

# Retrograde Axonal Transport of Transmitter Enzymes, Fucose-Labeled Protein, and Nerve Growth Factor in Streptozotocin-Diabetic Rats

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## SUMMARY

Rapid axonal transport was studied by several methods in rats with serum glucose levels above 300 mg/dl as a result of treatment with streptozotocin (45–50 mg/kg) 3 days, 1 wk, 4 wk, 8 wk, or 4 mo earlier. With untreated age-matched rats as controls, rapid anterograde axonal transport in the sciatic nerves of diabetic rats appeared entirely normal. Statistically significant differences were never observed between experimental and control nerves in the basal content of acetylcholinesterase (AChE) and dopamine- $\beta$ -hydroxylase (DBH) activity or in the rate of accumulation of these enzymes proximal or distal to a ligature. Therefore, the basic capacity for rapid anterograde and retrograde axonal transport of proteins was probably unimpaired in the diabetic nerves. The accumulation of labeled glycoproteins proximal to ligatures on the contralateral nerves of the same rats after injection of the L5 dorsal root ganglion with  $^3\text{H}$ -fucose was likewise normal. However, rats with 1, 4, and 8 wk of diabetes did show reduced accumulation of fucose-labeled protein distal to nerve ligations, indicating a long-lasting abnormality of retrograde axonal transport. Furthermore, this abnormality was reversed by daily insulin treatment during the second half of an 8-wk experimental period. It is therefore unlikely that the depression of retrograde transport reflects direct toxic effects of streptozotocin. We conclude that streptozotocin-induced diabetes leads to: (1) abnormal delay in the turnaround of transported proteins in distal nerve regions, perhaps combined with (2) an abnormal metabolism of glycoproteins. A delayed onset of retrograde transport is consistent with present observations of reduced accumulation of  $^{125}\text{I}$ -labeled nerve growth factor (NGF) below a midhigh ligature on the sciatic nerve after injection of this protein into

the hindfoot of rats with 3–5 wk of diabetes. Further work on the factors controlling rapid retrograde transport of proteins in diabetic nerve is warranted. **DIABETES 30:797–803, October 1981.**

**C**linical signs and symptoms of neuropathy in diabetes mellitus are accompanied by changes in nerve fiber structure, nerve conduction, and axonal transport.<sup>1–5</sup> Neurophysiologic changes are present even in patients with newly diagnosed diabetes,<sup>4</sup> although little is known about the morphology of the nerves and the intraaxonal transport of proteins at this stage of the disease.

Animal models have recently been used to gain information about the structure and function of peripheral nerve during the early stages of diabetes. In rats, the caliber of myelinated axons, the conduction velocity, and the rate of slow axonal transport are all reduced when experimental diabetes is induced by streptozotocin.<sup>6–9</sup> These changes appear within the first few weeks after the induction of diabetes, and the structural and neurophysiologic abnormalities can both be prevented by insulin treatment.<sup>10</sup>

Recently, a decreased accumulation of retrogradely transported glycoproteins in axons of rat sciatic nerve has been reported as early as 1 day after the establishment of a diabetic state.<sup>11,23</sup> It has not been shown, however, whether this effect involves abnormalities in the transport mechanism itself or whether it involves an altered metabolism of glycoproteins during their turnaround in the distal parts of the nerve cell. Nor has it been shown whether the abnormality can be reversed by insulin treatment.

In an attempt to elucidate these questions, we examined the axonal transport of fucose-labeled glycoprotein, the transmitter-associated enzymes acetylcholinesterase (AChE) and dopamine- $\beta$ -hydroxylase (DBH), and nerve growth factor (NGF) in streptozotocin-diabetic rats. Our results confirm the view that the early stages of diabetes are associated with disturbances in retrograde axonal transport. The transport disturbances reported here were reversed by

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insulin treatment but they proved to be more selective than was previously suspected.

#### MATERIALS AND METHODS

Male Sprague-Dawley rats were used for all experiments. For experiments of up to 2 mo in duration, rats 18–22 wk old weighing from 250–500 g were injected intravenously with streptozotocin, 45–50 mg/kg. The axonal transport of transmitter enzymes and fucose-labeled proteins was measured together in two groups of animals, with 1 and 4 wk of diabetes, respectively. Labeled protein was examined alone in a group with 8 wk of diabetes. Accumulation of nerve growth factor was determined in separate groups of rats with from 3 days to 5 wk of diabetes. All groups of diabetic rats were matched at the time of streptozotocin treatment with control groups of the same age and average weight.

Serum glucose determinations were made on tail vein blood samples withdrawn on the day after treatment with streptozotocin and on the day of the transport experiments. Glucose determinations were made by the Mayo Clinical Chemistry Unit on a Beckman Analyzer (Beckman Instruments, Fullerton, California) using the glucose oxidase procedure. All rats included in the study had serum glucose levels above 300 mg/dl in both samples.

The rats used for estimation of axonal transport of transmitter enzymes in long-term diabetes were treated with streptozotocin (50 mg/kg) at 10–12 wk of age (starting body wt. about 200 g). Age-matched animals of the same starting body weight were kept as controls. Only rats with serum glucose levels above 300 mg/dl on the day following injection were saved for study. After 20 wk, ligatures were placed on the sciatic nerves, which were later taken for measurements of the accumulation of transmitter enzymes.

**Insulin treatment.** One group of rats was treated with insulin, starting 4 wk after the induction of diabetes. A long-acting insulin preparation (modified Ultralente, pH 5.5) kindly provided by Dr. J. Schlichtkrull (Novo Research Institute, Copenhagen, Denmark) was used for this purpose. Insulin was given daily at noon and the dose was selected according to blood glucose determinations made just beforehand (average dose 3 International units). At 8 wk after induction of diabetes, these animals were used for determination of the anterograde and retrograde transport of fucose-labeled protein.

**Axonal transport of transmitter enzymes.** Our initial studies (on rats with 4 mo of diabetes) were made with single ligatures placed on the sciatic nerve for 6 h. At the end of this collection period, the nerves were removed and 3-mm segments were taken just proximal and distal to the ligatures. Three to four control segments were taken from more proximal regions of the nerve. All segments were homogenized and assayed for DBH or AChE activity (see below). Accumulation was defined as the enzyme activity of the ligated segments minus the mean enzyme activity of the control segments.

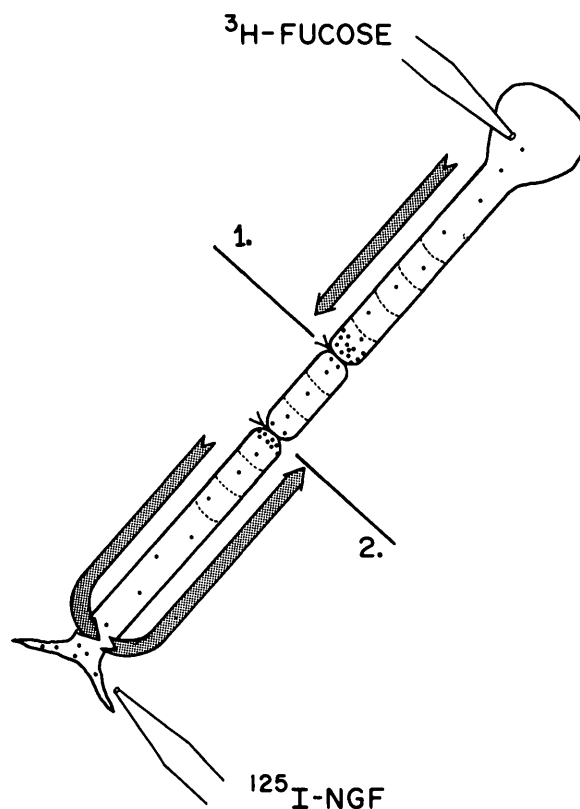
Since accumulation of enzyme activity in the distal segments was small after 6 h, further work was done to determine whether it would be enhanced by longer periods of ligation. The results showed that the distal accumulation of both enzymes was essentially linear for up to 24 h. For practical reasons, experiments on enzyme transport after 1 and 4 wk of diabetes were then designed with a 19-h ligation pe-

riod. These experiments were carried out in the same rats used to study transport of fucose-labeled glycoproteins (see below). Two hours after radioisotope injection on the right side, two ligatures were placed 9 mm apart around the midhigh portion of the left sciatic nerve (see schematic diagram of Figure 1). At 21 h after injection, that nerve was removed and cut into 3-mm segments. The mean enzyme activity in the interligature region ( $A_1$ ) was used as a baseline against which to measure accumulation in the segments above the proximal and below the distal ligature ( $A_{cc}$ ). Percentage accumulation of enzyme activity was then calculated by means of the formula:

$$\%A = 100[(A_{cc}/A_1) - 1]$$

**Enzyme assay.** Nerve segments were homogenized in glass homogenizers each containing 300  $\mu$ l of ice-cold buffer [0.05 M Tris HCl, pH 7.4, 0.2% (w/v) bovine serum albumin, and 0.1% (v/v) Triton X-100]. The homogenates were centrifuged for 10 min at 2500 rpm in a Sorvall GSA rotor at

**FIGURE 1.** Diagram of the sciatic nerve system showing sites of ligation and isotope injection (not to scale). For studies on the anterograde and retrograde transport of glycoconjugates,  $^3\text{H}$ -fucose was injected into the right L5 dorsal root ganglion. For studies on the retrograde transport of exogenous protein,  $^{125}\text{I}$ -labeled nerve growth factor ( $^{125}\text{I}$ -NGF) was injected into the right hind footpad. For studies of enzyme transport, the left sciatic nerve was used without injection. Ligatures were applied simultaneously in the midhigh. Dashed lines indicate the 3-mm segments typically analyzed. The three segments between the ligatures were used as an index of the baseline enzyme or glycoconjugate content of the nerve. At site 1, anterogradely transported material arriving during the experimental period was collected. At site 2, retrogradely transported material was collected. This material was expected to include exogenous proteins taken up by the nerve terminal as well as endogenous proteins that had moved anterogradely past the ligatures before their application and had then turned around in the distal part of the nerve.



4°C, and the supernatant fractions were saved for enzyme assay.

AChE activity was measured by the radiometric assay of Potter,<sup>12</sup> using 50- $\mu$ l portions of tissue extract, as previously described.<sup>13</sup> All assays were carried out at 37°C with <sup>14</sup>C-acetate-labeled acetylcholine as a substrate in a concentration of 1 mM (New England Nuclear Corp., Boston, Massachusetts; final specific activity 0.5 mCi/mmol). Ethopropazine HCl (kindly supplied by Warner Lambert Pharmaceuticals, Morris Plains, New Jersey) was routinely added in a final concentration of 10<sup>-4</sup> M to inhibit pseudocholinesterase.

DBH activity was measured in duplicate 50- $\mu$ l portions of nerve extract by the double enzyme assay of Molinoff et al.,<sup>14</sup> using phenylethylamine as a substrate. Optimal concentrations of CuSO<sub>4</sub> were added to overcome endogenous copper-sensitive inhibitors of this enzyme, as previously described.<sup>15</sup>

**Axonal transport of fucose-labeled glycoproteins.** The fifth lumbar dorsal root ganglion on the right side was exposed by a partial laminectomy. During ether anesthesia, 5  $\mu$ Ci of <sup>3</sup>H-fucose (110 Ci/mmol, New England Nuclear, or 24 Ci/mmol, Amersham, Arlington Heights, Illinois) dissolved in 1  $\mu$ l of 0.9% saline was injected through a glass micropipette into the ganglion; 19 h later, when the retrograde transport of labeled protein is maximal,<sup>16</sup> two ties were placed 9 mm apart in the midhigh portion of the sciatic nerve (see Figure 1). After a further 2 h, the sciatic nerve was removed, frozen on a brass block, and cut into 3-mm segments. The segments were treated overnight with 10% trichloroacetic acid - 2% phosphotungstic acid, dissolved in Soluene (Packard) and diluted in 5 ml of Dimilume (Packard) for scintillation counting.

The amount of background and blood-borne radioactivity was estimated by scintillation counting of a 3-mm segment of the sciatic nerve from the contralateral side. All segments were counted for 10 min, and estimates of background and blood-borne radioactivity (typically on the order of 25 cpm) were subtracted. The accumulation was expressed as a percentage of the interligature activity, the formula being the same as that used to calculate percentage accumulation of enzyme activity.

**Axonal transport of <sup>125</sup>I-nerve growth factor.** Nerve growth factor ( $\beta$ -NGF) was purified from male mouse submaxillary glands (Pelfreez) using the procedure of Mobley et al.<sup>17</sup> Purification was monitored by SDS gel electrophoresis and analytical isoelectric focusing. Optimal activity of the purified protein using the 8-day chick embryo dorsal root ganglion was 10 ng or less of protein per assay.<sup>18</sup> Radioiodinated NGF was prepared using the lactoperoxidase procedure of Sutter et al.<sup>19</sup> <sup>125</sup>I-NGF dissolved in 30  $\mu$ l 0.9% NaCl was injected into the subcutaneous tissues of the hindpaws.<sup>20</sup> In the experiments with a 3-5-wk and a 3-day duration of diabetes, 3.0-3.2  $\times$  10<sup>6</sup> cpm and 1.9  $\times$  10<sup>6</sup> cpm, respectively, were used for each injection. In those experiments designed for the study of transport in the axons, a collection crush was made immediately after the injection by a unilateral ligature on the midhigh portion of the sciatic nerve. On the contralateral side, the nerve was left intact for the study of transport to the ganglion.

Ganglia and nerve segments were exposed and removed at various intervals and the radioactivity of each sample was

measured in a gamma scintillation counter for 5 min. Accumulation in the sciatic nerve was defined as the activity in the 3-mm segment distal to the ligature minus the activity in the proximal 3-mm segment. Accumulation in the ganglion was defined as the activity on the intact side minus that on the ligated side.

**Statistical analysis.** Unless otherwise stated, the standard deviation was used as the measure of variation. Differences between paired means were assessed by means of the *t* test, with *P* < 0.05 taken to indicate statistical significance. Two-way analysis of variance among treatment means was performed on a Hewlett Packard Model 85 microcomputer, following established procedures.

## RESULTS

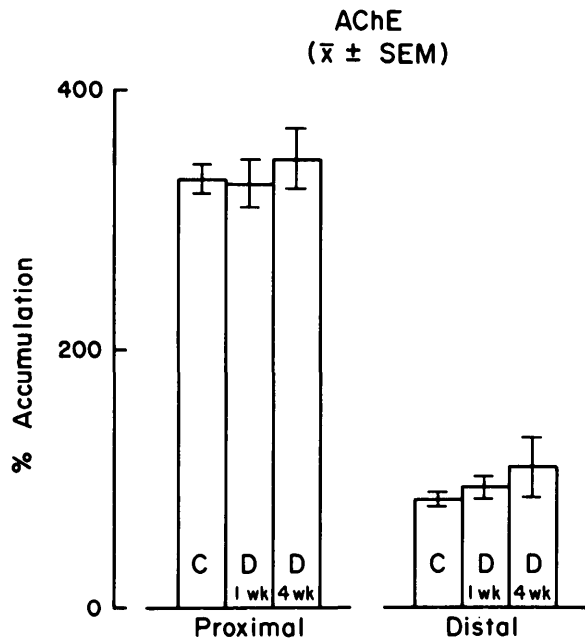
**Body weight and serum glucose.** At the time of the axonal transport experiment, serum glucose was raised substantially in all streptozotocin-treated rats. The average value was 423  $\pm$  61 mg/dl as compared with 134  $\pm$  12 mg/dl in controls. These large rats had lost 31  $\pm$  5 g of body weight after 3 days of diabetes, 50  $\pm$  16 g after 1 wk, 126  $\pm$  34 g after 4 wk, and 154  $\pm$  32 g after 8 wk. Over the same period, on the other hand, the age-matched control rats gained weight. At 4 wk, this weight gain averaged 10  $\pm$  11 g.

The insulin-treated rats had a starting body weight of 306  $\pm$  35 g and blood glucose levels of 458  $\pm$  27 g 4 wk after the administration of streptozotocin. During the next 4 wk of hormone injections, the 20th, 50th, and 80th percentiles of blood glucose levels were 55, 106, and 225 mg/dl, respectively. At the time of the transport experiment 8 wk after streptozotocin administration, these rats weighed 391  $\pm$  18 g. The final blood glucose determinations (made during the transport experiment, 13 h after ganglion injection) yielded an average value of 117  $\pm$  97 mg/dl.

The rats with diabetes for 4 mo had an average serum glucose of 415  $\pm$  29 mg/dl as compared with 137  $\pm$  23 mg/dl in the age-matched controls. At the time the rats were killed, the mean body weight of the diabetic rats was 266  $\pm$  32 g while the control rats weighed 489  $\pm$  40 g. Both the treated and the control rats gained weight, although at different rates. Cataracts developed in 80% of the diabetic rats but in none of the control rats.

**Retrograde axonal transport of endogenous transmitter enzymes.** Figures 2 and 3 show the accumulations of AChE and DBH activity in the proximal and distal collection segments of control rats and rats with diabetes for 1 and 4 wks. In control nerves, the amount of AChE in the distal accumulation segment doubled during the 19-h ligation period. The accumulation of DBH was almost three times larger. There were no significant differences from control in the distal accumulation of the two enzymes in either group of diabetic rats. Nor were there any differences in proximal accumulation nor in absolute amount of interligature activity among the three groups. A statistically significant difference did arise between the distal accumulation of DBH activity in rats with 1 wk of diabetes as compared with that in rats with 4 wk of diabetes.

In the group with 4 mo of diabetes, the ligatures were placed for 6 h only, and the accumulation of AChE and DBH activity in the proximal and distal collection segments was correspondingly lower (Figure 4). Nevertheless, none of the values differed significantly from those obtained in the age-

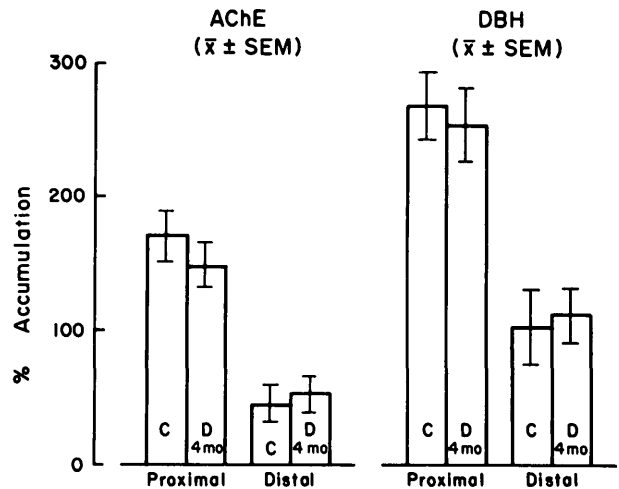
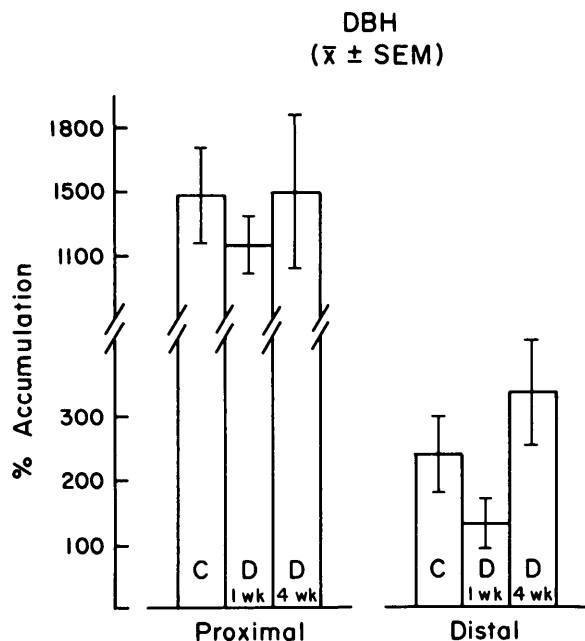


**FIGURE 2.** Effect of short-term diabetes on axonal transport of acetylcholinesterase in rat sciatic nerve. Double ligatures were placed for 19 h in the midhigh region. Accumulation of enzyme activity is calculated with reference to the mean activity of the interligature region, as described in METHODS (100% accumulation corresponds to doubled content). "C" indicates controls; "D 1 wk" and "D 4 wk" indicate rats with diabetes induced by streptozotocin, 50 mg/kg, 1 and 4 wk earlier, respectively. Data represent means of 5-6 experiments.

matched control rats subjected to ligation for the same period of time. It was concluded that experimental diabetes caused no consistent changes in the axonal transport of the measured enzymes.

**Retrograde axonal transport of <sup>3</sup>H-fucose labeled glycoproteins.** For technical reasons, the amount of label incorporated into transported protein after injection of <sup>3</sup>H-fucose

**FIGURE 3.** Axonal transport of dopamine-β-hydroxylase. Data represent simultaneous enzyme assays on the nerve samples shown in Figure 2.

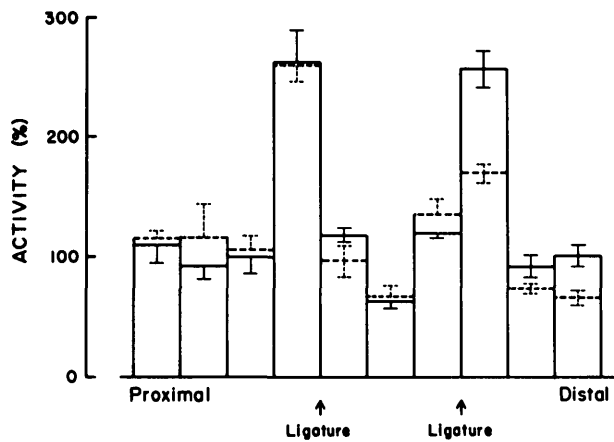


**FIGURE 4.** Effect of long-term diabetes on axonal transport of acetylcholinesterase and dopamine-β-hydroxylase. Single ligatures were placed on the sciatic nerve for 6 h in vivo. Accumulation of enzyme activity was calculated with reference to the mean activity of 3-4 nerve segments proximal to the collection segment above the ligature. "C" indicates controls; "D" indicates rats with diabetes induced by streptozotocin 4 mo earlier. Data represent means of 7 (control) and 10 (diabetes) experiments.

into the L5 dorsal root ganglion varies greatly from one animal to the next. Therefore, the radioactivity in the interligature segments was used as a reference value to normalize the accumulation of labeled material against the ligatures (see METHODS). In control rats, the total radioactivity in the 9-mm interligature region was  $263 \pm 147$  cpm. In rats treated with streptozotocin, interligature radioactivity was slightly lower ( $253 \pm 128$  cpm at 1 wk,  $232 \pm 137$  cpm at 4 wk, and  $218 \pm 67$  cpm at 8 wk) but none of the differences from control was statistically significant. Neither did the combination of streptozotocin and insulin treatment have a statistically significant effect on this quantity ( $196 \pm 21$  cpm).

In Figure 5, the normalized distribution of labeled protein along the sciatic nerve is shown at 21 h after injection of <sup>3</sup>H-fucose into the L5 dorsal root ganglia of control rats and rats with 1 wk of diabetes. The most striking abnormality in the diabetic animals was a reduction in the proportion of label in the segment just below the distal ligature. In the segment just above the proximal ligature, on the other hand, the proportion of label was the same in both groups of rats. This pattern of results was obtained in every diabetic group, as can be seen in Figure 6, which shows individual values for proximal and distal accumulation of labeled material in all rats. After 1, 4, and 8 wk of diabetes, the accumulation in the distal collection segment was reduced by 55% ( $P < 0.001$ ), 27% ( $P < 0.01$ ), and 51% ( $P < 0.001$ ) respectively. It is especially noteworthy that insulin treatment during the second half of the experimental period completely overcame the depression of distal accumulation measured after 8 wk of diabetes, even though it was associated with a small drop in proximal accumulation (Figure 6).

**Retrograde axonal transport of <sup>125</sup>I-NGF following subcutaneous injection.** The uptake and transport of nerve growth factor (NGF) was studied in several groups of rats with diabetes of 3-5 weeks duration, in comparison with age-matched controls. Figure 7 shows the accumulation of <sup>125</sup>I-labeled NGF in the ligated sciatic nerve at 3 times after

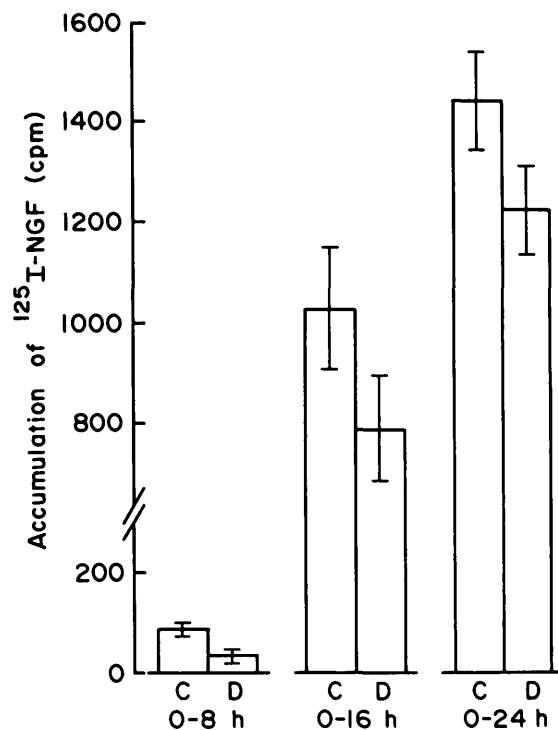
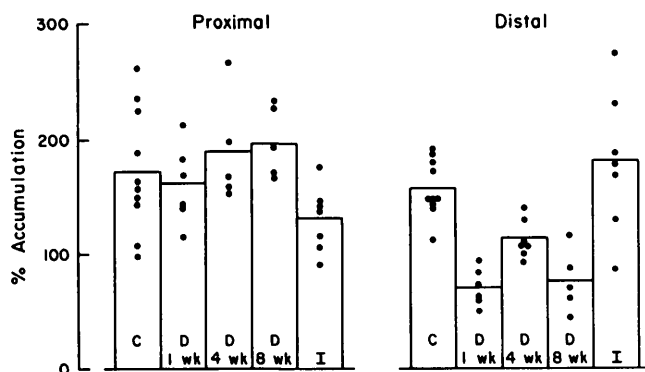


**FIGURE 5.** Effect of short-term diabetes on the distribution of  $^3\text{H}$ -fucose labeled protein in ligated rat sciatic nerve. Double ligatures were placed at the indicated points (arrows) 19 h after the L5 dorsal root ganglion was injected with  $^3\text{H}$ -fucose. A 2-h collection period was used. Labeled protein content is expressed as a percentage of the mean activity per 3-mm segment in the interligature region. Solid bars indicate control nerves; dashed bars indicate nerves from rats with 1 wk of streptozotocin-induced diabetes. Means and SEMs of 10 experiments are shown.

s.c. injection in the foot. After 8 h, the radioactivity in the distal collection segment was  $34 \pm 44$  cpm in diabetic rats versus  $88 \pm 22$  cpm in controls, when activity in proximal nerve segments ( $122 \pm 43$  cpm) was subtracted to correct for local uptake of blood-borne NGF. The reduction, which amounts to 61%, is statistically significant ( $P < 0.02$ ). After 16 h of collection, the accumulation in the diabetic nerves was reduced by 23%, and after 24 h, it was reduced by 15% as compared with control nerves. Neither of these differences was significant by themselves. However, a two-way analysis of variance supports the conclusion that accumulation of NGF in diabetic nerves was generally depressed ( $F = 5.5$  with 1 and 30 degrees of freedom,  $P < 0.05$ ).

No accumulation of labeled NGF was detected in the fifth lumbar dorsal root ganglion at 8 or 16 h after injection. By 24 h, however, labeling of the ganglia on the unligated side was definitely increased over that of the ganglia on the li-

**FIGURE 6.** Effect of short-term and intermediate-term diabetes on axonal transport of  $^3\text{H}$ -fucose labeled protein. Double ligations were for 2 h, as in the previous figure. Accumulation is calculated with reference to the mean radioactivity per segment in the interligature region. "C" indicates control rats; "D 1 wk", "D 4 wk" and "D 8 wk" indicate rats with 1, 4, and 8 wk of streptozotocin-induced diabetes, respectively. "I" indicates the rats with 8 wk of diabetes that were treated daily with insulin during the 4 wk preceding sacrifice. Symbols show individual values; mean values are indicated by the bar heights.



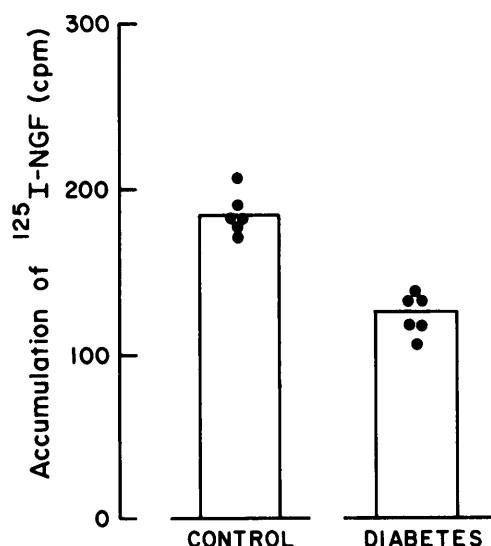
**FIGURE 7.** Effect of short-term diabetes on the retrograde axonal transport of labeled nerve growth factor. Ligatures were placed on the sciatic nerve at midhigh level at the time when  $^{125}\text{I}$ -NGF ( $1.3\text{--}1.4 \mu\text{Ci}$ ) was injected into the corresponding footpad. Accumulation is defined as the amount of label in the 3-mm nerve segment just below the ligature, minus the activity in the segment just above the ligature. Means of 6-7 experiments per group are shown. "C" indicates controls; "D" indicates rats with 3-5 wk of streptozotocin-induced diabetes.

gated side. The net accumulation during the 24-h collection period was  $185 \pm 13$  cpm in controls and  $124 \pm 13$  cpm in the diabetic rats (Figure 8). The difference amounts to 33% and is highly significant ( $P < 0.001$ ).

To determine if the onset of diabetes leads to immediate abnormalities in the transport of NGF, a further experiment was performed on rats treated with streptozotocin 3 days earlier. However, after 12 h of ligation, the accumulation of labeled NGF in eight diabetic nerves ( $545 \pm 89$  cpm) was almost identical to the accumulation in seven control nerves ( $564 \pm 89$  cpm). And in the dorsal root ganglia taken 24 h after NGF injection in the hindpaw, the same accumulation of radioactivity was seen in the diabetics ( $93 \pm 28$  cpm) and controls ( $97 \pm 14$  cpm).

## DISCUSSION

Our results show that experimental diabetes in rats is associated with selective abnormalities of retrograde axonal transport. Significant reductions in the distal accumulation of fucose-labeled material (mostly glycoproteins, presumably) were found to persist for up to 8 wk after administration of streptozotocin. This persistence makes it highly unlikely that the abnormalities of transport represent direct toxic effects of streptozotocin. On the contrary, it seems probable that the effects on retrograde transport follow from the metabolic derangements associated with the loss of pancreatic islet cells. This conclusion is strongly reinforced by the observation that retrograde transport of fucose-labeled material was restored to normal in diabetic rats whose blood



**FIGURE 8.** Accumulation of labeled NGF in dorsal root ganglia. The contralateral footpads of the animals represented in Figure 7 were also injected with 1.3–1.4  $\mu\text{Ci}$  of  $^{125}\text{I}$ -NGF. In the groups killed 24 h after injection, the amount of label in the corresponding dorsal root ganglia was measured. Accumulation was defined as the cpm in these ganglia minus cpm in the contralateral ganglia (to which retrograde transport was prevented by the ligature). Means and individual values for seven rats are shown.

sugar was reduced by daily insulin treatment during the second half of an 8-wk experiment.

By way of contrast, the present study also demonstrated that the accumulation of endogenous transmitter enzymes on both sides of a set of ligatures remains nearly normal for up to 4 mo of streptozotocin-induced diabetes. The only effect noted was a small reduction in the distal accumulation of DBH activity, which appeared only at 1 wk of diabetes and which was statistically significant only in comparison with the accumulation at 4 wk of diabetes. In view of the considerable reduction in retrograde transport of fucose-labeled material in the contralateral nerves of the same rats, it is remarkable that experimental diabetes had so little effect on the transport of DBH and AChE. We conclude that there was no generalized loss of fibers in the diabetic nerves and that the system for rapid axonal transport in these nerves was intact and probably had a normal capacity. This conclusion is at variance with an earlier published report on axonal transport of transmitter enzymes in rats with experimental diabetes. Perhaps a more severe diabetes explains the 20% decrease found by Schmidt et al.<sup>21</sup> in the accumulation of AChE activity in ligated nerves 1 mo after streptozotocin treatment. However, it should be emphasized that our own negative findings were obtained in the face of a threefold elevation of blood sugar. Furthermore, Bisby<sup>22</sup> has also recently failed to demonstrate any abnormality of rapid orthograde axonal transport in streptozotocin-treated rats.

The striking difference we observed in the retrograde transport of labeled and unlabeled molecules could have arisen from a delay in the turnaround from anterograde to retrograde transport. An abnormal delay would not necessarily affect the accumulation of endogenous enzymes which, because of continuous transport, should be at equilibrium throughout all parts of the nerve. But a delayed turnaround could account for reduced accumulation of pulse-labeled proteins during early collection periods. To estab-

lish definitively whether or not experimental diabetes leads to a delayed turnaround of transported proteins, the complete time course of the accumulation of labeled material will have to be examined. Meanwhile, one must realize that other effects could also explain the present observations, e.g., selective retention of particular glycoproteins in the distal part of the neuron, or selective loss of fucose residues from proteins returned by retrograde transport. The latter possibility especially deserves scrutiny.

The present behavior of  $^{125}\text{I}$ -NGF is also consistent with an abnormality of retrograde axonal transport in diabetic rats. Accumulation at the ligature was sharply reduced during the early period after NGF injection (when the wave of label was presumably just reaching the midhigh region) but the reduction in accumulation became proportionately smaller after longer collection periods. If the turnaround of intraneuronal molecules is delayed in diabetic nerve, it is possible that the factors responsible for this effect could also delay the onset of retrograde transport after uptake of extraneuronal proteins. However, reduced uptake of NGF or selective loss of NGF-sensitive neurons are other potential causes of the changes observed in the present series of experiments.

In view of the reduced accumulation of  $^{125}\text{I}$ -NGF in sensory ganglia after 3–5 wk of diabetes, it is worth considering whether a reduced trophic support from endogenous NGF could be responsible for the pathophysiology of diabetic nerve. Although unproven, it is reasonable to assume that the label accumulating in the present study was still associated with biologically active NGF. This trophic factor is essential for survival and outgrowth of adrenergic and sensory neurons *in vitro*, and its effect is thought to be exerted in the cell bodies after retrograde axonal transport from endogenous sources in the periphery.<sup>24,25</sup> However, it is questionable whether NGF is required for maintenance of mature sensory nerves *in vivo*.<sup>26</sup>

Since the ganglionic accumulation of NGF was not decreased after 3 days of diabetes, when retrograde transport of glycoproteins is already affected,<sup>11</sup> the later abnormalities in NGF transport are likely to be secondary. The accumulation of NGF in dorsal root ganglia is reputedly confined to the large and light-staining "A cells,"<sup>20</sup> which have been found to be reduced in number after 4 wk of streptozotocin diabetes.<sup>27</sup> Therefore, selective cell and fiber abnormalities could account for the small changes in NGF accumulation presently observed. We do not currently favor the idea that NFG plays a crucial role in the development of the very early abnormalities of peripheral nerve in experimental diabetes.

#### ACKNOWLEDGMENTS

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