lum" patterns, this transformation takes place within relatively late phases of leaf development. If there rises only one second epidermis, it will contain typical stomata and typical mother cells of hairs. In the interior of the leaf, however, these hairs do not develop further. In some cases this second epidermis is situated immediately below the normal one; in other cases it lines the much enlarged air chambers (Fig. 3*b*). In the first instance, the stomata of neither epidermis function. In the second case, the guard cells of the inner epidermis are lifted up under the influence of the high air moisture within the leaf. In most patterns with a double epidermis, one of the chlorenchyma layers is missing.

In some cases most layers of the spongy parenchyma have lost their normal chloroplasts and have changed into an epidermal tissue with intercellular spaces. Stomata were formed at the boundary between this and the normally green parenchyma amid the leaf (Fig. 3*c*). Other similar transformations of palisade parenchyma cannot be identified quite exactly as epidermal tissue because stomata are missing in them.

In the third type of "rhytidiophyllum" the following fact is of greatest interest: a plasmonic alteration, which can be proved as such, does not produce disturbances of normal development as in other cases of cytoplasmic inheritance, but a change of determination takes place. A meristematic tissue that should form chlorenchymatic cells develops instead into epidermal cells. This determination process differs from the normal formation of dermatogen only by the atypical moment of realization and by its abnormal localization. This difference, however, is caused by the time and locality of cytoplasmic segregation. If we start from the well-founded opinion that plasmonic segregation is produced by an unequal distribution of plasmagenes, we then logically arrive at the further conclusion that similar proceedings take place during the determination of the typical dermatogen as well, and that plasmagenes are distributed irregularly during the first cell divisions of the embryo (*4*), in which root and shoot and later on the layers of differentiated tissues are preformed.

Of course, cytoplasmic inheritance, as taking part in the processes of determination, is very difficult to prove by crossing experiments, and in many cases this task is impossible to solve at all. The aforementioned observations, however, show that the hypothesis of the significance of plasmagenes for determination possesses a high degree of probability. This hypothesis can be proved exactly by a more detailed investigation of the behavior of cytoplasm and its inheritance during ontogeny. One then will have to take into account that an analysis of intraindividual patterns is of the same importance for cytoplasmic inheritance as is an analysis of segregating crossings for chromosomal inheritance. Moreover, one should not forget that the fundamental principle of heredity means the identical reproduction and passing on of all genes during vegetative as well as during generative reproduction.

P. MICHAELIS
F. BARTELS

Max-Planck-Institut für Züchtungsforschung, Köln-Vogelsang, Germany

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Role of Fumarate in Formation of Stromata in "Vernalized" Ergot Fungus

It is well known that, for germination of *Claviceps purpurea,* an exposure of several weeks to cold, followed by a short period of exposure at higher temperature, is necessary. According to Kirchhoff (*1*), who studied this question in detail, the effect of cold seems to be similar to that of the vernalization of seeds. In studies of the respiration of germinating sclerotia and fully developed stromata of ergot of rye, I have found that fumarate plays a special role in this process.

The test ergot strain was Hungarian 12. An exposure to 0° to 3°C during 6 weeks was effective, causing 65 to 70 percent of the sclerotia to germinate after 3 weeks at 20°C on a double layer of wet filter paper in petri dishes. The sclerotia that had been treated as described were vacuum infiltrated for 1 hour with distilled water (control) or with $2 \times 10^{-2}M$ fumarate under 20 to 30 mm-Hg pressure. Infiltration with water does not influence the development of stromata, while infiltration with fumarate entirely inhibits the germination. This inhibition can be overcome to some extent by infiltration with $2 \times 10^{-2}M$ succinate. Succinate by itself has no effect on germination.

In order to gain a deeper insight into this question, conventional Warburg respirometers were used for the estimation of oxygen absorption (Q_{O_2}) of the sclerotia and stromata that had been infiltrated with the various compounds described in Table 1. Measurements were carried out on four occasions in triplicate. As shown in Table 1, the fumarate does not inhibit the respiration of sclerotia, while the oxygen consumption of stromata was strongly affected by it. Succinate added in concentrations equal to those of the fumarate is able to renew oxygen uptake to a considerable degree.

Table 1. Effect of fumarate and succinate on the respiration of sclerotia and stromata of ergot fungus.

Infiltration	Q_{O_2}		
	Sclerotia		Stromata
	Control	Cold treated	
Distilled water	25	27	346
Fumarate ($2 \times 10^{-2}M$)	22	25	20
Succinate ($2 \times 10^{-2}M$)	24	26	387
Fumarate + succinate	25	26	276

A consideration of these results has led to the following tentative conclusions and working hypothesis. The respiration of stromata follows a different pathway from that of the sclerotia. An explanation could be given for the inhibitory effect of fumarate on germination by the fact that, in the presence of fumarate, the respiration of stromata is inhibited. On the basis of the compensatory effect of succinate, it may be assumed that an unknown acid metabolism plays an important role in the organization of the stromata of ergot fungus. This is in agreement with the work of Cantino (*2*), who studied the relationship between cellular metabolism and morphogenesis in *Blastocladiella emersonii.*

A. ST. GARAY*

Research Institute for Medicinal Plants, Budapest, Hungary

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* Present address: Institute of Plant Breeding, Fertöd, Hungary.

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Conditioned Inhibition of Respiration and Heart Rate in the Goldfish

Conditioned inhibition of breathing rate and heart rate has been reported for various mammalian species, including man (*1*). Typically, the termination of a light, sound, or some other conditioned stimulus (CS) is repeatedly associated with noxious electric stimulation of some

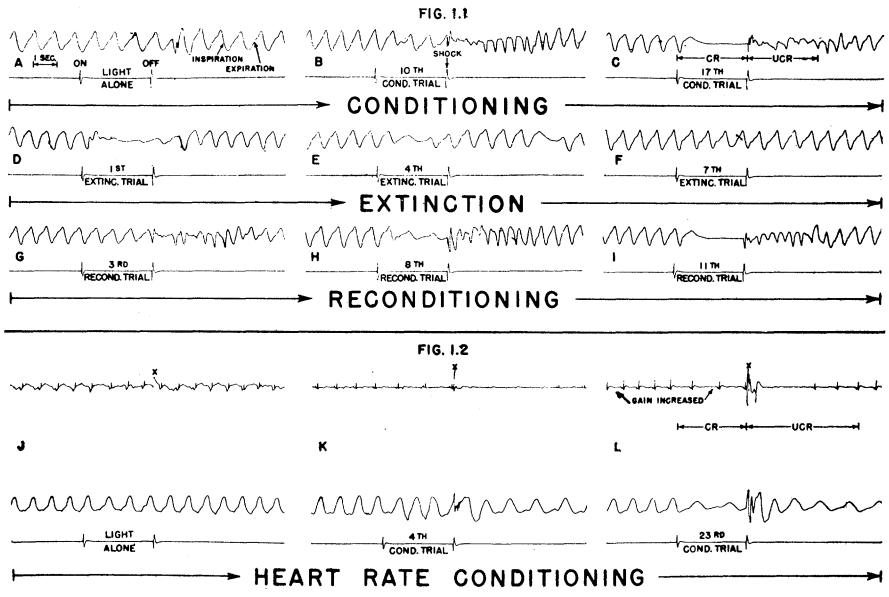

Fig. 1.1. Effect on breathing rate of light alone during preconditioning trials (A); of light paired with shock during conditioning trials (B, C); of light alone during extinction trials (D–F); and of light paired with shock during reconditioning trials (G–I). CR denotes conditioned response to light onset, and UCR denotes unconditioned response to shock. Fig. 1.2 (another fish). Effect on breathing and heart rate of light alone (J) and of light paired with shock (K, L). *X* denotes recording artifacts. Note that both frequency and amplitude of the EKG diminish during presentation of the CS (L). The EKG was recorded with a small time constant of amplification in order to avoid drift.

portion of the organism's anatomy. After sufficient pairings of the CS and shock, the CS acquires the power to elicit a slowing of the heart rate or inhibited respiration, or both, as a conditioned response. The conditioned inhibition disappears (that is, is extinguished) with successive presentations of the CS in the absence of continued shock reinforcement.

Although this has been amply demonstrated in higher forms, little evidence of this sort of conditioning exists for poikilotherms (*2*). The purpose of this report is to demonstrate similar breathing and heart-rate conditioning effects in goldfish (*Carassius auratus*) and to suggest some applications of the conditioning method that we have employed.

In our experiments (*3*), a fish was immobilized in a special clamp (which left the head and operculi free) and was submerged in a small, water-filled tank that was painted flat black. Two sheets of aluminum foil, placed along opposite sides of the tank, served as electrodes. Shock was delivered through the water (and the fish) by discharging, through the electrodes, a 0.3- to 1.0-μf condenser, charged at 45 v (*4*). The shock occurred simultaneously with the termination of the CS, which consisted of a 3- to 7-sec duration change in illumination through the milk-glass bottom of the tank. Two 6-v lamps (one at each end of the tank) were illuminated during the CS interval. The sequence of light-followed-by-shock was automatically timed (Hunter interval timer).

Respiratory movements were mechanically transmitted (by means of a lever arm that rested against one operculum) to a thin sheet of flexible steel (1.5 in. by 3 in.) upon which four strain gages were mounted and wired to form a Wheatstone bridge. The lever arm was soldered to the free end of the flexible steel. Opercular movements were translated into proportional direct-current voltages across the bridge, which was supplied by a 3-v battery. Potential changes were amplified and recorded on one channel of a Grass standard inkwriter; the largest time constant available was used to prevent distortion of the tracings. In some of the experiments, leads from thin steel needles introduced in the region of the heart were coupled to another channel of the inkwriter for recording the EKG. A signal marker recorded onset and termination of the CS. Each subject was permitted from 10 to 30 minutes' adaptation in the tank (that is, until breathing rate had become regular) before each experiment was started.

Figure 1 shows typical results. Figure 1.1 presents recordings from a fish in which respiratory inhibition was first conditioned, then extinguished (by nonreinforced presentation of the CS), and then reconditioned. Figure 1.2 shows the record of another fish in which inhibition of heart rate, as well as respiration, was conditioned.

It has been our experience that goldfish will show the conditioning effects illustrated in Fig. 1 within 15 to 40 trials, spaced from 1 to 2 minutes apart. Of the 30 fish conditioned, 16 showed marked inhibition of breathing within 20 trials, five within 30 trials, three within 40 trials, and six failed to condition reliably within 100 trials (*5*).

Respiratory inhibition does not appear to be a matter of pseudoconditioning or sensitization. Breathing fails to be inhibited by noise or by tactual stimulation after conditioning, and animals fail to show inhibition of respiration at CS onset after backward conditioning (that is, shock presented first, followed by the CS). Also, the reconditioning record of Fig. 1.1 indicates that extinction was not caused by fatigue, ataxia, and the like.

The technique which we have decribed is a convenient, quantitative method for studying the conditioning and retention of breathing and heart-rate inhibition in the goldfish—a readily accessible, inexpensive, and easily maintained species. Preliminary studies suggest that the method may have general application as a screening device for testing the effects of drugs on unconditioned breathing rate and heart rate and on learned emotional responses that involve these measures (*6*). The method, of course, is also suitable for behavioral studies involving the acquisition, retention, and extinction of learned responses in fish.

LEON S. OTIS*
JEAN A. CERF†
GARTH J. THOMAS
Neuropsychiatric Institute, University of Illinois, College of Medicine, Chicago

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4. A 20- to 25-v square pulse of 0.2-msec duration, delivered at a frequency of 400/sec and on for 0.25–0.50 sec. has been alternately used (Grass stimulator S-4). This stimulus is recommended for chronic preparations.
5. Occasionally fish are found which have a breathing rate so irregular that they are not fit subjects for this sort of conditioning. Also, some fish appear to react to shock stimulation with less disturbance of respiration than others. [see V. I. Gusel'nikov, *Fiziol. Zhur. S.S.S.R.* 38, No. 5, 612 (1952)].
6. Emotional responses are operationally defined here as changes in response measures which attend or follow the onset of a neutral stimulus previously associated with *noxious* stimulation.

* Present address: Department of Psychology, State University of Iowa, Iowa City.
† Chargé de recherches of the Belgian National Foundation for Scientific Research. Permanent address: Laboratoire de Pathologie générale, Faculté de Médecine, Brussels University, Belgium.

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