.5%)	17 (–86.5%)

[a] Values represent the mean of 3 experiments. The difference between control and experimental total lipid values is significant at 1% level.
[b] Polar lipids represent phospolipids and other polar materials of total lipid extract.



Effect of cholesterol photo-oxidation products on the respiratory rates of human skin. Values represent the mean of 3 experiments. Respiratory rates are expressed on an accumulative basis. ●, control; ○, UV-PP treated.

Fresh human skin specimens were obtained from the lower abdomen of male caucasians. The preparation of skin specimens has been previously described[5]. The prepared biopsies were placed in 15 ml manometric reaction flasks containing 1.0 ml of Krebs-Ringer phosphate buffer, pH 7.4, 1.0 ml tween 80 solution (86.6 mg/100 ml tween 80 in 1% ethanol), 1 µC of 1-^{14}C-acetate and 150 µg UV-PP. The concentration of UV-PP was calculated on the basis of the specific activity of radioactive cholesterol. This concentration is only approximate as radiolabelled cholesterol is known to be more susceptible to auto-oxidation than the unlabelled compound[2]. The flasks were incubated in a Gilson model GR-14 respirometer at 37°C for 6 h. After incubation, the total lipids of the specimens were extracted as previously described[5]. Radioactivity from the total lipids was determined with duplicate samples (10% of the total extract) by liquid scintillation spectrometry.

The remaining sample was reduced to approximately 50 µl under a stream of N_2. Polar lipids, fatty acids, sterols, triglycerides and sterol esters of the total lipid extract were separated on TLC plates, developed with 1, 2-dichloroethane. The different classes of lipids were visualized by the appearance of brown spots when the TLC plates were saturated with iodine vapors. The areas corresponding to the various lipid fractions were scraped from the plate directly into scintillation cocktail and radioactivity measured by liquid scintillation spectrometry.

Results and discussion. The Table shows that UV-PP actively inhibits the incorporation of 1-^{14}C-acetate into total lipids, polar lipids, fatty acids, free sterols, triglycerides and sterol esters of human skin. At least 53.7% inhibition was observed for each class of lipid. The level of inhibition reached 82.7% and 86.6% for sterols and sterol esters, respectively. The possibility that this significant inhibition might be the result of general cellular injury by UV-PP was considered. However, as seen in the Figure, the respiratory rates of UV-PP treated specimens were not greatly affected and were only 15% less than controls at the end of the experimental period.

BLACK and RAUSCHKOLB[5] demonstrated that a striking reduction in 1-^{14}C-acetate incorporation into lipids occurred in skin irradiated with xenon light. In their studies only about 17% inhibition of respiratory rates of irradiated tissues occurred as compared to the 50% reduction in 1-^{14}C-acetate incorporation. In our present studies a greater inhibition of radioiosotope incorporation was found, compared to their findings, with a similar effect on the respiratory rates. Although the present results could not be quantitatively correlated with their findings, the fact that the same type of inhibition occurrs with both xenon light and UV-PP suggests that the effect on skin lipogenesis is mediated via similar mechanism(s). Tentative identification has indicated that 5-α-cholestan-3α, 5β, 6α-triol, 7-α-OH-cholesterol, 7-β-OH-cholesterol, 7-ketocholesterol, cholesterol α-oxide and 4-cholesten-3-one are among the several constituents of UV-PP prepared in this study. These compounds have previously been reported as oxidation products of cholesterol[2,7]. Whether one constituent is responsible for the inhibitory effect of UV-PP or if the effect is a synergestic one produced by a mixture of these compounds is not known. Most, however, demonstrate structural similarities in regard to their polycyclic hydrocarbon structure. Of interest is the fact that aflatoxin B_1, a potent carcinogen, shares certain structural similarities as these oxidation products and has been shown to cause similar effects on acetate incorporation into skin lipids as those reported here for UV-PP[8].

In summary, the inhibition of 1-^{14}C-acetate incorporation into skin lipids by xenon light can be replaced by UV-PP. Preliminary studies also indicate that these photo-oxidation products are, indeed, formed in UV-irradiated skin. These data suggest a possible relationship between photoproduct formation and light induced inhibition of lipogenesis. It is conceivable that photo-oxidation products formed in UV-irradiated human skin may, in turn, motivate the responsive inhibitory effects on lipogenesis[9].

Zusammenfassung. Hautbestrahlung beim Menschen mit Xenon- oder UV-Licht und die dabei beobachteten chemischen Vorgänge (Hemmung von 1-^{14}C-Azetat und Einbau in die Gesamtlipide) legen eine mögliche Relation zwischen Photoproduktbildung und lichtinduzierter Hemmung der Lipogenese nahe.

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