The Effect of Colchicine on the Transport of Axonal Protein in the Chicken

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1. Small doses (1–10 μ g) of colchicine injected into the ventral horn of the spinal cord of the chicken caused paralysis in the legs. 2. Colchicine had no effect on the incorporation of leucine into proteins of the spinal cord but markedly decreased the total amount of protein flowing into the axons of the sciatic nerve. 3. This axonal flow of protein proceeded at two rates: a high rate (300 mm/day) and a low rate (2 mm/day). Although both groups of proteins were affected, the slow transport of protein was more profoundly blocked by colchicine. 4. The results suggest that axonal flow is dependent on the neurotubular system in the axon.

The phenomenon of the transport of protein along nerve axons and some other intracellular components has been well established over the past 20 years (see Weiss & Hiscoe, 1948; Miani, 1960; Droz & Leblond, 1963; Lubińska, 1964; Austin, Bray & Young, 1966; Ochs, Johnson & Ng, 1967; Bray & Austin, 1968), but little is known about its ultrastructural basis and physiological significance.

It has been shown that the transport of protein in axons occurs at two distinct rates (McEwen & Grafstein, 1968; Bray & Austin, 1969). In the chicken sciatic nerve (Bray & Austin, 1969) one group of proteins, which disperses as it moves down the nerve, travels at a rate of 200–300 mm/day; another group moves distally down the nerve at a rate of 1–2 mm/day. It has been suggested that the low rate of transport represents the growth of continuous structural elements such as neurotubules and neurofilaments, whereas the high rate of transport may be equated with the movement of mobile organelles (McEwen & Grafstein, 1968; Bray & Austin, 1969). Radioautographic electron-microscopy studies (Droz, 1966) support this hypothesis, since they suggest that the slowly transported protein appears to be associated with the neurotubules and neurofilaments.

The movement of organelles has also been studied in non-neural tissues. Cytoplasmic filaments in rat fibroblasts in tissue culture have an overall movement of 0.4 mm/day, but granular organelles, including mitochondria, move more rapidly at rates of up to 500 mm/day (Buckley & Porter, 1967). Organelles in neurons in tissue culture also move through the cytoplasm at high rates (Geiger, 1963; Pomerat, Hendelman, Raiborn & Massey, 1967).

Dahlström & Häggendal (1967) and Livett, Geffen & Austin (1968) have both demonstrated the rapid transport *in vivo* of either catecholamine-storage granules or noradrenaline and protein within sympathetic nerves.

Colchicine is known to interfere with a number of cellular processes dependent on cytoplasmic microtubules (Tilney, 1968; Rosenbaum & Carlson, 1969), and to produce characteristic deformations of neurotubular and neurofilamentous structures in the central and peripheral nervous system (Wisniewski & Terry, 1967; Wisniewski, Shelanski & Terry, 1968). When applied locally to adrenergic axons colchicine causes an accumulation of catecholamine-storage granules, thought to be due to inhibition of the fast-transport mechanism (Dahlström, 1968). Karlsson & Sjöstrand (1969) reported some preliminary investigations on the effect of colchicine on the axonal transport of protein in the optic nerve and tract of the rabbit. They concluded that colchicine causes a more complete inhibition of the rapid transport of protein than of the slow transport. Kreutzberg (1969) has shown that, when colchicine is injected under the perineurium of the rat sciatic nerve, the transport of acetylcholinesterase is interrupted whereas the transport of the mitochondrial enzyme, lipoamide dehydrogenase, is not affected.

The aim of this work was to determine the effect of colchicine *in vivo* on the rapid and slow components of the axonal transport of protein in the chicken. The results are discussed in relation to the ultrastructural basis of axonal flow.

MATERIALS AND METHODS

Chemicals. L-[U- 14 C]Leucine (specific radioactivity 311 mCi/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K.

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Colchicine was obtained from British Drug Houses Ltd., Poole, Dorset, U.K.; bovine serum albumin (Cohn fraction V) was from Commonwealth Serum Laboratories, Melbourne, Vic., Australia.

Animals. Female chickens (White Leghorn $\times$ Australorp strain) 17–18 weeks old and weighing approx. 1.5 kg were used.

Methods. Chickens were anaesthetized with sodium pentobarbitone by administration through a cannula into a wing vein. The lumbar region of the spinal cord was exposed and colchicine was injected into the ventral horn in the region of the motor neurons whose axons pass into the sciatic nerve as described by Bray & Austin (1968). Doses of 1, 3 or 10 μ g of colchicine in 8 μ l of 0.9% NaCl were used. Control chickens were injected with 8 μ l of 0.9% NaCl only. The animals were allowed to recover and after a further 72 h the spinal cord was re-exposed and either 5 μ Ci of [14 C]leucine in 6 μ l of saline (short-term experiments) or 4 μ Ci in 5 μ l of saline (long-term experiments) was injected into the same region previously injected with colchicine. The chickens were killed by an overdose of anaesthetic, either 5 h (short-term experiments) or 14 days (long-term experiments) after injection of the labelled amino acid, and both sciatic nerves, together with a portion of the spinal cord from the site of injection, were removed. The nerves were cut into 1 cm or 2 cm segments and these, together with pieces of cord, were stored at -20°C .

Determination of protein-bound radioactivity. Each piece of nerve or spinal cord was frozen in liquid nitrogen and finely crushed as described by Austin *et al.* (1966).

The crushed powder was extracted to remove acid-soluble material, lipids and nucleic acids (Bray & Austin, 1968), except that lipids were extracted with 90% (v/v) ethanol containing sodium acetate (2%, w/v) followed by ethanol-ether-chloroform (2:2:1, by vol.). The protein residue was air-dried for 15 min and dissolved in formamide by heating at 120°C for 2 h. Portions were transferred to scintillation vials, 10 ml of 'aqueous' scintillation fluid (Bruno & Christian, 1961) was added to each and radioactivities were measured in a Packard Tri-Carb (model 3003) liquid-scintillations pectrometer. The efficiency of counting, which was determined by the addition of [14 C]toluene standard to a random selection of samples, ranged from 65 to 70%.

Determination of protein. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with dried bovine serum albumin as standard.

RESULTS

Gross effects of colchicine. Within 24 h of injection of colchicine into the lumbar region of the spinal cord the chickens showed signs of ataxia. The degree of ataxia was difficult to estimate in the early stages as some control chickens showed transient unsteadiness in the legs, presumably as a result of the injections into the spinal cord. Chickens dosed with 10 μ g of colchicine showed pronounced signs of paraparesis at 48 h, and by 72 h were completely paralysed and remained so for the duration of the experiment. Differences between

Table 1. *Effect of colchicine on the rapid transport of ^{14}C -labelled protein in chicken sciatic nerve*

Length of nerve distal to spinal cord (cm)	Control			Colchicine (1 μ g)			Colchicine (3 μ g)			Colchicine (10 μ g)		
	Sp. radioactivity	% of total radioactivity	total d.p.m./segment total d.p.m./nerve $\times 100$	Sp. radioactivity	% of total radioactivity	total d.p.m./segment total d.p.m./nerve $\times 100$	Sp. radioactivity	% of total radioactivity	total d.p.m./segment total d.p.m./nerve $\times 100$	Sp. radioactivity	% of total radioactivity	total d.p.m./segment total d.p.m./nerve $\times 100$
0–2	298 $\pm$ 26	47.5	157 $\pm$ 19	109 $\pm$ 13	49.1	109 $\pm$ 13	94.7 $\pm$ 11.3	51.8	94.7 $\pm$ 11.3	49.5	94.7 $\pm$ 11.3	49.5
2–4	142 $\pm$ 20	17.1	76.0 $\pm$ 2.2	48.3 $\pm$ 5.0	19.2	48.3 $\pm$ 5.0	60.1 $\pm$ 8.7	19.4	60.1 $\pm$ 8.7	21.5	60.1 $\pm$ 8.7	21.5
4–6	128 $\pm$ 15	9.9	74.2 $\pm$ 7.6	41.6 $\pm$ 4.0	13.4	41.6 $\pm$ 4.0	45.5 $\pm$ 8.4	12.7	45.5 $\pm$ 8.4	12.4	45.5 $\pm$ 8.4	12.4
6–8	180 $\pm$ 40	12.2	56.4 $\pm$ 7.3	32.7 $\pm$ 2.4	10.3	32.7 $\pm$ 2.4	33.6 $\pm$ 3.0	8.7	33.6 $\pm$ 3.0	8.0	33.6 $\pm$ 3.0	8.0
8–10	175 $\pm$ 38	13.3	39.3 $\pm$ 2.7	26.8 $\pm$ 2.8	7.9	26.8 $\pm$ 2.8	32.2 $\pm$ 3.7	7.2	32.2 $\pm$ 3.7	8.6	32.2 $\pm$ 3.7	8.6

Colchicine was injected into the lumbar region of the spinal cord and 72 h later 5 μ Ci of [14 C]leucine was injected into the same region. After 5 h the sciatic nerves were removed and analysed for protein-bound radioactivity. Six nerves were analysed in each group. Specific radioactivities are expressed in d.p.m./mg of protein and are given as means $\pm$ s.e.m. In the same column of each group the value for the percentage of total radioactivity is given by

chickens dosed with 1 or 3 μg of colchicine were not easily observed although they suffered a less severe paraparesis. Many birds in this group could walk with some difficulty.

Effect of colchicine on the rapid transport of protein. Table 1 shows that colchicine decreased the amount of labelled protein along the entire length of nerve examined. Because of variations in the site of injection of the amino acid, the results for the total radioactivity in the nerve segments were adjusted by expressing the total d.p.m. in each segment as a percentage of the total d.p.m. in the nerve. The adjusted results demonstrate that the labelled protein in distal regions of the nerve was decreased by colchicine to a greater extent than was the labelled protein in the proximal regions.

Effect of colchicine on the slow transport of protein. The distribution of labelled protein in the sciatic nerve is shown in Table 2. The control nerves in this experiment showed a well-defined peak of labelled protein at a distance of 1–2 cm from the spinal cord. The results presented in Table 2 show that colchicine decreased the amount of labelled protein along the nerve, although, as emphasized by the adjusted results, a small but clearly defined peak of labelled protein was retained 1–2 cm distal to the spinal cord after injection of 1 μg of colchicine. By contrast the peak was abolished after injection of 3 or 10 μg of colchicine and a gradient of labelled protein occurred along the nerve.

Effect of colchicine on total protein-bound radioactivity in sciatic nerve. Total protein-bound radioactivity in the nerve was obtained by summing the radioactivity in the protein of the individual segments. The results, presented in Table 3, show that colchicine decreased the total protein-bound radioactivity in both types of axonal flow. The effect was more pronounced on the slow transport of protein, where the percentages of the control, for a given dose of colchicine, were 20–36% of the effect on the fast transport.

Effect of colchicine on protein-bound radioactivity in chicken spinal cord. Protein from the pieces of spinal cord taken from the injected area was extracted and the specific radioactivity determined. The results, presented in Table 4, show that colchicine had no marked effect on the incorporation of [^{14}C]leucine into protein of the cells of the anterior horn of the spinal cord.

DISCUSSION

Colchicine, when injected into the spinal cord, inhibits the slow phase of protein transport in the sciatic nerve more completely than it does the rapid phase of protein transport. These results differ from the findings of Karlsson & Sjöstrand (1969),

Table 2. *Effect of colchicine on the slow transport of ^{14}C -labelled protein in chicken sciatic nerve*

Length of nerve distal to spinal cord (cm)	Control		Colchicine (1 μg)		Colchicine (3 μg)		Colchicine (10 μg)	
	Sp. radioactivity (10)	% of total radioactivity	Sp. radioactivity (6)	% of total radioactivity	Sp. radioactivity (8)	% of total radioactivity	Sp. radioactivity (6)	% of total radioactivity
0–1	377 $\pm$ 76	8.4	112 $\pm$ 25.7	17.5	61.1 $\pm$ 5.7	21.6	46.6 $\pm$ 4.5	21.0
1–2	989 $\pm$ 150	23.8	168 $\pm$ 52.4	22.6	62.3 $\pm$ 9.8	19.2	42.8 $\pm$ 0.9	16.5
2–3	920 $\pm$ 174	18.1	112 $\pm$ 35.7	14.0	48.9 $\pm$ 7.3	14.0	39.7 $\pm$ 2.7	14.3
3–4	603 $\pm$ 98	10.8	103 $\pm$ 24.5	10.7	36.6 $\pm$ 6.8	9.0	36.1 $\pm$ 1.9	11.7
4–6	676 $\pm$ 116	20.5	100 $\pm$ 23.0	19.2	40.1 $\pm$ 7.1	18.0	37.7 $\pm$ 1.5	19.7
6–8	580 $\pm$ 107	18.4	79 $\pm$ 18.5	15.9	42.6 $\pm$ 10.9	18.1	32.3 $\pm$ 2.0	16.8

Colchicine was injected into the lumbar region of the spinal cord and 72 h later 4 μCi of [^{14}C]leucine was injected similarly. After 14 days the nerves were removed and analysed for protein bound radioactivity. The number of nerves analysed is shown in parenthesis. For definition of specific radioactivity and percentage of total radioactivity, see Table 1.

Table 3. *Effect of colchicine on total protein-bound radioactivity in chicken sciatic nerve*

Chickens were injected with colchicine and [^{14}C]leucine as described in the Materials and Methods section. The nerve segments were analysed for labelled protein and the total protein in the nerve was obtained by the summation of the individual segments. Numbers in parentheses indicate the number of nerves analysed in each group.

Dose of colchicine	Rapid protein transport Total protein-bound radioactivity		Slow protein transport Total protein-bound radioactivity	
	Total radioactivity	% of control	Total radioactivity	% of control
Control	2345 $\pm$ 178 (6)	—	6300 $\pm$ 1045 (10)	—
1 μg	1170 $\pm$ 135 (6)	49.8	1115 $\pm$ 265 (6)	17.7
3 μg	646 $\pm$ 68 (6)	27.6	544 $\pm$ 77 (8)	8.6
10 μg	731 $\pm$ 27 (6)	31.2	385 $\pm$ 20 (6)	6.1

Table 4. *Effect of colchicine on incorporation of [^{14}C]leucine into protein in the spinal cord*

Chickens were injected with colchicine and [^{14}C]leucine as described in the Materials and Methods Section. Protein was extracted from the spinal cord at the times indicated and the specific radioactivity (d.p.m./mg of protein) determined. R.S.A. is specific radioactivity relative to that of the control value.

Dose of colchicine (μg)	5 h after injection of [^{14}C]leucine		14 days after injection of [^{14}C]leucine	
	Sp. radioactivity	R.S.A.	Sp. radioactivity	R.S.A.
None (control)	83 200	1.00	19 500	1.00
1	72 300	0.87	16 800	0.86
3	75 300	0.91	16 000	0.82
10	121 000	1.45	25 000	1.28

who suggested that rapid transport of protein in the optic nerve of the rabbit is more completely inhibited by colchicine. However, our results are in agreement in that colchicine does have an effect on both rapid and slow axonal transport of protein, and they complement the work of Dahlström (1968), which shows that the transport of amine-storage granules is inhibited by colchicine. Dahlström found that when high doses of colchicine (100 μg or 1 mg) were injected under the perineurium of the rat sciatic nerve there was a pronounced accumulation of noradrenaline at the site of injection. This was interpreted as being due to the blockage of the rapid proximo-distal flow of catecholamine-storage granules. In our experiments 10 μg of colchicine caused a 69% inhibition of the rapid transport of labelled proteins in the nerve in 5 h.

The incorporation of labelled amino acid into spinal-cord protein does not appear to be affected by colchicine, indicating that protein synthesis is not affected, although the higher specific radioactivities obtained 5 h or 14 days after injection of 10 μg of colchicine may be due to an accumulation of protein in the cell bodies arising from the inhibition of the protein-transport mechanism. There

was no marked inhibition by colchicine of protein synthesis in the rabbit retina (Karlsson & Sjöstrand, 1969).

The finding that colchicine affects both slow and rapid transport of protein means that some modification of the suggestion that slow protein transport reflects the outgrowth of neurotubules into the axon may be necessary. It appears that the two transport systems may be intimately linked to each other and are not separate processes as was first envisaged. The results presented here are in accord with the hypothesis of Schmitt (1968), who has suggested that neurotubules and neurofilaments mediate the fast transport by a process of mechano-coupling.

Although it is generally accepted that colchicine forms a non-covalent bond with microtubule protein *in vitro*, the exact nature of the cytological lesions caused by the administration of this compound *in vivo* remains to be defined. Colchicine may cause the depolymerization of microtubules or alternatively prevent the polymerization from the sub-units, although Stephens (1968) has reported that in flagellar microtubule protein colchicine does not have a direct effect on the polymerization process.

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