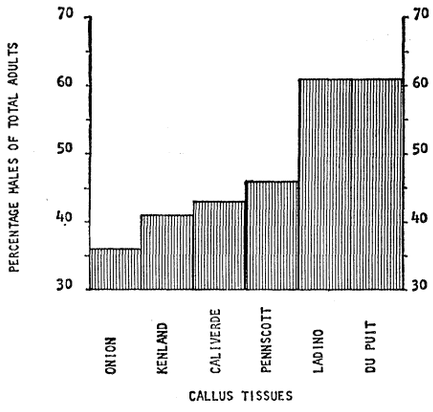


Fig. 1. Plant callus tissue varieties, arranged in order of increasing percentages of males of the total number of adult nematodes found after the tissues were cultured for 6 weeks at 27.5°C.

adult females in the garlic race of *D. dipsaci* is 1:2. (This holds for adults from onion or garlic plants or preadults from dried garlic scales.) In the cultures of callus tissue the overall ratio of males to females is standard (1:2), but the ratio of adult males to adult females differs markedly among cultures (Fig. 1).

In these host-parasite systems, resistance appeared to be expressed in the relative unbalancing of the sex ratio in favor of maleness, though there was no indication of sex reversal or the formation of intersexes.

The apparent trend to adult maleness was a result of the differential development between male and female stages, depending upon host suitability. Males were able to develop normally to adulthood, whereas the females were less able to do so. However, preadult females were normally active and feeding. The tendency of diverse nematode populations to increased maleness under environmental stress (*2*, *3*) may be partially explicable in terms of arrested female development.

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## Histochemical Fluorescence after Application of Neurochemicals to Caudate Nucleus and Septal Area in vivo

Abstract. *The movement of carbachol, norepinephrine, and dopamine from cannula sites in caudate nucleus and septal area of freely moving rats was traced by means of biogenic amine fluorescence. Fluorescent patterns seen after application of carbachol and norepinephrine to brain tissue did not appear to differ from controls. Three types of movement from the cannula site after administration of dopamine were observed. There was a spherical distribution approximately 2 millimeters in diameter. Fluorescence also followed axonal pathways in the orthodromic direction, suggesting that dopamine may have been transported by "axonal streaming" or by some other unknown mechanism in periaxonal spaces. Because fluorescence was present in both the ependymal lining and the choroid plexus, it was inferred that the cerebral ventricles were also involved in the movement of chemical. Any attempt to ascribe anatomical localization to behavioral effects resulting from chemical stimulation of the brain should take into account the widespread movement of chemicals after their local application to brain tissue.*

In the study of the effects of neurochemicals applied to specific anatomical sites on behavior, it has been presumed (*1*) that the chemicals act at the site of stimulation, diffusing less than a millimeter from the cannula tip. Routtenberg (*2*) questioned this assumption on the basis of certain paradoxical results. Studies in which the movement of dyes have been traced (*3*) are only approximations since there is no way to be certain that the neurochemical would not behave somewhat differently from the dye.

We have studied the movement of chemicals from the cannula tip using the histochemical technique (*4*) for demonstration of biogenic amines. Although this technique demonstrates the presence of norepinephrine, dopamine, and serotonin, it will not show the diffusional movements and distribution of carbachol. However, since carbachol might cause release of biogenic amines (*5*), we observed the pattern of fluorescence after its application as well as after that of norepinephrine and dopamine.

Using stereotaxic methods, we implanted a stainless steel cannula into each of 25 female adult albino rats anesthetized with barbiturate (*6*). Ten additional rats served as operated and unoperated controls. Cannulas (*7*) were placed in either the caudate nucleus or the septal area. After a 2-day recovery period, approximately 10 $\mu$g of chemical were applied to each animal; fourteen animals received carbamylcholine chloride (carbachol), four received DL-norepinephrine hydrochloride, and six received dopamine hydrochloride. The rat was decapitated after 10 minutes, and the brain was rapidly removed (1 to 2 minutes) and immediately frozen in liquid Freon 22, chilled first with liquid nitrogen to −150°C. An additional animal, receiving dopamine, was decapitated 1 minute after application. The tissue was then processed for the histochemical demonstration of biogenic amines (*4*).

The clearest demonstration of fluorescence was obtained with dopamine. With this chemical, three major types of movement from the cannula tip were seen. The first, seen in two cases, was spherical diffusion, which appeared as a circular pattern of fluorescence surrounding the chemical probe. Near the site of the cannula tip was a bright yellow fluorescence which became yellow-green and then green with increasing distance from the probe. This bright yellow fluorescence is believed to be related to the presence of high concentrations of the catecholamines (*8*). The spherical diffusion from the probe site was about 1 to 2 mm, variations likely depending upon the amount of dopamine applied.

A second type of transport from the cannula was associated with the axon (Fig. 1). This was seen in five of the seven cases. Dopamine applied to the caudate nucleus moved into the corpus callosum, traveling several millimeters to the contralateral side (Fig. 1A). A similar axon-associated movement was also demonstrated in the anterior commissure. In cases where dopamine entered this latter system, one could trace fluorescence on successive sections to the side contralateral to chemical application. Dopamine applied to the caudate nucleus also entered into the internal capsule and moved in the orthodromic direction (Fig. 1, B–D). A section slightly anterior to the probe site (Fig. 1B) showed no fluorescence in the in-

ternal capsule, whereas at the site of stimulation (Fig. 1C) and posterior to the site of stimulation (Fig. 1D) there was bright fluorescence. These results suggest that a chemical applied to brain does not only follow a simple spherical pattern of diffusion, but can also become associated with nerve tracts and actively be transported along these tracts.

It may be of some interest that these tracts, as observed in control animals, do not appear to show any biogenic amine fluorescence. In sum, it does not seem unreasonable to suppose that chemicals applied to the brain may be transported relatively long distances either by active "axonal streaming" (*9*), which normally transports various substances manufactured in the perikaryon to nerve terminals, or by diffusing along periaxonal membranes (*10*). "Axonal streaming" is not an entirely satisfactory explanation since rates of movement so far described (*9*) are slower (maximum of 5 mm/day) than occurred in the present study (maximum of 0.5 mm/min).

A third type of diffusion involved the ventricles, and was seen in two cases. It was not entirely clear whether this involvement was due to some active ventriculopetal force, such as hygroscopicity, or whether this was merely due to diffusion into the ventricle; analysis of nonspherical fluorescent patterns near the ventricle, seen in five of seven cases, suggested that dopamine may have diffused preferentially to ventricular sites. Two lines of evidence suggest ventricular involvement. First, although the ependymal wall in control subjects did not fluoresce (Fig. 2A), after a 10-minute application of dopamine, the apical portion of the ependymal cells, both on the side of chemical application (Fig. 2C) and on the other side of the ventricle, was fluorescent (Fig. 2B). Dopamine applied to septal area entered the corpus callosum and appeared to move along callosal fibers via axonal movement. The dopamine then entered the ventricle and was taken up by the apical portion of the ependymal cells on both sides. Second, although the choroid plexus normally does not fluoresce (Fig. 2D), after a 1-minute application of dopamine, both the ipsilateral (Fig. 2F) and contralateral (Fig. 2E) choroid plexus demonstrated marked fluorescence. Thus, it may be inferred that the chemical not only entered the ventricle on the ipsilateral side but also moved to the contralateral side.

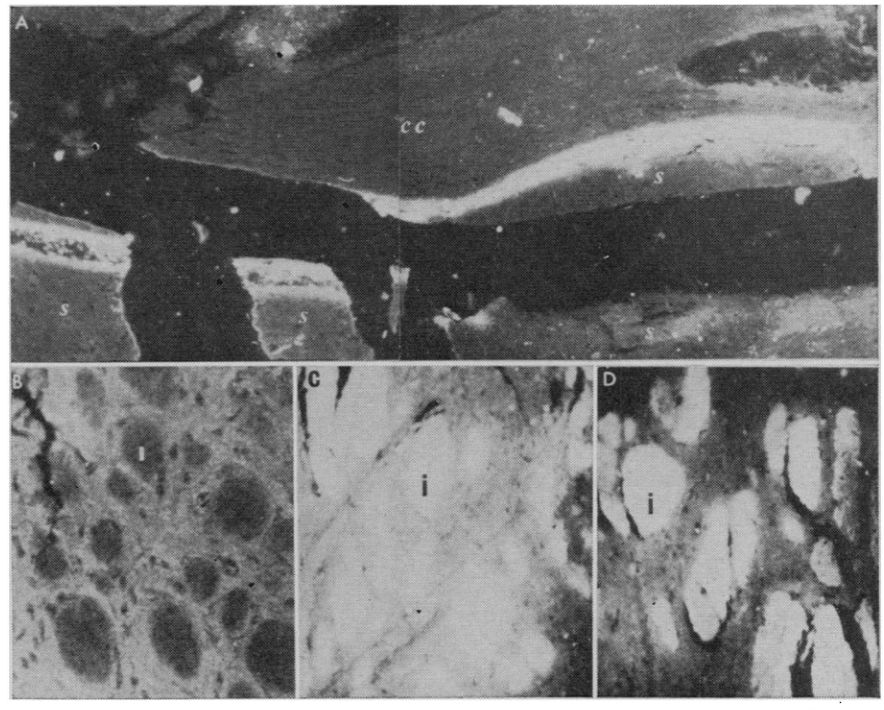


Fig. 1. Fluorescence after application of dopamine to caudate nucleus. (A) Band of fluorescence seen in ventral corpus callosum (*cc*; note that placement of label is in midline) immediately dorsal to septal region (*s*). Black regions resulted from cracks in tissue. If we take this fixation artifact into account, the fluorescent band is continuous from extreme right to extreme left of the picture (× 300). All sections in this report were taken in the frontal plane. (B–D) Internal capsule (*i*) rostral to site of stimulation (B), at site of stimulation (C), and caudal to site of stimulation (D) (× 200).

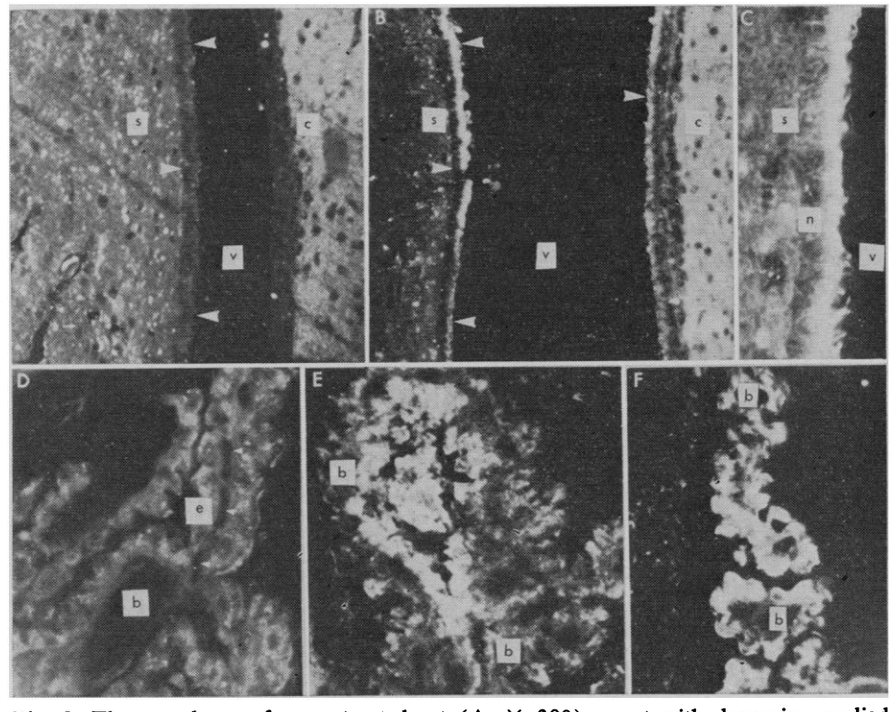


Fig. 2. The ependyma of an untreated rat (A, × 300), a rat with dopamine applied to septal area in both lower and higher magnification ( B, × 300; C, × 1080). Note in (A) and (B) the arrows which delimit the ependyma on the septal (*s*) side of the ventricle (*v*). Note the fluorescence in the apical portion of the ependyma on both the septal and the caudate (*c*) side of the ventricle. Note in (C) the characteristic basal nucleus (*n*) of the ependyma. (D–F, × 1500) The choroid plexus of an untreated subject (D); note the blood vessels (*b*), epithelial cells (*e*), and arrows demonstrating a capillary in longitudinal section. Choroid plexus of animal treated with dopamine in the contralateral (E) and ipsilateral (F) side.

Our results support the view (*2*) that the ventricle transports chemicals applied to brain tissue. Such transport may bring the chemical to distant sites which, in turn, may mediate certain observed behavioral effects. The recent report of Baxter (*11*) is consistent with this view.

Because of the inability to fluoresce carbachol, we have not been able to assess its pattern of diffusion directly. Although it does not seem unreasonable to presume that carbachol, like dopamine, also enters the ventricle (*2*), autoradiographic studies may assist in evaluating this possibility. The inability to find any significant alteration of the normal fluorescent pattern after application of norepinephrine is somewhat puzzling but perhaps comprehensible in terms of mechanisms of rapid uptake recently described on the basis of autoradiographic techniques (*12*).

Our results demonstrate that chemicals are transported distances considerably greater than have been heretofore assumed. Such results are clearly relevant to discussions of widespread behavioral effects of neurochemicals applied to brain (*1*). A combination of the techniques of fluorescence and chemical stimulation of brain may be valuable in studying the way in which neurochemicals are transmitted. Additionally, such information may shed light on the interactions among different neurochemicals. For example, if carbachol does become associated with the axon, it may subsequently cause the release of the transmitter located in the end button of the axon (*13*). This view might explain the occurrence of 2- to 5-minute latencies of behavioral effects which are sometimes observed after chemical stimulation of the brain.

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## Conformation and Activity of Chymotrypsin: The pH-Dependent, Substrate-Induced Proton Uptake

Abstract. *Hydrogen ion uptake by chymotrypsin during reversible binding of specific substrate is shown to be due to an ionizing group of the enzyme with a* pK(*apparent*) ~ *9 in the free enzyme. This* pK(*apparent*) *is shifted to a higher value in the enzyme-substrate complexes. Previous results indicating an equilibrium, controlled by this ionizing group, between active and inactive conformational forms of chymotrypsin are confirmed.*

The experiments reported are part of an investigation of the mode of enzymic action of chymotrypsin (CT), which belongs to a group of hydrolytic enzymes including thrombin and acetylcholine esterase (*1*). The amino acid sequence of CT is known from the work of Hartley and of Keil and Sörm (*2*), and the conformation of crystalline $\alpha$-CT has recently been deduced from x-ray diffraction studies by Blow and collaborators (*3*).

We have previously presented data which indicate that the reaction of CT with inhibitor (diisopropyl fluorophosphate) at alkaline *p*H is accompanied by an uptake of hydrogen ion by the enzyme (*4*), and this phenomenon has been confirmed by inhibition experiments in a number of laboratories (*5–7*). We have shown by both titration experiments (*8*) and kinetic studies (*9*) that an ionizing group of the enzyme with *pK*(apparent) ~ 9 is implicated in this process. There is evidence (*10*) that this ionizing group is the amino-terminal isoleucyl $\alpha$-amino group that is liberated in the conversion of chymotrypsinogen to active enzyme. We have, further, presented data which indicate the existence of *p*H-dependent equilibria between catalytically active and inactive conformations of the enzyme (*9–13*), the active conformation being stabilized by a positively charged $\alpha$-amino group with *pK*(apparent) ~ 9. These equilibria between enzyme conformations are schematically presented in Fig. 1. The *p*H dependence of the enzyme-substrate dissociation constant (*12, 14, 15*) indicates that substrate binds better to the active conformation. Implications of these observations are that substrate binding must result in a stabilization of that enzyme conformation in which the crucial ionizing group

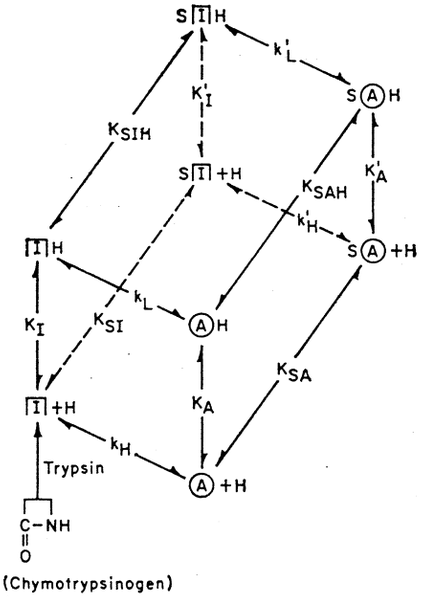


Fig. 1. Scheme showing equilibria between two main conformations of the enzyme, A (active) and I (inactive), in the free state and in complexes with substrate, S, and in protonated and unprotonated states of a critical ionizing group. A, AH, I, IH, SA, SAH, SI, and SIH represent the various protonated and unprotonated forms of enzyme and enzyme-substrate complexes. $K_A$ and $K_I$ are the acid dissociation constants of this ionizing group in the A and I conformations, respectively. This ionizing group controls the equilibria between enzyme conformations, equilibria characterized by the constants $k_L$, $k_H$, $k'_L$, and $k'_H$, where the subscript L refers to the conformation of the enzyme when the critical ionizing group is protonated (at *p*H 7), the subscript H refers to the high-*p*H form of the enzyme, and the prime refers to enzyme-substrate complex rather than free enzyme. $K_{SA}$, $K_{SI}$, $K_{SAH}$, and $K_{SIH}$ are the various enzyme-substrate dissociation constants.