finements to distinguish BB from MB may eventually be desirable, BB activity is generally not present in plasma from patients with acute myocardial infarction and accordingly displacement binding will, under almost all circumstances, reflect MB exclusively. These impressions are corroborated by parallel radioimmunoassay and enzyme measurements in serial determinations performed in 60 samples from three patients with infarction; the results from one patient are illustrated in Fig. 3.

The high level of specificity in the radioimmunoassay system for creatine kinase isoenzyme suggests that a similar approach may be useful in differentiation of other clinically important enzymes which exist in multiple forms. Previous assays for plasma enzymes and isoenzymes have been based on enzymatic activity. Studies evaluating the disappearance of the enzymes from the circulation have been restricted to determining the loss of activity. However, since this assay detects the concentration of molecules, one can determine the actual rate of isoenzyme turnover independent of activity. The assay should help to elucidate mechanisms responsible for disappearance of individual creatine kinase isoenzymes from the circulation as well as aid in elucidating the relative importance of inactivation, denaturation, or removal of creatine kinase molecules under various clinical circumstances.

Even more important, because of its sensitivity and potential for detecting enzymatically inactive MB creatine kinase isoenzymes in the circulation, the creatine kinase radioimmunoassay may lead to improved enzymatic estimates of infarct size as well as earlier detection of acute myocardial infarction. The mean activity of MB creatine kinase in the plasma of normal subjects is only 0.002 IU/ml. Thus, even a two- to threefold increase would be difficult to detect reliably with conventional methods whereas the present method detects 0.0001 IU/ml. In view of the recent evidence that ischemic myocardium can be protected in patients with acute infarction, it is important that a definitive diagnosis be made as soon as possible, since agents that can potentially decrease infarct size would be more effective if they were administered soon after the development of the initial symptoms.

ROBERT ROBERTS
BURTON E. SOBEL
CHARLES W. PARKER
Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

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16. We thank A. Painter and M. Baumann for technical assistance.
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Early Eye Removal Produces Excessive Bilateral Branching in the Rat: Application of Cobalt Filling Method

Abstract. *When one eye of a rat is removed at birth, axons from the remaining eye form an excess of branches which are directed to both sides of the brain. This finding, which is based on a novel application of cobalt tracing methods, provides an explanation for previous reports of expanded uncrossed projections after early eye removal.*

The formation of organized pathways in the central nervous system must include both the directed growth of an appropriate number of axons, sometimes over long distances, and the spatial ordering of their synaptic connections within target nuclei. Considerable attention has been devoted to the problem of the formation of connections in the brain, but relatively little is known about the factors which direct the growth of a normal complement of axons to their appropriate target nuclei. One aspect of this question concerns the rather discrete pathways of the central nervous system with bilateral projections—that is, those in which some of the component axons cross the midline axis while others remain on the same side. The optic decussation of mammals provides a good model of such a pathway because the normal complement of crossed and uncrossed axons has been well defined for many mammalian species.

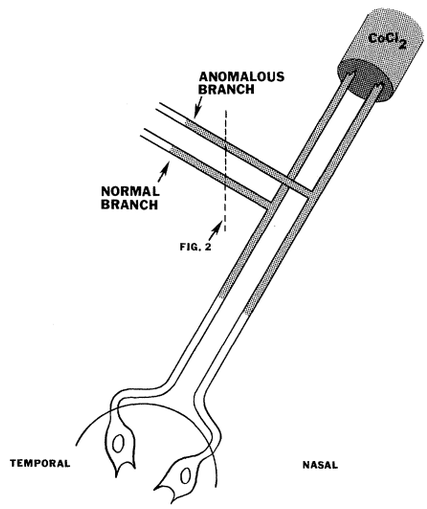

Fig. 1. Schematic diagram showing the site of cobalt injection and both the normal and anomalous uncrossed projections becoming filled, as would be expected if these projections from the retina were formed by branches of crossed axons. The dashed line through the ipsilateral optic tract illustrates the location of the section shown in Fig. 2.

In the normal hooded rat, part of each retina (the peripheral temporal segment) projects to restricted regions of both the left and right subcortical visual centers (*1*). Recent anatomical and electrophysiological studies have shown that a substantial component of these normal bilateral retinal projections consists of ganglion cells whose axons branch, presumably at the optic chiasma, to supply both sides of the brain (*2*). If one eye is removed from the rat at birth, the whole remaining retina now appears to project bilaterally. The result is an expanded distribution of the uncrossed pathway from the remaining eye; instead of the normal restricted distribution in the visual centers the uncrossed pathway occupies almost the total volume of these nuclei, although this projection is not as dense as the remaining crossed projection (*3*). These observations have indicated that there is some modification of the growth of remaining ganglion cell axons at the optic chiasma. The fibers which are normally restricted to the contralateral side have either: (i) been redirected to the ipsilateral side, or (ii) formed atypical uncrossed branches.

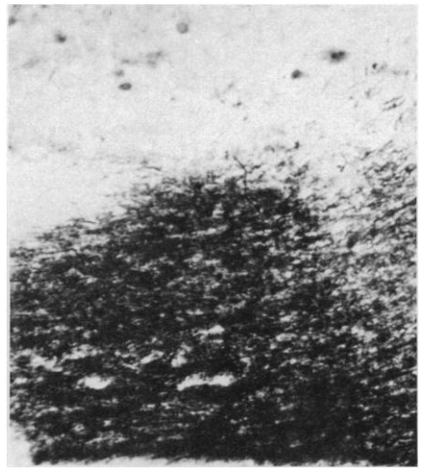

Fig. 2. Bright-field photomicrograph through the optic tract ipsilateral to the remaining eye in a rat which had one eye removed at birth. The tract contralateral to the remaining eye was injected with cobalt. The black-filled axons occupy the entire optic tract which appears in a parasagittal section at the ventrolateral margin of the thalamus (× 75).

The present experiments, in which the relatively new cobalt filling methods were used (*4*), were designed to distinguish between these alternatives and to provide more data about the formation of the more limited bilateral projections of normal mammals. Cobalt filling techniques were used because cobalt travels intra-axonally and is capable of demonstrating discrete axonal pathways (*4*). In this system, I expected that injection of cobalt in one optic tract would fill axons in a retrograde direction toward the eye, and if the axons had branches in the opposite optic tract, these would fill in the orthograde direction (Fig. 1).

Four hooded rats (3 to 8 months old), each with one eye removed on the day of birth, were perfused with cold saline (4°C). After decapitation, the head was placed in a dish of saline in a cold room (4° to 7°C). The optic tract contralateral to the remaining eye was reflected from the lateral margin of the thalamus and isolated with Vaseline. The rostral cut end of the tract was positioned in a suction electrode containing 0.3*M* cobaltous chloride, and 10 μa of current was applied for 48 hours. After this procedure the brain was removed, immersed in ammonium sulfide for 60 minutes, and then fixed for 1 to 3 weeks. Sections (30 μm in width) were cut and mounted, and then the cobalt-filled profiles were intensified with Timm's silver developer (*5*).

In all of the rats, the cobalt injected into the tract contralateral to the remaining eye appeared to fill all the axons of the opposite or ipsilateral optic tract (Fig. 2). In one of the rats the filled axons penetrated the opposite lateral geniculate nuclei (*6*) and showed an expanded uncrossed pathway which was similar to that previously described with anterograde degeneration methods after eye removal at birth (*3*). In regions of the nucleus where terminal staining would be expected with other methods, the cobalt method revealed irregular clusters of undirected profiles (Fig. 3). It is possible that these represent the filling of optic terminals.

The apparent completeness of filling of the ipsilateral optic tract indicates that both the normal uncrossed projection and the expanded uncrossed projection are principally composed of branches of crossed axons. The results of these experiments do not rule out the existence of some nonbranched and separate uncrossed axons in this pathway. It is possible that some fibers were not filled in the ipsilateral tract and these would not be readily apparent under the light microscope. However, because of the density of filling, the number of such axons is expected to be small.

The experiments also demonstrate that removal of the optic axons from one eye results in increased branch formation by axons from the opposite eye and this increase contributes to an expanded distribution of the uncrossed pathway in the subcortical visual centers. Previously it was shown that the amount of central expansion of the uncrossed projection from one eye depends on the postnatal age at which the opposite eye is removed (*3*). Thus, there is a progressive limitation of the expansion from birth to day 12. The fact that a larger effect is found in the younger rats has been interpreted as reflecting an anomalous routing of developing ganglion cell axons even though it is not known at what age all the axons reach the optic chiasma. The present results indicate that at least a part of this anomaly consists of exaggerated branch formation by crossing axons. This excess could include the formation of more branches from axons which normally have bilateral projections and new branches from axons which normally are restricted to the contralateral side. Furthermore, because the retinal projection is still developing at the time the lesion is made, it seems most likely that the branches are formed from the growing tips of the axons as they pass through the optic chiasma. Another possibility is that axons which have already grown through the chiasma have the capacity (in neonatal rats) to form branches from regions other than the growing tip.

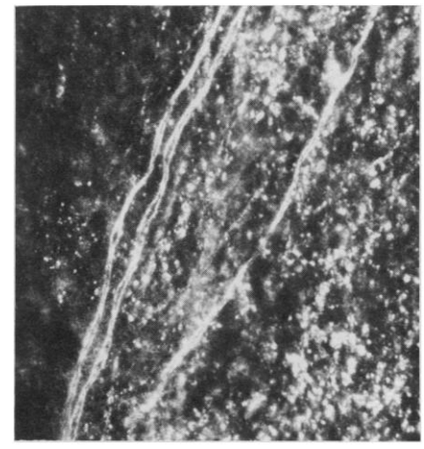

Fig. 3. Dark-field photomicrograph through the ventral lateral geniculate nucleus after cobalt injection of the optic tract on the opposite side. The section is from an animal which had one eye removed at birth. The nucleus is bounded by two filled axons to the left and to the right is a zone of optic termination (× 525).

In either case, the presence of intact axon populations from both eyes must limit the number of bilateral branches during normal development. Other work in this laboratory, in which silver degeneration methods have been used, shows that even the normal bilateral branches from the temporal retina are quite separate at the optic chiasma (*2*). The crossed branches run from dorsal to ventral at the chiasma while the uncrossed branches maintain a dorsal position and travel for a short distance in what appears to be a separate bundle. This separation may imply that uncrossed branch formation cannot occur in the proximity of crossed axons—that the presence of crossed axons in some way inhibits uncrossed branch formation—even in normal rats. Therefore, one factor which may be important in controlling the bilaterality of axons, or branch formation in general, is the way in which functionally related axons interact during development.

T. J. CUNNINGHAM

Department of Anatomy,
Medical College of Pennsylvania,
Philadelphia 19129

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6. The cobalt ions never traveled more than 15 to 16 mm in any of these experiments or in concomitant experiments where the optic nerve was filled instead of the tract. This limitation indicates that the branches are formed at the optic chiasma: from the point of injection on one side to the geniculate nuclei on the opposite side (by way of both optic tracts through the optic chiasma) is about 15 mm.
7. I thank M. Segraves for expert histological assistance, R. Malinsky for photographic work, and M. Murray and M. Goldberger for valuable criticism. This work was supported by NSF grant BMS 74-24088.

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Carrying Behavior in Humans: Analysis of Sex Differences

Abstract. *Behavioral differences between the sexes include methods of carrying books. Females clasp books against their chests; males carry them at their sides. In kindergarten and the first grade, both sexes carry like mature males. Sex-typical carrying appears before adolescence. Behavioral differences seem to be primarily a consequence of morphological differences and social modeling.*

Male and female college students differ consistently in their methods of carrying books. The results of our studies on carrying behavior of college students in Montana, Ontario, New York, El Salvador, and Costa Rica all show similar sex differences (*1*) and are in agreement with studies in Tennessee (*2*). College females usually wrap one or both arms around their books, which they rest on their hips or clasp against their chests. College males carry books in one hand at the side of the body. This behavioral difference must be widely known and recognized (*3*), but it has usually been ignored as a subject of study.

We classified book-carrying behavior into eight categories, but it is sufficient here to combine them into two basic types (Fig. 1) and to group the other categories as "other." In type I, one or both arms wrap around the books; the forearm, on the outside of the books, supports them. The short edges of the books rest horizontally against the body on top of the hip or in front of the body in line with or higher than the hips. When books are carried in one arm, the fingers wrap around the long edges. When they are carried in both arms, the fingers wrap around contralateral edges or grasp contralateral forearms or wrists. In type II, books are supported by one arm and hand at the side of the body, with the long edges approximately horizontal. The hand may be above the books, pinching them between the thumb and the fingers, or on the outside of the books with the fingers wrapped around the lower edges. When the elbow is flexed and the books are raised, the long edges sometimes rest on both the hand and the forearm or wrist. Other methods include a variety of unusual, infrequent methods such as resting the books on the shoulder or head.

Our initial discovery that 92 percent of females at the University of Montana carried their books according to type I and 95 percent of males carried according to type II was confirmed in widespread locations throughout North and Central America (*1*). Sex differences in strength or in load size do not explain the behavioral difference. Both females and males carry both large and small loads typically for their sex (*1*). A detailed study of book carrying, weight of books, and grip strength failed to reveal any causal relationship among these variables (*4*).

Differences in morphology of physically mature individuals may contribute to the differences in carrying. Ratios of hip width to shoulder width are different for males and females. Not only is the female hip relatively wider than that of the male, but fat over the iliac crest gives it a more shelf-like quality (*5*). In most females, the carrying arm could not hang vertically but would have to angle outward. Males and females also differ in the angle at which the forearm attaches to the arm.

The differences in book carrying might also be an expression of sex differences in body postures and in the way the limbs are held. Females tend to assume more closed positions than males; they more often fold their arms in front of the body and cross their legs or keep them together. Male positions tend to be more open or exposed (*6*). Similar postural differences were found in a comparative study of 480 human cultures (*7*) and in studies of other primates (*8*). This raises the possibility that these differences may in part be genetically determined. Certainly the female carrying methods result in positions that are more closed, with the arms and books partially covering the front of the body; male carrying methods result in open positions that leave the body unobstructed.

First-grade school children in Montana lacked both the sex differences and the rigid stereotypy typical of college students. The differences developed most rapidly during the junior high school years. To determine more precisely when these changes in behavior occur, we recorded the spontaneous book carrying methods of 2256 individuals from kindergarten through old age in Ithaca, New York, between October and December 1975. We made a single record of each individual's carrying method as he entered an arbitrarily defined space. All data were combined into types I, II, and other, and into ten levels: kindergarten through grade 1; grades 2 and 3, 4 and 5, 6, 7, 8, 9, and 10 through 12 (high school); college; and mixed-age adults.

In the kindergarten and grade 1, there are no significant differences in carrying behavior between males and females

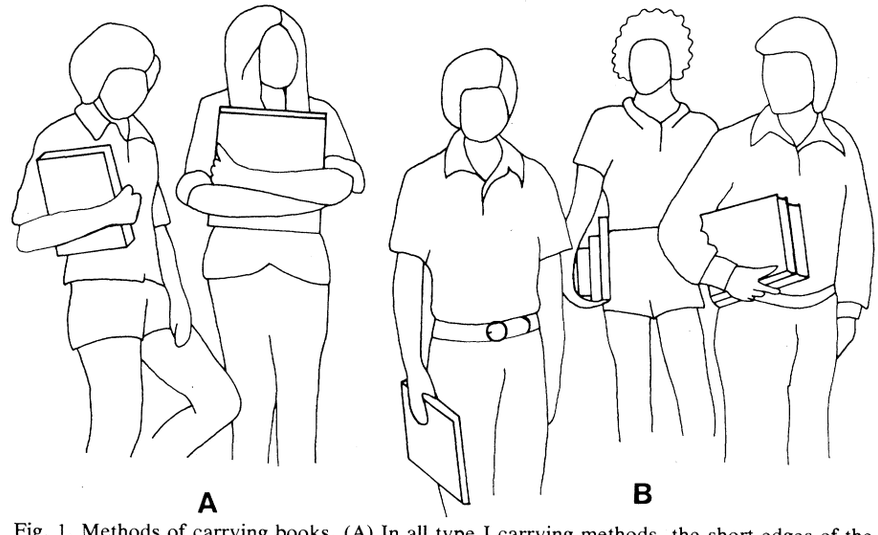

Fig. 1. Methods of carrying books. (A) In all type I carrying methods, the short edges of the books rest on the hip or in front of the body. (B) In type II methods, the books are either pinched from above or supported from below by the hand or the hand and arm.