on Gas Chrom Q. The major peak (95% of the mass of the sample) had the same retention time as cholesterol acetate. A minor peak was present with a retention time of 0.18 relative to cholesterol acetate. This component behaved in a manner similar to cholestane, leading to the conclusion that it was a hydrocarbon of some sort.

Mass spectrometry of the brine shrimp sterol acetate was performed by the Morgan-Schaefer Corp. (Montreal, Quebec). The spectrum obtained, agreed well with a spectrum of cholesterol acetate[11]. A second parent ion was found at *m/e* 430 indicating that cholestanol (cholestane-3-ol) was present. This is not surprizing as cholestanol acetate is poorly separated from cholesterol acetate in most gas chromatographic systems[12].

On the basis of these studies, we have concluded that the sterol fraction of *A. salina* from Mono Lake is composed primarily of cholesterol with a significant percentage of cholestanol also present. The minor component on the gas chromatograph may have been canthaxanthin or another caroteniod commonly present in brine shrimp[13].

It is difficult to compare the results of the present study with those of other workers as the population of brine shrimp used here is physiologically distinct[1,2] from those used in other studies. In addition, TYSON[14] has observed a spirochete-like organism in the tissues of some specimens of *A. salina*. As our shrimps were harvested from a natural source, we cannot rule out the possibility that infected individuals were present.

TESHIMA and KANAZAWA[15] have demonstrated that *A. salina* can convert dietary ergosterol into cholesterol. In this study gas chromatographic data was presented showing cholesterol to be the only sterol in *Euglena*-fed brine shrimp. However, their system, like ours, would have separated cholesterol and cholestanol with difficulty[12]. Assuming that cholestanol was also present in their sterol sample, it now appears evident that the extreme environment of Mono Lake has not required a change in the qualitative sterol composition of *A. salina*.

Zusammenfassung. Artemia salina vom Mono-See, Kalifornien, U.S.A., enhält Cholesterin und Cholestanol als ihr hauptsächlichstes Sterin. Diese Zusammensetzung ist ähnlich wie die jenige von im Laboratorium erzeugten Salz-Krabben und zwar trotzdem das Wasser des Mono-Sees ein pH von 9.6 und einen Salzgehalt von 2.23 *M* hat[16].

TH. PAYNE and S. S. KUWAHARA[17]

Department of Chemistry,
California State College at Long Beach,
Long Beach (California 90801, USA), 22 February 1972.

[11] B. A. KNIGHTS, J. Gas Chromat. *5*, 273 (1967).
[12] G. W. PATTERSON, Analyt. Chem. *43*, 1165 (1971).
[13] B. H. DAVIES, W.-J. HSU and C. O. CHICHESTER, Comp. Biochem. Physiol. *33*, 601 (1970).
[14] G. E. TYSON, J. Invert. Path. *15*, 145, (1970).
[15] S. TESHIMA and A. KANAZAWA, Comp. Biochem. Physiol. *38 B*, 603 (1971).
[16] Acknowledgment. This work was supported, in part, by a grant from the Long Beach (California) Heart Association.
[17] Present address: Department of Developmental and Cell Biology, University of California, Irvine (California 92664, USA).

A Site of Action of Light on ^{14}C-Acetate Incorporation into Human Skin Sterols

It was recently shown that broad spectrum light caused marked reductions in the level of ^{14}C-acetate incorporated into sterols of human skin[1]. Incorporation of acetate into other classes of lipids was similarly affected by light. Examination of the sterol biosynthetic pathway revealed that light had no effect on the incorporation level of mevalonate, the committed step in sterol synthesis. Nor were there significant effects upon respiratory rates of irradiated tissues. It was suggested, from these observations, that the inhibitory effect of light was at a common point in the lipid synthetic pathways and probably involved acetate activation or the availability of acetyl Coenzyme A pools of sufficient size to sustain endogenous lipogenesis. Because sufficient quantities of fresh human skin were not available for isolation and direct measurement of acetate activating enzyme, indirect lines of evidence were sought to elucidate the specific site(s) of action of light upon skin lipogenesis and to assess their physiological importance.

Materials and methods. Fresh human skin was obtained, irradiated, and processed exactly as previously described[1]. After irradiation the skin specimens were placed in 15 ml manometric flasks containing 2.0 ml of Krebs-Ringer phosphate buffer, pH 7.4, 2 m*M* glucose, and the appropriate radio-labelled intermediate or precursor. In

[1] H. S. BLACK and E. W. RAUSCHKOLB, J. Invest. Derm. *56*, 387 (1971).

Table I. Incorporation of exogenous ^{3}H-acetyl Co A into skin sterols[a]

Incubation (min)	cpm/100 mg tissue wt.
45	237
90	261
180	388
360	1,394

[a] Free sterols were isolated from total lipid extracts by thin layer chromatography. The chromatograms were developed in a 1,2-dichloroethane solvent system.

Table II. Effects of broad spectrum light on acetyl Co A incorporation into skin sterols

	cpm/100 mg tissue wt.	
	^{14}C-acetate	^{3}H-acetyl Co A
Control	6,240 (−79%)	1,075 (−25%)
Irradiated	1,292	0,808

Values represent the mean of 3 experiments. Final concentration of ^{3}H-acetyl Co A was $2.5 \times 10^{-6} M$.

some cases this consisted of ^{3}H-Acetyl Coenzyme A (1.2 Ci/mmoles) at a final concentration of $2.0 \times 10^{-6} M$ or 1 μC of ^{14}C-acetate (52.9 mCi/mmoles). Other flasks contained non-labelled acetyl Co A at concentrations ranging from 1×10^{-4} to $2.5 \times 10^{-6} M$ and, in addition, 1 μC of ^{14}C-acetate. Pyruvate-2-^{14}C was used in some studies (1 μC; 4.04 mC/mM). The flasks were shaken (120 strokes/min) on a Gilson respirometer for 6 h at 37°C. After the incubation period the reactions were halted, the tissue sectioned on a freezing stage microtome, and total lipids extracted[2]. Free sterols were isolated as the digitonides[3]. Aliquots of a chloroform-methanol solution (2:1 v/v) of the digitonides were dried under nitrogen and levels of radioactivity determined by liquid scintillation spectrometry. Counting efficiencies for ^{14}C and ^{3}H were 83% and 34%, respectively.

Results and discussion. The synthesis of acetyl Co A is known to result from the degradation of carbohydrates or glucogenic amino acids, via pyruvic acid, as well as from the oxidation of fatty acids. In rodent skin the principal sources of substrates for endogenous oxidation are lipids[4]. These oxidations occur intramitochondrially and result in formation of acetyl Co A, the precursor for lipogenesis. On the other hand most lipid synthesis occurs extramitochondrially and probably requires mitochondrial produced acetyl Co A. It was shown that, in the case of rat liver, acetyl Co A diffuses across the mitochondrial membrane very slowly and the rate of diffusion of acetyl Co A could not account for the rates of lipogenesis observed. The alternative possibilities for formation of lipogenic sustaining levels of extra-mitochondrial acetyl Co A have been discussed[5].

Table III. Effects of acetyl Co A on ^{14}C-acetate incorporation into sterols by irradiated skin

Treatment	cpm/100 mg tissue wt.	
	^{14}C-acetate	^{14}C-acetate + acetyl Co A
1 Control	4,811	6,251
Irradiated	1,367 (−70%)	0,712 (−89%)
2 Control	5,183	4,970
Irradiated	2,204 (−57%)	2,743 (−45%)
3 Control	4,975	3,183
Irradiated	1,270 (−75%)	0,772 (−76%)

Values are from single experiments. Concentrations of non-labelled acetyl Co A are $2.5 \times 10^{-6} M$, $5 \times 10^{-6} M$, and $1 \times 10^{-4} M$ for treatments 1–3, respectively.

Table IV. A comparison of the effects of broad spectrum light on incorporation of ^{14}C-acetate and ^{14}C-pyruvate into human skin sterols

cpm/100 mg tissue wt.			
Acetate		Pyruvate	
Control	Irradiated	Control	Irradiated
2844 (−76.6%)	667	587 (−69.8%)	194

Values represent the mean of 3 experiments.

Regardless of the mechanism involved for the transfer of mitochondrial acetyl Co A to the sites of sterologenesis, we have shown that exogenous acetyl Co A does diffuse into human skin slices and is incorporated into sterols (Table I). When irradiated skin specimens were incubated in the presence of tritiated acetyl Co A only 25% inhibition of isotope incorporation occured as compared with 79% inhibition obtained with labelled acetate (Table II). If availability of acetyl Co A is the rate-limiting step for sterologenesis in irradiated skin then the slight inhibition (about 30% of that obtained with acetate) observed with labelled acetyl Co A could represent inability to overcome this step due to low diffusion rates of exogenous acetyl Co A into the tissue. Alternatively the residual inhibition could represent a second minor site of inhibition from acetyl Co A to mevalonate. The fact, however, that 70% of the usual inhibitory effect was not present when sterologenesis was traced with acetyl Co A, indicates that the principal inhibitory site(s) of light on sterologenesis is prior to the formation of acetyl Co A. With the knowledge that 70% of the inhibitory effects of light were negated when sterologenesis was followed by incorporation of exogenous acetyl Co A, skin slices were incubated in the presence of several concentrations of non-labelled acetyl Co A in addition to ^{14}C-acetate. As shown in Table III the inhibitory effect of light on ^{14}C-acetate incorporation into the sterol fraction was not affected by any of the concentrations of acetyl Co A tested. These data indicate that acetate activation is the rate-limiting step of sterologenesis in irradiated skin rather than acetyl Co A pool size.

The incorporation rates of ^{14}C-acetate should parallel, but not necessarily reflect, the actual rate of lipogenesis[6,7]. It has been argued that glucose is the primary substrate for lipogenesis in skin[8]. In our earlier studies the experimental conditions excluded the addition of glucose to the incubation medium of the skin slices and hence should reflect, after 1 to 2 h incubation when endogenous glucose would be exhausted, lipogenic levels supported from the oxidation of lipids. We have found, as have others, that the addition of glucose to the incubation medium of excised skin greatly enhances the incorporation levels of acetate. However, the addition of glucose does not alter the effect of light upon acetate incorporation. Furthermore, when pyruvic acid is used to trace sterologenesis, the effects of light upon incorporation of this glycolytic end-product is very similar to that of acetate (Table IV). The effect of light upon pyruvic acid incorporation into sterols could result from inhibition of pyruvic acid decarboxylation. Alternatively, being only a few steps removed from acetyl Co A it might reflect the importance of acetate as an intermediate in sterologenesis. In skin, if extramitochondrial acetyl Co A is formed by the activation of acetate derived from mitochondrial acetyl Co A, the inhibitory effect of light on acetate activation could

[2] J. FOLCH, M. LEES and G. H. SLOANE STANLEY, J. biol. Chem. *226*, 497 (1957).
[3] W. M. SPERRY and M. WEBB, J. biol. Chem. *187*, 97 (1950).
[4] C. N. D. CRUIKSHANK, M. D. TROTTER and J. R. COOPER, J. Invest. Derm. *39*, 175 (1962).
[5] J. M. LOWENSTEIN, Biochem. Soc. Symp. *24*, 57 (1963).
[6] R. J. EMERSON and J. T. VAN BRUGGEN, Arch. Biochem. Biophys. *77*, 467 (1958).
[7] W. G. DUNCOMBE, Biochem. J. *106*, 179 (1968).
[8] V. R. WHEATLEY, L. T. HODGINS and W. M. COON, J. Invest. Derm. *54*, 288 (1970).
[9] Supported in part by a grant from the Morrison Trust of San Antonio (Texas, USA).

result in a substantial effect upon lipogenesis. Although the present data indicate that one inhibitory site of action of light is upon acetate activation they do not preclude an effect on other susceptible sites prior to the formation of acetyl Co A[9].

Zusammenfassung. Die Synthese von Sterolen in menschlicher Haut, welche im langwelligen Spektralbereich bestrahlt worden war, wurde mit radioaktiv markiertem Acetyl-Co A, Acetat and Pyruvat geprüft. Von Acetyl-Co A konnte nur geringe Hemmung nachgewiesen werden, während Licht eine merkbare Hemmung von Acetat und Pyruvatinkorporation verursachte. Die Resultate lassen vermuten, dass die Acetataktivierung die von Licht beeinflussbare Stufe in der Biosynthese von Sterolen ist.

H. S. Black, J. D. Smith,
B. J. Cumbus and W. B. Lo

*Veterans Administration Hospital
and Departments of Dermatology and Biochemistry,
Baylor College of Medicine,
Houston (Texas 77031, USA),*
6 March 1972.

Keratin Decomposition by Dermatophytes: Evidence of the Sulphitolysis of the Protein

Dermatophytes are capable of decomposing keratin, resistant scleroprotein very rich in cystine. Nevertheless, the metabolism of sulphur in these fungi has been given small attention so far [1–4]. Therefore, we examined the growth of the dermatophyte *Microsporum gypseum* on media containing 0.1% cystine. As basic nutrients, the media contained 1% gelatin, serum albumin or casein or 0.8–1.0% glucose combined with 0.1–0.4% peptone, glutamin, urea or $(NH_4)_2HPO_4$. On all media, cystine was intensively metabolized and its sulphur almost quantitatively converted to sulphate excreted into the culture fluid. Apart from sulphate as final oxidation product, we have also found sulphite in some culture filtrates. It used to appear in amounts of several tens up to hundreds μg/ml. In most of the media, no free sulphite could be detected in any phase of the growth. However, considerable amount of this compound was found after treatment by alkaline cyanide (NaCN, final concentration 0.5%; EDTA $10^{-2}M$; pH 10–11, 50 °C, 30 min). This 'bound sulphite' obviously represents the S-sulphocysteine, which originated by a nonenzymic reaction between cystine and sulphite[5]:

$$R\text{-}S.S\text{-}R + HSO'_3 \rightleftarrows R\text{-}SH + R\text{-}S.SO'_3.$$

S-sulphocysteine (as well as other S-thiosulphate esters) can be split by cyanide to thiocyanate and sulphite[5]:

$$R\text{-}S.SO_3H + CN' \rightarrow R\text{-}SCN + HSO'_3.$$

Therefore we concluded that on our media it was always also sulphite that was produced besides sulphate. In most cases this sulphite disappeared due to the reaction with excess cystine. The capacity of producing sulphite by oxidation of cystine was later proved by us also in other species of dermatophytes.

The findings described may be of importance for elucidating keratin decomposition by dermatophytes. Since sulphite is also produced from protein-incorporated cystine, the fungus could use it for splitting disulphide bonds of keratin. Keratin denatured by 'sulphitolysis' would then be easily accessible to the proteinases of the fungus.

The results corroborating this hypothesis were obtained by the analysis of culture filtrates of *Microsporum gypseum* growing on human hairs in mineral medium (Table). In stationary culture, the fungus managed to digest about 32% of the substrate in 60 days. Even here, the main product of cystine oxidation was sulphate, whose concentration in the medium amounted to 1.6 mg/ml. Free sulphite was not present in the filtrate. It could however be demonstrated, that S-thiosulphate esters ('bound sulphite') were present. The gel filtration on Sephadex G-50 and G-10 proved that thiosulphate ester groups are bound to compounds of molecular weight amounting to several thousands, i.e. obviously, to polypeptides. This is in agreement with the presumption that the compounds under study are peptides containing combined S-sulphocysteine. The quantity found (corresponding to 15–20 μg/ml of S-sulphocysteine) is, taken absolutely, not large. However, it represents 25–60% of all combined cysteine and its derivatives in the medium. This demonstrates that

[1] W. H. Stahl, B. McQue, G. R. Mandels and G. H. Siu, Arch. Biochem. *20*, 422 (1949).
[2] G. E. Mathison, Mycopath. Mycol. appl. *27*, 225 (1965).
[3] H. Ziegler and G. Reichmann, Mykosen *11*, 903 (1968).
[4] H. G. Schaper und H. Ziegler, Vortrag, 5. Tagung der Gesellschaft für Medizinische Mykologie der DDR, Leipzig, 7.–10. 5. 1970. Kurzreferat: Mykosen *14*, 589 (1971).
[5] B. Milligan and J. M. Swan, Rev. pure appl. Chem. *12*, 72 (1962).

Keratin decomposition by *Microsporum gypseum. Analysis of the culture fluid*

Days	3	11	17	24	32	40	50	60
Substrate digestion (%)	0	4	16	18	25	26	29	32
pH	6.4	8.0	8.3	8.4	8.2	8.1	8.2	8.1
Proteins (μg/ml)	110	560	365	310	465	440	440	380
Sulphate (μg/ml)	18	147	490	538	842	1187	1499	1641
Bound sulphite (μg/ml)	0	4.1	10.0	13.4	16.7	19.0	19.0	18.5

Cultures with 400 mg of ethylene oxide-sterilized human hairs in 20 ml of simple mineral solution, 29 °C. Substrate digestion was calculated from total dry weight (substrate + mycelium). Proteins (Lowry method) are expressed as μg/ml bovine serum albumin, sulphate and sulphite as μg/ml of anhydrous natrium salts. No free sulphite was found; 'bound sulphite' was determined after treatment with cyanide (see text).