Individual flashes were also observed and recorded. With only one exception, which may have been a small ctenophore which penetrated the baffle and screen cover of the sample cell, all of the hundreds of flashes observed were extremely short bursts lasting only 0.1 or 0.2 second. The decay time of some representative flashes is shown in Fig. 3*a* to be first order with a mean life of 0.05 second. A complete flash is shown in Fig. 3*b*. The rise time of 0.01 second is the limit of the high frequency response of the d-c amplifier used in the equipment. The actual rise time may be much shorter (*4*).

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Production of Biologically Active Compounds by Isolated Lichenized Fungi

Abstract. Fungi which were separated from their lichenized associations and cultured independently produced several unusual and highly pigmented compounds. Certain of these compounds exhibited marked inhibitory activity against test strains of gram-positive bacteria and free-living molds; others showed a growth stimulatory effect on selected bacterial species.

The fact that lichens form many unique and interesting chemical compounds (so-called lichenic acids) which possess strong antibiotic activity has been firmly established (*1*). Unfortunately, lichens are notoriously slow growers and are not amenable to laboratory cultivation. Thus, practical exploitation of such compounds is limited only to lichens growing naturally in sufficient abundance.

Few workers have investigated the possibility of the lichen fungal component's synthesizing such active compounds apart from the algal association. Thomas (*2*) isolated many lichen fungi, several of which produced lichen acids. Castle and Kubsch (*3*) showed the presence of several lichen acids in a culture of the mycobiont of *Cladonia cristatella*. The activity of these acids was not tested. Zehnder (*4*) demonstrated a strong inhibition of the mold *Penicillium* sp. by the mycobiont of *Lecanora subfusca*; he did not describe the active compound.

In our investigation, 11 pure cultures of mycobionts were tested for active compounds. The mycobionts were separated from the following lichens: *Acarospora fuscata, A. smaragdula, Baeomyces roseus, Cladonia cristatella, C. pleurota, C. subcariosa, Graphis* sp., *Lecanora chlarotera, Physcia stellaris, Sarcogyne simplex*, and *Stereocaulon dactylophyllum* var. *flabellatum*. The mycobionts were isolated by means of the cultivation of spores ejaculated onto the surface of a soil-extract agar medium (*5*). Small portions of the agar which were free from contamination and showed a high percentage of germinating spores were then transferred to tubes which contained a malt extract and yeast extract agar (*p*H 5.8).

After 3 to 4 months of growth in complete darkness or low light intensity (20 to 25 ft-ca) at 17° to 18°C, the fungal colonies assumed various shapes, sizes, and pigmentation, each species passing through an initial white mycelial stage. In mycobionts *Acarospora fuscata* and *A. smaragdula* a bright red, water soluble pigment, which colored both the colony and surrounding medium, was produced by the hyphal cells. This single pigment showed a high degree of variation in color, ranging from bright red and purple to yellow and brown; the color changes and the amount of pigment produced appeared to be influenced by variations of temperature, *p*H, light intensity and carbon dioxide concentration. In *Cladonia cristatella, C. pleurota, C. subcariosa*, and *Stereocaulon dactylophyllum* var. *flabellatum*, the maturing fungal colonies changed color from white to pure yellow to red to reddish-brown or dark brown. Conditions and compounds which caused the color changes of these mycobionts were not investigated. In *Cladonia pleurota* a bluish pigment also developed in some parts of the colony. The mycobiont of *Baeomyces roseus* produced an orange pigment, and the hyphal cells were filled with abundant oil droplets. The mycobiont of *Lecanora chlarotera* was bright yellow in color and produced a water-soluble, dark brown pigment.

Acetone extracts of the fungal colonies, picked directly from tubes of a malt extract and yeast extract agar, were assayed with standard paper disk-agar plate techniques. Test organisms were selected strains of *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Aspergillus niger*, and *Penicillium chrysogenum*.

Results of the assay tests can be summarized as follows: Extracts of *Acarospora fuscata, A. smaragdula*, and *Cladonia cristatella* produced definite zones of inhibition against *Bacillus subtilis* and *Staphylococcus aureus*, the degree of inhibition being greater against *Bacillus subtilis*. The extract of *Acarospora smaragdula* was alone in inhibiting the two test molds. Acetone extracts of the remaining mycobionts showed no activity against the test organisms. None of the extracts showed activity against *Escherichia coli*.

The mycobiont of *Acarospora smaragdula* seemed to produce three types of compounds. During its initial growth phase, the fungus excreted large quantities of the water soluble, red-yellow-brown pigment mentioned above; this pigment was formed both on solid and in liquid media. The pigment showed strong inhibition of the two test fungi, no inhibition of the test bacteria, but an enhancement of growth of *Bacillus subtilis*. In later growth stages of the mycobiont, abundant prismatic plate-like crystals were found in the regions of agar where the pigment had diffused and also on the hyphal cells. These crystals were insoluble in water and acetone and found only on solid media. In older cultures of the mycobiont, bright yellow, plate-shaped structures appeared directly on the fungal colony and also on the agar surface in areas of the greatest pigment concentration. These yellow structures, which were produced only on solid media and which were readily soluble in acetone and insoluble in water, appeared amorphous under microscopic examination but were easily crystallized by the method of Asahina (*6*). Disks soaked in an acetone solution of such structures and then air dried to remove the solvent showed strong inhibition of *Bacillus subtilis, Staphylococcus aureus, Streptococcus fecalis, Aspergillus niger*, and *Penicillium chrysogenum*, with no activity against *Escherichia coli* and *Pseudomonas aeruginosa*. Evidence obtained from crystallization, chromatography, color reactions, and absorption spectra showed a similarity between this compound and a sample of known usnic acid produced commercially by Nutritional Biochemicals Corporation.

Study of a few lichen fungi has given several interesting and fruitful results. If one considers the fact that some 15,000 unique and diverse lichen species occur in nature, the research possibilities on the physiology and chemistry of the mycobionts alone seem limitless. (*7*).

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Delayed Alternation in Hemicerebrectomized Monkeys

Abstract. Monkeys subjected to extensive unilateral brain extirpation learned a delayed alternation task, although their rates of learning were significantly lower than those of a control group of normal animals. Visual field defects did not seem to account for the deficit.

It was recently reported that impairment on the delayed alternation task could be ". . . turned on and off . . ." by unilateral electrical stimulation to the sulcus principalis of the monkey brain (*1*). Earlier, deficit on this task had often been reported in monkeys as a result of bilateral damage to brain tissue, specifically the midlateral frontal cortex (*2*). Few studies, however, reported impairment after unilateral lesions (*3*). In each case of unilateral lesion the deficit was temporary, and in none did the animals undergo unilateral extirpations of the brain to the extent of those to be reported here.

Table 1. Number of days to criterion on delayed alternation. The data on delayed alternation performance has been supplemented by scores from another group of hemicerebrectomized monkeys with a similar background, which was not discussed in the text.

Subject No.	Days
Normal group	
126	10
127	17
129	13
130	13
135	20
Mean	14.6
Operated group	
106	31
116	24
121	31
123	42
124	37
Mean	33.0
Other operated animals	
109	54
112	27
113	18
120	27
122	46
Mean	34.4

The surgical procedure, hemicerebrectomy (*4*), involves the unilateral removal of all cerebral structures lateral to the hypothalamus and rostral to the midbrain (Fig. 1). Since a similar but less extensive operation has been applied to the treatment of certain neurological disorders in humans—for example, tumor, infantile hemiplegia, and epilepsy (*5*)—the resulting effects of hemicerebrectomy upon learning and retension abilities of primates are of considerable importance. Consequently, a series of experiments was designed to evaluate the behavior of hemicerebrectomized monkeys. The experiment discussed in this report utilized delayed alternation performance to measure one aspect of learning ability.

The subjects were immature rhesus monkeys, five normal and five hemicerebrectomized, that had received previous training on object-quality discrimination problems. Feeding was scheduled so that the subjects had been deprived of food for about 22 hours at the time of testing and, therefore, were well motivated for the raisin rewards.

An adaptation of the Wisconsin general test apparatus was located in a room in which normal laboratory noises were greatly muffled. The stimuli consisted of a single pair of identical objects (plain, black, wooden blocks, measuring 4¼ by 2¾ by ¾ in.), which covered two food wells that were located about 11½ in. apart in a light gray presentation tray. The wells were baited during a 10-second delay, while the opaque screen was interposed between the subject and objects. To serve as a starting point for the alternation sequence, displacement of either object was rewarded on the first trial, and subsequently 50 more trials were given each day.

The problem required the subject to alternate from a just previously rewarded choice to the object on the other side. By following a noncorrection procedure, a criterion of at least 86 percent correct responses over two consecutive days was used.

Normal animals required significantly fewer days to reach criterion than did the operated subjects (*6*). The latter, in fact, took over twice as long (Table 1). Figure 2 presents graphic data for the course of learning in each group. A pronounced overlap may be noted for the first 4 days, but after that time the curve of the normal group remains higher.

Although homonymous hemianopsia is an expected result of this surgery, the visual field defect did not seem to account for the delayed alternation deficit in these subjects. The animals demonstrated past high performance levels on visual discriminations between two dissimilar objects. Because the possibility existed that the field defect might be related to side preferences, the numbers of both total responses and errors made by each subject to each side were tabulated. In neither case did the operated subjects show a side preference significantly different from that of the normal subjects (*7*).

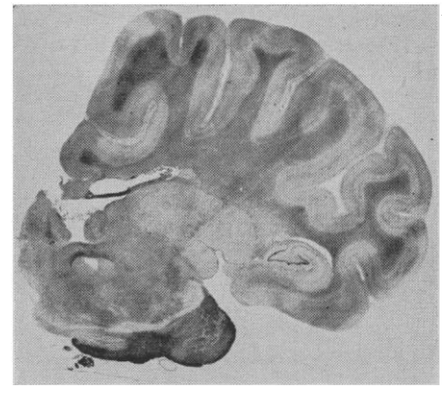

Fig. 1. This section of brain tissue, at about the level of the posterior commissure, is from a hemicerebrectomized monkey. The Kluver and Barrera double stain technique was used.

Even though the hemicerebrectomized group exhibited a level of performance lower than that of the normal group, it is believed more important to stress that the operated monkeys did meet the criterion of learning. This accomplishment emphasizes the observation that, several months after surgery, the hemicerebrectomized monkeys still retained structures necessary to the learning of this task.

The hemicerebrectomized monkey provides a useful preparation in which to study the function of remaining structures with an increased certainty, prior to histology, regarding the bilaterality

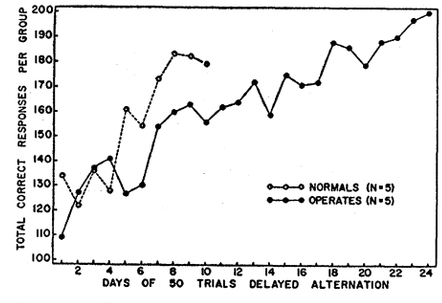

Fig. 2. Total correct responses, summed for each group daily, have been plotted. Totals for only the period during which each group remained intact are shown; that is, before any subject in each group met the criterion.