

The Hypnotic Constituent of *Stipa vaseyi*, Sleepy Grass

The plant, *Stipa vaseyi*, also known as *Stipa viridula*, *Stipa viridula* var. *robusta*, and commonly as sleepy grass, has been reported¹⁻³ to produce a hypnotic effect in animals, particularly horses which graze on the grass. The best substantiated work is that of MARSH and CLAWSON¹ who concluded that the plant, either green or dry, produces a distinctly hypnotic effect in horses. MUENSCHER³ states that chemical examination has not revealed any toxic substances.

We wish to report the isolation, identification, and some of the pharmacological properties of diacetone alcohol, an active constituent of sleepy grass.

Samples of sleepy grass were collected from mountain valleys on the Mescalero Apache Indian Reservation near Ruidoso (New Mexico). The dried grass was powdered and extracted with a sequence of non-polar to polar solvents. The ether, acetone, and methanol extracts which were tested in mice and rats all showed activity. Distillation of the crude extracts through a spinning band column gave a colorless liquid, b.p. 45–46° (6 mm), which had all the activity of the crude extracts. The active material, diacetone alcohol, was identified by a comparison of b.p., infrared spectra, m.p., m.m.p. of the DNP derivatives (m.p. 203–204°), and pharmacological properties of the active principle with known diacetone alcohol. It is not understood why earlier investigators failed to isolate diacetone alcohol from the plant, unless the low yield (1.2% on a dry weight basis) was responsible.

Preliminary pharmacological studies show that diacetone alcohol has central depressant properties. Other investigators have also reported on some central effects of this compound^{4,5}. The central nervous system effects in the present study were observed in both rats (Sprague-Dawley males) and mice (Carworth Farms males).

In rats we noted that doses of 0.1 to 0.2 ml (93–186 mg) per 250 g produced depression which was most conspicuous if the animal was not stimulated; the treated animals

displayed a great reduction in motor activity and a closing or semi-closing of the eyelids. Upon stimulation by either touch or sound, the animals responded with an increase in motor activity and appeared normal. Higher doses (0.5 ml/250 g rat), in contrast, produced what appeared to be a mixture of depression and stimulation. The animals exhibited a marked increase in motor activity, including running, and a moderate degree of ataxia.

In mice 0.1 to 0.2 ml/25 g produced a conspicuous respiratory as well as motor depression. Lower doses (0.05 ml) produced a curious posture: the animals stretched and arched their backs concavely, so that their abdomens touched the bottom of the cage⁶.

Zusammenfassung. Der aus *Stipa vaseyi* isolierte wirk-same Bestandteil (depressive Wirkung auf das Zentral-nervensystem von Tieren) wurde chemisch und pharma-kologisch als Diacetonalcohol identifiziert.

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¹ C. D. MARCH and A. B. CLAWSON, U.S. Dept. Agr. Tech. Bull. No. 114 (1929).

² A. W. SAMPSON and H. E. MALMSTEN, *Stock-Poisoning Plants of California*, Univ. of Calif. Agr. exp. Sta. Bull. No. 593, 74 (1935).

³ W. C. MUENSCHER, *Poisonous Plants of the United States* (The Macmillan Co., New York 1951), p. 40.

⁴ R. L. DRIVER, Proc. Soc. exp. Biol. Med. 64, 248 (1947).

⁵ D. G. WENZEL and G. Y. KOFF, J. Amer. pharm. Assoc. 45, 669 (1956).

⁶ This investigation was supported by U.S. Public Health Service research grant NB-04313-1, National Science Foundation undergraduate summer research award, and National Institutes of Health research career award 5-K3-NB-8641-02.

Protection of *Vicia faba equina* Against X-Rays by Serotonin¹

It seemed interesting to us to examine the radio-protective effect of serotonin on plants. To this end we used the germinating beans of *Vicia faba equina*. We made two series of experiments: (1) as preliminary experiments we irradiated germinating beans of *Vicia faba* with increasing doses of X-rays, as JÜNGLING et al.² had already done in 1920, and GLOCKER, HAYER and JÜNGLING³ in 1929, in their studies of dosage and the biological effect of X-rays. The test which we used in the course of these experiments (prompted by the work of JÜNGLING et al.²) was the appearance and the growth of secondary roots in germinating *Vicia faba*. (2) We tried to show, in *Vicia faba*, the protective effect of cysteamine already established by BACQ et al.⁴⁻⁷ on the pea (*Express* of Alaska) and the effect of serotonin.

First series of experiments. In the first experiments we sought to find out which was the dose of X-rays necessary in *Vicia faba* to prevent the appearance of secondary

roots (Figure 1). From these results it is evident that the appearance and growth of accessory roots diminishes progressively in proportion to the dose of X-rays applied. Plants which have received 400 R do not develop accessory roots. We therefore used this dose for experimenting on the protection of plants against irradiation.

¹ The research reported in this document has been made possible through the support and sponsorship of the US Department of Army, through its European Research Office. Contract number (DA-91-591-EUC-2696).

² O. JÜNGLING, Münch. med. Wschr. 40, 1141 (1920).

³ R. GLOCKER, HAYER, and O. JÜNGLING, Strahlenther. 32, 10 (1929).

⁴ Z. M. BACQ and A. HERVÉ, Bull. Acad. Roy. Méd. Belg. série 6, 17, 13 (1952).

⁵ Z. M. BACQ and A. HERVÉ, Schweiz. med. Wschr. 82, 1018 (1952).

⁶ P. ALEXANDER, Z. M. BACQ, S. F. COUSSENS, M. FOX, A. HERVÉ, and J. LAZAR, Radiation Res. 2, 292 (1955).

⁷ Z. M. BACQ, Triangle 6, 2 (1961).

Second series of experiments. In this second series of experiments we experimented on the protective effect of serotonin in *Vicia faba* against an X-ray dose of 400 R (Figures 2 and 3) and we compared this protective effect with that afforded by cysteamine.

The following points can be drawn from these experiments: (1) The control plants developed well and the accessory roots were abundant. (2) The plants in the non-irradiated cysteamine and serotonin solutions also developed very well and the toxic effect of these substances showed itself only in a very slight retardation of the appearance of accessory roots. (3) The plants plunged into cysteamine and serotonin solutions for $\frac{1}{2}$ h and then irradiated, developed accessory roots to a certain extent after an inhibitory phase of 6 to 8 days. (4) Plants which received 400 R without protection did not develop any accessory roots at the end of the experiment, 25 days after the application of the X-rays.

Conclusions. *Vicia faba* (tested by the appearance and growth of accessory roots by the method of JÜNGLING et

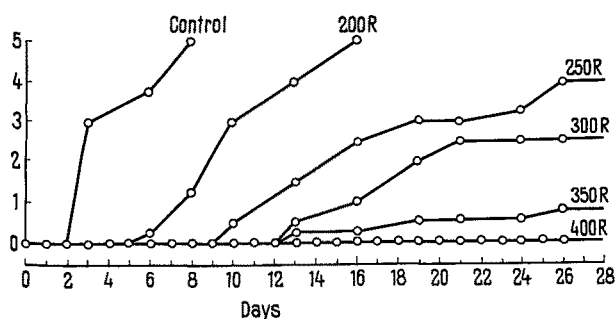


Fig. 1. The effects of X-rays in increasing doses. Abscissae: time from the beginning of irradiations. Ordinate: evaluation of the development of secondary roots.

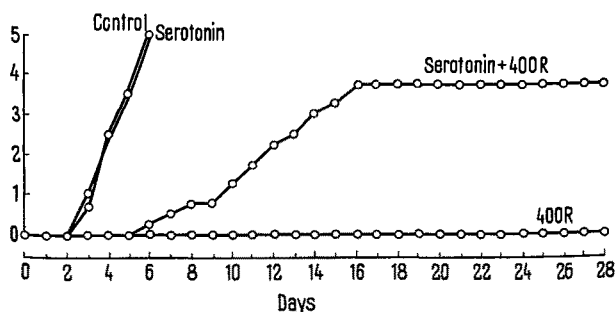


Fig. 2. Protection provided by serotonin against 400 R.

al.²) gave – in our experimental conditions – excellent results. Increasing doses of X-rays (200, 250, 300, 350 R) diminish the appearance and growth of accessory roots in proportion to the X-ray dose applied. 400 R completely impedes the appearance of accessory roots. We were able to confirm the protective effect of cysteamine. With this test serotonin has a very clear radioprotective effect. Cysteamine and serotonin have, in this test with *Vicia faba*, a protective effect like the one they have in tests on mammals³.

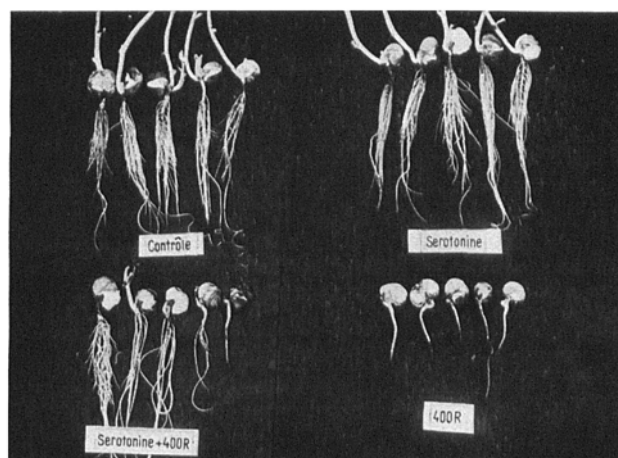


Fig. 3. Protection provided by serotonin against 400 R.

Résumé. La *Vicia faba* se prête bien, non seulement au dosage biologique des rayons X, mais également à des expériences sur la radioprotection. Avec le test utilisé lors de ces expériences, il a été possible de démontrer l'effet protecteur non seulement de la cystéamine, mais aussi celui de la sérotonine.

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² The new way of writing R for r should not be confused with the German unit 'R' which was used from 1924 to 1928 to indicate 1 cm³ of air at 17°C ('R' = 1,066 r). The French roentgen energy unit R*, introduced by SOLOMON and based on radium γ -radiation, is no longer in use (1 R* = 0,44 r).

Iron Pigmentation of the Gut of Germ-Free and Conventionalized Guinea-Pigs¹

In the course of some studies of healthy conventionalized and germ-free guinea-pigs, an unexpected difference was consistently observed in concentrations of pigment in the tunica propria of the intestinal tract. We are not aware of any report of this in the literature. The pigment was visible with the routine hematoxylin and eosin

staining method and a special stain (Perls) revealed that the pigment contained iron.

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