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20 March 1959

Differentiation of Activity of Three Mouse Strains with Magnetic Pickup Apparatus

Abstract. An activity pickup, when used with a high-gain amplifier, permits records to be made of essentially all the activity of a mouse. The sensitivity is such that the differences in the activity patterns of strains of mice can be determined.

When the activity of small animals is appraised, the animal is usually required to cause movement of a relatively large mass, most commonly a pan or an entire cage, which is then connected to a recording device of some sort (*1*). Problems frequently encountered in such apparatus are incomplete recording of activity, artifacts due to resonance, and feedback to the animal due to pan or cage inertia and noise in the recording apparatus.

The apparatus described in this report (*2*) apparently circumvents all these difficulties. The activity pickup employs the principle of movements of a small iron plate which induces electric current in a coil with a bar magnet core. The currents thus produced are amplified electronically and recorded as activity.

Figure 1 shows the apparatus schematically. Movements of the mouse or other small animal cause micromovements of the galvanized iron plate *A*. Magnetic lines of force flowing between the poles of the magnet *NS* are altered by the movement of the plate, and thus current proportional to the movement is induced in the windings; the current is fed (*C*) to a preamplifier, amplifier, and recorder. Specifications are as follows: *A*, galvanized iron plate, 22 gauge, 3 by 4 in.; *B*, latex tubing, ½ in., ¼ in. I.D., cemented to the plate by rubber-to-metal cement. *NS*, 3-in. bar magnet wound with one layer of magnet wire. The specifications are for a mouse apparatus; with slight changes the apparatus could be adapted to larger or smaller animals. A Grass EEG machine was used for amplification and recording.

The plate is doubly damped, by the latex tubing and the attraction of the magnet, and resonance is kept to a minimum without friction. Use of larger plates tended to cause resonance. Three such plates were used, the leads being connected in parallel. For multiple-plate recording, adjacent magnets should be arranged with like polarity (or repulsion) for maximum sensitivity. Clear plastic walls of dimensions that allow 1/16-in. clearance of the plates act as the cage and permit observation of the mouse. All exposed wire is covered with rubber tubing to protect it from urine. The plate is built up to allow ½-in. clearance between the magnet and the plate. The apparatus is mounted on firmly damped ¼-in. mesh screening material.

Sensitivity is such that virtually all activity can be recorded, from shaking and trembling to running and jumping. Artifacts appear to be minimal or absent. On the records obtained, activity resolves into three major components: (i) large discrete deflections, correlated with jumping, jerking, and sudden movements in general; (ii) moderate, continuous deflections, correlated with walking, shaking, trembling, and scratching; (iii) quiet periods.

Figure 2 shows representative records of three mouse strains. Ten male mice were tested in each strain, and the activity patterns illustrated are characteristic for the given strains. The top record is of *Peromyscus gracilis*, a wild mouse exhibiting much running and jumping behavior. The middle line shows activity of strain C57BL/6, an aggressive, fighting mouse, which is characterized by many sudden movements with a moderate amount of continuous activity. Strain A/JAX, a standard laboratory albino, shows less activity of all kinds than the other two strains.

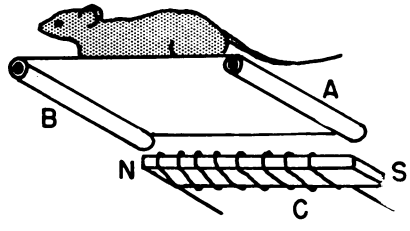

Fig. 1. Schematic representation of pickup apparatus.

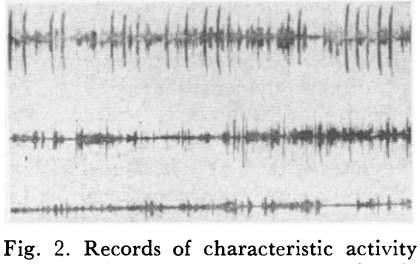

Fig. 2. Records of characteristic activity patterns of three different strains of male mice: (top) *Peromyscus gracilis*; (middle) C57BL/6; (bottom) A/JAX.

The sensitivity of the activity pickup apparatus described suggests possible application in psychopharmacological studies.

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2 April 1959

Tartaric Acid Metabolism of Neurospora crassa

Abstract. The growth of wild type *Neurospora crassa* is stimulated by various organic acids including tartaric, tartronic, and mesoxalic acids. Evidence is presented that this organism converts *d*- or *l*-tartaric acid to tartronic and mesoxalic acid, probably by fixation of CO_2.

The function of tartaric acid has been a biochemical enigma since Pasteur initiated research on this substance. Recently its metabolism by several organisms or tissues has been reported with varying results. Cleland and Johnson (*1*) find that it is converted quantitatively to oxalate by *Aspergillus niger*. Nomura and Sakaguchi (*2*) find that the principal product is succinate with *Pseudomonas incognita*, while Kun and Hernandez (*3*) propose that, with animal mitochondria, the first product is oxaloglycolic acid which can then be converted to a number of products, including diketosuccinic acid and glyoxylic acid. When, therefore, we found that *Neurospora crassa* is stimulated by tartrate as well as by several other organic acids, we made an effort to determine the mechanism of this stimulation and the fate of the tartrate.

Conidia were taken from cultures of *Neurospora crassa*, strain 74A (wild type), grown on minimal medium (*4*) with 2 percent (wt./vol.) sucrose and 1.5 percent (wt./vol.) agar. Aliquots were inoculated into 20-ml portions of Westergaard-Mitchell medium (*5*) containing 2 percent (wt./vol.) sucrose and supplements. Pads were harvested after 72 hours, dried, and weighed.

Among the organic acids which were

found stimulatory were tartaric, mesoxalic, and tartronic acids, and all the acids of the citric acid cycle that were tested. It was thought possible that tartrate might be decarboxylated to tartronate and the latter oxidized to mesoxalate, as has been observed in other organisms (*6*). Fresh mycelial pads, grown in 10 m*M* tartrate, were shaken in 0.1 *M* phosphate buffer of *p*H 5.0, alone or with 10 m*M* tartrate, tartronate, or mesoxalate, for up to 5 hours. The buffer was exhaustively extracted with ether, and the organic acids were chromatographed with three different solvents. When the pad was shaken with buffer alone, only citrate appeared in the extract. When it was shaken with *d*-tartrate, then citrate, tartrate, tartronate, and mesoxalate appeared. When it was exposed to tartronate; citrate, tartronate and mesoxalate appeared on the chromatograms, and when it was shaken with mesoxalate, only citrate and mesoxalate could be detected.

This hypothesis was also checked by using Stanier's theory of simultaneous adaptation (*7*). If the enzymes involved in the utilization or conversion of tartrate, tartronate, or mesoxalate are adaptive in character, then a culture adapted by previous exposure to one of these compounds should give a more vigorous response to subsequent addition of this organic acid than an organism not so preadapted. Furthermore, if the organism has, for instance, the capacity to convert tartrate to tartronate, then a previous exposure to tartrate should adapt it to tartronate. If it does not have this capacity, then previous exposure to tartrate would not be expected to increase the magnitude of its response to tartronate.

Strain 74A was grown for 1 day in the presence of 2 m*M* concentration of an organic acid. After this, further organic acid was added in 6 m*M* concentration, and the dry weight attained after 3 days was assayed. An adapted response was defined as the growth attained when the same acid was added as was initially present. If the growth attained was significantly below this value, that was considered an unadapted response. The results appear in Table 1.

Table 1. Adaptation of *Neurospora crassa* strain 74A to various organic acids. Conidia were inoculated into 20 ml of Westergaard-Mitchell medium containing 2 m*M* of the "initial" acids indicated below. After 24.5 hours, 0.2 ml of a *p*H-5.5 aseptic solution of other acid was added (or in the case of "None," 0.2 ml of sterile water). Mycelium was dried and weighed after 3 days' growth. Weights reported are averages of four replicates.

Acid added after 24.5 hr	Wt. of mycelium (mg)	Standard deviation
Initial acid: d-*Tartrate*		
None	33.1	0.2
Malate	41.6	0.3
d-Tartrate	47.5*	1.2
l-Tartrate	47.5*	0.0
meso-Tartrate	41.4	0.0
Tartronate	47.7*	0.4
Mesoxalate	48.2*	0.9
Initial acid: l-*Tartrate*		
None	34.5	0.6
Malate	41.6	1.0
d-Tartrate	50.1*	0.1
l-Tartrate	47.8*	1.7
meso-Tartrate	46.5*	1.0
Tartronate	48.7*	0.4
Mesoxalate	49.8*	1.0
Initial acid: meso-*Tartrate*		
None	33.6	0.0
Malate	44.3	0.9
d-Tartrate	49.6	1.6
l-Tartrate	46.7	3.1
meso-Tartrate	46.1	1.2
Tartronate	54.3	3.6
Mesoxalate	48.2	2.1
Initial acid: Tartronate		
None	38.1	0.5
Malate	42.4	0.8
d-Tartrate	40.4	0.5
l-Tartrate	42.2	0.8
meso-Tartrate	38.1	1.6
Tartronate	48.1*	0.2
Mesoxalate	47.2*	0.8
Initial acid: Mesoxalate		
None	36.0	1.2
Malate	41.0	0.9
d-Tartrate	41.5	0.1
l-Tartrate	41.1	0.1
meso-Tartrate	42.2	0.1
Tartronate	43.3	0.8
Mesoxalate	48.5*	1.9

* Weight significantly greater than that obtained by adding malate after 24.5 hr (adapted response).

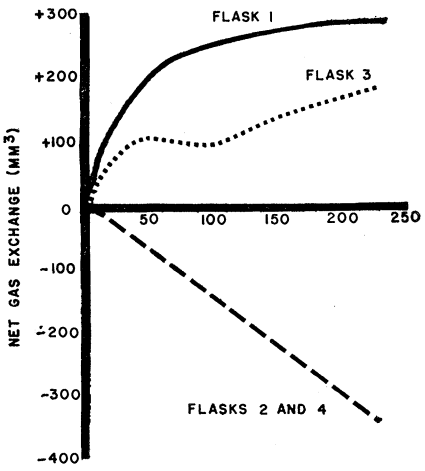

Fig. 1. Effect of *d*-tartaric acid on the respiration of *Neurospora crassa*. All flasks contained fresh mycelium and 0.1*M* phosphate buffer at *p*H 5.0. Flasks 2 and 4 contained 20 percent NaOH to absorb the CO_2 evolved. Flasks 3 and 4 contained 0.7 ml 2.8 m*M* *d*-tartrate (1.9 μmole). The results have been corrected to constant mycelial weight. The difference between the CO_2 evolved by flasks 3 and 4 is 3.8 μmole and was constant for the last 75 minutes of the experiment.

Fresh mycelium was shaken with tartrate and *p*H-5 buffer in a Warburg apparatus with the results shown in Fig. 1. The CO_2 output was reduced by 3.8 μmole, 2 mole/mole of tartrate, the same results being obtained with *d*- and *l*-tartrate, and with fresh mycelium and mycelium which had been soaked in acetone and dried. *meso*-Tartrate had no significant effect on CO_2 output or O_2 consumption. These results suggest that tartrate is converted to mesoxalate and tartronate by the following mechanism:

$$\text{HOOC—CHOH—CHOH—COOH} + 2CO_2 \rightarrow \text{HOOC—CO—COOH} + \text{HOOC—CHOH—COOH}$$

and that tartronate is oxidized to mesoxalate:

$$\text{HOOC—CHOH—COOH} + \tfrac{1}{2}(O_2) \rightarrow \text{HOOC—CO—COOH} + H_2O$$

An investigation of the possible mechanism of stimulation of growth in *Neurospora crassa* by tartronate and mesoxalate was also made (*9*).

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9. A description of the results of this investigation is in preparation.

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3 March 1959