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 topochemical methods in the attacked substrate proper[6].

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.

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Zusammenfassung. Es wird wahrscheinlich gemacht, dass die Denaturierung des Keratins durch Sulfitausscheidung und Sulfitolyse 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 system[1–4]. 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 metabolism[7], and bioassay methods for testing the information content of material extracted from brain[8–10].

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 DOMAGK[11] 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 colors[12]. 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 ($26 \times 50 \times 35$ 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 volume[14]. 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

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Table I. Net avoidance (B–G) in recipients of brain extracts from blue-avoidance-trained (BA), green-avoidance-trained (GA) and untrained donors (C), tested before and for 3 consecutive days after injection

Donor training →	BA		GA		C		P
Day	B–G ± S.D.		B–G ± S.D.		B–G ± S.D.		
0	0.3	1.8	0.3	1.2	0.25	1.3	N.S.
1	1.85	2.2	−1.25	2.2	0.6	1.2	<0.001
2	2.0	1.8	−1.5	1.4	0.15	1.0	<0.001
3	1.6	1.8	−0.5	1.9	0.55	1.2	<0.001
1 to 3	1.8	1.7	−1.1	1.3	0.4	1.0	<0.001
N	48		48		24		

All recipients were given the equivalent of 80 mg of donor brain (crude RNA extract) by intracranial injection. *P* values were calculated by *t*-test between BA and GA injected groups.

Table II. Statistical analysis of results obtained in BA-injected and GA-injected recipients by χ^2 method

Days	BA		GA		P
	(B–G)>0	(B–G)<0	(G–B)>0	(G–B)≤0	
0	26	22	27	21	N.S.
1	36	12	11	37	<0.001
2	40	8	2	46	<0.001
3	36	12	10	38	<0.001

Table III. Avoidance-inducing effects of different doses of the dialyzable fraction of brain extract (D) and of its non-dialyzable fraction (ND) taken from blue-avoiding (BA) and green-avoiding (GA) donors

Donor training →		BA		GA		P
Extract		B–G ± S.D.		B–G ± S.D.		
D	40 mg	0.9	1.4	0	1.8	N.S.
	60 mg	1.3	2.0	−0.4	1.1	<0.05
	80 mg	2.6	1.3	−0.9	1.1	<0.001
	120 mg	1.9	1.3	−1.25	1.0	<0.001
ND	80 mg	0.75	1.3	1.1	1.3	N.S.

Dose indicated in equivalents of wet weight of brain. Each group consisted of 8 animals. Calculated with mean values obtained on first and second day after injection.

Table IV. Effect of trypsin and chymotrypsin on avoidance-inducing effects of brain extracts

Donor training →	BA		GA		P
Enzyme	B–G ± S.D.		B–G ± S.D.		
0	1.5	1.1	−0.8	1.1	<0.01
Trypsin	−0.1	1.5	−0.5	1.1	GA v. control BA<0.02
Chymotrypsin	1.4	1.2	0.5	2.8	BA v. control GA<0.01

All recipients received dialyzate equivalent to 80 mg donor brain. Groups of 8 fish. For incubation with enzymes see text.

was scored when the fish swam to the opposite side within 30 sec. The trials were separated by a 30 sec dark interval. All testing was done under blind conditions.

Table I summarizes the pooled results of 6 experiments, in each of which 8 fish were injected with BA extracts, 8 with GA preparations and 8 with similarly prepared extracts from untrained fish (in 3 experiments only). In any given test each fish could make a maximum of 5 avoidances in each direction. The results are expressed in terms of the mean net avoidance (blue avoidance minus green avoidance) which expresses the opposite changes in color preference induced by the injection of the brain extracts in the 2 experimental groups. The data were analyzed by the *t*-test evaluating the significance of the difference between the 2 means. Table II shows the same data tested by the χ^2 method, the recipients being classified according to whether their net avoidance was in the expected direction or not.

In the next series of experiments, we dialyzed the crude RNA preparations adjusted to pH 3.7 by ammonium acetate buffer against distilled water for 48 h at 2°C. The dialyzate was lyophilized and redissolved in a small volume of water for intracranial injection. Table III shows that the dialyzate was active at doses equivalent to 60 to 120 mg of brain. The optimal dose for the blue avoidance factor was 80 mg and for the green avoidance factor 120 mg. The non-diffusible fraction was inactive.

These results suggested that the active substances may be peptides that form dissociable complexes with RNA, as in the case of a behavior-inducing peptide previously isolated from rat brain[13]. We incubated the dialyzed extracts with trypsin or with chymotrypsin (both at 100 µg ml at pH 7.8) at room temperature for 2 h and redialyzed the preparations. Control samples were submitted to the same procedure but without enzymes. Doses equivalent to 80 mg of fresh weight of brain were injected into groups of 8 fish. The results of the experiments shown in Table IV indicate that BA is inactivated by trypsin and GA by chymotrypsin.

It is planned to gather sufficient material for the isolation and identification of the 2 substances. In order to accomplish this in a reasonable time, donors are beintrained in groups of 20 to 30. They reach criterion in approximately the same time as with the previously described procedure and show satisfactory performance when tested individually at the end of their training. It is anticipated that 20,000 to 30,000 brains will have to be collected and it is hoped that this can be accomplished within a year. Elucidation of the structure of these substances could supply important clues to the possible role of the behaviour-inducing peptides in the coding of information by the brain[15].

Résumé. Des comportements provoqués par la discrimination de couleurs ont pu être observés chez des poissons ayant reçu par injection intracranienne des extraits de cerveau de poissons donneurs entrainés. Cette expérience est utilisée comme essai biologique pour la purification et l'identification des substances actives.

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