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SHORT COMMUNICATION

Evidence for RNA transport in rat optic nerve

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A NUMBER of biochemical and autoradiographic studies have been made on the distribution and movement of labelled substances within the neuron. Proteins labelled after *in vivo* administration of radioactive precursors have been shown to be conveyed from the original site of synthesis down the nerve fibres at an average daily rate of a few millimetres (GRAFSTEIN, 1967; LASEK, 1968; TAYLOR and WEISS, 1965 and WEIS and HOLLAND, 1967). Furthermore, recent studies on the uptake of radioactive amino acids from isolated axons have shown that the nerves can carry on a significant local protein synthesis (EDSTRÖM, 1966; GIUDITTA, DETTBARN and BRZIN, 1968 and KOENIG, 1967). The discovery within axons of small amounts of RNA, mainly of the ribosomal type (EDSTRÖM, EICHNER and EDSTRÖM, 1962; KOENIG, 1965 and KOENIG, 1967), would indicate the presence of a protein-synthesizing machinery which operates independently from the cell body. At present the origin and form of this RNA are unknown. It may either be synthesized by the axon or by satellite cells, or perhaps be transported down from the neuronal perikaryon. In the present experiments the possibility of axonal flow of RNA from the cell body has been studied by measuring the distribution of radioactivity along the optic nerves at various times after injection into the eyeball of radioactive precursors.

EXPERIMENTAL

Rats were used in all experiments. The cornea of the ether-anaesthetized animal was punctured near the limbus with a 30 gauge needle connected to a Hamilton microsyringe. Each eye received 5 μc of [^{3}H]uridine (20 c/m-mole) or 50 μc of [^{32}P]orthophosphate in a volume of 10 μl. The retinas were excised after cutting the eyeball and removing the lens and the vitreous. The optic nerves, about 10 mm long, were cut into segments and immediately frozen. The tissue samples were homogenized in 1 ml of glass distilled water with a tight glass-to-glass homogenizer. After addition of 1 ml of cold 10% trichloroacetic acid (TCA) and rehomogenization, the sample was centrifuged in an International centrifuge at 3500 rev./min for 10 min. The precipitate was washed with 1 ml of 5% TCA and centrifuged as before. The two TCA supernatants were pooled and assayed for radioactivity. RNA in the precipitate was hydrolysed with 2 ml of 1 N-KOH at 37° for 4 hr. After acidification with 0·4 ml of 6 N-perchloric acid and centrifugation, the supernatant (RNA hydrolysate) was analysed for ultraviolet absorption and radioactivity. Portions (0·1 ml) of TCA-soluble and RNA fractions were counted in 10 ml of XDC (BRUNO and CHRISTIAN, 1961). The amount of quenching was determined in each sample with an internal standard.

Retinas and optic nerve segments were homogenized with 2 ml of 0·25 M-sucrose (in 50 mM-Tris, pH 7·4; 25 mM-KCl; 5 mM-$MgCl_2$) in the presence of 1% sodium dodecyl sulphate. To the homogenate, 3 ml of a postmitochondrial supernatant fraction from a 10% rat brain homogenate and 5 ml of water-saturated phenol were added. The mixture was shaken in the cold for 30 min and at room temperature for 10 min. The aqueous phase was separated by centrifugation in a Sorvall centrifuge at 28,000 *g* for 15 min. Three millilitres of 0·25 M-sucrose buffer were added to the interphase and phenol phase and the mixture was shaken for 10 min at 55°, then cooled and centrifuged as previously. The supernatants were combined and treated once more with phenol for 5 min at room temperature and centrifuged. From the resulting aqueous phase, the RNA was precipitated by the addition of 2 vol. of cold 95% ethanol containing 0·2 M-potassium acetate. After standing at −15° for 24 hr, the final RNA precipitate was dissolved in 1 ml of 50 mM-Tris buffer and layered over 12 ml of a 4–20% sucrose gradient (in 50 mM-Tris buffer). Centrifugation of the gradients was carried out in the SW 40 rotor of a Spinco centrifuge at the average centrifugal force and time indicated in the figures. At the end of the centrifugation the tubes were punctured and the contents were continuously analysed for optical density in a flow-through cuvette and recording spectrophotometer (Gilford). Samples of 0·7 ml were collected and counted directly in 10 ml of XDC. Alternatively, RNA in each portion was precipitated by adding an equal volume of 10% TCA in the presence of 200 μg of bovine serum albumin. The TCA-insoluble radioactivity was collected on glass fibre filters which were counted in 10 ml of toluene fluor.

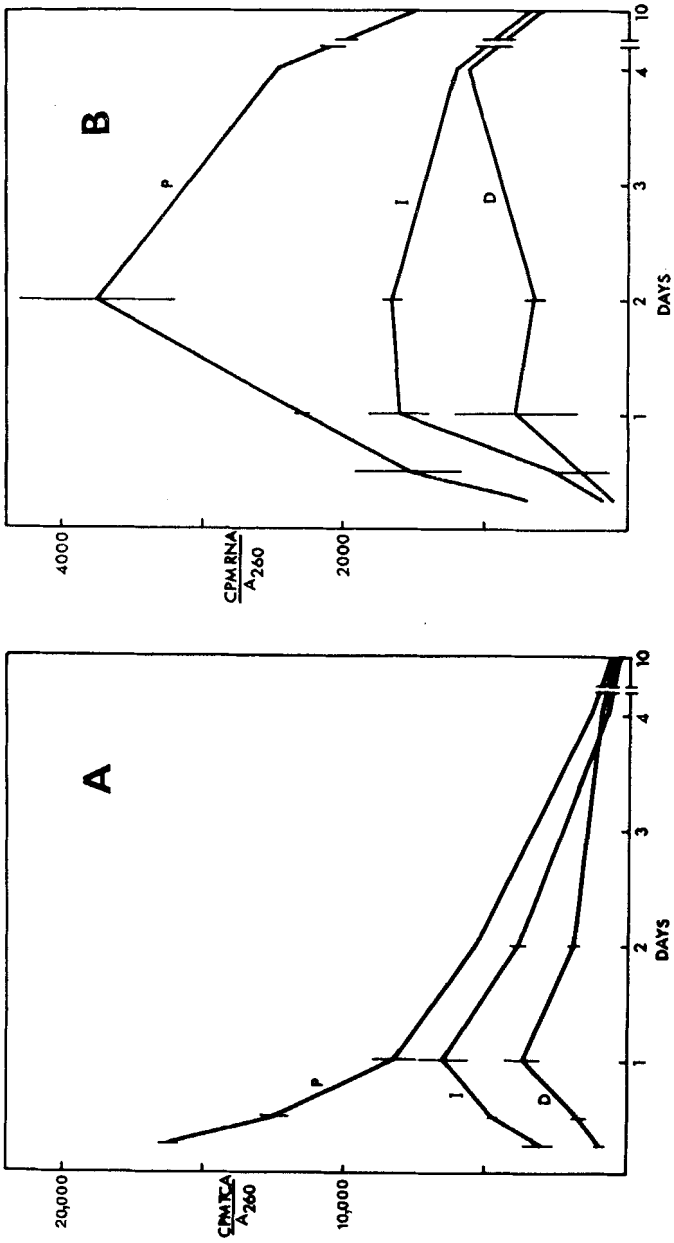

FIG. 1.—Time course of [^{3}H]uridine incorporation into TCA-solube (A) and RNA fraction (B) of optic nerve segment. The optic nerve between the eyeball and the chiasma was divided into 3 equal segments, proximal (P), intermediate (I) and distal (D) to the eyeball. For each point the segments from four animals were pooled. Points with bars are the means of two experiments; the bars show the range. For (A) and (B) the radioactivity values have been divided by the total optical density at 260 mμ of the RNA hydrolysate. CPM = counts/min.

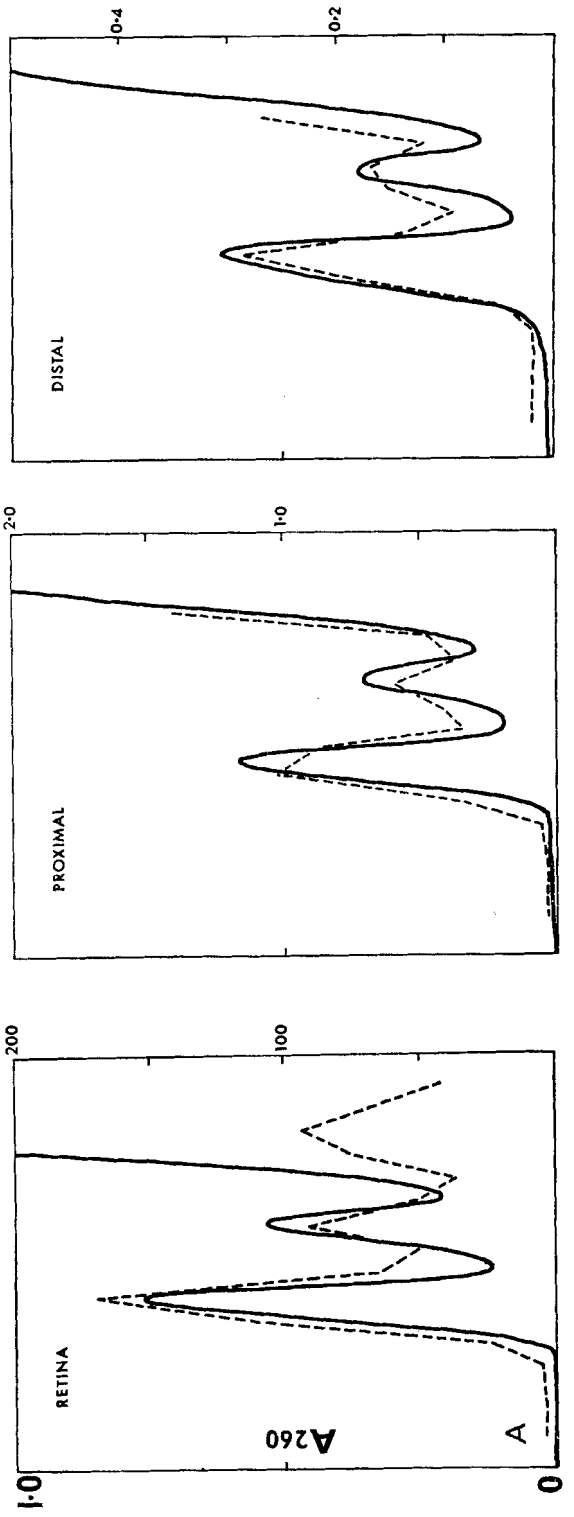

FIG. 2a.

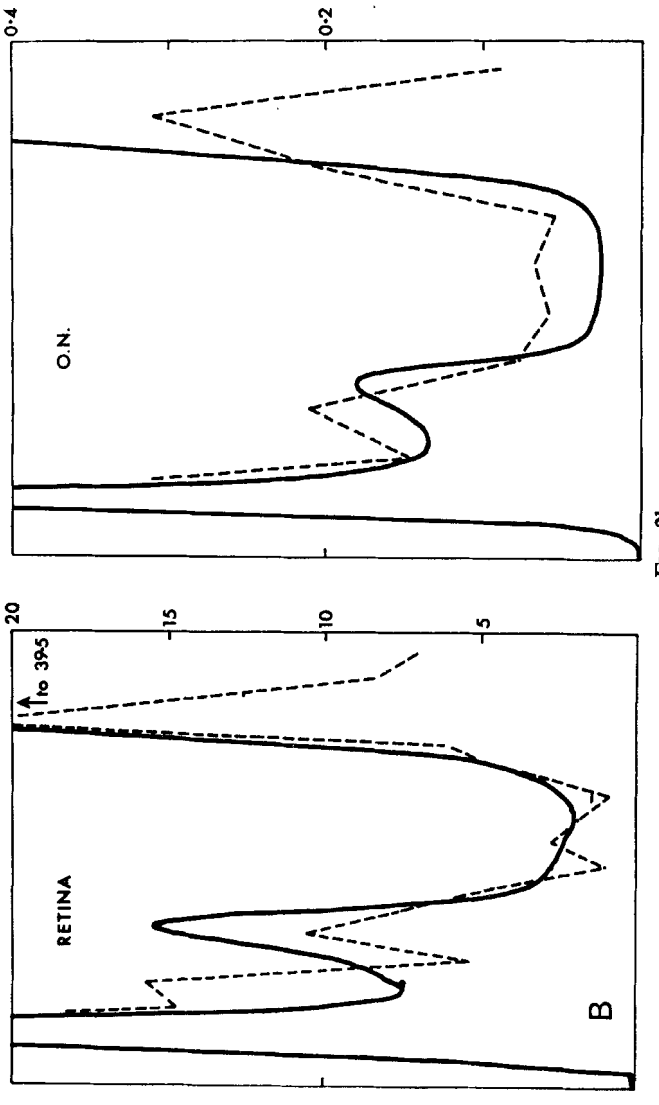

FIG. 2b.

FIG. 2.—Sucrose density gradient centrifugation of [^{32}P]RNA from retina and optic nerve segments. (A) Four rats were given 50 μC into each eyeball and killed 3 days later. Optic nerves from the eyeball to the chiasma were divided into two segments. RNA from the segments and the retinas was sedimented on a 4–20% sucrose gradient in the SW 40 rotor at 70,000 *g* for 17 hr at 5°. Fractions were counted directly in XDC. (B) Ten animals received a 24 hr pulse with [^{32}P]. RNA extracted from retinas and undivided nerves was layered over a 4–20% sucrose gradient with a 1·5 ml cushion of 60% sucrose. Centrifugation was performed in the SW 40 rotor at 140,000 *g* for 17 hr. Each fraction was analysed for TCA-insoluble radioactivity.

For all gradients, the radioactivity (dashed line) is expressed on the right-hand scale as counts/min $\times 10^{-3}$. The scale for optical density at 260 mμ (solid line) is 0 to 1·0 in all cases.

RESULTS AND DISCUSSION

The radioactivity in the TCA-soluble and RNA fractions of optic nerve segments at various times after intraocular injection of [^{3}H]uridine is shown in Fig. 1. It is apparent from Fig. 1A that there is diffusion or transport of TCA-soluble material down the optic nerves. This material, which is probably free uridine, does not appear to reach the optic nerve from the general circulation, since cerebral cortex taken from the same animal showed minimal radioactivity. RNA also appears to flow down the nerve (Fig. 1B) but with a different and slower time course than the TCA-soluble material. The increase in labelled RNA, particularly in the proximal segment, concurrent with the sharp decline in the TCA-soluble radioactivity indicates that at least some of the RNA is transported from the retina. The sharp peak of RNA radioactivity apparent in the proximal segment was not detected in the distal segment at later times.

To investigate further the nature of the labelled RNA, retinas and optic nerves were extracted with phenol 3 days after intraocular injection of [^{32}P]orthophosphate. Figure 2A shows the presence of two peaks of radioactivity coinciding with the optical density at 260 mμ of the ribosomal RNA markers in the retina as well as in the proximal and distal segments of the nerves. In the two segments of the optic nerve however, a fair amount of radioactivity was found in the light region of the gradient. This radioactivity, as shown in Fig. 2B, is at least in part TCA-insoluble and sediments in the 3–6 s region. MIANI, DI GIROLAMO and DI GIROLAMO (1966) have also found a considerable amount of light material in RNA extracted from vagus and hypoglossal nerves. In conclusion, our results demonstrate the presence in optic nerve of ribosomal RNA together with some slower sedimenting RNA species. In addition, we have presented evidence that RNA may be transported from the retina, though some local synthesis may also occur. The presence in nerve of RNA species required for protein synthesis supports the evidence of others for a local synthesis of protein in nerves. A report (PETERSON, BRAY and AUSTIN, 1968) which appeared after this paper had been prepared also demonstrates a flow of RNA along the optic nerve. The authors show, with autoradiography, that a significant portion of the RNA radioactivity of the nerve located within axons.

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International Laboratory of Genetics and Biophysics, Naples, Italy. L. CASOLA

Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan 48104. G. A. DAVIS, R. E. DAVIS

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