the "vulcanized" cross-linked wall fabric into a form capable of plastic deformation.

As is revealed by the electron microscope, polymer formation in the direction of growth develops as a densely intermeshed fibrillar network appropriate to an expanding prolate spheroid. It is important to note that covalent bonding between fibrils of the wall components that are not linearly ordered very likely serves to increase the modulus of elasticity in proportion to the number of covalent bonds (*8*). This feature of the wall fabric is undoubtedly essential for the expanding spheroid. The "ring type" of ordered fibrillar structure observed in "bud scars" serves to constrict the base of the bud in a plane normal to the direction of growth of the bud. This arrangement of fibrils is beautifully illustrated in electron microscope photographs of the yeast glucan layer by Houwink and Kreger (*9*). It is in the ordering of these fibrils that covalent bonding is believed to interfere. This may be a second stage in the division process during which maintenance of a sulfhydryl condition in a structural polymer is temporarily essential. Covalent (—S—S—) bonds might be formed, however, after the ordered arrangement has been achieved.

The molecular events in cellular division that have been uncovered thus far bring into fold many scattered, apparently unrelated, observations of the environmental control of cellular division. It is now intelligible, for example, that sulfhydryl substances applied externally to growing, but nondividing, cells might induce division in such cells (*10*). It is becoming increasingly clear that a cell possesses a variety of systems, each with its degree of specificity, for maintaining functional —SH groups. Those employed in growth may be operative while a disulfide reductase essential for division may have failed. In fact, this exact situation is met in the filamentous strain of *C. albicans,* which possesses both an active glutathione reductase and cystine reductase (*11*) but is deficient in a protein disulfide reductase.

WALTER J. NICKERSON
G. FALCONE*

Institute of Microbiology, Rutgers University, State University of New Jersey, New Brunswick

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* Waksman-Farmitalia postdoctoral fellow; present address: Institute of General Pathology, University of Naples, Naples, Italy.

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"Permanent" Alteration of Behavior in Mice by Chemical and Psychological Means

We wish to report a behavioral response in mice which may be induced by both chemical and psychological means and which responds in some measure to drugs useful in mental illness. We had previously demonstrated (*1*) that fish exposed to LSD (lysergic acid diethylamide), upon return to a normal environment, exhibited an unusual and characteristic behavior pattern, and that this pattern could be rendered "permanent" by appropriate chemical treatment of the fish. In an attempt to extend this type of study to mice, we first searched for alterations in behavior that might be consistently attributed to treatment with LSD. We found that the behavior pattern described by Woolley (*2*) could be confirmed and that it was reasonably reproducible, but in the meantime Lars Flataker had pointed out to us a behavior response that was easier to determine.

The response consists of a rapid and violent head shaking when any area about the back of the head is touched very lightly with a small stick or pencil point. The head-twitch response does not occur in normal mice, and with a little experience the response is easy to detect. It is only rarely that one is uncertain whether a particular animal possesses the head twitch or not, and usually, if such an animal is followed, a clear-cut answer is found on the subsequent days. Independent observations by different workers are remarkably consistent, so that this provided a suitable tool for the behavioral studies.

If mice are injected intravenously with from 5 to 100 μg LSD (0.25 to 5 mg/kg), this characteristic head shake response appears in from 5 to 10 minutes and lasts for intervals of from 10 minutes to 2 hours, the length of time it persists being roughly proportional to the amount of LSD used. A variety of other substances (for example, mescaline, yohimbine, serotonin) do not elicit this response. The response is consistently reproducible in 90 to 100 percent of the animals injected and seems to occur in several strains of mice tested.

In an effort to render the head-twitch response "permanent," mice were injected intravenously with 30 μg indole (1.5 mg/kg), followed almost immediately by 30 μg LSD. All such mice showed the head-twitch response, but, in the majority of the cases, it subsided in 1 to 2 hours, and the mice were subsequently normal. However, in from 5 to 30 percent of the mice so treated (housed in groups of 10 each) the response remained for periods as long as a week, and in some cases for a few months. We have apparently therefore produced a "permanent" alteration in a particular aspect of mouse behavior by chemical treatment, in analogy to the alterations in fish (*1*), but such "chemical imprinting" is apparently more difficult in the mouse. Injection of either indole alone or saline produced no response, while injection of LSD alone produced only the usual temporary response.

It was also found that the identical response was produced in a larger percentage of mice by solitary confinement. The mice were placed in separate cages (one mouse to a cage) in which they had access to light, sound, and so forth, but were unable to see any other mice. Under these circumstances roughly 30 percent of the mice developed the head-shake response in 2 days (this percentage varied from 30 to 80 percent, depending on the strain of mouse employed). If such mice were kept in solitary confinement for 3 weeks (2 weeks is not adequate) and were then returned to groups (8 to 10 in a group), generally about 80 percent retained the response for weeks and even months. One has, under these circumstances, apparently rendered the response permanently "implanted." If such mice, in which the head-shake response has been permanently established, are treated with reserpine (5 mg/kg – p.o. for 3 days) about half become symptom free in 2 to 3 days and remain in this state as long as reserpine is supplied. When the reserpine is withdrawn, the head-shake response gradually reappears, even though the mice are kept in groups.

About another third of the mice do not develop the symptom until they have been kept in solitary confinement for at least a week. When these are grouped after 3 weeks of solitary confinement, the response is retained in most of the animals but is slowly lost with time. If such animals are given reserpine, most of them promptly lose the

head-shake response, and it does not return when the reserpine is withdrawn.

A third group does not develop the symptom on solitary confinement. This group generally consists of 30 to 40 percent of the population of most mouse strains that we have tried, but in some cases it is much less.

It appears from the foregoing, that a head-twitch response may be produced in mice by solitary confinement. This response is in all known respects identical to that produced by LSD injected intravenously. The response can be made "permanent" either by treating the mice with indole followed by LSD or by long exposure (3 weeks) to solitary confinement. When it is produced in a "permanent" fashion, the response may be temporarily relieved or may be cured by treatment with reserpine.

DORIS L. KELLER
WAYNE W. UMBREIT
Merck Institute for Therapeutic Research, Rahway, New Jersey

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Distinction between Effects on Metabolic Transport and Passive Transfer of Ions

Studies showing that drugs, ions, metabolic inhibitors, and substrates induce or alter the net movement of sodium and potassium in nerve fibers have led to the recognition that such effects are brought about either by altering the metabolic reactions responsible for "active transport" or by modifying membrane permeability or the other electrochemical conditions for ion exchange and diffusion involved in "passive transfer" (*1–3*). A reliable method for distinguishing between effects on metabolism and on the passive properties of biological systems is increasingly necessary in the light of growing evidence for an intimate relationship between ionic movement and alterations in the physiological functioning of many cells (for example, *2, 4*).

It was previously found that while the net movements of ions are suggestive, they do not suffice as a definitive basis for such discrimination (*3*). On the other hand, research currently in progress with M. D. Berman on the unidirectional fluxes of ions in metabolically inhibited and uninhibited sciatic nerves of the toad (*5*) appears to provide one satisfactory approach to this problem. The purpose of this preliminary report is to provide two examples of the technique that demonstrate different types of results and to call attention to erroneous conclusions that may be drawn if metabolic and physical effects are not both considered as possibilities.

The approach consists of a comparison of the action of a given experimental agent or condition before and after metabolic inhibition on ionic flux. Anoxia combined with iodoacetate poisoning has been used as the standard procedure for inhibition because of evidence that this causes cessation of energy turnover (*6*). If, now, the result of treatment of an otherwise normal tissue duplicates that of metabolic inhibition, and this effect is absent when the tissue has been previously inhibited by anoxia and iodoacetate, then the original result is considered to be a consequence of interference with metabolic reactions. The effect of lowering the sodium content of the medium on potassium influx is in this category and is shown in Table 1.

The data in Table 1 were obtained by first exposing desheathed toad (*Bufo marinus*) sciatic nerves for 2 hours to normal or low-sodium Ringer's solution in the presence or absence of oxygen and 1 mmole/lit of sodium iodoacetate, then replacing these solutions for another 2 hours with similar solutions, except for the presence of K^{42}. Glass units identical with those previously described (*5*) assured good stirring and replacement of solutions without oxygen contamination when necessary. Conventional extraction and radioisotope-counting procedures served for measurement of the activity gained by individual nerves. The activity taken up, corrected for that in the extracellular space and for the small backflux, was converted to the equivalent uptake of potassium and divided by the time of exposure to K^{42} to give the influxes in Table 1 (and Table 2).

Our earlier studies demonstrated that metabolic inhibition depresses potassium influx in the desheathed toad sciatic nerve to one-third or one-fourth of that of controls (*5*). This can be verified in Table 1 by comparing the influx in oxygen with that in helium combined with iodoacetate treatment. In addition, Table 1 shows that reduction of the sodium content of the medium to 10 percent of the normal level under aerobic conditions also reduces the influx of potassium. This is true whether choline replaces sodium or whether sucrose replaces both sodium and chloride. The extent of the reduction of influx is about one-half as great as that produced by the combination of anaerobiosis and iodo-

Table 2. Potassium influx (μmole/g min) in paired desheathed toad sciatic nerves compared in Ringer's and in Ringer's with 0.1-percent cocaine with and without prior metabolic inhibition. As in Table 1.

Additional conditions	K^{42} exposure period	Control	Cocaine	Δ
O_2	2 hr	0.031 ± 0.0015	0.027 ± 0.0032	−0.0041 (6) ± 0.0025
O_2	4 hr	0.032 ± 0.0023	0.027 ± 0.0028	−0.0045 (6) ± 0.0028
He + IAA	2 hr	0.0113 ± 0.0005	0.0082 ± 0.0005	−0.0030 (8) ± 0.0004

Table 1. Potassium influx (μmole/g min), on a wet-weight basis, in desheathed toad sciatic nerves compared in Ringer's with normal sodium content and with 90 percent of the normal sodium replaced with choline or sucrose with or without metabolic inhibition. Δ is the mean difference with its standard error based on the differences of individual paired nerves on the same horizontal line. All variability is expressed as the standard error of the mean. The parenthesized figures give the number of experiments. The data in the last row are the only unpaired sets for Δ given to the right.

Other conditions	Choline chloride replacing sodium chloride			Sucrose replacing sodium chloride		
	100% Na	10% Na	Δ	100% Na	10% Na	Δ
O_2	0.042 ± 0.0019	0.030 ± 0.0022	−0.012 (16) ± 0.0024	0.048 ± 0.0019	0.034 ± 0.0021	−0.014 (6) ± 0.0022
He + 1 mmole/lit IAA	0.014 ± 0.0005	0.019 ± 0.001	+0.0054 (16) ± 0.0011	0.013 ± 0.0004	0.026 ± 0.001	+0.013 (4) ± 0.0008
		0.016 ± 0.0013			0.022 ± 0.0007	+0.0064 (4) ± 0.0018
		0.019 (20) ± 0.0011			0.024 (8) ± 0.0009	+0.005 ± 0.0014