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Senescence Inhibition and Respiration

Abstract. Freshly harvested asparagus spears treated with N^6-benzyladenine and then held in the dark at 21°C for 136 hours showed a lower respiration rate, as measured by CO_2 evolution, than nontreated spears. Associated with the lower respiration rate were proportional decreases in postharvest spear elongation and weight loss through desiccation.

Since the discovery of the biological activity of kinetin (6-furfurylaminopurine) in cell division (*1*), many studies have been made in which this and similar compounds have been evaluated in cell division of tobacco tissue, germination of lettuce seeds, and cell enlargement in leaf disks of radish (*2*). In addition, some rather striking effects on protein metabolism, chlorophyll breakdown, and the capacity of excised leaves to withstand stress have been reported (*3*). N^6-benzyladenine, a compound in which the furan ring in kinetin has been replaced by a benzene ring, has shown some effect in preventing postharvest breakdown of head lettuce when applied shortly before harvest or as a postharvest dip (*4*). This compound reportedly has a similar effect on other green vegetables, including asparagus (*5*).

Freshly harvested asparagus spears were obtained from a commercial processor and held for 24 hours at 5°C; the apical ends were trimmed to 5-inch length and then dipped in a $5 \times 10^{-5}M$ solution of N^6-benzyladenine or water. Eight 500-g samples of the treated spears and like samples of nontreated spears were placed in respirometers (*6*) and held in the dark at 21°C. The CO_2 evolution was measured every 8 hours for 136 hours, whereupon the spears were removed and their weight loss and length were determined.

The observed mean values and the fitted regression lines describing CO_2 evolution in treated and nontreated asparagus spears (Fig. 1) indicate that treatment with N^6-benzyladenine materially decreased respiration. Theoretical values of total CO_2 evolution during the 136-hour interval, obtained by integration of the regression equations, were 303 mg/kg for the nontreated and 257 mg/kg for the treated spears. These calculated values correspond very closely to the observed values of 295 and 253 mg/kg. The area between the curves constitutes the total respiration inhibition. This calculation yielded a value of 48 mg of CO_2 per kilogram, indicating about a 16-percent reduction in respiration through the first 120 hours in the N^6-benzyladenine-treated samples. Observed values for these differences were 47 mg of CO_2 per kilogram and 16 percent, respectively. After 120 hours, the nontreated samples evolved CO_2 at a lesser rate than did the treated, as a probable consequence of an initially more rapid depletion of the metabolites in the nontreated samples.

Weight losses in 136 hours were 7.25 and 5.95 percent in the nontreated and treated samples, respectively. Though the differences in weight loss were largely a function of the degree of desiccation, it is noteworthy that the decrease in water loss of approximately 18 percent in the treated samples was comparable to the decrease in respiration. Furthermore, nontreated spears elongated an average of 8.2 percent, and treated spears, of 7.0 percent. This difference (15 percent less elongation in the N^6-benzyladenine-treated spears) was again directly proportional to the amount of respiration inhibition.

These data suggest that respiration as measured by CO_2 evolution, desiccation as measured by weight loss, and growth as measured by elongation are all proportionally inhibited in harvested asparagus spears following postharvest application of N^6-benzyladenine.

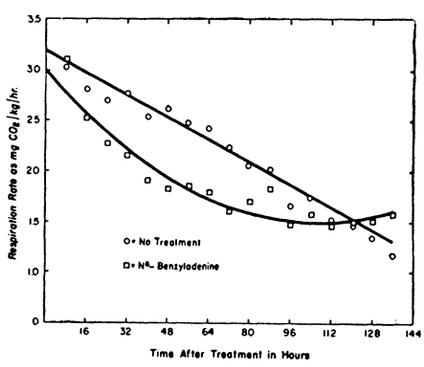

Fig. 1. Respiration of treated and nontreated asparagus held in the dark at 21°C.

It is further suggested that many of the phenomena observed by others, such as chlorophyll retention, changes in nitrogen metabolism, and general inhibition of senescence in green plant tissues subsequent to treatment with kinetin and related compounds, may be a consequence of respiration inhibition of the type described in this report (*7*).

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3 July 1961

Explanation of Cocaine Desensitization of Blood Pressure Responses to Ephedrine

Abstract. It is suggested that cocaine desensitizes ephedrine blood pressure responses by depletion of norepinephrine depots. This was shown by reversing cocaine desensitization through the administration of agents such as bretylium, which has a sparing effect on norepinephrine, and by the infusion of norepinephrine itself.

A number of workers (*1*), since the original discovery by Tainter (*2*), have shown that cocaine in large doses diminishes or abolishes the pressor responses to ephedrine. The exact mechanism of this antagonism has not been explained. We therefore attempted in the present study (*3*) to elucidate this phenomenon according to the theory of one of us (T.K.). This theory postulates that large doses of cocaine exhaust the sympathetic neurohumor depots and, owing to this mobilization, may change physicochemical parameters of cellular membranes (*4*). Therefore, three alternatives were considered: ephedrine should be protected from cocaine desensitization by the administration of (i) substances which prevent such neurohumoral release, or (ii) substances which protect the neurohumor from metabolic destruction, or (iii) the neurohumor itself.

Male mongrel dogs, weighing from 6 to 10 kg, were anesthetized intraven-

ously with sodium pentobarbital (30.0 mg/kg). Blood pressure was recorded from one carotid artery with the usual hemodynamic setup. Both femoral veins were canulated for either drug injection or infusion. Polygalacturonic acid glycoside (Mepesulfate, 20.0 mg, total dose) was injected intravenously as an anticoagulant. Additional doses of 10.0 mg each were given at hourly intervals, as needed; the arterial pressure system was filled with a 0.25 percent solution. All dogs were given atropine sulfate (2.0 mg/kg), intravenously, after this procedure, to prevent reflexes mediated by parasympathetic nerves. Doses of *dl*-ephedrine sulfate were administered at hourly intervals, in volumes constant for each experiment, and flushed in with 2.0 ml of physiological salt solution. All other drugs were administered intravenously excepting cocaine (20.0 mg/kg), which was injected subcutaneously exactly 30 min after the first or third control injection of ephedrine. An interval of 30 min was allowed to insure sufficient time for the absorption of this drug. Additional drugs used in these experiments were given either 15 min before or 45 min after the injection of cocaine. A nontachyphylactic dose of racemic ephedrine salt, as reported by Tainter (*2*), was 0.5 mg/kg, and, therefore, was the dosage chosen for all of our experiments. Our findings, though, indicate a reduction in the pressor responses to ephedrine up to 33 percent after three consecutive hourly injections. The first three hourly responses to ephedrine showed no significant difference. Nevertheless, the reduction in ephedrine pressor effects caused by such a "tachyphylaxis" is not comparable to the depotentiation observed after the administration of cocaine, when pressor responses to ephedrine are reduced between 84 and 100 percent (Fig. 1).

In order to test the previously described theory of cocaine depotentiation of ephedrine pressor responses, we first tested the effect of two drugs, namely, bretylium tosylate (10.0 mg/kg), which is reported to inhibit norepinephrine release from storage sites (*5*), and chlorisondamine dimethochloride (0.3 mg/kg), a ganglionic blocking agent with prolonged duration of action (*6*), which inhibits passage of central sympathetic impulses, and, therefore, continuous release of norepinephrine from the nerve endings. Both drugs were administered to the animals exactly 15 min after the third hourly injection of ephedrine and before the subcutaneous cocaine. Both drugs produced an inhibition of cocaine desensitization of ephedrine pressor responses (Fig. 1). The differences represented in the bar graph are statistically significant; the probability of such a phenomenon being observed due to chance is less than 1 in 1000 for bretylium, and much less than 1 in 1000 for chlorisondamine.

Second, we injected the cocaine 30 min after the first control injection of ephedrine and then observed the depotentiation of the second hourly ephedrine injection, which was comparable to the fourth hourly injection after cocaine, as shown in Fig. 1. Pyrogallol (20.0 mg/kg), reported to be an O-methyltransferase inhibitor (*7*), was injected 15 min after this depotentiated second hourly injection of ephedrine. It progressively increased the subsequent hourly ephedrine responses (5th hour shown in Fig. 1). This reversal of ephedrine depotentiation by cocaine could have been observed by chance less frequently than 1 in 1000.

We finally tested whether or not infusion with the generally postulated sympathetic neurohormone itself could reverse cocaine desensitization. Norepinephrine (26 μg/kg per hour) was infused 15 min after the observed cocaine-desensitized ephedrine response, as described for pyrogallol. It can also be seen in Fig. 1 that norepinephrine itself counteracted cocaine desensitization to a highly significant extent (the chance probability of such an event is less than 1 in 1000).

Therefore, our theory that cocaine desensitization of ephedrine is caused by depletion of endogenous norepinephrine was confirmed. Lately, also, von Euler (*8*) stated that cocaine, in such large amounts, causes a depletion of this sympathetic neurohumor.

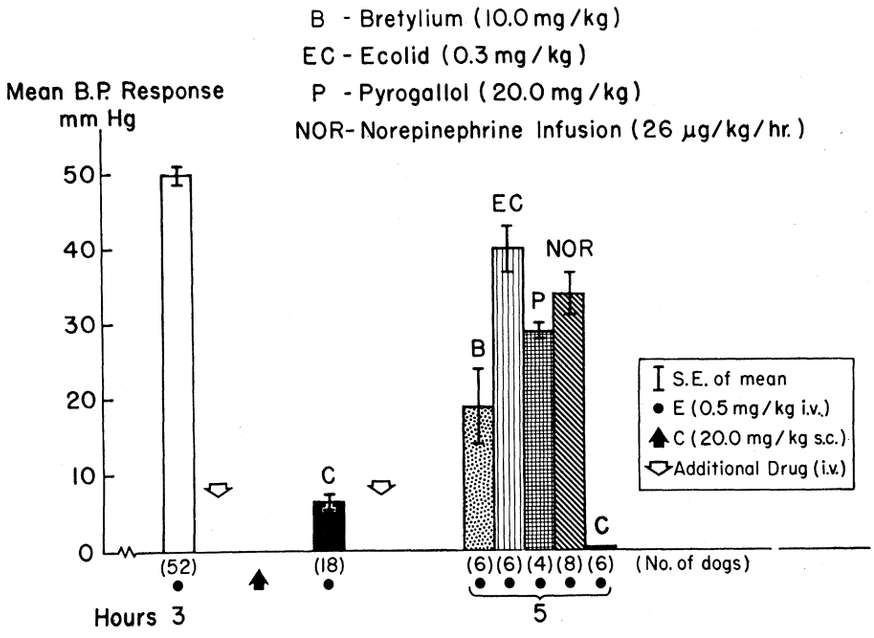

Fig. 1. Antagonism of cocaine (*C*) desensitization of ephedrine (*E*) blood-pressure (*B.P.*) responses. On the ordinate, mean blood pressure responses and the standard error of the means are indicated in millimeters of mercury. The abscissa indicates the time of the hourly injections of ephedrine sulfate (0.5 mg/kg), the black dot representing the injection time. The first bar represents the mean (52 animals) of the third hour response to ephedrine sulfate. The first downward white arrow indicates the time of injection (15 min before cocaine—given 30 min after the third hourly injection of ephedrine) of bretylium tosylate or chlorisondamine dimethochloride (Ecolid); the second downward white arrow indicates the time of administration 45 min after cocaine (given 30 min after the first hourly ephedrine injection) of pyrogallol and of the start of the infusion of norepinephrine hydrochloride (26 μg/kg per hour). The heavy upward black arrow indicates the time of cocaine injection (30 min after either the first or the third hourly injection of ephedrine). In the second black bar, the pooled mean responses to the first injection of ephedrine sulfate 30 min after cocaine administration are indicated. The last bar, labeled C, indicates the mean of the fifth hour responses to ephedrine (0 mm-Hg) in six animals treated with cocaine only. The statistical significance of the results is discussed in the text. Note the unequivocal reversal of cocaine inhibition when the black *C* bars are compared with the experimental *B*, *EC*, *P*, and *NOR* bars.

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7 July 1961

Absence of the Diego Antigen, a Genetic Characteristic of Early Immigrants to South America

Abstract. The Diego blood group is an exclusive Mongoloid gene marker, although it is not present in all Mongoloid populations. The absence of the gene in Waica Indians and its very low frequencies in Warrau and Yaruro Indians of South America suggest that it represents a genetic characteristic of Marginal American Indians. Since Marginal Indians are considered to be early comers to the New World, we suggest that Diego-negative populations were the first to arrive and to extend throughout South America, while the Diego-positive tribes came later.

The Diego blood group antigen was first discovered in a Venezuelan family of Carib Indian ancestry (*1*). Subsequently it proved to be an exclusive Mongoloid gene-marker, which is not present in Caucasoids and Negroids. This evidence, however, does not imply that the gene is carried by all Mongoloid populations or that it is carried by them in a more or less constant frequency. On the contrary, studies during the last 6 years have shown that there exist populations which, while classified as Mongoloid, do not carry the gene at all—for example, the Oceanic populations, the Eskimo, and several Amerindian tribes (*2*).

In a recent review of all the populations examined for this antigen, it was observed that populations with related languages tend to show similar Diego frequencies. One of the best documented cases studied is that of three groups of Venezuelan Cariban tribes which showed a Di (a +) range between 20 and 34 percent (mean 28 percent), in spite of being separated from each other by some 3000 years (30 m.c.) in time and over a distance of 1500 km (*2*, *3*). Thus, linguistic affiliation, together with several specific culture aspects of the various tribes, has proved to be useful in predicting the Diego frequencies in Indian populations. Since the identification of extreme culture types is less complicated than that of intermediate ones, we directed our studies toward those tribes of South America which are conventionally classified as Marginal. Combining the physical anthropologists' notion of Marginal Indians with that of the cultural anthropologists, we understand here as being Marginal those aborigines of South America who are predominantly dolichocephalic, of medium or stocky build, with varying degrees of Mongoloid features, nonagricultural, with little developed technology, and a simple social structure based on kinship rather than on class. The purpose of our study was to find out whether Diego antigen frequencies would show marked differences between Indian populations of very different culture types.

Last April we had an opportunity to study the Waica Indians of southern Venezuela, whose identification as Marginals can hardly be doubted (*4*). As a subtribe of some 10,000 individuals, they belong to the Yanoama, who also include the Sanemá, Samatari, Kasapare, Surára, and Pakidái of southern Venezuela and northwestern Brazil; these various languages form a linguistic stock of 6 to 35 m.c. of internal separation.

Without going into the details of the results of antigen analysis of the ten blood group systems, we would like to report here the absence of Diego in 142 samples. They were taken from less closely related individuals living in six settlements within an extensive area. We consider, therefore, that this sample is a genetic representative of the entire Waica population and, in consequence, that it rules out the possibility that the Diego result is due to gene drift.

This finding is considered to be of major importance, because it raises the figure of Diego-negative Marginal tribes of Venezuela to the number of three—that is, Warrau, Yaruro, and Waica (Table 1).

The Warrau (*5*) occupy the Orinoco Delta and the adjacent swampy regions of the coast of British Guiana (latitude, 8°–10°N; longitude, 59°–62°W). Two different subtribes were tested, one of them a peripheral community, called Guayo, in which some admixture with the neighbouring Indian tribes was expected to have occurred (Carib, Arawak). The other one, the Winikina, live in the center of the Orinoco Delta, where they have been living in complete isolation until very recently. The Guayo-Warrau showed a Di^a gene frequency of 1.9 percent, and the Winikina-Warrau were negative.

The Yaruro (*5*) Indians inhabit the savannahs or *llanos* of southwestern Venezuela (7°N, 68°W). They have been living in this alternately desert-like and flood-covered territory for many centuries, and there are reasons to assume that they took possession of it long before the discovery of America. From 102 samples tested, only five demonstrated the Di^a, of which four were derived from 11 samples, taken from a single band. This indicates that they do not represent actually a homogenous pool of Di^a genes.

For the reasons mentioned above, we suggest that the Warrau as well as the Yaruro were Diego-negative originally, and that their low Di^a frequencies are due to admixture with the neighbouring tribes who exhibit a relatively high frequency of the gene.

Contrasting with the absence of the Diego gene among Marginals are the elevated frequencies of 10 to 45 percent of Diego among peoples of a higher culture level and different linguistic affiliation—that is, Saliva, Arawak, Tupí, Carib, Chibcha, Quechua, and Aymara—and who, according to their culture and physical type, fit into the scheme of Coon *et al.* (*6*), as either Tropical Forest Indians or Central American Indians. Exceptions, which expectedly have come up, were discussed by the authors in the case of the Tunebo, Irapa, Colla, and Omaguaca

Table 1. Distribution of the Di^a genotype in Marginal Indian tribes.

Tribes	No. tested	Phenotypes (%)		Genotypes (%)	
		Di (a+)	Di (a−)	Di^a	Di
Winikina-Warrau	72	0.00	100.00	0.00	100.00
Guayo-Warrau	81	3.69	96.31	1.9	98.1
Yaruro	102	4.91	95.09	2.49	97.51
Waica	142	0.00	100.00	0.00	100.00