

Coat 'Color' Difference Between Castrated and Intact Male Mice

In a previously reported experiment concerned with genotype and sex drive¹, 12 of 24 male mice of the hybrid strain B6D2F₁ were castrated at 16 weeks of age. The animals were housed in groups of 6 so that each cage contained both castrated and intact males. Daily tests for sexual behavior were continued until a criterion for loss of the ejaculatory reflex was met by all castrates. A total of 11 weeks was required to meet this criterion.

About 4 weeks after castration, we discovered that it was possible to select from each cage the castrated, or the non-castrated males, on the basis of their 'coat color' alone. Animals of this strain are black, but there was a qualitative difference in coat blackness between castrates and non-castrates. Comparisons with animals of various ages in the laboratory indicated that the non-castrates had undergone a normal moult which changed their coats from a glossy black to a rather rusty black. The castrates, on the other hand, had maintained a more 'juvenile' coat condition. Crude microscopic examinations indicated a lower pigment content in hairs of the intact males.

10 naive observers were asked to look at the mice in each cage and to divide them into 2 groups on the basis of coat color. In every case, the observers placed all castrates in 1 group and all intact males in the other.

After all males had met the criterion for decline of sex drive, injections of 1 mg testosterone propionate² were given for 21 consecutive days. These injections failed to

change the coat condition of the castrates, although sex drive returned in all animals.

A search of the relevant literature has failed to reveal previous reports of such a major effect of castration on coat color. Since we do not plan to pursue the matter further, we decided to present our observations in this short note with the hope that they might be of interest to those concerned with hormone-integument interactions³.

Zusammenfassung. Es wird festgestellt, dass kastrierte männliche Mäuse der Kreuzung B6D2F₁ sich von nicht-kastrierten männlichen Mäusen gleichen Alters durch einen qualitativen Unterschied der Fellfärbung unterscheiden.

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¹ T. E. MCGILL and G. R. TUCKER, *Science*, **145**, 514 (1964).

² The hormone preparations were generously supplied by Dr. R. R. McCORMICK, Schering Corporation, Bloomfield (New Jersey).

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A New Method for Recording the Swimming Activity in Flatfishes

Several methods for recording the diurnal activity of fishes have been developed in recent years, some more or less adapted to a single species. For a review of the main principles described in the literature, see the Table.

For our study of the diurnal activity of flatfishes under nearly natural conditions, the use of a large tank (8.5 · 3.5 · 1.5 m) seemed essential. First, several recording methods were tested without satisfactory results. The mechanical method used previously for plaice⁴ failed in a large basin with turbots. The wires easily became entangled and the leaden balls were bitten off. The main objection, however, was that the levels at which the fishes passed could not be clearly separated.

Next, a thermoconductive method was employed¹³. The difficulty with this method was that large scale eddies caused by the circulation system and by the movements of fishes of the size used were persistent in large tanks and were recorded together with the real displacements of the fishes.

In the wake of the fish there are a sufficient number of smaller eddies. These are less permanent²². Small eddies can be selectively measured with a rapid thermic system. The thermic resistance of the glass envelope of the thermistor, however, proved to be too large for our purpose. In seawater, an unprotected thermistor, on the other hand, deteriorated in the course of a few weeks and the initially successful method became unreliable.

Finally, the following method was worked out and yielded reliable results. This inductive method reported here uses the induction voltage which is generated in a fixed, oblong, rectangular coil, when a flatfish provided

with a permanent magnet crosses the coil (Figure 1). The magnet is a plastic covered ferrite (size: 15 · 9 · 5 mm; mass: 5.5 g), attached by means of a fishtag according to PETERSEN.

For the recording of the activity on and along the bottom, one coil was buried in the sand, while for the surface activity a second coil was placed in the same position just above the surface. The length of the coil was determined by the width of the passage to be controlled.

The actograph was to operate when the magnetic flux ϕ through the coil reached a certain threshold value. This was achieved by the integration of the voltage induced over the coil ($d\phi/dt$). The range of the actograph will then become, in principle, independent of the swimming speed of the passing fish. Only with very small swimming velocities is the range impaired owing to the bad ratio between the induction tension and the tensions as a result of the magnetic perturbations in the environment. As they have no swimbladder, flatfish cannot, however, reduce their swimming speed unlimitedly without sinking.

The range of the actograph is determined by the dipole moment²³ of the magnet M ($= 2.2 \cdot 10^{-7}$ Vsm), the number of windings n (2 · 1250; 0.22 mm Cu), the dimensions of the coil (1 · 0.1 m) and the adjustment of the range (Figure 2a). The range applies to a fish swimming horizontally. A fish swimming obliquely upward will cause a smaller difference in flux through the coil, resulting in a slight decrease of the range. For the case drawn in Figure 1, the magnetic flux ϕ is about equal to:

$$\phi \approx \frac{n M a}{\pi z^2} \left\{ \frac{2l}{(z^2 + l^2)^{3/2}} - \frac{l^3}{(z^2 + l^2)^{5/2}} \right\} \frac{1}{1 - (a^2 - y_M^2)/z^2} \quad (1)$$

provided that: $a \ll z$ and $y_M \ll z$.

Methods used for activity recording in fishes. (R), Round fish;
(F), Flat fish

Principle	References
Physical methods	
Mechanical: Fish is connected to recorder by a wire (R)	1
Displacements of unstable constructions (wire, pendulum, or frame) as a result of swimming cause the closing of electrical contacts (R), (F).	2-8
Disturbance of the equilibrium of a balance carrying a false bottom is caused whenever a flat fish rises.	9,10
Optical: Fish interrupts an IR-lightbeam.	11,12
Thermoconductive: Swimming motions disturb the convection pattern around an NTC resistance (thermistor) and cause resistance variation owing to the changed cooling (Crustaceans).	13
Electrical: The electrical resistance between a pair of electrodes changes when a fish passes (freshwater, R).	14-17
The discharge rate of special species of electrical fish may be used for the activity recording, (R).	18
Acoustic: Whenever a fish moves in a beam of ultrasonic sound from or towards the transmitter, the reflected sound shows a Doppler shift in frequency.	19
Chemical methods	
Analysis of differences in the consumption of oxygen during 24 h	20,21

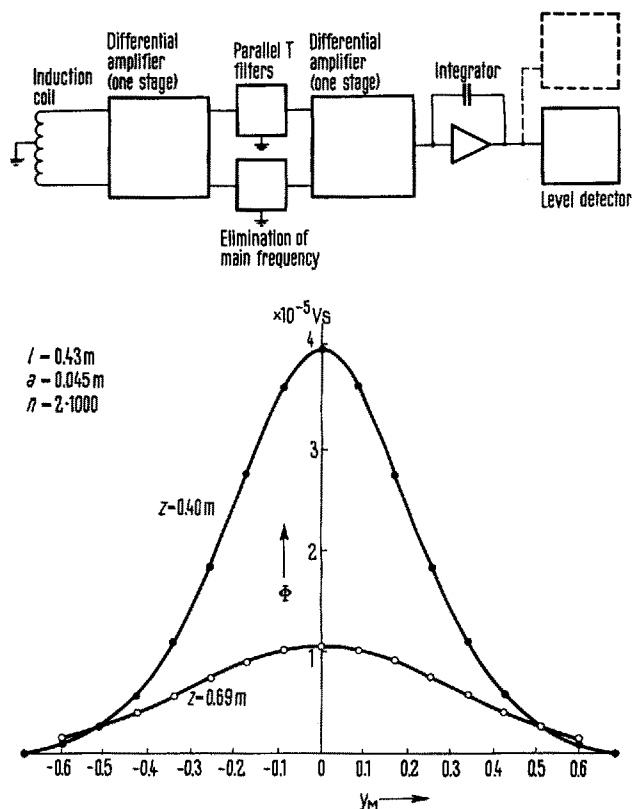


Fig. 2a. Diagram of the actograph. With a potentiometer the output signal of the integrator can be attenuated whereby the range can be diminished, if necessary. The difference in range between several units, if any, can thus be compensated.

Fig. 2b. The measured instantaneous flux Φ through a coil as a function of the position of the magnet ($0, y_M, z$). The values of Φ for $y_M = 0$ are in agreement with those calculated according to formula (1).

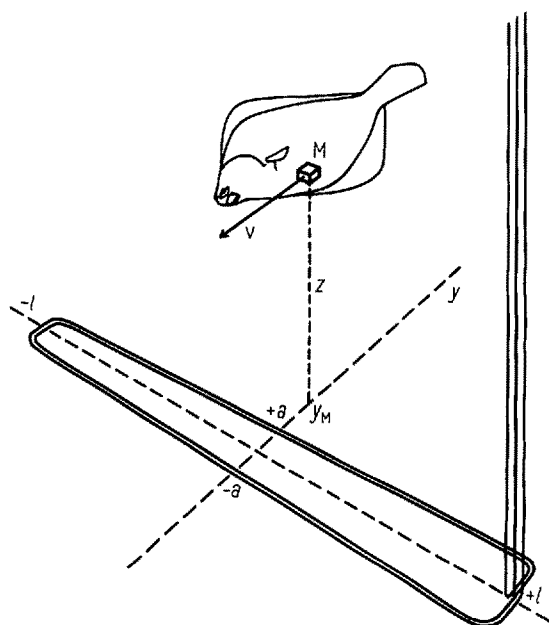


Fig. 1. The magnetization is perpendicular to the greatest surface of the magnet, i.e. in the dorso-ventral direction. All magnets are to be orientated with the same pole to the back of the fish.

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In the derivation the undulating movement of the flatfish is left out of account. For $z = 0.4$ m and $z = 0.69$ m the measured progress of the flux with time is given in Figure 2b.

Neither the plaice nor the turbot seemed to be hampered by the weight of the magnet. The mass of the magnet may be diminished, however, at the cost of the range of the actograph. For instance, if the mass is reduced to one-fifth of the value mentioned above, the range becomes according to (1) $5^{-1/2} 2^{1/4} \approx 0.5$ times the original value of about 0.5 m ($= l$), as M is roughly proportional to the volume.

For the bottom unit a large range as well as a small range may be desirable in the case of flatfishes, to be able to distinguish between swimming and creeping along the bottom. With 1 coil as detector, both movements may be detected simultaneously (second parallel channel dotted in Figure 2a).

Résumé. Une nouvelle méthode pour enregistrer le rythme nycthémeral des poissons plats (Pleuronectiformes) est décrite. Elle fait usage du voltage de l'induction produite dans une bobine, quand un poisson plat muni d'un aimant permanent passe dans son voisinage. Le dispositif fonctionne indépendamment de la vitesse de natation.

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La chronologie d'invagination chez le Poulet: Etude à l'aide de la thymidine tritiée

La thymidine tritiée est couramment employée dans les problèmes qui impliquent des migrations de cellules. En effet, ce radio-isotope s'incorpore spécifiquement dans l'ADN et ne passe que dans les cellules-filles qui dérivent de cellules initialement marquées. Le principe consiste à échanger une région donnée d'un embryon non marqué par une région similaire prélevée sur un germe marqué. Après une période de développement déterminée, on fixe l'embryon et on étudie la répartition des cellules marquées sur les autoradiogrammes.

Dans un premier travail (NICOLET¹), nous avons appliqué cette technique, en nous limitant à l'étude de la destination des cellules contenues dans le nœud de Hensen de la ligne primitive achevée. Comme les résultats ainsi obtenus étaient beaucoup plus précis que ceux qui ont servi de base à l'établissement des cartes de localisation des ébauches présomptives déjà publiées chez le Poulet, nous avons décidé de l'utiliser systématiquement pour soumettre le problème de la gastrulation chez les Oiseaux à une réanalyse globale.

Technique. Toutes les opérations sont effectuées sur des blastodermes de White-Leghorn cultivés in vitro selon une variante de la technique de NEW. L'échange d'un fragment de la ligne primitive ne présente, du point de vue méthodique, que des inconvénients mineurs dans la mesure où les fragments excisés n'excèdent pas certaines dimensions. Notons toutefois que la capacité de cicatrisation est d'autant plus grande que l'embryon est plus jeune. En pratique, les fragments excisés mesurent 0,4 sur 0,4 mm, mais seulement 0,2 sur 0,2 mm, si le blastoderme est opéré après le stade de la ligne primitive achevée.

Pour établir avec précision quelle est la chronologie d'invagination des différentes ébauches et à quel niveau de la ligne primitive s'invagine le matériel qui leur est destiné, nous aurons à échanger diverses parties de la ligne primitive de son apparition à sa disparition. Pour l'instant, nous avons pratiqué l'échange dans les parties suivantes, conformément au schéma de la figure: échange des parties antérieures et postérieures de la ligne primitive aux stades 2, 3 et 3⁺ selon les tables de HAMILTON et

HAMBURGER², échange de quatre régions de la ligne primitive au stade 4 (fragments A, B, C et D), enfin échange de la région post-nodale au stade 5.

En général, les blastodermes opérés sont incubés jusqu'à ce qu'ils forment un corps embryonnaire comprenant 6-7 paires de somites, mais 10-15 paires de somites, s'il s'agit d'embryons opérés au stade 5.

Résultats expérimentaux. Si la partie antérieure de la ligne primitive est échangée aux stades 2, 3 et 3⁺, nous retrouvons les cellules marquées en grande majorité dans l'endoblaste de l'aire transparente et en moindre quantité dans l'intestin céphalique. Une minorité de cellules participe, semble-t-il indifféremment, à la constitution de toutes les ébauches chordo-mésoblastiques, à l'exception du mésoblaste extra-embryonnaire. La partie postérieure de la ligne primitive aux stades précités ne fournit au contraire que du mésoblaste extra-embryonnaire.

En pratiquant l'échange du greffon A au stade 4, nous retrouvons du marquage dans toutes les cellules de la chorde, dans une grande partie des cellules de l'intestin céphalique et assez souvent dans les cellules endoblastiques réparties sur toute la longueur de l'axe embryonnaire. Certaines cellules marquées ne s'invaginent pas. Elles s'incorporent au plancher du tube neural ou restent

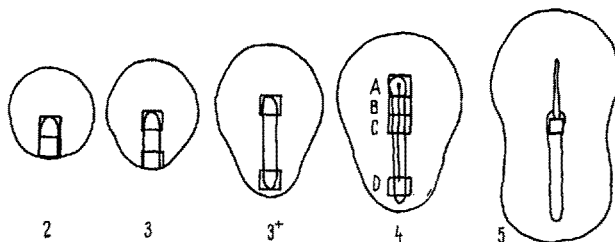


Schéma des diverses régions de la ligne primitive soumises à l'échange aux stades successifs de son évolution.

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