A detailed report on the isolation and characterization of polycyclic aromatic substances from barnacles will be given elsewhere.

References

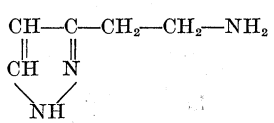
1. SHIMKIN, M. B., and BRYAN, W. R. *J. Natl. Cancer Inst.*, **4**, 25 (1943).
2. SHIMKIN, M. B., and ANDERVONT, H. B. *J. Natl. Cancer Inst.*, **1**, 57 (1940).
3. BRYAN, W. R., and SHIMKIN, M. B. *J. Natl. Cancer Inst.*, **3**, 503 (1943).

An Analog of Histamine that Stimulates Gastric Acid Secretion without other Actions of Histamine

C. E. Rosiere and M. I. Grossman

Department of Clinical Science,
University of Illinois, College of Medicine, Chicago

During a survey of the stimulatory and inhibitory actions of chemical analogs of histamine on gastric secretion, one compound was found that possessed the unique property of stimulating gastric acid secretion without producing any of the other pharmacologic actions of histamine. The compound is 3-(β ethylamine) pyrazole (compound XXIV). Its structural formula is:



It will be seen that this is an isomer of histamine in which the ring has a pyrazole instead of an imidazole configuration. The relationship of the side chain to the =N— is the same as in histamine. The compound was first synthesized and studied by Lee and Jones (*1*), who found that it did not contract the isolated guinea pig ileum and that it did not lower blood pressure in cats. In the anesthetized dog we have found compound XXIV to be about 1/700th as potent as histamine in depressing blood pressure.

Compound XXIV stimulates acid secretion in dogs with pouches of the entire stomach when the hydrochloride is administered subcutaneously, intramuscularly, intravenously, or orally. By the subcutaneous route the ED_{50} of the hydrochloride (dose required to produce 50% of the maximal secretory rate) is 8 mg/10 min in comparison with 0.12 mg/10 min for histamine diphosphate. The maximal secretory rate attainable with compound XXIV is the same as with histamine. The pepsin concentration of the juice secreted in response to compound XXIV is not significantly different from that stimulated by histamine.

The hydrochloride of compound XXIV has been injected into 20 human subjects by the subcutaneous and intramuscular routes in doses of 10–50 mg. In no instance have any of the characteristic side effects of histamine been observed, including triple response at site of injection, headache, or flush. The 50-mg dose produced a greater output of HCl than 0.01 mg/kg of histamine diphosphate. Doses of 100 mg produce side effects similar to those seen with histamine.

The existence of this histamine analog with specificity for site of action is in keeping with the known differences between the action of histamine on the gastric glands and its action at other sites. These include (*a*) failure of antihistaminic drugs to counteract action of histamine on gastric glands, and (*b*) inhibition by xanthine alkaloids of actions of histamine at other sites but potentiation of the gastric secretory action.

Compound XXIV may prove useful for routine clinical testing for achlorhydria, not only because it would eliminate the undesirable side reactions that follow histamine, but also because it would be possible to produce stronger stimulation of acid secretion and thus provide more clear-cut results in borderline cases. It may also prove useful for the investigation of the maximal secretory capacity of the human stomach in health and disease.

Reference

1. LEE, H. L., and JONES, R. G. *J. Pharmacol. Exptl. Therap.*, **95**, 71 (1949).

Recovery of Tumor Cells from Effects of the Tumor-inducing Principle in Crown Gall

Armin C. Braun

Laboratories of the Rockefeller Institute for Medical Research, New York

When the tumor-inducing principle associated with the crown-gall bacterium acts on cells of plant species such as *Helianthus annuus* (*1*) and *Vinca rosea* (*2, 3*), an abrupt and irreversible change in the behavior of these cells occurs. Following the transformation process, proliferation of the altered host cells becomes an automatic process that is independent of the inciting bacteria. The cells of the resulting neoplasm are characterized by excessive powers of proliferation and limited powers of differentiation. Competence for organization appears to have been lost as a result of the action of the tumor-inducing principle. Tumor cells of this type are transplantable, and when bacteria-free fragments are implanted into a healthy host they develop into typical uncoordinated crown-gall tumors.

When, on the other hand, this same tumorogenic principle acts on cells located close to the top of a plant species such as *Kalanchoe daigremontiana,* the altered cells appear at first to be of an undifferentiated type. As the tumors grow, however, there results, in place of the characteristic neoplasm, an overgrowth composed not only of uncoordinated tumor cells but of cells that are organized into morphologically complex structures (*4*). The question as to whether these structures result from the growth of normal cells that have been stimulated to develop by the expanding tumor, or whether the morphologically abnormal shoots are composed of altered cells that have acquired a ca-

pacity for differentiation and organization, has been a subject for speculation (*4–8*). It is with this problem and its relation to the recovery of tumor cells that this paper is concerned.

Turkish tobacco (*Nicotiana tabacum*), which responded to bacterial inoculation as did *Kalanchoe,* was used as the test object in these investigations. The plants were grown in 4- or 6-in. pots. When they reached a height of 8–12 in., a single cut was made with a sharp razor through an internode close to the top of the plants. Bacterial inoculations of the freshly cut stem surfaces were made with the T-37 strain of the crown-gall bacterium. The inoculated areas were covered for several days with a rubber tape known as "Sterilastic" to prevent drying.

After a suitable incubation period undifferentiated tumors developed in the region of the cambium. As the tumors expanded, small areas of organized tissue that later developd into teratological structures appeared on their surfaces. Thereafter, the new growth developed rapidly into moderate to large complex overgrowths of a character similar to those previously described as developing on this host (*9*), on *Kalanchoe* (*4*), and on certain other plant species (*7, 8*).

Early in the study of the growth patterns of normal and tumor cells *in vitro* it was observed that, although bacteria-free sunflower tumor cells grew rapidly and in a completely uncoordinated manner on White's medium, normal tissue isolated from the same host developed very slowly on this medium (*1*).

The growth habits of normal cells, crown-gall tumor cells, and cells isolated from the teratological structures that developed from the complex overgrowths on tobacco were studied *in vitro*. More than 200 fragments of normal tobacco tissue were isolated from newly formed as well as from somewhat older axillary and adventitious shoots, from internodes of young and mature stems, from the region of petiolar attachment as well as from immature tissue just below the developing tip, and were planted on White's medium. The normal tissue fragments themselves increased in volume only slightly in a 12-week period. Adventitious buds that developed from many of these fragments frequently grew into small but otherwise normal-appearing plantlets. Bacteria-free tobacco tumor cells, on the other hand, grew rapidly and in a completely uncoordinated manner on White's medium. This culture medium appeared suitable, therefore, for differentiating normal cells from crown-gall tumor cells of tobacco.

Fragments of tissue were removed aseptically from morphologically distorted but organized stem tissues that developed from the teratomata and were planted on the culture medium. The rate of growth of these bacteria-free tissue fragments, although somewhat slower than that of true tumors, was nevertheless far greater than the rate of growth of normal tobacco cells. These cells differed from the tobacco tumor cells studied in the past, however, in that they possessed a well-developed capacity for differentiation and organization. Large areas of the surfaces of the cultures were covered with small adventitious buds and leaves that showed varying degrees of structural development. Some buds appeared quite normal, whereas others were highly distorted. The buds never developed appreciably in culture. Roots were not formed. Histologically the cellular mass was composed of a firm core of disorganized hypertrophied and hyperplastic parenchymatous cells. Areas of organization arose for the most part at the edges of the growth. Attempts to isolate fragments from these masses that would develop in culture in a completely undifferentiated and unorganized manner have thus far failed. One clone of this tissue has now been maintained in culture for 16 months. Other clones isolated from different teratological structures have been cultured for shorter periods. The tissues were divided at 6- to 8-week intervals and planted on fresh media. In all instances these cells have maintained their characteristic properties unaltered. It thus appears that the morphologically distorted but organized stems that develop from the teratomata are composed of cells whose metabolism is fundamentally different from that found in normal tobacco cells isolated from comparable but normal stem segments.

When fragments of these abnormal tissues that had been carried in culture for more than 10 months were implanted in healthy hosts, they developed into teratomatous overgrowths that reached a diameter of about 1 cm in a 6- to 8-week period. In their ability to grow profusely on a medium free of added growth substances and in their ability to develop independently of the growth-restraining influences of the host, these cells resemble crown-gall tumor cells of the type previously described. These tumor cells differ from those isolated and studied in the past in that they retain indefinitely *in vitro* and *in situ* a well-developed capacity for organizing leaves and buds.

It has been demonstrated (*10, 11*) that certain self-duplicating cytoplasmic entities can be eliminated from cells under conditions that favor the increased multiplication of those cells relative to that of the self-duplicating factor. Such entities have been postulated (*12*) as being responsible for the continued abnormal proliferation of crown-gall tumor cells.

Rapid multiplication of plant cells can be effectively encouraged by forcing the growth of a bud. The meristematic cells of an actively growing bud divide with far greater frequency than do most crown-gall tumor cells. Since it has been demonstrated that certain crown-gall tumor cells retain a capacity for organizing buds, it seemed possible to test the hypothesis indicated above. In order to induce a maximum stimulus for regeneration in the host, all normally produced axillary buds were removed from tobacco plants that had been inoculated at the cut stem surface with crown-gall bacteria. This resulted in the production of a complex tumor that contained adventitious buds composed of tumor cells. When these buds produced shoots large enough to be used as scions, the shoots were removed and grafted to

healthy tobacco plants from which again all normal buds had been removed. It should be recalled that these shoots were composed of tumor cells and, hence, were organized tumors. The new growth that resulted from these rapidly growing shoots gradually became more and more normal in appearance. The tips of the shoots were again removed and grafted to healthy plants. They developed rapidly and appeared normal in every respect, ultimately flowered, and set seed. Fragments of tissue isolated from the normal-appearing stems derived from tumor tissue buds grew poorly in culture, as does healthy tobacco tissue. In these instances recovery appeared to have been complete. It was a gradual process that progressed in the direction of the normal as affected shoots developed and were forced into rapid growth.

Our findings suggest that the factor which causes crown-gall tumor cells to develop abnormally becomes diluted in, and is eventually lost from, affected cells that are forced to grow and divide with sufficient rapidity. Details will be published elsewhere.

References

1. WHITE, P. R., and BRAUN, A. C. *Cancer Research*, **2**, 597 (1942).
2. BRAUN, A. C. *Am. J. Botany*, **30**, 674 (1943).
3. WHITE, P. R. *Ibid.*, **32**, 237 (1945).
4. BRAUN, A. C. *Ibid.*, **35**, 511 (1948).
5. LEVIN, I., and LEVINE, M. *J. Cancer Research*, **5**, 243 (1920).
6. LEVINE, M. *Bull. Torrey Botan. Club*, **46**, 447 (1919).
7. SMITH, E. F. *Bull. Johns Hopkins Hosp.*, **28**, 277 (1917).
8. ———. *J. Agr. Research*, **21**, 593 (1921).
9. LEVINE, M. *Phytopathology*, **13**, 107 (1923).
10. KUNKEL, L. O. *Bull. Exptl. Sta. Hawaiian Sugar Planters' Assoc., Botan. Ser.*, **3**, 115 (1924).
11. SONNEBORN, T. M. *Growth*, **11**, 291 (1947).
12. DEROPP, R. S. *Am. J. Botany*, **34**, 248 (1947).

Structural and Insecticidal Relationships in Rotenone, Methoxychlor, and DDT

R. W. Hummer and E. E. Kenaga

The Dow Chemical Company, Midland, Michigan

In the course of investigating the relationship of chemical structure to insecticidal activity, molecular models of known insecticides were constructed, using Fisher-Hirschfelder-Taylor atom models. In this way an interesting structural similarity among rotenone, DDT,[1] and methoxychlor[2] was encountered. This similarity is not at all obvious without the use of the models.

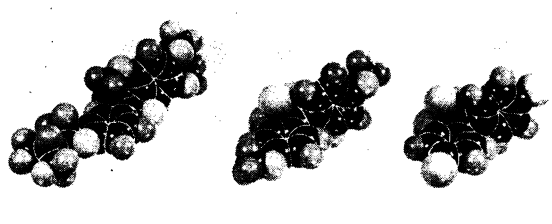
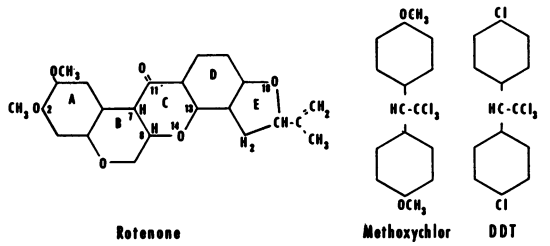


FIG. 1. Molecular models of rotenone, methoxychlor, and DDT.

Fig. 1 shows the molecular models of rotenone, methoxychlor, and DDT. The configuration of rotenone, (*1*) shown is *cis* at the 7–8 bond. The structural formulas of these compounds are:



La Forge *et al.* (*2*) have discussed a series of reactions of rotenone involving hydrolysis of the 13–14 bond followed by oxime formation and ring closure with a nitrogen between the 11 and the 14 positions. Study of the models indicates that this ring closure is strongly hindered sterically for the *trans* position and can be effectuated readily only if the configuration is *cis* at the 7–8 bond. Moreover, the ease of dehydrogenation of rotenone at the 7–8 bond would also seem to indicate that the configuration of the natural product is *cis*.

For the three molecules pictured in Fig. 1, the notable points of similarity are the angles of intersection of the axes of the benzenoid rings and the comparable slopes of the planes of these rings. In rotenone the benzenoid rings are essentially fixed with relation to each other, because of the interconnecting ring structures. In DDT and methoxychlor also, steric hindrance by the trichloromethyl group restricts the benzene rings to a relatively fixed position. In general one of the rings will be quite fixed, whereas the other may oscillate but not perform a complete, free rotation. The configuration of DDT and methoxychlor shown in the figure is, of course, only one phase in the oscillatory movements of these molecules. The rotation-hindering trichloromethyl group forms a "bump" on the molecule which coincides with a similar "bump" in the rotenone molecule resulting from puckering of ring B.

Further comparison of the molecular models indicates a close correspondence in intramolecular distances in these three substances. For example, the distance between the oxygens at positions 2 and 19 in rotenone is very close to the distance between the 2 methoxyl oxygens in methoxychlor and between the *p, p′* chlorines in DDT. The models are made to approximate scale, and these distances correspond to within 1 A. It is interesting to note that, regardless of rotation or oscillation in the DDT-type molecule, the distance between atoms in the *p, p′* positions remains constant. The importance of such molecular

[1] 1,1,1-Trichlor-2,2-bis(*p*-chlorophenyl) ethane.
[2] 1,1,1-Trichloro-2,2-bis(*p*-methoxyphenyl) ethane.