their separating and assuming a spherical shape. This is illustrated in Fig. 5—a stage where complete dissolution of the middle lamella had occurred, resulting in the centripetal progression of abscission.

The hypothesis that auxin inhibits abscission through its effect on the maintenance of membrane-integrity was further supported by experiments in which 1 percent IAA in lanolin-water emulsion was applied distally on debladed petiolar stumps. Hand sections of these petioles, 3, 5, and 10 days after deblading, revealed a continuity of air in the intercellular spaces traversing the abscission zone.

A similar mechanism may be operative, attending a drop in auxin level, during tissue senescence in certain fleshy fruits as well as in abscission of determinant organs in other plants.

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25 March 1957

Differential Responses to Population Pressures by Normal and Dwarf Lines of Maize

Dwarf or semidwarf variants in such species as sorghums, apples, beans, and peas are of economic importance. Suggestions have been made that one of the numerous, genetically different, semidwarf mutants of maize might also be useful agriculturally. The rationale has been that the shortened stalk of such dwarf types would markedly reduce the incidence of stalk breakage and root lodging which make machine harvesting difficult. Therefore, these types would be acceptable if their yield were equal to or only slightly below normal. Leng (*1*) recently reported that single crosses made from inbreds which had been converted to the recessive mutant *brachytic* 2 were satisfactory in yield.

Field observations in our laboratory of dwarf types had indicated that they might actually possess yielding ability beyond their normal counterparts. These observations motivated the start of a testing program in 1954 designed to characterize the response of both dwarf and normal types to population pressures at high levels of fertility and with adequate water available. Work during the past 3 years has shown that at least one recessive semidwarf mutant *compact* (*ct*) has a significantly different response to population pressures from the normal inbred Hy or two other semidwarf mutants *reduced* and *brachytic* 2 (*2*).

The *compact* mutant arose by spontaneous mutation in a stock of Hy2 and has previously been designated as Hy2 (*dwarf*). It has been compared for 3 years in replicated yield tests to the normal Hy inbred, for 2 years to a Hy conversion to *reduced*, and for 1 year to a Hy conversion to *brachytic* 2. Thus all types under test were inbreds which had roughly comparable genotypes except for the loci conditioning plant height. Data on grain and stover yields, leaf areas, mineral content of the leaves, flowering dates, and ear characteristics were collected for each strain at various population levels. Figure 1 shows the yield in bushels per acre at four populations for 1956, the only year in which all four genetic strains were compared. Note the attainment of a yield optimum at 26,000 plants per acre by the *compact* strain and only slight decreases in yield at the higher populations; this is in marked contrast to the other types tested. Note, too, that at 26,000 plants per acre, the *compact* strain yields more than the normal strain yields at 13,000 plants per acre. Such a superiority in yield for the *compact* strain at higher populations over the normal strain at any population tested was also noted in the 1954 and 1955 tests.

The test reported here was made with inbred material, Hy, and various semidwarf mutants inserted into a Hy background. It would be unwarranted to extrapolate from the responses of inbreds to population pressures to the responses of hybrids. Other tests have shown, however, that normal hybrids with Hy as one parent react similarly to Hy with regard to population increases. Further, *reduced* hybrids respond in much the same manner as *reduced* Hy while *brachytic* 2 hybrids are similar to *brachytic* 2 Hy.

The *compact* strain clearly has a different response to high populations from three other comparable strains carrying other genes affecting plant size. This response enables *compact* to yield slightly more at high populations than the normal type yields at any population tested. Further, inbreds and hybrids carrying the same dwarfing gene (either *rd* or *br*2) react similarly to population pressures. These findings suggest that yield increases over normal hybrids can be obtained by the use of hybrids converted to the *compact* gene. Preliminary tests of partially converted hybrids will be made in 1957 (*3*).

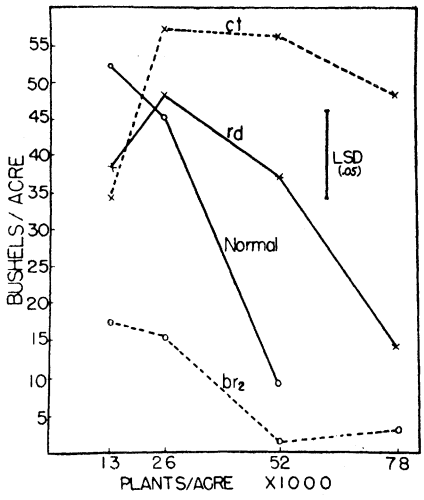

Fig. 1. Yields in bushels per acre for four inbreds which are genetically similar except for major genes that affect plant size (1956).

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2. We are indebted to W. R. Singleton for seed of the *reduced* types and to E. R. Leng for seed of the *brachytic* types.
3. This article is journal paper No. 1101 of the Purdue University Agricultural Experiment Station.

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Selective Blockade of Excitatory Synapses in the Cat Brain by γ-Aminobutyric Acid

γ-Aminobutyric acid (GABA) has been identified (*1*) as an active principle in the inhibitory substance (factor I) that can be extracted from the mammalian brain (*2*). Both the extract and the compound have been tested, chiefly on the crayfish stretch receptor (*1–3*), and both diminish the depolarizing electrogenesis caused by stretch of its mechano-sensitive dendrites. GABA also appears to augment the inhibitory postsynaptic potential of the receptor (*3*). On the dog brain, both "excitatory" and "inhibitory" effects by GABA and other amino acids have been reported (*4*).

The mode by which a synaptic drug exerts its overt effects in the central nervous system is often difficult to determine (*5, 6*). For example, although

strychnine and Metrazol are both classified as "stimulants of the central nervous system" (*7*), only the latter is truly a synaptic excitant (*6*). The overt excitatory activity produced by strychnine is caused by selective blockade of inhibitory synapses. Methods, developed in this laboratory, that can distinguish the modes of action of synaptic drugs have been used to analyze the effects of GABA in the mammalian central nervous system.

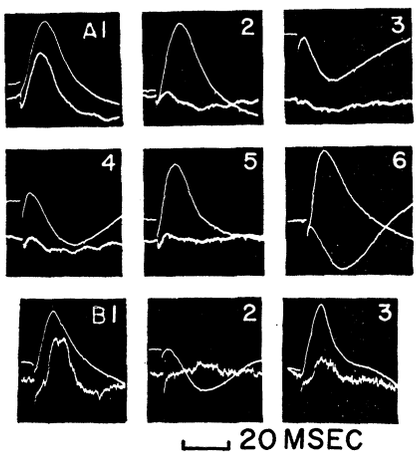

Fig. 1. Effects of GABA on postsynaptic potentials evoked in cerebral and cerebellar cortex of cat by surface electric stimuli. The stimuli were 0.1 msec square pulses, applied through a pair of closely spaced 100-μ silver wires, Teflon-insulated except at their cross sections, which rested lightly on the cortical surface. The active recording electrode was a silver ball about 1 mm distant from the stimulating electrodes. (*A*) Surface-negative responses in cerebral cortex (suprasylvian gyrus) shown on upper cathode-ray oscillograph traces: 1–5, lower trace, simultaneously registered the activity recorded with a 100-μ wire electrode, insulated except at the tip; 1 and 2, responses before application of GABA; 1, both recording electrodes on surface; 2, wire inserted 0.4 mm below the surface. In 3–5, recording conditions were as in 2; 3, reversal of surface negativity developing 20 sec after application of GABA to the region of the electrodes (3 drops, 10^{-2} w/v); 4, 30 sec later, full reversal; 5, 1 min after flushing the cortex with saline solution; 6, superimposed dendritic responses recorded from the surface before and after applying GABA, showing relative time relations. (*B*) Upper trace, from cerebral; lower trace, from cerebellar cortex, simultaneous oscillographic recordings: 1, surface-negative responses produced by a stimulus to each cortical surface; 2, topical applications of GABA (5 drops, 10^{-3} w/v) to each site reversed the response of the cerebral activity but diminished and abolished that of the cerebellar cortex. The cerebellar effect, blockade of its excitatory axodendritic synapses, developed more slowly than did the action on the cerebral cortex; 3, recovery of both cortical responses 2 min after flushing with Ringer's solution.

Injected into unanesthetized succinylcholine-paralyzed cats, even in high concentrations (100 mg/kg, intravenously, in one dose), GABA has only minimal, transient effects on responses evoked in the cerebral or cerebellar cortex by diverse pathways. When applied topically, buffered at *p*H 7.4, GABA in dilutions of 10^{-5} weight for volume exerts marked effects on evoked cortical responses. Rapid, pronounced actions occur with application of strong solutions (Fig. 1). These are quickly reversible upon flushing of the cortical surface with Ringer's solution, and the cycle may be repeated many times.

The effects of GABA differ characteristically in the cerebral and cerebellar cortex, thereby defining one mode of its action. The surface-negative postsynaptic potentials of the apical dendrites produced in the cerebral cortex by various stimuli (*6*) are reversed by GABA (Fig. 1, A1-6). The consequent surface-positivity (Fig. 1, A3, 4, 6) is almost a mirror image of the previous negativity (Fig. 1, A6). This large change in surface potentials has no correlate anywhere below the cortical surface. The reversed activity, therefore, is a "standing" potential characteristic of postsynaptic potentials (*5*), and the positivity induced by GABA represents hyperpolarizing synaptic electrogenesis of the apical dendrites.

Applied to the cerebellar cortex (Fig. 1, B1-3) GABA also eliminates the surface negative dendritic postsynaptic potentials, but does not induce positivity (Fig. 1, B2). The difference in action at the two sites is ascribable to the relative paucity of inhibitory synapses in the cerebellar cortex (*6*). It also indicates that at least one mode of action of GABA is to block, selectively, the electrogenesis of depolarizing, excitatory synapses. The reversal of potential observed in the cerebral cortex may be accounted for as the disclosure of hyperpolarizing postsynaptic potentials that are normally masked by countervailing synaptic depolarizations (*6*).

Of a number of substances tested thus far, including various amino acids, only β-alanine exerts effects similar to, but weaker than, those of GABA, a relationship that also obtains for the crayfish stretch receptor (*2*). Cytidine and uridine, which sustain electrocortical activity of the perfused brain (*8*), caused no marked effects. Therefore, these nucleosides probably act on metabolic processes of the brain, not directly on synaptic electrogenesis.

Picrotoxin, *l*-carnosine, and strychnine "antagonize" the effects of GABA but by two characteristically different modes (Fig. 2). Carnosine, the "excitin" of Hayashi (*9*), and picrotoxin are, like Metrazol, excitants of synaptic activity

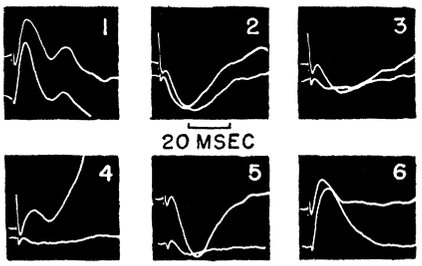

Fig. 2. Interaction of GABA with picrotoxin and strychnine. Simultaneously recorded responses to independent stimulations of homologous points of right (upper trace) and left (lower trace) anterior suprasylvian gyrus, the cerebral hemispheres being disconnected by callosal section: 1, initial dendritic postsynaptic potentials; 2, 30 sec after application of GABA (3 drops, 10^{-2} w/v) to each side. Picrotoxin (2 drops of 3×10^{-3} w/v solution) was then applied to right cortex and strychnine sulfate (2 drops of 5×10^{-3} w/v) to the left; 3, 20 sec later; 4, 1 min later, after another application of the drugs; 5, 2 min later and 30 sec after another application of GABA. Whereas the effects on the strychninized (left) cortex were minimal, marked antagonism to picrotoxin is seen; 6, 15 min after repeated washing of the cortex with Ringer's solution. Recovery of previously inactive strychninized side is the more rapid, probably denoting persistent blockade of inhibitory synapses.

and thus (*5*) act as competitive antagonists of GABA. Strychnine merely eliminates the surface positivity by blocking the inhibitory synapses that remain after GABA has blocked the excitatory synapses.

The foregoing experiments demonstrate, therefore, that GABA blocks depolarizing, excitatory synaptic electrogenesis in the mammalian brain as it also blocks that of the mechanosensitive receptor (*1–3*). The tests carried out thus far do not, however, preclude the possibility that GABA may also augment inhibitory postsynaptic potentials of the brain (*10*).

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* Scholar, Sister Elizabeth Kenny Foundation; work supported in part by Donner Foundation.
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25 February 1957

Serotonin and Histamine in Mast Cells

Previous investigations have demonstrated the presence of heparin (*1*) and histamine (*2*) in mast cells of various animal species. Asboe-Hansen (*3*) has presented evidence that these cells also produce hyaluronic acid. Recently, Benditt *et al.* (*4*) identified serotonin (5-hydroxytryptamine) in mast cell suspensions prepared from peritoneal washings of rats and found serotonin in rat skin in proportion to its mast cell content. Similar studies by Parratt and West (*5*) suggested that much of the serotonin, as well as histamine, in rat skin is held in the mast cells. The studies of Rowley and Benditt (*6*) in rats indicate that the edema-producing action of agents which damage mast cells may be mediated by "release" of both of these potent amines.

This is primarily a study of serotonin and histamine in mast cells of three animal species: mouse, dog, and man. Mice of the inbred strain DBA/2 and (BALB/c by DBA/2)F_1 hybrids bearing transplantable mast cell neoplasm P185 in subcutaneous solid tumor and ascitic forms (*7*) were made available for study during the 26th to 30th transfer generations. Several other reticular neoplasms in mice were obtained for assay. A first- and second-generation transplantable subcutaneous mast cell tumor of the dog was also used (*8*). Urine specimens were obtained from two patients with urticaria pigmentosa, a condition characterized histologically by dense accumulations of mast cells in the skin. In addition, a skin biopsy was obtained from one of these patients (*9*). Portions of skin from other patients were also studied.

The mouse tumor was composed chiefly of closely packed mast cells, whereas the dog tumor contained a considerable amount of fibrous tissue and a few eosinophils. Details of the discovery, technique of transplantation, and morphology of the mouse tumor have been presented by Dunn and Potter (*7*). One of the patients with urticaria pigmentosa also had skeletal involvement. A report of this case has been made by Zak, Covey, and Snodgrass (*10*). Chemical methods for the measurement of histamine, serotonin, and the serotonin metabolite, 5-hydroxyindoleacetic acid (5HIAA), were those developed previously in this laboratory (*11*).

The serotonin and histamine levels of various tissues are shown in Table 1. The solid mast cell tumors in mice contained large amounts of both serotonin and histamine, while in the dog tumor only the histamine concentration was elevated. Catechol amines could not be detected in the mouse tumor, and paper chromatographic studies showed serotonin to be the only 5-hydroxyindole present. Skin from the patient with urticaria pigmentosa contained a high level of histamine but insignificant amounts of serotonin. Measurements of urinary 5-hydroxyindoleacetic acid in the two patients with urticaria pigmentosa gave values of 5.1 to 7.2 mg/day (normal—2.0 to 9.0), confirming the finding that human mast cells do not contain serotonin.

It is known that carcinoid tumors, derived from the chromaffin cells of the intestinal tract, contain large amounts of serotonin and that patients with metastatic carcinoid excrete excessive amounts of 5-hydroxyindoleacetic acid in the urine (*12*). Recently, Waldenström *et al.* (*13*) reported an elevated urinary excretion of histamine in some of these patients and suggested the possibilities that some tumors might produce both serotonin and histamine or that the serotonin produced might liberate histamine from other tissues. Three carcinoid tumors were found to contain the usual large amount of serotonin but only a small amount of histamine (Table 1). If this is characteristic of all carcinoid tumors, then the increased histamine excretion by carcinoid patients must be a secondary phenomenon.

Table 1. Serotonin and histamine content of various tissues.

Material	Serotonin (μg/g)	Histamine (μg/g)
Tumors		
Mouse mast cell*	80–180	470–560
Dog mast cell	< 0.2	315, 160
3 human carcinoids	360, 570, 800	3.4, 2.0, 0.8
Other mouse tumors†	< 0.2	< 1
Human skin		
Urticaria pigmentosa	< 0.25	44
3 normals	< 0.7	4.7, 5.4, 5.0

* Several assays were done on pooled solid tumor specimens from a total of 20 DBA/2 mice.
† Several transplantable tumors (transfer generation indicated in parenthesis) were analyzed. In strain DBA/2: one type-A reticulum cell sarcoma, P228 (39) and one type-B, P195 (19), two lymphocytic, P228 (90) and P388 (63), and one granulocytic, P1081 (3); in strain C57BL: one lymphocytic, P1162 (5); in strain C3H: one well-differentiated plasma cell neoplasm, X5563 (8). A somewhat elevated amount of serotonin (22 μg/g) was found in a pooled sample of four poorly differentiated plasma cell neoplasms, 70429 (36) in strain C3H.

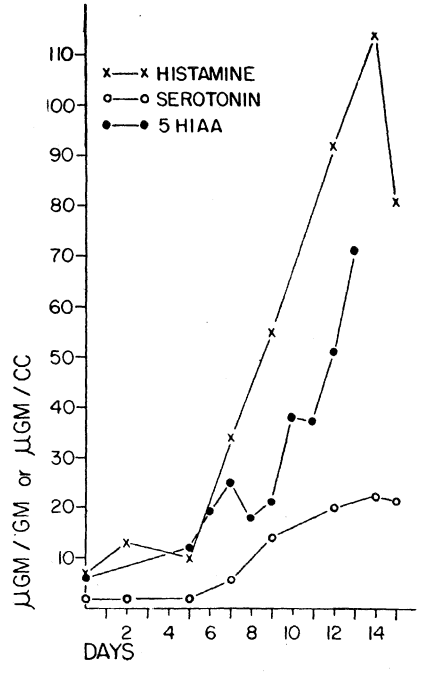

Fig. 1. Effect of mast cell tumor transplantation (ascitic form) on serotonin and histamine contents of whole mice (μg/g) and urinary excretion of 5-hydroxyindoleacetic acid (μg/ml). The animals usually died suddenly at 14 to 16 days.

The increased production of serotonin and histamine in tumor-bearing mice was studied in two ways. First, a group of 16 mice, (BALB/c by DBA/2)F_1, were placed in a metabolism cage after intraperitoneal injection of tumor cells, and urine was collected for assay for 5-hydroxyindoleacetic acid. Control values were obtained on a group of eight nontumor mice of the same strain, which were studied for 2 weeks. Second, serotonin and histamine assays were done on homogenates of pairs of whole mice at various intervals after tumor transplantation. The results, shown in Fig. 1, indicate that there was a marked production of both substances in association with growth of tumor. A simple test for excretion of excessive amounts of urinary 5-hydroxyindoles, useful in the clinical diagnosis of carcinoid (*14*), also became positive in the tumor-bearing mice.

Assuming that the abnormal mast cells in the three species studied produce serotonin or histamine, or both, in a manner comparable to that of normal mast cells, a marked species variation is clearly