Reports

Monoamine Oxidase, Psychoenergizers, and Tranquilizers

Abstract. 1-Benzyl-2-methyl-5-methoxytryptamine (BAS) inhibits monoamine oxidase in man. This finding confirms the findings of Woolley in mice. Since BAS, a tranquilizing agent, and Marsilid, a psychoenergizer, are both monoamine oxidase inhibitors, doubt is cast upon the hypothesis that the stimulatory effect of Marsilid is due to its ability to inhibit monoamine oxidase.

Marsilid has been shown to be an inhibitor of monoamine oxidase (*1*). The attractive hypothesis has been put forth that the antidepressant action of Marsilid is due to its ability to inhibit the enzyme (*2, 3*) and, more specifically, that the increased level of brain serotonin or norepinephrine is responsible for the central stimulatory effects of Marsilid (*4*). A parallelism between the enzymatic inhibition and clinical effectiveness of Marsilid and its analogs has been reported (*3*); Resnick (*5*), however, reported a lack of correlation between the degree of enzymatic inhibition and the clinical effectiveness of Marsilid in several patients.

Woolley *et al.* (*6*) reported that 1-benzyl-2-methyl-5-methoxytryptamine (BAS) is a monoamine oxidase inhibitor in mice. We have confirmed this finding in man. In our first study, BAS (100 mg/day) was administered orally to four schizophrenic patients for the first 2 weeks. During the third and fourth weeks, BAS and D,L-5-hydroxytryptophan (5-HTP) (30 mg/day) was administered intramuscularly. BAS alone did not affect the excretion of endogenously formed 5-hydroxyindoleacetic acid (5-HIAA), but it did prevent the expected increase of 5-HIAA due to the metabolism of 5-HTP in three of the four patients; only 40 percent of the L-5-HTP was recovered in the fourth patient. In our second study, two patients received BAS for 1 week and BAS and 5-HTP (100 mg/day), intramuscularly, for the next 3 weeks. Again the BAS prevented the expected increase in urinary 5-HIAA. This was confirmed by a two-dimensional paper chromatogram which showed the usual amount of 5-HIAA and a large increase in serotonin and 5-HTP, presumably the D-form.

Although BAS and Marsilid are both monoamine oxidase inhibitors, BAS, unlike Marsilid, has tranquilization properties. Woolley and his co-workers (*7*) first observed the tranquilizing action of BAS in mice. Wilkins *et al.* (*8*), in a study of BAS on patients with hypertension, noted a state of sedation and tranquilization. Rudy and his co-workers (*9*) administered BAS to 24 moderately disturbed, chronic psychotic female patients and reported "a strong tranquilization action not unlike that of reserpine."

Thus we have a new and very interesting situation of two monoamine oxidase inhibitors, one a psychoenergizer (Marsilid), the other a tranquilizer (BAS). This finding casts some doubt on the hypothesis that Marsilid exerts its central stimulatory action by virtue of its ability to inhibit monoamine oxidase. It should be noted, however, that although BAS does possess central activity, it does not readily pass into the brain (*7*) and, further, that we have measured the inhibition of monoamine oxidase in the whole organism and not in the brain specifically.

It is relevant that isoniazid (Rimifon) is not a monoamine oxidase inhibitor (*1*) but that it nevertheless possesses central stimulatory properties in man (*10*) and has been used successfully in the treatment of depression (*11*). Since Rimifon is closely related structurally to Marsilid, it seems likely that a common mechanism underlies the psychoenergizing properties of Marsilid, Rimifon, Marplan, and other analogs. The possibility that the central stimulatory action of these compounds is due to their ability to produce a pyridoxine deficiency (*12*), to inhibit decarboxylase activity (*13*), and to inhibit transaminase activity (*14*) should be considered (*15*).

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References and Notes

1. E. A. Zeller, J. Barsky, E. R. Berman, J. R. Fouts, *J. Lab. Clin. Med.* 40, 965 (1952).
2. H. P. Loomer, J. C. Saunders, N. S. Kline, paper presented at regional research conference, Am. Psychiatric Assoc., 6 Apr. 1957, Syracuse, N.Y.; N. S. Kline, *J. Clin. Exptl. Psychopathol. & Quart. Rev. Psychiat. Neurol.* 19, 72 (1958).
3. ———, S. C. Saunders, S. A. Bailey, E. Gosline, M. Vaisberg, paper presented at meeting of N.Y. Acad. Sci., 20–22 Nov. 1958, New York, N.Y.; A. Voelkel, *ibid.*
4. P. A. Shore, *J. Clin. Exptl. Psychopathol. & Quart. Rev. Psychiat. Neurol.* 19, 56 (1958).
5. O. Resnick, J. M. Wolfe, H. Freeman, F. Elmadjian, *Science* 127, 1116 (1958).
6. D. W. Woolley and P. M. Edelman, *ibid.* 127, 281 (1958).
7. D. W. Woolley, E. Van Winkle, E. Shaw, *Proc. Natl. Acad. Sci. U.S.* 43, 128 (1957).
8. R. W. Wilkins and W. Hollander, *Circulation* 16, 256 (1957).
9. L. H. Rudy, E. Costa, F. Rinaldi, H. E. Himwich, *J. Nervous Mental Disease* 126, 284 (1958).
10. R. H. Reilly, K. F. Killam, E. H. Jenney, W. H. Marshall, T. Tansig, N. S. Apter, C. C. Pfeiffer, *J. Am. Med. Assoc.* 152, 1317 (1953).
11. J. A. Flaherty, *Delaware State Med. J.* 24, 198 (1952); J. Delay and J. F. Buisson, *J. Clin. Exptl. Psychopathol. & Quart Rev. Psychiat. Neurol.* 19, 51 (1958); H. M. Salzer and M. L. Lurie, *A.M.A. Arch. Neurol. Psychiat.* 70, 317 (1953); ———, *Ohio State Med. J.* 51, 437 (1955).
12. J. P. Biehl and R. W. Vilter, *J. Am. Med. Assoc.* 156, 1549 (1954); *Proc. Soc. Exptl. Biol. Med.* 85, 389 (1954).
13. F. Rosen, paper presented at meeting of N.Y. Acad. Sci., 20–22 Nov. 1958; A. N. Davidson, *Biochim. et Biophys. Acta* 19, 131 (1956).
14. H. Weissbach, W. King, A. Sjoerdsma, S. Udenfriend, *J. Biol. Chem.* 234, 81 (1959).
15. We thank D. W. Woolley for suggesting the study and for supplies of BAS and 5-hydroxytryptophan. We acknowledge completion of the work under a grant from the Ford Foundation and the Scottish Rite Committee.
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Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

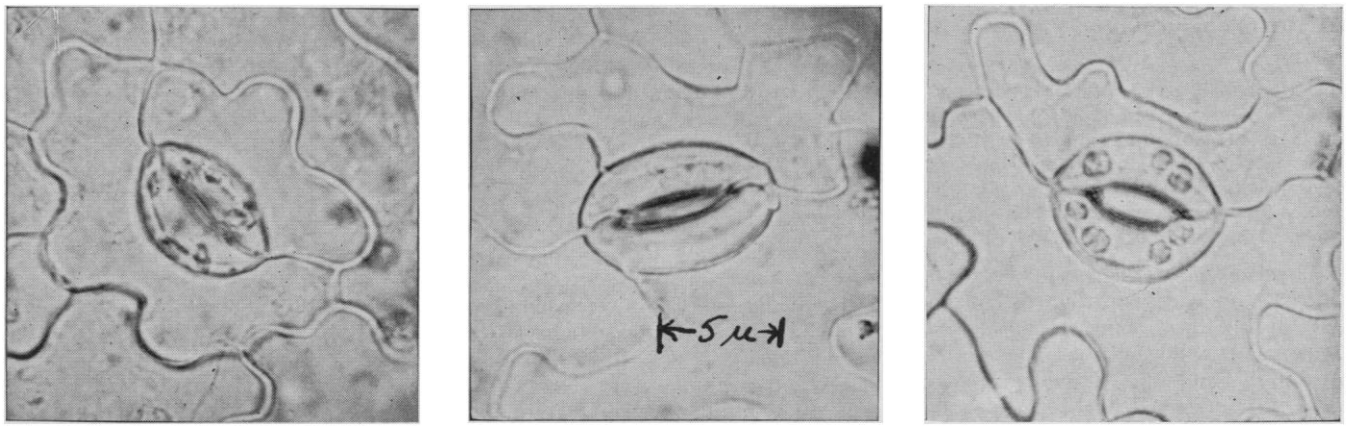
Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

Disappearance of Guard Cell Chloroplasts in Ultraviolet-Irradiated Leaves

Abstract. Ultraviolet irradiation of kidney-bean leaves results in the disappearance of chloroplasts from guard cells. The evidence indicates that ultraviolet irradiation causes plastid breakdown indirectly through an effect on guard-cell metabolism.

In some earlier experiments (*1*) the disappearance of guard-cell chloroplasts in ultraviolet-irradiated bean leaves was observed, but not reported. Cells of irra-



Stomata of red kidney bean leaf epidermis after incubation in the dark: Fig. 1 (left), 3 hours after ultraviolet irradiation; Fig. 2 (middle), 24 hours after ultraviolet irradiation; Fig. 3 (right), unirradiated control.

diated epidermis left in the dark die (*2*), and in our experiments, after 4 days, when the epidermal strips were made, the epidermis had undergone considerable decomposition. The disappearance of chloroplasts could therefore have been related to this decomposition rather than to more immediate effects of ultraviolet treatment. Experiments were therefore designed to obtain more information on the mechanism of ultraviolet-induced disappearance of guard-cell chloroplasts (*3*).

Primary leaves were detached from greenhouse-grown Red Kidney bean seedlings just before expansion of the first trifoliate leaf. Half-leaves were cut free of the midrib and placed, lower surface up, on moist filter paper in petri dishes. A glass slide was placed across the center of each half-leaf, and the leaves were irradiated for 2 minutes at 36 cm with a General Electric (G8T5) germicidal lamp, giving 130 $\mu w/cm^2$ (2537 A) at the leaf surface. The glass slides were then removed, and the leaves were either placed immediately in the dark at 22°C for varying periods or illuminated with 680 ft-ca of light from "daylight" fluorescent tubes for 4 hours before incubation in the dark.

In the unprotected portions of leaves, the glazing which is a characteristic external symptom of injury (*1*) never was evident until 24 hours after incubation in the dark. However, epidermal strips taken from irradiated surfaces as early as 3 hours after treatment already showed indications of chloroplast damage in guard cells when examined microscopically. As shown in Fig. 1, the outlines of such plastids are extremely irregular or angular in appearance. At the end of 24 hours most guard cells have no chloroplasts, although an occasional guard cell shows faintly visible structures with the general outlines of plastids. Both types are represented around the stoma in Fig. 2. In Fig. 3, guard cells with normal chloroplasts are pictured. These were characteristic of epidermal strips taken from leaves prior to irradiation, of portions of irradiated leaves protected by the glass slide, or of unprotected portions of such leaves which were illuminated with visible light for 4 hours after irradiation. All the photographs were made in ordinary light, but examination of chloroplast-free guard cells with phase optics did not reveal structures resembling chloroplasts; this indicates that ultraviolet irradiation leads to an actual disintegration of chloroplasts, rather than merely to changes in light absorption or index of refraction.

Along with lysis, chloroplasts lost starch, as indicated by iodine staining before and after the structural changes occurred. In one experiment, epidermal strips rather than leaves were irradiated. This also resulted in the disappearance of chloroplasts, but not in strips floated in 0.1-percent $HgCl_2$ after exposure to ultraviolet irradiation.

Guard-cell chloroplasts in bean, as in other species studied (*4*), are lighter in color than chloroplasts in the mesophyll; they are also less stable, since aside from the effect of ultraviolet irradiation, when leaves are boiled or strips are steamed for 1 minute, or even if leaves are kept at 33°C for 24 hours, guard-cell chloroplasts break down, while mesophyll chloroplasts show no visible damage immediately after such treatment.

Some preliminary experiments were also done with geranium, with results similar to those in bean, and with calla lily, which showed no visible effects after ultraviolet irradiation equivalent to that used in studying guard-cell chloroplasts.

The disappearance of guard-cell chloroplasts so soon after ultraviolet treatment obviously cannot be attributed to secondary effect of tissue decomposition. Whether ultraviolet irradiation acts directly to disrupt chloroplasts or indirectly by influencing cell metabolism cannot be finally decided on the basis of our work. Ultraviolet irradiation produces a depolymerization of nucleic acid (*5*) and proteins (*6*) and, were it acting similarly on plastids, the diffusion of fragments would be expected to be relatively independent of subsequent treatment. Use of $HgCl_2$ prevented chloroplast breakdown in our experiments; this might have been due to its action as a fixative. However, the disappearance of starch along with chloroplasts suggests that ultraviolet irradiation acts indirectly by at least temporarily favoring degradative over synthetic metabolism (*6*), and that the Hg ion inhibits the metabolism associated with injury from irradiation.

Pertinent to our findings are some cytological effects of ultraviolet irradiation reported by others. These effects include the disruption and subsequent coalescence of the large mitochondria of *Saccharomyces* (*8*), the protoplasmic fragmentation of the ciliated protozoan *Spirostomum ambiguum* (*9*), and the contraction and breaking up of the ribbon-like chloroplast of *Spirogyra* (*10*). In none of these cases, however, was total disappearance of the damaged structure reported.

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References and Notes

1. M. Chessin, *Ann. Appl. Biol.* 46, 88 (1958).
2. F. C. Bawden and A. Kleczkowski, *Nature* 169, 90 (1952).
3. This study was supported by National Institutes of Health grant E-596(C4).
4. F. E. Lloyd, *Carnegie Inst. Wash. Publ. No. 82* (1908), p. 139.
5. A. Hollaender, J. P. Greenstein, W. V. Jenrette, *J. Natl. Cancer Inst.* 2, 23 (1941).
6. T. Svedberg and S. Brohult, *Nature* 143, 938 (1939).
7. P. C. Owen, *ibid.* 180, 610 (1957).
8. A. Sarachek and G. F. Townsend, *Science* 117, 31 (1953).
9. E. S. Shirley, Jr., and H. E. Finley, *Trans. Am. Microscop. Soc.* 68, 136 (1949).
10. E. N. Harvey, *J. Gen. Physiol.* 25, 431 (1942).

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