Regeneration of Olfactory Paths in Carp (*Cyprinus carpio* L.)

Previous investigations[1] have demonstrated the regenerative capacity of spinal cord, optic pathways and even whole tectal[2] and forebrain[3] lobes in Teleost fish.

In this study regeneration of the olfactory tract and central olfactory connections has been demonstrated in carp histologically and behaviourally. In fourteen carp the entire olfactory tract (both medial and lateral portions) was completely severed bilaterally at operation. The wounds were closed and regeneration and recovery of olfaction was permitted for up to 20 weeks.

Behavioural evidence of regeneration was obtained using a simple test[4] for olfaction in fishes, in which two identical cheesecloth sacks, one containing concealed minced earthworms or tubifex and the other stones, were suspended in an aquarium with the fish. Normal fish lost their ability to locate and select the sack containing worms when olfaction was impaired (olfactory tracts severed, olfactory sacs occluded or olfactory mucosa anaesthetized, etc.) but not if blinded. Our methodological refinement (see Figure) to record potentials induced when a coil to which the sack is attached moves through a magnetic field with responses registered directly by a penwriter, yielded much more quantitative information about olfaction in our fishes and enabled us to trace the course and extent of its recovery during regeneration of the olfactory pathways.

Using this method, the results (Table, A) from 20 normal fish compared to results from eight fish with olfactory tracts regenerating, show re-establishment of olfaction with approximately normal responsiveness to the worm-sack in the latter group. No olfactory responses were observed sooner than 40 days post-operatively. In both these groups the responses to worm-filled sacks showed significantly higher frequency of strike groups[5], more touches per strike, and longer duration of each strike. This difference may be considered a function of the olfactory attraction of the worm-sack because of a standardized 24 h pre-test food deprivation for all groups.

Fourteen normal fish were then conditioned to respond positively to the odour of morpholine[6] (strongly repellant for naive fish in the concentration used) by daily administration of morpholine to the aquarium several minutes prior to and during feeding, and required an average of 30 days training. The responses of these fish (Table, B) to a sack soaked in morpholine $1 \cdot 10^{-2}$ solution differ significantly from those to the control sack.

Four fish whose bilaterally severed tracts were regenerating (using the test method already described) were also conditioned to respond positively to this normally very repellent odour of morpholine and a second similar group was similarly conditioned to another repellant odour, coumarin. The difference between the responses to the morpholine sack or coumarin sack on one hand, and their controls on the other, is significant (Table, B).

The higher response level of the olfactory-tract-regenerated carp to both worm and morpholine sacks (Table, A and B), compared to the response rate of normal fish, may most likely be due to impaired localization of the source of odour with consequent increase in the number of strikes at both positive and control sacks (Table, A and B). However, in spite of this possible impairment of function, recovery of some degree of sophistication in olfaction[7] is evident when fish with regenerated olfactory

[1] D. Hooker, in W. F. Windle (Ed.), *Regeneration in the Central Nervous System* (Charles C. Thomas, Springfield, Ill. 1955). – D. G. Attardi and R. W. Sperry, Exper. Neurol. *7*, 46 (1963).

[2] W. Kirsche and K. Kirsche, Z. mikr.-anat. Forschung *67*, 140 (1961).

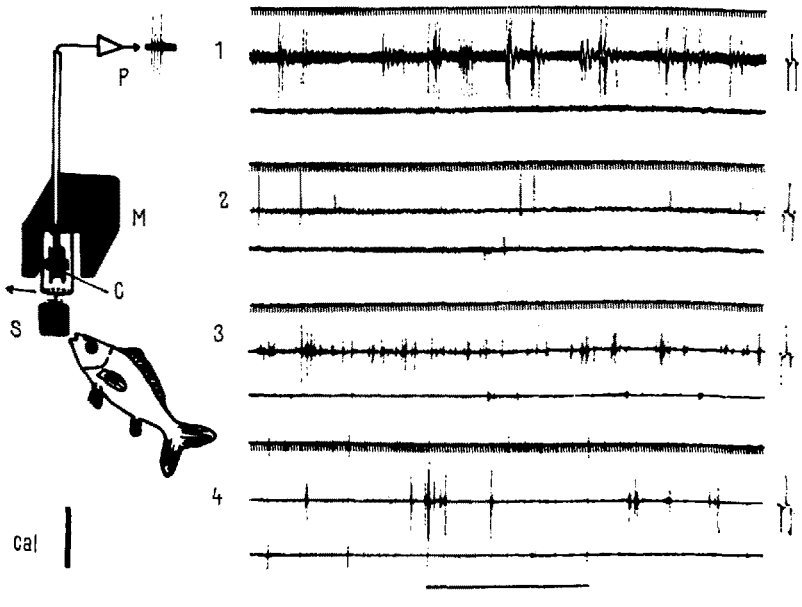
[3] J. Segaar, Acta morph. neerlando-scand. *5*, 49 (1962). – J. Segaar and R. Nieuwenhuys, Animal Behaviour *11*, 331 (1963).

[4] G. H. Parker, J. exp. Zool. *8*, 535 (1910). – G. H. Parker, *Smell, Taste, and Allied Senses in the Vertebrates* (Lippincott, Philadelphia 1922).

[5] The sensitivity of the recording method enabled measurement of the duration in sec and the exact number of sack-touches by the fish. Each so-called 'strike-group' is a group of closely-spaced touches separated by not more than an arbitrary 6 sec, and probably corresponds to what Parker[4] describes as the seizing of the sack.

[6] A. D. Hasler and W. J. Wisby, Trans. Am. Fisheries Soc. *79*, 64 (1950). – A. D. Hasler, J. Fish. Res. Bd. Canada *11*, (2) 107 (1954).

[7] F. K. Sanders, J. exp. Biol. *17*, 416 (1940).



As illustrated in the diagram at the left, fish touching and striking at the gauze sack (S) produce small potentials across the coil (C) when this moves through the magnetic field of the magnet (M). Potentials so produced are amplified and recorded by a penwriter (P).

Three-minute sections of typical records 1–4, shown at the right, each registers: above – a time mark (sec), centre – a sack containing worms (1–3) or morpholine (4), and lower – a sack containing stones.

Record 1 is that from four normal fish together in an aquarium. Record 2 from four fish whose olfactory tracts, severed bilaterally 47 days previously, are regenerating and who are just regaining their olfactory sense. Record 3, from the same four fish as record 2, was obtained 14 days later after further recovery of olfaction. The same group of four fish after full olfactory recovery were conditioned to respond positively to the smell of morpholine, and record 4 shows (centre) their responses to an empty sack soaked in 10^{-2} morpholine solution. Calibration 100 μV for 1 and 500 μV for 2, 3 and 4.

Group	Sack contents	Strike groups per fish per h	Touches per strike group	Duration (sec)
(i)	(ii)	(iii)	(iv)	(v)
A				
(20) Normal fish	worms	9.4[b]	5.3[b]	3.7[b]
n = 10	stones	3.0	2.5	1.6
(10) Both olfactory tracts regenerated	worms	13.0[b]	5.8	4.3
n = 20	stones	4.2	2.5	1.5
B				
(8) Normal fish	morpholine, $1 \cdot 10^{-2}$	4.5[b]	3.8[a]	2.3[a]
n = 10	stones	1.5	1.5	0.8
(4) Both olfactory tracts regenerated	morpholine, $1 \cdot 10^{-2}$	11.8[a]	4.6[b]	2.8[a]
n = 6	stones	3.6	2.1	1.2
(4) Both olfactory tracts regenerated	coumarin	5.4[a]	2.2	1.5
n = 7	stones	0.6	1.8	1.2

[a] $P < 0.05$. [b] $P < 0.01$.

In column (i) the number in parenthesis refers to the number of fish tested for each group; 'n' is the number of separate 1-h-long observations (on different days) from which were obtained the mean figures in columns (iii)–(v) for each group.

tracts learn and display clear positive responses to morpholine and to coumarin in an unambiguous test situation.

Histological confirmation of tract regeneration and central reconnections was obtained.

Further olfactory discrimination, histological and electrophysiological investigations are in progress.

Zusammenfassung. Wie mit einer neuentwickelten Methode gezeigt werden konnte, erlangen Karpfen 40 Tage nach Durchschneidung der Olfaktoriusbahnen wieder weitgehende Riechfähigkeit. Auch histologisch tritt Regeneration ein.

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Alteration of Resistant *Staphylococcus aureus* Cultures by Contact with Yeast Products

From the residue of the brewing process we extracted two products: yeast protein, Malucidin, having antibiotic properties *in vivo* and purified yeast antibiotic having antibiotic properties *in vivo* and *in vitro*[1–9]. Yeast antibiotic in 30 min contact with resistant and non-resistant staphylococcus killed about 95% of bacteria in doses of 0.75 mg and 0.035 mg/ml, respectively. Higher doses of yeast antibiotic, 1–1.5 mg/ml producing up to 98% mortality of resistant staphylococcus, have an alternative effect on this organism. Cultures grown from surviving cells were sensitive to penicillin and streptomycin and produced no coagulase or hemolysin. The effect of this treatment is highly increased sensitivity to standard antibiotics; original cultures of resistant staphylococcus which survived contact with 10 μg/ml of streptomycin after the treatment with yeast antibiotics were sterilized by 0.019 μg of streptomycin.

COHEN[10] tested our cultures of resistant staphylococcus for penicillinase after we had treated them with the yeast antibiotic and found no detectable penicillinase activity.

A solution of Malucidin, in combination with soap and merthiolate, produced a similar alteration in resistant staphylococci. They became sensitive to penicillin, streptomycin, tetracyclin and chloromycetin, produced no hemolysin or coagulase, and became non-typable for the phage pattern. Acquired new characteristics were inheritable and after eight transfers the new variant remained susceptible to standard antibiotics, and produced

Table I. Bactericidal effect of yeast antibiotic No. 260 on resistant and non-resistant staphylococci

Dose of yeast antibiotic mg/ml	Resistant strain SA		Non-resistant strain C-1	
	No. of colonies	% mortality	No. of colonies	% mortality
2	0	100	0	100
1.5	1	99.9	0	100
1.2	3	99.6	0	100
1	3	99.6	0	100
0.75	2	97.6	0	100
0.6	77	91.2	0	100
0.4	750	84	0	100
0.35	750	84	12	99.6
0.035	1450	63.2	122	96