marked inhibition of the enzyme was found after the administration of 0.6 m*M* PLP plus the same amount of L-DOPA. Such an enzyme inhibition was possibly caused by condensation products formed from L-DOPA and PLP in the incubation chamber, such as L-DOPA-PLP complex[7]. It is well established that L-DOPA and m-hydroxyphenylethylamines react nonenzymatically with aldehydes including PLP to corresponding tetrahydroisoquinoline derivatives[8]. These products are stable[7,8] and can account for the inhibition of enzymic decarboxylation activity obtained in our in vitro studies. Such an inhibition of DOPA decarboxylase by L-DOPA plus PLP may not be caused by an enlarged pool of L-DOPA subsequent to the addition of carrier L-DOPA to the enzyme mixture, since no change in the decarboxylation of DOPA was found with such a very small amount of L-DOPA (Figure).

The fact that DOPA decarboxylase activity is inhibited by L-DOPA plus PLP, possibly by condensation products formed from these substrates[7,8] may support previous clinical[9] and experimental[10] studies showing that pyridoxine antagonized the activity of L-DOPA in the treatment of parkinsonism. Interestingly, the salutary effects as well as the serious side effects observed in patients treated with L-DOPA were also reversed by pyridoxine[10,11].

*Sommaire.* Les résultats obtenus démontrent une inhibition de l'activité de la DOPA decarboxylase par de haute concentration de PLP ou de faible concentration de L-DOPA plus PLP. Ceci pourrait expliquer les observations cliniques et expérimentales précédentes démontrant que la pyridoxine antagonise l'effet de L-DOPA utilisé dans le traitement de la maladie de Parkinson.

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## Sterols of the Brine Shrimp, *Artemia salina*, from Mono Lake, California

Mono Lake, California, has no outlet for its waters, consequently its salinity is almost twice that of the sea. In addition to their high salt content (ca. 2.23 *M*) and high content of dissolved organic matter (ca. 62 mg/l), the waters of Mono Lake have a fairly basic pH (ca. pH 9.6) with 95% of the buffer capacity contributed by the carbonate-bicarbonate system and the remaining 5% contributed mainly by borate. As a result of this extreme environment, the biological composition of the lake is relatively simple and the highest organism found in the lake waters is the brine shrimp, *Artemia salina* (L.). MASON[1] who obtained the data given above, has surveyed the limnology of Mono Lake showing the extreme environmental conditions in the habitat of these shrimp. Others[1,2] have shown that the brine shrimp in Mono Lake are physiologically distinct from the populations found in San Francisco Bay, California, and the Great Salt Lake of Utah.

Sterols have been implicated as having major roles in maintaining the stability of biological membranes[3–5] and variations in the permeability of erythrocyte membranes have been observed as a result of removing part of their sterol compliment or replacement of their cholesterol content with other sterols[6]. Since cellular membranes serve as an interface between an organism and its environment and, since sterols are an important component of these membranes, it was possible that the sterol composition of *A. salina* from Mono Lake would be altered as a result of its adaptation to this unusual environment.

Fresh-frozen brine shrimp, harvested from Mono Lake, were obtained from the California Koi Company (Glendora, California), thawed in tap water, and washed several times with cold 0.1 *M* KCl to remove debris and adhering material. The shrimp were initially extracted with diethyl ether-ethanol (2:1, v/v), and the residue was re-extracted 3 times with diethyl ether, after which the combined extracts were concentrated on a rotary evaporator and taken up in petroleum ether (B.P. 30–60 °C).

The concentrate was chromatographed on silicic acid[7], and the sterol containing fractions were collected. The sterol ester fraction was saponified and the sterols were combined with the free sterol fraction from the silicic acid column. The combined sterol fraction was further purified on Florisil[8]. A yellowish contaminant was eluted with the sterol fraction from Florisil.

Preparative thin-layer chromatography on Silica Gel G was used for the final isolation of brine shrimp sterols. The developing solvent contained: petroleum ether, diethyl ether, and acetic acid (50:50:0.25; v/v/v). This sterol fraction was completely precipitated by digitonin[9] and gave a positive Lieberman-Burchard reaction[10]. There was no evidence for 'fast-acting' sterols.

Sterol acetates were prepared by reacting the sterols with acetic anhydride in pyridine, and stored in petroleum ether. Cholesterol acetate prepared from commercial cholesterol gave m.p. 114°–115°. The brine shrimp sterol acetate gave m.p. 111°–112° and a mixture of the shrimp sterol acetate and cholesterol acetate gave m.p. 113°–114°. The brine shrimp sterol acetate had an IR-spectrum virtually identical with that of cholesterol acetate.

Gas chromatography of the brine shrimp sterol acetate was performed on a glass column containing 1% XE-60

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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].

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## 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

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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 $2.5 \times 10^{-6} M$.