Identification of a Cyanogenetic Growth-Inhibiting Substance in Extracts from Peach Flower Buds

Abstract. A cyanogenetic substance capable of completely inhibiting growth of pea sections has been isolated from dormant peach flower buds. This substance was identified as mandelonitrile (*dl*-benzaldehyde cyanohydrin) by infrared spectographic analysis and paper chromatography.

The presence of a cyanogenetic substance capable of completely inhibiting growth of pea sections has been reported to occur in peach flower buds (*1*). Recently, Jones (*2*) followed the level of total cyanide in peach leaves and peach flower buds from July through February. Total cyanide was found to increase rapidly, beginning about the time rest ended, and was highest in buds in the balloon stage. It was proposed that cyanide might be associated with rest in peach flower buds.

Earlier attempts by us to isolate and identify the cyanide compound resulted in failure, primarily because they were directed toward obtaining a sample of pure crystalline material. When it had been determined that the material was a liquid, it was isolated in a relatively pure form by the following method.

Dormant peach flower buds were collected and extracted for 24 hr in two changes of diethyl ether. The ether extracts were combined, and the ether was evaporated off at room temperature. The residue was then dissolved in ethyl acetate and filtered. The material left by the evaporation of the ethyl acetate was picked up in distilled water and filtered. The water fraction, which was extracted with diethyl ether in a separatory funnel, was discarded. Upon evaporation of the ether fraction, a yellow oily liquid remained, which had an odor similar to that of mandelonitrile.

On the assumption that this substance might be mandelonitrile (*dl*-benzaldehyde cyanohydrin), we obtained a sample of the latter, for comparison with the unknown substance, from the K & K Laboratories, Jamaica, N.Y.

The unknown material and mandelonitrile were chromatographed on Whatman No. 1 filter paper in a butanol, ethanol, water solvent (1:2:3). The unknown material had an R_F of 0.95, identical with that of mandelonitrile. Both the unknown material and mandelonitrile gave a rusty red color when the chromatograms were sprayed with sodium picrate solution. Both materials caused complete inhibition of growth of pea sections when the spots were cut out of the chromatogram and used in growth tests. As little as 10^{-3} mole of mandelonitrile was sufficient to completely inhibit growth of pea sections.

Mandelonitrile and the unknown substance were chromatographed in a hexane, ethanol, water solvent (1:1:2). The unknown material had an R_F of 0.78, identical with that of mandelonitrile.

Samples of the unknown material and of mandelonitrile were sent to Sadtler Research Laboratories, Philadelphia, for infrared examination. The spectrum of mandelonitrile and that of the unknown were very similar (Fig. 1). It was proposed that the differences in the spectra might be due to the presence of prunasin (*d*-mandelonitrile glucoside), a closely related compound. Spectographic analysis of a commercial sample of prunasin and of a mixture of prunasin and mandelonitrile indicated that the unknown sample sent in for analysis did contain a small quantity of prunasin. Prunasin is reported to be the first breakdown product of amygdalin, mandelonitrile being formed upon the hydrolysis of prunasin.

The results of these investigations indicate that the cyanogenetic growth-inhibiting substance isolated from peach flower buds is mandelonitrile, a powerful growth-inhibiting substance. To our knowledge, neither mandelonitrile nor prunasin has been previously reported to occur in peach flower buds (*3*).

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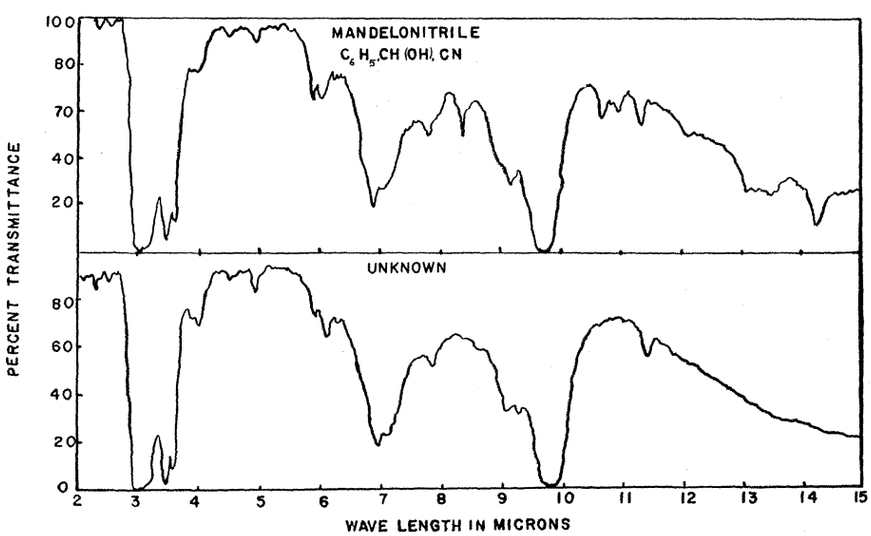

Fig. 1. Infrared absorption spectra of mandelonitrile (top) and of the material isolated from dormant peach flower buds (bottom).

References and Notes

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3 April 1961

Reaction of Human Sera with Mammalian Chromosomes Shown by Fluorescent Antibody Technique

Abstract. Certain human sera, including sera from five patients with lupus erythematosus, react with mammalian chromosomes. If chromosomal preparations are exposed first to the serum and then to horse antihuman globulin conjugated with fluorescein, the chromosomes will fluoresce. Sera having this activity appear to react with all the chromosomes of the cell.

Sera from patients with certain diseases, particularly lupus erythematosus, react with the nuclei of human cells. When serum with antinuclear activity is incubated with human cells, gamma globulin from the serum reacts with the nuclei of the cells. If the cells are then incubated with rabbit or horse antihuman globulin, which has been conjugated with fluorescein, the nuclei will fluoresce under ultraviolet light (*1*). Sera from a number of patients with lupus erythematosus, Sjögren's syndrome, and several other diseases

Table 1. Nuclear fluorescence after treatment of cells with certain sera and staining with fluorescein-labeled horse antihuman globulin.

Lupus erythematosus	Nephrosis	Sjörgen's syndrome	Normal donors
Sera tested			
6	2	3	3
Sera positive against acetone-fixed mouse liver nuclei (1:8 dilution)			
6	2	3	0
Sera positive against ITA nuclei (chromosomal preparation)*			
5	1	0	0
Sera positive against CHA† nuclei (chromosomal preparation)			
5	1	0	0

* ITA, a strain of human euploid fibroblasts developed from skin of a normal donor. † CHA, a line of Chinese hamster fibroblasts (see text).

have been shown to possess this activity in high titer (*2, 3*). In some cases, discernible antinuclear activity has been found at titers in excess of 1:4000. Antinuclear activity is uncommon in sera from normal donors and is very rare at titers greater than 1:8. A titer of 1:8 has therefore been arbitrarily designated as diagnositic of abnormal sera.

The antinuclear activity when present is demonstrable against the patient's own cells, cells from other donors, and other mammalian cells. The significance of this activity in the diseases in which it is commonly found is not fully understood. The activity may be related in some instances to the pathogenesis of the disease.

The material within the nucleus with which the serum reacts has been studied in the case of patients with lupus erythematosus. It would appear that the serum reacts with deoxyribonucleic acid and with nuclear histones (*4*). Some other nuclear antigens may also be involved.

Since the reaction is between the serum and the nucleus of the cell, and since it is histologically demonstrable, we have inquired whether the chromosomes are involved in the reaction. One might expect them to be, partly because of the character of the known antigens referred to above and partly because of the extent of the nuclear reactions—that is, the reaction is not confined to the nuclear membrane. We were particularly interested in the following questions.

1) In lupus erythematosus sera which have antinuclear activity, will the chromosomes fluoresce? If so, do all of them fluoresce, or some of them, or only certain segments?

2) Do sera from patients with Sjögren's syndrome and other diseases where antinuclear activity is sometimes present show the same pattern of chromosomal fluorescence as lupus erythematosus sera?

Sera from six patients with lupus erythematosus, three with Sjögren's syndrome, and two with nephrosis were used. All the sera had antinuclear activity, detectable at a dilution of at least 1:8 when tested by the fluorescent antibody technique with acetone-fixed mouse liver sections (*5*). Sera from three normal controls were also used. These sera had no detectable antinuclear activity when tested under the same conditions used for the abnormal sera.

Chromosomal preparations from two strains of euploid human fibroblasts and human peripheral blood from a normal donor were used. The lines were developed by the usual technique (*6*). In addition, an established line of Chinese hamster fibroblasts (*7*) and the S-3 subline (*8*) of the Hela group (*9*) were used. Chromosomal preparations of tissue culture cells were made by the technique of Tjio and Puck (*10*), and those of peripheral blood cells were prepared by the method of Moorhead *et al.* (*11*). After fixation and air drying of the cultured cells and mouse liver sections, the test sera were allowed to react with the preparations for 20 min and subsequently were stained with fluorescent antibody by the technique of Goodman *et al.* (*5*). The specimens were stimulated with ultraviolet light at wavelengths of 3900 to 4400 A and 3600 to 3700 A, and fluorescence photomicrographs were taken as described by Tobie (*12*).

In Table 1 the reactions of the undiluted sera used with the nuclei of two lines of cultured cells are given. Similar results were obtained with the other lines used and with peripheral blood cells in short-term culture. Note that although the sera from three patients with Sjögren's syndrome had antinuclear activity against acetone-fixed mouse liver cells, they did not react with the nuclei of the cultured cells (Carnoy-fixed).

The sera which did show antinuclear activity against the cultured cells appeared to react with all the chromo-

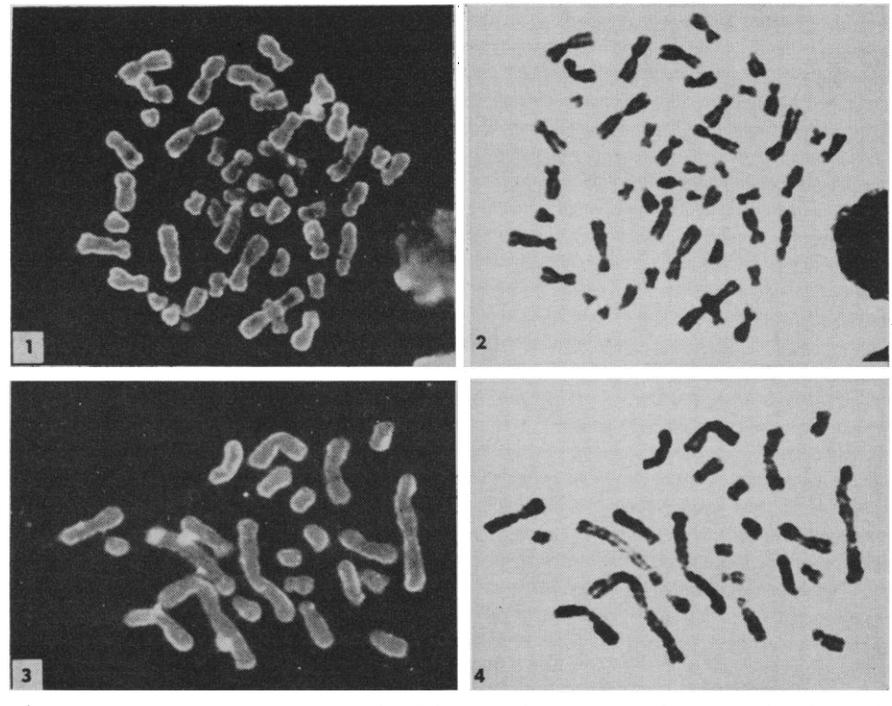

Fig. 1. Fluorescence photomicrograph of human chromosomes from peripheral blood culture stimulated with ultraviolet light (3900 to 4400 A). Chromosomes were treated with a dilution of a lupus erythematosus serum (1:50) and stained with fluorescein-labeled horse antihuman globulin. Fig. 2. Photomicrograph of the chromosomes in Fig. 1 taken with visible, white light; orcein stain. Fig. 3. Fluorescence photomicrograph of Chinese hamster chromosomes from tissue culture stimulated with ultraviolet light (3900 to 4400 A). Chromosomes were treated with a dilution of a lupus erythematosus serum (1:50) and stained with fluorescein-labeled horse antihuman globulin. Fig. 4. Photomicrograph of the chromosomes in Fig. 3 taken with visible, white light; orcein stain.

somes of the cells. When incubated first with these sera and then with horse antihuman globulin conjugated with fluorescein, the euploid fibroblasts and the peripheral blood cells (Fig. 1) showed 46 discrete fluorescent chromosomes. When, after fluorescent staining, the same cell was restained with acetic acid–orcein and photographed in visible light, every chromosome staining with fluorescent antibody also stained with orcein (Fig. 2). Similar results were obtained with the Chinese hamster line (Figs. 3 and 4). The sera from the normal donors, one patient with lupus erythematosus, one patient with nephrosis, and from the three patients with Sjögren's syndrome did not lead to any chromosomal fluorescence. Easily detectable chromosomal fluorescence was obtained with one lupus erythematosus serum at a dilution of 1:320.

These investigations suggest that certain human sera react with mammalian chromosomes and that, moreover, the reaction is with the full chromosomal complement of the cell. It may be of interest to see whether sera which are more specific in their chromosomal reactions can be found. It may also be useful to try to absorb some of the antinuclear activity with nuclear fractions or with nuclei from one species before incubating the sera with chromosomes from another species.

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15 May 1961

Possible Mode of Antidepressive Action of Imipramine

Abstract. Imipramine augmented and prolonged methamphetamine-induced increases in the rate of responding of rats working for "rewarding" hypothalamic and midbrain stimulation. In contrast, chlorpromazine antagonized the effects of methamphetamine on self-stimulation. These opposite psychopharmacological effects are consistent with the different clinical effects of these drugs and suggest a mechanism for the antidepressive action of imipramine.

Despite the similarity between their chemical structures, imipramine and chlorpromazine differ in certain of their clinical actions. As a rule, chlorpromazine calms agitated patients, while imipramine elates depressed ones (*1*). Pharmacological tests have provided no basis as yet for the qualitative difference in these clinical actions. Most often, the same pattern of results is seen with the two drugs in the laboratory (*2*).

As a basis for our experiments, we have tentatively taken the view that agitations and depressions result from abnormalities in motivational and reward processes—agitation from pathological overactivity of reward processes (*3*), and depression from underactivity. On this view, it may be supposed that drugs effective against agitation inhibit an excessive reward activity, and that drugs effective against depression enhance a deficient reward activity.

The brain system that subserves these functions of motivation and reward has been made accessible to experimental investigation by the development of precise methods both for stimulating deep in the brain and for measuring changes in behavior. The self-stimulation technique, in which an intact and unanesthetized animal is trained to obtain brief electrical stimulations of its own brain by performing an arbitrary response (such as pressing a lever), is the method of choice for these studies (*4*). In this paper, we report the effects, and particularly the interactions, of imipramine, chlorpromazine, and amphetamine on self-stimulation.

Adult male rats were implanted with permanent bipolar platinum electrodes in the posterior hypothalamus or midbrain tegmentum. After they recovered, they were trained to stimulate their brains electrically by pressing a lever. Each response delivered a 0.15-sec current train of moderately rewarding intensity (about 0.4 ma). The stimulating wave form was a square pulse of 0.2-msec duration presented at 100 pulses per second through a cathode follower output stage and a shielded, low-impedance isolation transformer to the electrodes (*5*). These stimulating conditions are relatively noninjurious and thus allow the self-stimulation base lines to be stable for many months. With properly placed electrodes, the training often requires only a few minutes.

After the rats became expert at self-stimulation, the stimulating current was lowered individually for each rat, to a level in the vicinity of the threshold for self-stimulation (0.1 to 0.25 ma). Drug tests were begun after many sessions under the minimal current conditions, after the response rates had stabilized at a low level. At least one week intervened between drug dosings. All doses reported are expressed in terms of the total salt.

Our first experiments compared the effects of chlorpromazine and imipramine. The results were somewhat disappointing, as both drugs were found to inhibit self-stimulation. Chlorpromazine was about ten times more potent as an inhibitor than imipramine. These results coincided with published pharmacological findings.

We then learned of work of Carlton (*6*) who found that imipramine augments the facilitating action of amphetamine on conditioned avoidance behavior. We knew from earlier studies that amphetamine is a highly active agent in the self-stimulating test; specifically, it lowers the threshold for electrical reinforcement, indicating a facilitating action on structures of the reward system (*7*). We therefore set about to determine the effects of imipramine and chlorpromazine on the amphetamine or methamphetamine) response in the self-stimulation test.

Figure 1 summarizes our main findings. Six weekly sessions of self-stimulation performance under various drug conditions are depicted for a rat implanted in a reward area of the midbrain tegmentum. The depressed base line rate of self-stimulation generated by the threshold current intensity is seen in Fig. 1*A*. A small dose of *d*-methamphetamine hydrochloride (0.25 mg/kg) produced a clear increase in rate beginning about 15 min after the injection (Fig. 1*B*). The methamphetamine dose was carefully selected to provide a moderate, but unequivocal, effect. Pretreatment with 3 mg/kg of