Protection of Synaptic Transmission Against Block by Nicotine

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During the past year we have studied the effects of various drugs on synaptic transmission in the crayfish; the results will be described elsewhere. In the course of this investigation many drugs were found to have no effect. In such cases a check was made on the preparation by applying a dose of nicotine (10^{-5} grams/cc.) 10 minutes after the original drug. Control tests had shown that nicotine in this concentration blocks transmission in 1–2 minutes. As expected, the presence of most drugs did not influence this action. With some, however, either the nicotine was without effect or the block was greatly delayed. Evidently under certain conditions the preparation is protected against the action of nicotine. Since this situation seems to be of considerable interest, the following preliminary report is presented at this time.

The synapse used in these investigations is between a giant fiber in the central nervous system and a motor fiber in an abdominal root of *Cambarus clarkii* (*3*). The preparation was kept in a bath containing 100 cc. of crayfish perfusion fluid, the drugs being added to this bath. The giant fiber was stimulated between the 5th and 6th abdominal ganglia, while the postganglionic response was led off the 3rd root of the 3rd ganglion. Stimuli were delivered throughout the experiment at 30/minute, at which rate, in the absence of drugs, the postganglionic response remains unchanged for several hours.

The protecting effect was first noted with two drugs, anabasine and nornicotine,[1] which are closely related in structure to nicotine. In a typical experiment, anabasine 10^{-4} was administered. After a transient facilitation, transmission returned to normal. At the end of 10 minutes, nicotine 10^{-5} was given. This had no effect. Ten minutes later, nicotine 10^{-4} was administered, but even this high concentration had no effect for at least 10 minutes.

The results of similar experiments, made with a number of other drugs, permit the following classification to be made:

(a) Drugs which in low concentration protect the crayfish synapse from the action of nicotine, but which block the synapse in higher concentration: anabasine, nornicotine.

(b) Drugs which in subthreshold doses show no protection against nicotine and which block transmission in higher concentrations: alcohol, DFP, eserine.

(c) Drugs which neither protect against nicotine nor block synaptic transmission: acetylcholine, adrenalin, atropine, coramine, picrotoxin, prostigmine, strychnine.

An unexpected result was obtained in experiments in which the nicotine was left in the bath after transmission was blocked. Complete recovery of the root potentials occurred within 40 minutes. Renewed application of nicotine in the same or even higher concentration was now without effect. The same result was obtained when the preparation was washed with fresh perfusion fluid after block by nicotine.

[1] We wish to thank R. C. Roark, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Beltsville, Maryland, for supplying these drugs.

A similar effect has been observed with eserine. After block by this drug, transmission rapidly returns to normal on washing with fresh fluid. Another dose of eserine, of even higher concentration, is now without effect. The preparation is also protected against the action of nicotine.

Protection against nicotine has been reported by other workers. Novocaine antagonizes the effects of an intravenous injection of nicotine on the heart, blood pressure, and intestine of the dog (*1*). Sulfathiazole inhibits the action of nicotine in the isolated intestine (*2*). The present study, however, has the advantage of confining drug action to synaptic transmission.

Biological competition between structurally related compounds is well known (*4*). In this type of action it is generally considered that the compounds involved compete for attachment to a specific receptor. Such a mechanism is not completely adequate to explain the phenomena described in the present paper, however. Nicotine evidently has a twofold action on synaptic transmission. When first applied, it blocks transmission. As recovery occurs, this blocking action is lost, but a protecting action remains, since further doses of nicotine are without effect. This shift from a blocking to a protecting action suggests that a change takes place in the nicotine molecule by which it becomes ineffective as a blocking agent but remains attached and thereby prevents the action of fresh nicotine. The attachment must involve a firm bond, since washing with perfusion fluid does not interfere with this protection.

Anabasine and nornicotine, which have much less blocking action, seem to attach as readily as nicotine and therefore protect against it. During recovery from nicotine block, nicotine may be transformed into a compound like these or into a related compound with a pure protective action.

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Role of Inhibitors in Soybean

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It is now recognized that cooking or autoclaving increases the nutritive value of the protein of soybean. The general opinion is that cooking increases the availability of cystine and methionine. Almquist, *et al.* (*1*) and Hayward and Hafner (*8*) demonstrated that the limiting amino acid in soybean is methionine, and heating under pressure increases the availability of this amino acid. Evans and McGinnis (*4*) found that the retention of organic sulfur is increased from 34 to 52 per cent and that of methionine from 58 to 74 per cent in autoclaved soybean. But Melnick, *et al.* (*9*) found no increase in the retention of methionine in autoclaved soybean, and from their experiments they concluded that the higher biological value of autoclaved soybean was due to an increased rate of release of methionine rather than a higher degree of its availability. Ham and Sandstedt (*6*) found that a dilute acid extract of raw

[1] Our thanks are due to V. Subrahmanyan for his keen interest in the work.

soybean contained a substance which greatly reduced the activity of trypsin *in vitro*. Ham, *et al.* (*7*) found that a solution of the partially purified inhibitor produced lowering of growth in chicks fed on a well-balanced diet. In a previous communication from this laboratory (*3*) it was stated that glycinin is more nutritive than autoclaved soybean. It was believed that the enhanced nutritive value of glycinin was due to elimination of the proteolytic inhibitor, as also any toxic factor which may be present in raw soybean.

The present investigation was undertaken to see whether the presence of the inhibitor would affect the nutritive value of soybean protein digested with enzyme so that the amino acids are in free form. According to the hypothesis of Melnick, *et al.*, the biological value of the digest is expected to be higher than the raw bean, because all the amino acids, including methionine will be simultaneously available for utilization.

The digest was prepared by subjecting soybean meal to digestion by papain. Acid hydrolysis was not resorted to because it would destroy tryptophane and other possible essential peptide factors like strepogenin, as suggested by Woolley (*5*). Papain was chosen for the proteolytic digestion, since the raw soybean meal contained the tryptic inhibitor. The soybean meal was finely ground and suspended in about four times its weight of water and digested with an active preparation of papain. The digestion was carried out at 50°C. for 48 hours. A control was run, using a boiled solution of the enzyme which was maintained at the same temperature. Both samples were then dried in a current of warm air. It was found that the inhibitor was destroyed to a small extent in the course of drying. A requisite amount of the inhibitor solution extracted from raw bean was added to both the extracts in order to restore the original activity.

The biological value and the digestibility coefficient of the protein in the digested and control samples were determined by the usual nitrogen balance method. The proteins were fed at a level of 10 per cent (nitrogen × 5.7). The results are as follows:

	Average digestibility coefficient	Average biological value
Digested sample	89.6	45.9
Control sample	89.2	44.7

The nitrogen in the digested sample was analyzed, and it was found that 91.6 per cent of the total nitrogen of the meal was in the nonprotein form, as estimated by its solubility in 7 per cent trichloroacetic acid, and 90 per cent of the total methionine of the protein was in the free form in the digest.

From the above results it is evident that the role of the proteolytic inhibitor in deciding the nutritive value of raw soybean is not in diminishing the degree of availability or rate of release of methionine. It is highly probable that its action is of the nature of an antigrowth factor affecting the usefulness of proteins in general. This statement is supported by the findings of Ham, *et al.* that a purified solution of proteolytic inhibitor caused growth retardation in chicks fed on a well-balanced diet.

It is thought that, apart from the tryptic inhibitor, there may be other toxic factors associated with the raw bean which are destroyed on heating. Evidence for the existence of a separate factor apart from the proteolytic inhibitor was adduced from the following experiment. Everson, *et al.* found that the growth-promoting value of soybean protein increases to a marked extent if the bean is germinated. This observation was also confirmed here, and on examining the concentration of the proteolytic inhibitor in germinated soybean (48 hours) it was found that it was not altered from that in the original bean. The concept of the proteolytic inhibitor cannot explain this increase in nutritive value after germination. That there was no change in the amino-acid composition of the protein after germination was proved by Block, *et al.* (*2*), who could detect no change in the contents of tyrosine, tryptophane, phenylalanine, cystine, and methionine in the protein of soybean after germination.

From the above findings it can be postulated that apart from the proteolytic inhibitor there is a separate factor which affects the nutritive value of the soybean protein. Further work on the isolation of this factor and the respective roles of these inhibitors in the nutritive value of soybean is in progress.

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Effect of Benzene Hexachloride on the Flavor of Poultry Meat

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Tests conducted during the summer of 1947 show that the meat of chickens fed, sprayed, or retained in a house previously sprayed with benzene hexachloride has a distasteful flavor. This flavor, which makes the meat nearly inedible, persists for a period of 6–10 weeks.

The absorption of a benzene hexachloride flavor and odor was first discovered accidentally. In the fall of 1946 and spring of 1947 a number of chemicals were tested for the control of chicken mites. A paper on the results of these tests is in press for the *Journal of Economic Entomology*. During the course of this study several chemicals, including benzene hexachloride, showed promise, and a test to determine their toxicity to poultry was undertaken. A 5 per cent concentration of wettable benzene hexachloride containing 5 per cent of the γ isomer was applied to several chickens, the house, and the litter in December 1946. This test was concluded, and the house was not cleaned up or used until May 1947. At this time market birds were placed in the house and fed grain in the litter as well as mash in a feeder for a period of 1 week. When cooked, the meat of these birds was not edible because of a distasteful flavor apparently caused by the absorption of benzene hexachloride.

Since many manufacturers and promoters of insecticides had used and are now recommending benzene hexachloride

[1] With the cooperation of the Departments of Poultry Husbandry and Home Economics.