ing individual amino acids required by the recipient. The ability of the selected recombinants to produce enterotoxin was tested with the modified Elek technique previously described (*9*). Briefly, each recombinant was streaked on a brain-heart infusion agar plate perpendicular to a strip of filter paper soaked in specific equine antitoxin and embedded in the medium. If the recombinant was *tox*+, an antigen-antibody precipitin line formed in the agar at an angle to the filter paper and the growth from the streaks. This technique facilitated the testing of hundreds of recombinants for production of enterotoxin.

Parker *et al.* have described three clusters of linked genes in *V. cholerae* which they have designated linkage groups I, II, and III (*8*). In order to determine whether or not *tox* belongs to any of these three linkage groups, we performed and analyzed matings between the prototrophic, *tox*+ donor strain 569B (P+) and the multiply auxotrophic, *tox*− recipient strain RV-31, which has mutant alleles (*his*−, *arg*−, *ilv*−) representing each of the three linkage groups. The results of several mating experiments are presented in Table 1.

Among 1465 *his*+ recombinants tested in ten experiments, 46 were *tox*+ (3 percent). In contrast cotransfer of *tox*+ with *ilv*+ occurred at a frequency of 2 in 665 (0.3 percent), and cotransfer of *tox*+ with *arg*+ was not detected (0 of 603). All *tox*+ recombinants were serotyped by slide agglutination tests, and all were Ogawa like the parental recipient strain. Therefore, the *tox*+ marker in strain 569B shows weak but significant linkage to the *his* gene of linkage group I, but little or no linkage to the *ilv* and *arg* genes of linkage groups II and III.

As an additional control to establish that the *tox*+ genotype of the recombinants arose by transfer of the *tox*+ allele from the donor to the recipient, a cross was performed using the *tox*−, prototrophic donor strain RV-102 and RV-31 as the recipient. No *tox*+ isolates were found among 100 *his*+, 100 *arg*+, and 100 *ilv*+ recombinants tested. The possibility of reversion from *tox*− to *tox*+ in strain RV-31 was also lessened by the observation that more than 500 single colonies of RV-31 tested were *tox*− by the Elek technique.

The results presented here demonstrate that a gene controlling toxinogenesis in *V. cholerae* can be transferred by conjugation from a *tox*+ donor to a *tox*− recipient strain. The linkage of the *tox* gene to the *his* gene, but not to genes *arg* and *ilv*, is inconsistent with control of toxinogenesis by a plasmid and provides strong evidence for the chromosomal location of the *tox* gene. Thus, in spite of the striking immunological and physiological similarities between the enterotoxins from *E. coli* and from *V. cholerae*, synthesis of enterotoxin in *V. cholerae* 569B is controlled by a chromosomal gene and differs from the plasmid-regulated system in *E. coli*.

We have recently isolated and characterized several different classes of mutants of *V. cholerae* strains 569B Inaba and 3083-2 Ogawa that are altered in toxinogenicity (*10*). Formal genetic analysis of such mutants should provide a feasible approach for the identification of the structural and regulatory genes that control toxinogensis in *V. cholerae*.

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Mapping of Functional Neural Pathways by Autoradiographic Survey of Local Metabolic Rate with [^{14}C]Deoxyglucose

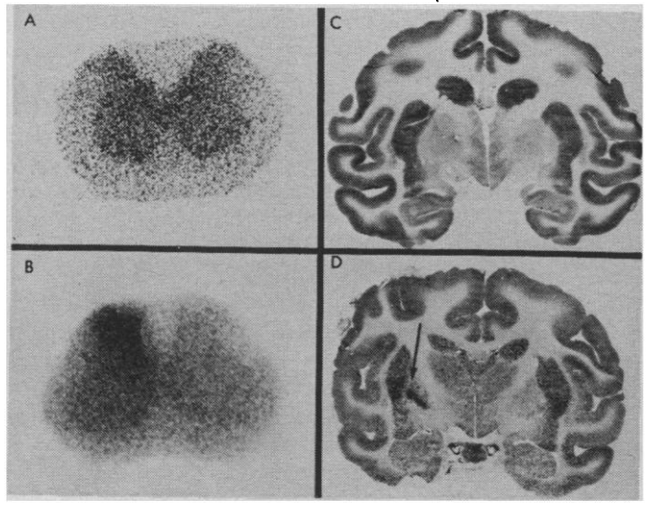
Abstract. *If sufficient time has elapsed following an intravenous pulse of [^{14}C]deoxyglucose, the carbon-14 contents of the tissues of the central nervous system represent mainly the accumulated phosphorylated derivative of [^{14}C]deoxyglucose and reflect the rates of glucose consumption of the tissues. Altered functional activity alters metabolic activity and the uptake of [^{14}C]deoxyglucose in the tissues. By autoradiographic survey of sections of the central nervous system it is then possible to map all the regions with altered functional and metabolic activities in response to experimentally induced changes in functional state.*

A method has been developed for quantitative estimation of the rates of glucose consumption in the various structural components of the brain (*1*). The method is based on the uptake of ^{14}C in the various cerebral tissues following an intravenous tracer pulse of [^{14}C]deoxyglucose. If sufficient time is allowed after the pulse for the free [^{14}C]deoxyglucose to have been cleared from the tissue, then the ^{14}C concentration of the tissue represents the [^{14}C]deoxyglucose-6-phosphate content which, in turn, equals the integrated rate of [^{14}C]deoxyglucose phosphorylation with respect to time. With appropriate consideration of the time course of the relative concentrations of glucose and [^{14}C]deoxyglucose in the plasma, of the rate constants for the turnover of the free glucose and [^{14}C]deoxyglucose pools in the tissue, and of the ratios of the kinetic constants for the transport and phosphorylation of glucose and [^{14}C]deoxyglucose, the rate of glucose utilization in the tissue can be computed from its [^{14}C]deoxyglucose-6-phosphate concentration (*1*).

In order to achieve as fine a resolution and as broad a representation of the various cerebral structures as possible,

tissue ^{14}C concentration is measured by a quantitative autoradiographic technique similar to the one used for the assay of tissue [^{14}C]antipyrine content in the measurement of local cerebral blood flow (*2*). Even without the quantitation, the autoradiographs provide a pictorial representation of the relative rates of glucose consumption in the various cerebral structures—the darker the area the higher the rate of glucose consumption (*1*). If, as is generally believed, the energy metabolism of a tissue is more or less coupled to its functional activity, then the autoradiographs could serve to identify the various components of the brain which exhibit altered metabolic and functional activities in response to alterations in the functional state of the animal. This report demonstrates that the [^{14}C]deoxyglucose autoradiographic technique is a useful, indeed powerful method for mapping functional neural pathways.

The experiments were performed with Sprague-Dawley adult male rats and mature rhesus monkeys. Polyethylene catheters were inserted into the femoral vein under Fluothane anesthesia, and the animals were allowed to recover from the anesthesia for at least 4 to 24 hours before the administration of [^{14}C]dexoyglucose. [1-^{14}C]Deoxyglucose (specific activity, 53 mc/mmole) was administered as a pulse via the femoral venous catheter. Rats received a dose of approximately 15 to 20 μc per 100 g of body weight in a total volume of 1.5 ml of physiological saline; the monkeys received 120 μc per kilogram of body weight in 3 ml of saline. Additional procedures specifically related to the experimental condition being examined are described when these experiments are discussed. The animals were killed 45 minutes after the pulse, the rats by decapitation and the monkeys by an intravenous dose of sodium thiopental and saturated KCl solution followed by decapitation. With rats the brains were immediately removed and frozen in Freon 12 chilled to −75°C with liquid nitrogen. In the case of monkeys the entire heads were frozen, and the brains were dissected out in a cold room maintained at 2° to 4°C. The frozen brains were then sectioned in 20-μm sections and autoradiographed as previ-



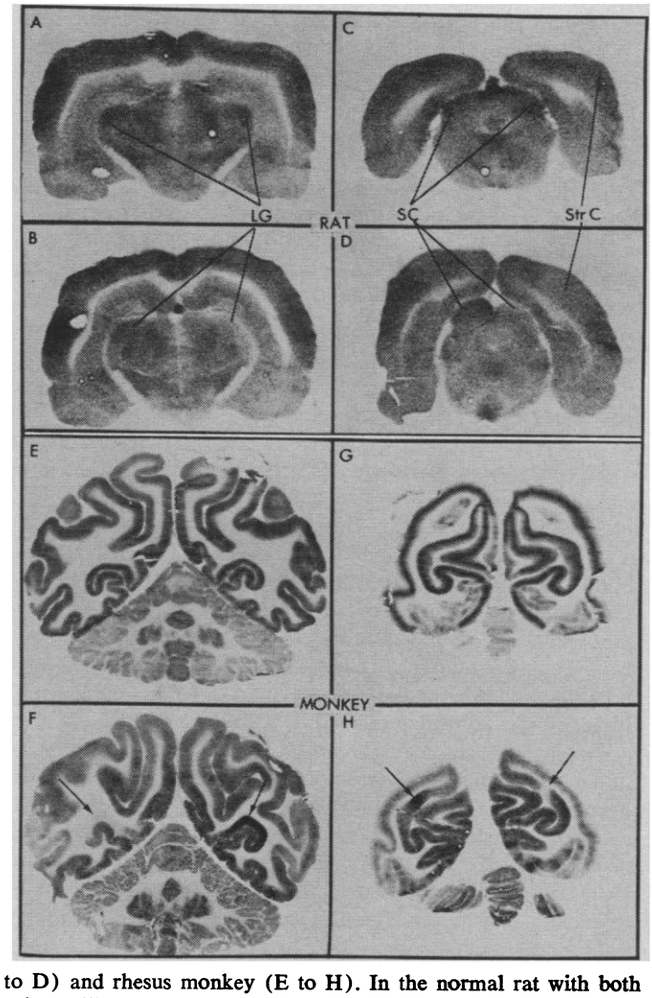


Fig. 1 (left). Effects of localized increases in functional activity on uptake of [^{14}C]deoxyglucose into neural tissues. (A and B) Autoradiographs of sections of the lumbar spinal cord of a control rat (A) and of a rat with unilateral electrical stimulation of the sciatic nerve (B). Note the bilateral symmetry of optical densities in control rat and the asymmetrical increase in optical density in the dorsal horn ipsilateral to stimulation in the animal with sciatic stimulation. (C and D) Autoradiographs of comparable brain sections from a normal rhesus monkey (C) and from a monkey in which seizures were induced by application of penicillin to the motor cortex of one side (D). Note the unilateral increases in optical density in discrete areas of the putamen and globus pallidus ipsilateral to the locus of penicillin application in (D). In autoradiographs of other brain sections from the animal with seizures (not shown), areas of increased uptake of [^{14}C]deoxyglucose are seen in the motor cortex adjacent to the site of penicillin application and in parts of the caudate nucleus and thalamus.

Fig. 2 (right). Effects of unilateral enucleation on [^{14}C]deoxyglucose uptake in components of the visual system in the rat (A to D) and rhesus monkey (E to H). In the normal rat with both eyes intact the uptakes in the lateral geniculate bodies (*LG*), superior colliculi (*SC*), and striate cortex (*Str C*) are approximately equal on both sides (A and C). In the unilaterally enucleated rat, there are marked decreases in optical density in the areas corresponding to these structures on the side contralateral to the enucleation (B and D). In the unilaterally enucleated monkey, areas of asymmetry are seen only in regions with monocular input, namely, the rostral portion of the calcarine cortex (F) and the loci corresponding to the blind spots of the visual fields (H). Autoradiographs from corresponding sections of brain from a control monkey with both eyes intact exhibit no asymmetry in these regions (E and G). The section in (H) is only one of many serial sections demonstrating the loci of the blind spots, which appear to extend approximately 3.4 mm from front to back; the gross appearance of the section in (G) is somewhat different from that of the section in (H) because of a slight difference in the plane of sectioning, but it includes the portions of cortex containing the loci of the blind spots.

ously described (*2*). In experiments in rats with sciatic nerve stimulation, the spinal cord was removed, frozen, sectioned, and autoradiographed instead of the brain.

Figure 1, A and B, illustrates the effects of increased neural activity resulting from electrical stimulation of the sciatic nerve in the rat under barbiturate anesthesia. Such stimulation caused increased [^{14}C]deoxyglucose uptake (that is, increased optical density in the autoradiographs) in the ipsilateral dorsal horn of the lumbar spinal cord (Fig. 1, A and B). The local injection of 25,000 units of potassium benzyl penicillin into the hand-face area of the motor cortex of the rhesus monkey has been shown to induce electrical discharges in the adjacent cortex and to result in recurrent focal seizures involving the face, arm, and hand on the contralateral side (*3*). When seizure activity so induced occurred following the pulse of [^{14}C]-deoxyglucose, there was a selective increase in uptake of tracer in areas of motor cortex adjacent to the penicillin locus and in small discrete regions of the putamen, globus pallidus, caudate nucleus, and thalamus of the same side (Fig. 1, C and D). The identical regions have previously been shown to have increased blood flow under the same experimental conditions and are believed to be selectively activated in the course of seizure activity (*4*).

Decrements in functional activity result in reduced uptake of [^{14}C]deoxyglucose. In the rat, the visual system is 80 to 85 percent crossed at the optic chiasma (*5*), and unilateral enucleation removes most of the sensory input due to either retinal stimulation by light or spontaneous retinal cell potentials to the central visual structures of the contralateral side. In the conscious rat studied 24 hours after unilateral enucleation, there are marked decrements in [^{14}C]deoxyglucose uptake in the contralateral superior colliculus, lateral geniculate body, and visual cortex as compared to the same structures on the ipsilateral side (Fig. 2, B and D). These effects are observed whether the remaining eye is stimulated repetitively with a photoflash or the animal is maintained in normal room light. In the rat with both eyes intact, no asymmetry in the autoradiographs is observed (Fig. 2, A and C).

In the monkey, in which the visual pathways are approximately 50 percent crossed (*6*), unilateral enucleation produces no asymmetry in [^{14}C]deoxyglucose uptake in the superior colliculi, lateral geniculates, and most areas of the visual cortex. There are, however, two small areas of the striate cortex which do exhibit asymmetry. One is situated in the rostral portion of the deep calcarine cortex, which receives only monocular input from the extreme nasal portion of the contralateral retina (*6*). Unilateral enucleation, therefore, removes all input to this area on the contralateral side, and, indeed, the autoradiographs demonstrate a marked reduction in [^{14}C]deoxyglucose uptake at this site compared to the ipsilateral side (Fig. 2F). The other area of asymmetry is located in a portion of striate cortex which may correspond to the "blind spots" of the visual fields. This area of cortex receives no input from the portion of the contralateral retina occupied by the optic disk but is fully innervated from the area of the ipsilateral retina that corresponds to the same spot in the visual field. Unilateral enucleation, therefore, does not alter the input to the contralateral cortical locus but removes all input to the ipsilateral locus. On the other hand, the surrounding striate cortex loses 50 percent of its input when either eye is removed. The neuroanatomical relationships are such that unilateral enucleation would be expected to reduce the input to the striate cortex of both sides by one-half, except in the loci for the blind spot, where input is unaffected on the contralateral side and completely interrupted on the ipsilateral side. The [^{14}C]deoxyglucose technique dramatically demonstrates these relations. In autoradiographs prepared from monkeys studied in the conscious state 24 hours after unilateral enucleation, a contralateral cortical locus in the deep, folded portion of the calcarine cortex appears very dark compared to its surrounding striate cortex, whereas the same locus on the ipsilateral side is almost blanked out, indicating markedly lower [^{14}C]deoxyglucose uptake compared to its surrounding cortex (Fig. 2H). Other evidence suggests that the cortical locus of the blind spot is approximately in this area (*7*). Qualitatively similar although quantitatively less pronounced differences between the two sides are obtained when visual input to one eye is blocked with an opaque plastic disk instead of by enucleation; the lesser contrast between the two sides probably reflects the persistence of spontaneous discharges from the retinal ganglion cells of the occluded eye (*8*).

The [^{14}C]deoxyglucose has also proved effective in mapping the central auditory components of the rat. The autoradiographs from normal conscious rats exposed to the ambient noise of the laboratory demonstrate high optical density in regions corresponding to the structures mediating hearing, that is, the auditory cortex, medial geniculate body, inferior colliculus, lateral lemniscus, and superior olive, and a narrow band in the auditory cortex which appears to correspond to layer 4. If the external auditory canals are occluded with wax and the experiments conducted in a quiet, soundproof room, the optical densities of the regions corresponding to these structures are markedly reduced, and they can hardly be distinguished from the adjacent gray matter. Unilateral occlusion of an auditory canal results in marked asymmetry of tracer uptake in the inferior colliculus, which has a lower optical density on the contralateral side. This observation is consistent with neuroanatomical knowledge of the central organization of the auditory system (*9*).

These studies demonstrate that [^{14}C]-deoxyglucose can be used to map the regions in brain with altered glucose utilization in response to alterations in local functional activity. This capability can be applied to the mapping of functional neural pathways. When combined with autoradiography, this mapping technique has a major advantage over conventional mapping methods in that it permits a survey of all the structures of the brain simultaneously. It is possible that the use of [^{3}H]deoxyglucose rather than [^{14}C]-deoxyglucose as the tracer may extend the structural resolution of the technique even further.

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Tryptoline Formation by a Preparation from Brain with 5-Methyltetrahydrofolic Acid and Tryptamine

Abstract. *An enzymatic preparation from human brain converts tryptamine to tryptoline (9H-1,2,3,4-tetrahydropyrido[3,4-b]indole) in the presence of 5-methyltetrahydrofolic acid. Similarly, N-methyltryptamine and 5-hydroxytryptamine yield 1-methyltryptoline and 5-hydroxytryptoline, respectively. Neither in vitro nor in vivo formation of these compounds by human tissues has been described.*

Administration of the amino acids tryptophan and methionine to schizophrenic patients exacerbates their illness, giving rise to suggestions that endogenous hallucinogens might be formed after methylation of indole derivatives (*1*). Methylated products such as the hallucinogen dimethyltryptamine (DMT) have been investigated as possible causative agents of schizophrenia (*2*). Several investigators have reported the enzymatic synthesis of DMT by human brain, lung, and blood (*3*). In these studies *S*-adenosylmethionine, a major methyl donor in methylations of biogenic amines, was used (*4, 5*). There have been recent reports, starting with those of Laduron, that 5-methyltetrahydrofolic acid (5-MTHF) might also serve as a methyl donor for reactions of 5-hydroxytryptamine (serotonin) and tryptamine with a previously undescribed enzyme (*6, 7*). Indirect evidence suggested *O*- or *N*-methylation; however, the identity of the products was not established in all cases. We describe here the formation in vitro of tryptolines, a class of tricyclic compounds. They are the major products from the incubation of tryptamines and 5-MTHF with an enzymatic preparation from human brain. The products were identified by thin-layer chromatography, cocrystallization, gas-liquid chromatography, and mass spectrometry.

The enzymatic preparation from brain was prepared as follows. Autopsied human brain, primarily cortex, was homogenized at 4°C in five volumes of water that was distilled in quartz vessels. The homogenate was then centrifuged at 30,000*g* for 30 minutes. The supernatant was subjected to fractionation with ammonium sulfate; the material precipitating in 30 percent to 60 percent ammonium sulfate was taken up in 0.02*M* potassium phosphate buffer (*p*H 6.5) and dialyzed overnight against the same buffer. Incubation mixtures for the enzymatic assay contained, at final concentrations: 0.2*M* potassium phosphate buffer (*p*H 6.5); 0.015*M* amine substrate; 6 m*M* pargyline; 11 μM [^{14}C]5-MTHF (Amersham/Searle, 60 mc/mmole in 0.01*M* mercaptoethanol); and tissue extract, 1 to 2 mg protein. Human brain enzyme, heated to 95°C for 5 minutes, was used as a blank. The mixtures (final volume, 0.45 ml) were incubated for 60 minutes at 37°C. The reaction was terminated with 0.5 ml of 0.5*M* borate buffer (*p*H 10). Then 5 ml of toluene and isoamyl alcohol (97 : 3) was added, the mixture was shaken vigorously for 15 seconds, 3 ml of the organic phase was transferred to a counting vial, and the solvent was removed by evaporation (*5, 8*) at 40°C in a vacuum oven. The residue was dissolved in 2 ml of ethanol, and its radioactivity was determined by liquid scintillation counting in 10 ml of toluene phosphor. Each substrate yielded a single major radioactive product, as determined by thin-layer chromatography.

Table 1. Relative substrate specificity of a partially purified, enzymatic preparation of human brain with tryptamine activity at 100 percent (59 pmole per milligram of protein per hour). Products were examined only for tryptamine, *N*-methyltryptamine, and 5-hydroxytryptamine. Incubation products were extracted into 5 ml of toluene and isoamyl alcohol (97 : 3), except for 5-hydroxytryptamine, for which 5 ml of ethyl acetate was used.

Substrate	Relative activity
Tryptamine	100
N-Methyltryptamine	61
N,N-Dimethyltryptamine	3
5-Hydroxytryptamine	99
5-Methoxytryptamine	78
1-Methyltryptamine	100
Tryptoline	113
1-Methyltryptoline	5

Under these conditions enzymatic activity is linear with time for at least 2 hours and with protein up to 9 mg/ml, when a boiled tissue blank is used. The ratio of the reaction product to the boiled enzyme blank is at least 5 : 1. The optimal *p*H for the reaction is 6.5, and phosphate buffers of increasing strength enhance the reaction. As has been reported (*7*) methylcobalamin (0.025*M*) and flavin adenine dinucleotide (0.01*M*) also increased formation of the product. At 1 m*M* concentrations, ascorbic acid enhances the reaction by 30 percent, while 1 m*M* cupric ion inhibits it by 77 percent. The relative activities of several substrates (Table 1) demonstrate that tryptamine and 5-hydroxytryptamine are the most active substrates among the indolealkylamines tested. Methylation of the indole nitrogen does not affect the reaction rate; in contrast, methylation of the side chain nitrogen decreases activity. The apparent Michaelis-Menton constant (K_m) for the enzyme from brain was 4 m*M* for tryptamine; 6 m*M* for *N*-methyltryptamine; 2 m*M* for 5-hydroxytryptamine; and 3 m*M* for 5-MTHF when tryptamine was used as a substrate. A comparison of enzymatic activity in a supernatant preparation of several regions of human brain was made with tryptamine as substrate. The relative activity, in units per gram of tissue, was: hypothalamus, 100; medial thalamus, 0; septal region, 20; amygdala, 64; occipital cortex, 14; temporal cortex, 62. Enzymatic activity was also present in human platelets (*9*) and liver, but not red cells or plasma.

We have been particularly concerned with identifying the reaction products formed from tryptamine, *N*-methyltryptamine, and 5-hydroxytryptamine. The reaction has generally been thought to be a methyl transfer in which tryptamine would yield *N*-meth-