

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 A⁹.

Zusammenfassung. Die Synthese von Sterolen in menschlicher Haut, welche im langwelligen Spektralbereich bestrahlt worden war, wurde mit radioaktiv markiertem Acetyl-Co A, Acetat und Pyruvat geprüft. Von Acetyl-Co A konnte nur geringe Hemmung nachgewiesen werden, während Licht eine merkbare Hemmung von Acetat und Pyruvatinkorporation verursachte. Die Re-

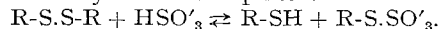
sultate lassen vermuten, dass die Acetataktivierung die von Licht beeinflussbare Stufe in der Biosynthese von Sterolen ist.

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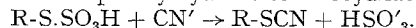
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Keratin Decomposition by Dermatophytes: Evidence of the Sulphitolysis of the Protein

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 far¹⁻⁴. 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 (NH₄)₂HPO₄. 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 10⁻²M; pH 10–11, 50°C, 30 min). This 'bound sulphite' obviously represents the S-sulphocysteine, which originated by a nonenzymic reaction between cystine and sulphite⁵:



S-sulphocysteine (as well as other S-thiosulphate esters) can be split by cyanide to thiocyanate and sulphite⁵:



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 sodium salts. No free sulphite was found; 'bound sulphite' was determined after treatment with cyanide (see text).

a part of the disulphide bonds of keratin was obviously cleaved by sulphite.

Strongly acidic groups with the properties identical to S-thiosulphate esters were also found by means of topographical methods in the attacked substrate proper⁶.

The results described are in compliance with the assumption of the sulphitolysis of protein as one of the basic reactions of keratin decomposition by dermatophytes.

⁶ J. KUNERT, *Sabouraudia* 10, 6 (1972).

Zusammenfassung. Es wird wahrscheinlich gemacht, dass die Denaturierung des Keratins durch Sulfitausscheidung und Sulfitolys des Cystins eine wichtige Rolle beim Keratinabbau durch Pilze spielt.

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Evidence for Chemical Coding of Color Discrimination in Goldfish Brain

During the last decade, stimulated by the successful elucidation of the molecular code of genetic information, attempts have been made to discover evidence for a similar code for the processing of acquired information in the nervous system¹⁻⁴. There have been three main experimental approaches to the problem: detection of chemical changes in the brain associated with the acquisition of information^{5,6}, impairment of information processing by inhibitors of RNA and protein metabolism⁷, and bioassay methods for testing the information content of material extracted from brain⁸⁻¹⁰.

The bioassay approach has been the most controversial because its purposes and the interpretation of its results were widely misunderstood, and because the unreliability inherent in all bioassays is further increased by the use of behavioral criteria. Enough results have, however, been published in the last six years by 33 laboratories to establish the validity of the method. One of the points that still remains controversial is the specificity of the information supplied by the brain material. The experiments of ZIPPEL and DOMAGK¹¹ on transfer of color discrimination in the goldfish gave the best evidence for stimulus specificity within the same sensory modality. In preliminary experiments we confirmed these results and extended them from red and green, used by ZIPPEL and DOMAGK, to other colors¹². The present paper deals with experiments in which donor fish were trained to discriminate between blue and green, and avoidance of one or the other color was transferred to naive and unreinforced recipients.

Goldfish (*Carassius auratus*) 6 to 10 cm long were obtained from Ozark Fisheries, Stoutland, Missouri. They were trained in a shuttle-box consisting of a rectangular tank (26 × 50 × 35 cm) divided in the middle by an opaque partition leaving a clearance of 4.5 cm at the bottom allowing the fish to cross from one compartment to the other. The depth of water was 12.5 cm. At each end of the tank was a 30 watt light bulb and a frame for the colored filters. The following Eastman Kodak gelatin filters were used in the experiments: green (No. 58) 480–630 nm and blue (No. 47) 370–510 nm.

Each of the compartments was provided with electrodes made of stainless steel wire mesh. Shocks of 2 mA of 50 msec duration were delivered every 2 sec during presentation of the unconditioned stimulus.

One group of fish was trained to avoid blue and escape into green. Each trial consisted in presenting blue light in one compartment and green in the other for 30 sec. At the end of 20 sec, the current was turned on in the blue compartment for 10 sec so that, if the fish was on that side, it received electric shocks. This was repeated for each fish 15 times daily with an intertrial interval of 30 sec during

which neither color was presented. Another group of fish was trained to avoid green and take refuge in the blue. The paired colors were presented on each side in random order. Training was continued until 13 correct avoidances were observed on 3 consecutive sessions. This took usually 8 to 12 days.

Twelve to 24 h after the last trial, the fish were decapitated and their brains were placed on dry ice. They were kept frozen until preparation of the extract. Three pools of brain, from blue-avoiding donors (BA), green-avoiding donors (GA) and untrained fish (C), were extracted according to a procedure previously used for rat brains^{8,13}. A crude RNA extract was used in the first series experiments, replaced later by a dialyzate. The extracts were injected into the recipient fish intracranially under a 40 µl volume¹⁴. The fish were tested once before injection for their color preferences. They were further tested 1, 2 and 3 days after injection. Preliminary experiments had shown that the peak of the effect was over by the 3rd day. In later experiments testing was not extended beyond the 2nd day because the results obtained on the 3rd day supplied no significant additional information. Each testing session consisted of 10 trials. In 5 trials, the fish was in the blue compartment and in 5 trials in the green compartment, the colors being presented in random order. The recipients were never shocked and avoidance

¹ W. L. BYRNE, *Molecular Approaches to Learning and Memory* (Academic Press, New York 1970).

² G. UNGAR, *Molecular Mechanisms in Memory and Learning* (Plenum Press, New York 1970).

³ G. ADÁM, *Biology of Memory* (Akadémiai Kiadó, Budapest 1971).

⁴ E. J. FJERDINGSTAD, *Chemical Transfer of Learned Information* (North-Holland Publishing Company, Amsterdam 1971).

⁵ E. GLASSMAN, *A. Rev. Biochem.* 38, 605 (1969).

⁶ D. A. BOOTH, in *Molecular Mechanisms in Memory and Learning* (Ed. G. UNGAR; Plenum Press, New York 1970), p. 1.

⁷ H. D. COHEN, in *Molecular Mechanisms in Memory and Learning* (Ed. G. UNGAR; Plenum Press, New York 1970), p. 59.

⁸ G. UNGAR, in *Methods in Pharmacology* (Ed. A. SCHWARTZ; Appleton-Century-Crofts, New York 1971), vol. 1, p. 579.

⁹ G. UNGAR, in *Handbook of Neurochemistry* (Ed. A. LAJTHA; Plenum Press, New York 1971), vol. 6, p. 241.

¹⁰ J. A. DYAL, in *Chemical Transfer of Learned Information* (Ed. E. J. FJERDINGSTAD; North-Holland Publishing Company, Amsterdam 1971), p. 219.

¹¹ H. P. ZIPPEL and G. F. DOMAGK, *Experientia* 25, 938 (1969).

¹² G. UNGAR, L. GALVAN and G. CHAPOUTHIER, *Fedn. Proc.* 30, 265Abs (1971).

¹³ G. UNGAR, D. M. DESIDERIO and W. PARR, *Nature, Lond.* 238, 198 (1972).

¹⁴ E. J. FJERDINGSTAD, in *Chemical Transfer of Learned Information* (Ed. E. J. FJERDINGSTAD; North-Holland Publishing Company, Amsterdam 1971), p. 199.