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In vivo Effect of Methylmercury on Protein Synthesis in Peripheral Nervous Tissues of the Rat*

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Abstract. The in vivo rates of protein synthesis in the peripheral nervous tissues of methylmercury-treated rats (10 mg/kg/day, for 7 days) have been estimated with improved methods by the injection of a large amount of [l-^{14}C]valine of low specific activity. Protein synthesis activity in the dorsal root ganglia was inhibited to the extent of 60% of the control as early as day 5 and this continued to the symptomatic period (day 15) on which crossing of hind limbs, a typical sign of organomercurial poisoning, was observed in the animals. The sciatic nerves and dorsal roots increased protein synthesis by 56% at the symptomatic period. These increases in protein synthesis may be due to the stimulation of reactivity of Schwann's cells. On the contrary, the protein synthesis in the ventral roots showed a gradual decrease as the intoxication proceeded and decreased to 73% of the control at the symptomatic period, being similar to the case of the brain. The double-labeling studies with sodium dodecyl sulfate/polyacrylamide gel electrophoresis exhibited that methylmercury inhibited the synthesis of the dorsal root ganglion proteins non-uniformly in various apparent molecular sizes, especially on day 10.

Key words: Methylmercury – Protein synthesis – Peripheral nerve – Rat

Introduction

From the point of view that defect of protein synthesis in the nervous system may have a deep correlation to the toxic effects of methylmercury (Brown and Yoshida 1965; Miyakawa and Deshimaru 1969; Chang and Hartmann 1972; Klein et al. 1972), several studies have been carried out to date to show that

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methylmercury could produce a disturbance to protein synthesis activity in the rat brain (Yoshino et al. 1966; Cavanagh and Chen 1971a; Farris and Smith 1975; Verity et al. 1977; Syversen 1977; Omata et al. 1978, 1980a). On the other hand, when methylmercury compounds are given to rats, there is both morphological and functional evidence that the primary site of action of methylmercury is in the peripheral nerves (Hunter et al. 1940; Miyakawa et al. 1970; Cavanagh and Chen 1971b; Chang and Hartmann 1972b; Klein et al. 1972; Herman et al. 1973; Somjen et al. 1973a; Jacobs et al. 1975). Some of these studies demonstrated the ultrastructural pattern of early onset of disruption of protein synthetic apparatus. However, little attention has been paid to the analysis of the underlying biochemical mechanism of the toxic effect of methylmercury on the protein synthesis activity of the peripheral nervous system. Earlier studies by Cavanagh and Chen (1971a) suggested that a decrease in ^{14}C-glycine incorporation in vivo into protein occurred more markedly in dorsal root ganglia than elsewhere early in the course of methylmercury intoxication, while the sciatic nerves showed a decrease in the incorporation at early phase followed by a sharp increase above normal at later phase.

We investigated protein synthesis in the peripheral nerves of the methylmercury-poisoned rats with improved methods for measuring the rate of protein synthesis in vivo (Dunlop et al. 1975; Reith et al. 1978). Our measurements confirmed the findings of Cavanagh and Chen (1971a) noted above and also demonstrated that dorsal roots, but not ventral roots, exhibited the alteration in the protein synthesis activity analogous to the sciatic nerves.

Material and Methods

Animal Treatment. Female Wistar rats weighing 170–190 g were maintained in plastic boxes in a room of constant temperature. Methylmercury chloride (MMC) was administered according to the dosage schedule of Klein et al. (1972). Rats were given daily s.c. injections of MMC (10 mg/kg) in 10 mM $NaHCO_3$-Na_2CO_3, pH 9.2, for 7 consecutive days. Day 1 means the day on which the first injection was given. Evolution of methylmercury intoxication in the rats was as described in our previous paper (Omata et al. 1978). Day 15 corresponds to the symptomatic phase.

In vivo Amino Acid Incorporation. Rats were injected i.p. with 3 ml of 500 mM valine aqueous solution, equivalent to 1.5 mmol valine containing 10 μCi [l-^{14}C]valine (New England Nuclear, Boston, USA) per 100 g body weight. One and two hours after the injection, the rats were killed by decapitation. The brain was removed and dissected into cerebral cortex, cerebellum, and the remainder which was termed "brain stem" in this paper. Dorsal root ganglia from L_2-L_6, together with their dorsal and ventral roots were excised quickly. Sciatic nerves from below the greater trochanther were also removed. These operations were done as quickly as possible in the room of 0–2° C. The samples were homogenized in 6 vol. of 3% (w/v) sulfosalicylic acid. The homogenate was centrifuged at 3,000 *g* for 10 min and the supernatant was separated for liquid scintillation counting. The pellet was processed as described previously to determine the radioactivity incorporated into the protein (Omata et al. 1980a).

In vitro Labelling of Proteins of Dorsal Root Ganglia. Dorsal root ganglia from a rat were incubated in 0.25 ml of Krebs-Ringer-bicarbonate, pH 7.4, containing 10 mM glucose, oxygenated by bubbling O_2 gas. Each ganglion was sectioned in two pieces with razor blade. Incubation was carried out at 37° C for 60 min in the presence of 30 μCi of [4,5-^{3}H] leucine (55 Ci/mmol, Radiochemical Centre, Amersham, GB) for ganglia from control rat or 10 μCi of [U-^{14}C]leucine (354 mCi/mmol,

Radiochemical Centre, Amersham, GB) for ganglia from methylmercury-treated rat. After incubation, the ganglia were washed on Millipore filter (0.45 μm), homogenized with the same buffer, and kept at −80° C until use.

Double-Labelling Experiments. This method afforded a sensitive means for assessing difference in the labelling profiles between ganglia proteins from control and methylmercury-treated rats. ^{3}H-Labelled homogenate of ganglia from control rat was mixed together with ^{14}C-labelled homogenate of ganglia from methylmercury-treated rat ($^{3}H/^{14}C = 6 \times 10^{5}/3 \times 10^{4}$, in dpm). The resulting mixture was added with an equal vol. of 10% (w/v) trichloroacetic acid (TCA) and the insoluble material was subjected to gel electrophoresis. The acid-insoluble material was dissolved in a sample buffer (0.075 M Tris-HCl, pH 6.8, 7% sucrose, 2% sodium dodecyl sulfate (SDS), 1% 2-mercaptoethanol, 0.005% bromophenol blue) and heated for 2 min in boiling water. Electrophoresis was carried out on polyacrylamide slab gel (11−15% polyacrylamide gradient) in the presence of 0.1% SDS according to Laemmli (1970). After completion of electrophoresis, the slab gel was fixed overnight in 12% TCA, stained overnight in 0.1% Coomassie brilliant blue G-250 in 12% TCA and cleared in several washes of water. The stained gel was scanned on a microdensitometer (Joyce Loebl, Gateshead, GB), photographed, and dried. The dried gel was cut to 2 mm-wide segments. The segments were placed into vials containing 1 ml 2% SDS-1 N NaOH and incubated at 60° C for 13 h, then 10 ml of ACS-II (Radiochemical Centre, Amersham, GB) and two drops of formic acid were added. Radioactivity was determined in a liquid scintillation counter (Aloka LSC-903, Tokyo, Japan). The radioactivity extracted from the gel was about 70% for ^{3}H and 85% for ^{14}C of that applied to the gel. The $^{14}C/^{3}H$ ratio in each segment is an index of the extent to which synthesis of a given size of proteins in the ganglia of methylmercury-treated rats is altered relative to that of control rats. If the protein populations synthesized in vitro in the ganglia of control and methylmercury-treated rats were similar in composition, the $^{14}C/^{3}H$ ratio would be constant throughout the gel. On the contrary, if the synthesis of particular protein types was selectively suppressed in the dorsal root ganglia of methylmercury-treated rats, such proteins would be characterized by a low $^{14}C/^{3}H$ ratio. Another validation of the procedure came from analyzing gels in which ^{3}H- and ^{14}C-labelled proteins of the same experimental group were combined and comigrated. For this purpose, dorsal root ganglia were excised from left and right sides of a normal rat, and the former group was labelled with ^{3}H-leucine while the latter group was labelled with ^{14}C-leucine. Such experiments allow us to determine the errors inherent in comparing two separately prepared protein samples, and provide the confidence limits for the $^{14}C/^{3}H$ ratio measurement.

Accumulation of Mercury in the Tissues. A group of rats were divided into two sub-groups each consisting of three animals. Each rat received daily s.c. injection of a mixture of ^{203}Hg-labelled and nonlabelled MMC (50 μCi and 10 mg/kg, respectively) for 7 days. Animals of the two sub-groups were killed on days 10 and 15, respectively. Whole-body perfusion was carried out as described previously (Omata et al. 1978). Tissues from three rats were pooled, weighed, and homogenized with a medium containing 20 mM HEPES buffer, pH 7.6, 25 mM KCl, 10 mM $MgCl_2$, and 0.25 M sucrose. Radioactivity for each aliquot of the homogenates was determined as described previously (Omata et al. 1980b).

Results

By flooding the precursor pool with a large amount of valine, the concentration of valine in the brain became around 30 times higher than the initial concentration and there were no significant differences in the concentration of valine between control and methylmercury-treated rats (data not shown). The specific activity of free valine showed also no significant difference between control and methylmercury-treated rats and was maintained nearly constant during 1- to 2-h period (data not shown). These agreed with the results which were obtained previously under experimental conditions identical to the present

study except using [l-^{14}C]valine instead of [U-^{14}C]valine (Omata et al. 1980a).

In Table 1, the acid-soluble radioactivities in peripheral nervous tissues are shown. These radioactivities are almost dependent on valine, since carboxy-labelled valine was used here (Banker and Cotman 1971). Dorsal roots exhibited roughly a half of the value of the brain, whereas dorsal root ganglia was approximately twice that of the brain. It seemed that there were no meaningful differences in the level of acid-soluble radioactivity between control and methylmercury-treated rats, except a slight increase on day 15 of sciatic nerves, and that the acid-soluble radioactivity was maintained roughly constant during 1- to 2-h period.

The incorporation of ^{14}C-valine into proteins of peripheral nervous tissues, as well as the brain regions, increased linearly up to 2 h after the injection of the isotope (data not shown). Table 2 shows the results of 2 h incorporation of ^{14}C-valine into proteins of peripheral nervous tissues. For the comparison, the results of the brain regions are shown in Table 3. In the control rats, the protein synthesis activity of dorsal root ganglia was highest among the peripheral nervous tissues and was about 4-fold higher than that of the brain and approximately as active as that (272 dpm/mg protein) of the liver. In contrast, the other three tissues showed rather low protein synthesis activity as compared to that of the brain. The most striking effect of methylmercury was observed in dorsal root ganglia (Fig. 1). This tissue showed a marked reduction in ^{14}C-valine incorporation as early as day 5, which continued up to the symptomatic period. On the contrary, sciatic nerves as well as dorsal roots tended to show a slight reduction in ^{14}C-valine incorporation on day 5 followed by return to normal level on day 10 and a marked enhanced rate of the incorporation at the symptomatic period (Fig. 1). Ventral roots also tended to show a slight reduction in ^{14}C-valine

Table 1. Acid-soluble radioactivity in peripheral nervous tissues after gross injection of valine

Tissues	Incorporation time (h)	Acid-soluble radioactivity (dpm/mg tissue)[a]			
		Control	Day 5[b]	Day 10	Day 15
Ventral roots	1	17.3 (100)	14.5 (84)	17.4 (101)	13.3 (77)
	2	18.9 (100)	19.9 (105)	15.5 (82)	16.2 (86)
Dorsal roots	1	21.3 (100)	22.1 (104)	23.8 (112)	20.9 (98)
	2	24.6 (100)	24.2 (98)	23.7 (96)	25.0 (101)
Dorsal root ganglia	1	95.6 (100)	82.6 (86)	82.8 (87)	89.8 (94)
	2	74.5 (100)	108 (145)	76.4 (103)	98.7 (132)
Sciatic nerves	1	33.2 (100)	32.0 (96)	39.0 (117)	65.4 (197)
	2	37.9 (100)	37.7 (99)	55.8 (147)	74.7 (197)

[a] Rats were injected i.p. with 1.5 mmol valine containing 10 μCi [l-^{14}C]valine per 100 g body weight. Values for 1 h incorporation were obtained with the tissues polled from four rats. Values for 2 h incorporation were the mean of two experiments in each of which the tissues from two rats were pooled for the assay. Numbers in parentheses are % of control value

[b] Number of day after onset of daily s.c. injections of MMC (10 mg/kg/day) for 7 days

Table 2. Protein synthesis in peripheral nervous tissues of methymercury-treated rats

Tissues	Specific activity (dpm/mg protein)[a]			
	Control	Day 5[b]	Day 10	Day 15
Ventral roots	45.5 [45.3, 45.6] (100)	37.3 [35.1, 39.5] (82)	35.7 [40.8, 30.5] (78)	33.4 [36.6, 30.2] (73)
Dorsal roots	46.0 [46.4, 45.6] (100)	38.9 [39.4, 40.4] (87)	50.3 [50.4, 50.2] (109)	71.6 [80.7, 62.4] (156)
Dorsal root ganglia	244 [231, 257] (100)	145 [146, 143] (59)	147 [148, 145] (60)	131 [134, 128] (54)
Sciatic nerves	37.4 [35.0, 39.7] (100)	36.5 [36.0, 36.9] (98)	42.5 [47.9, 37,0] (114)	58.3 [50.9, 55.6] (156)

[a] Rats were injected i.p. with 1.5 mmol valine containing 10 μCi[l-^{14}C]valine per 100 gr body weight 2 h before the sacrifice. Each value is the mean of two experiments shown in the square brackets. In each experiment, the tissues from two rats were pooled for the assay. Numbers in parentheses are % of control value

[b] Number of day after onset of daily s.c. injections of MMC (10 mg/kg/day) for 7 days

Table 3. Protein synthesis in central nervous tissues of methylmercury-treated rats

Tissues	Specific activity (dpm/mg protein)[a]			
	Control	Day 5[b]	Day 10	Day 15
Cerebral cortex	66.7 ± 3.5 (100)	62.3 ± 6.7 (93)	56.6 ± 4.2* (85)	49.2 ± 3.3**** (74)
Cerebellum	68.5 ± 3.8 (100)	62.6 ± 3.7 (91)	58.5 ± 3.6** (85)	50.9 ± 1.7* (74)
Brain stem	61.8 ± 1.9 (100)	56.4 ± 3.0 (91)	50.4 ± 3.9*** (82)	46.1 ± 2.9**** (75)
Spinal cord	51.6 ± 2.6 (100)	48.8 ± 0.3 (95)	44.4 ± 5.2 (86)	38.9 ± 4.8*** (75)

[a] Rats were injected i.p. with 1.5 mmol valine containing 10 μCi[l-^{14}C]valine per 100 g body weight 2 h before the sacrifice. Each value is mean ± SD of four rats. Numbers in parentheses are % of control value

[b] Number of day after onset of daily s.c. injections of MMC (10 mg/kg/day) for 7 days
* $0.02 < p < 0.05$; ** $0.01 < p < 0.02$; *** $0.001 < p < 0.01$; **** $p < 0.001$

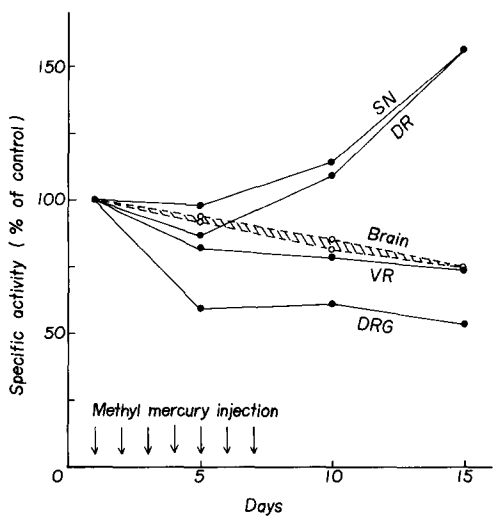

Fig. 1. Incorporation in vivo of [l-^{14}C]valine into proteins of brain and peripheral nervous tissues of methylmercury-treated rats. Rats received daily s.c. injections of MMC (10 mg/kg/day) for 7 days. Rats were injected i.p. with 1.5 mmol valine containing 10 μCi [l-^{14}C]valine per 100 g body weight 2 h before the sacrifice. Each point represents the mean of four rats for brain and the mean of two experiments in each of which the tissues from two rats were pooled for peripheral nervous tissues

incorporation on day 5, but as the intoxication proceeds, they showed an increasing reduction to a similar extent to the case of the brain (Fig. 1).

Table 4 shows mercury accumulation in the brain and peripheral nervous tissues of methylmercury-treated rats. Dorsal root ganglia showed the highest value, particularly on day 10.

Table 4. Accumulation of mercury in brain and peripheral nervous tissues[a]

	μg Hg/g wet tissue[b]	
	Day 10	Day 15
Ventral roots	13.6	16.2
Dorsal roots	13.6	15.2
Dorsal root ganglia	30.2	33.2
Sciatic nerves	12.4	15.8
Spinal cord	10.8	14.0
Cerebral cortex	20.2	30.8
Cerebellum	22.5	27.7
Brain stem	16.9	24.0

[a] Rats received daily s.c. injections of a mixture of ^{203}Hg-labelled and nonlabelled MMC (50 μCi and 10 mg/kg, respectively) for 7 days and killed on days 10 and 15

[b] Values were obtained with the tissues pooled from three rats

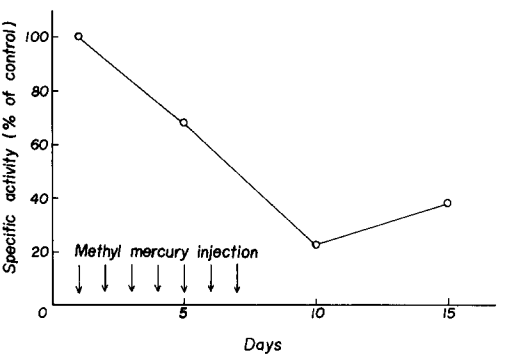

Fig. 2. Incorporation in vitro of ^{14}C-leucine into proteins of dorsal root ganglia. Rats received daily s.c. injections of MMC (10 mg/kg/day) for 7 days. Dorsal root ganglia from three rats were incubated as described under Materials and Methods

To know whether methylmercury selectively suppress the synthesis of some kind of protein species, the double-labelling technique was used. Figure 2 shows ^{14}C-leucine incorporation in vitro by dorsal root ganglia which was carried out as described in Material and Methods. Difference in pattern of decrease in protein synthesis from that of Table 2 might be due to difference in experimental conditions; in vitro incorporation with a tracer amount of ^{14}C-leucine versus in vivo incorporation with a large amount of ^{14}C-valine. Figure 3 shows a representative photograph and corresponding densitometric scan of protein staining pattern from control dorsal root ganglia separated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. Over 50 major and minor bands are resolved using the present polyacrylamide system. No significant difference was detected in the pattern between control and methylmercury-treated rats (data not shown). Figure 4 shows control studies in which ganglia from left and right sides of one normal rat were incubated in the presence of ^{3}H- and ^{14}C-leucine, respectively, and both were combined and comigrated. All gel slices had a ^{14}C/^{3}H ratio varying with standard deviation of ± 4.3% of the mean value. This

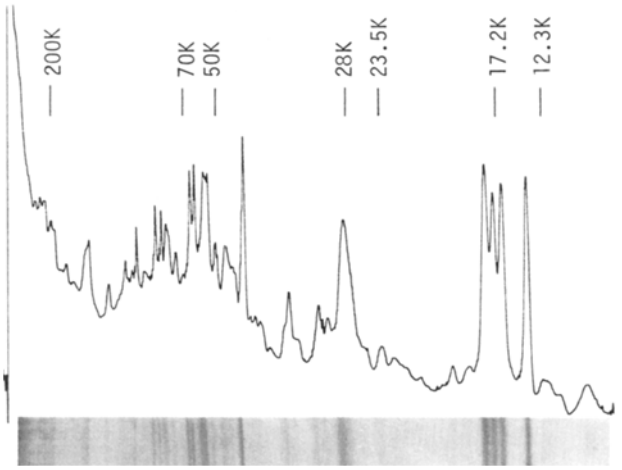

Fig. 3. Sodium dodecylsulfate/polyacrylamide gel electrophoresis of the homogenate of dorsal root ganglia from control rats. Electrophoresis, staining, and densitometry were carried out as described under Materials and Methods

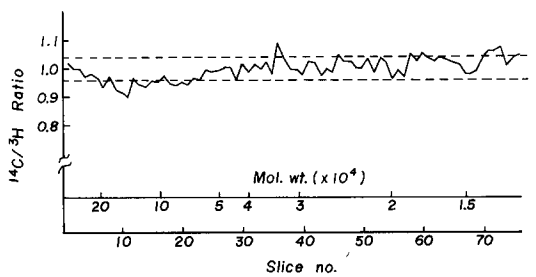

Fig. 4. Variation of the $^{14}C/^{3}H$ ratio in a control study. Ganglia from left and right sides of one normal rat were incubated in the presence of ^{3}H- and ^{14}C-leucine, respectively, and both were combined and comigrated in a sodium dodecylsulfate/polyacrylamide gel. The average $^{14}C/^{3}H$ value for the gel is normalized to 1.0. The standard deviation of the $^{14}C/^{3}H$ value is indicated by broken lines

provides the confidence limits for deviations of the $^{14}C/^{3}H$ ratio in the subsequent experiments in which the normal ganglia labelled with ^{3}H-leucine were mixed with the poisoned ganglia labelled with ^{14}C-leucine and the resulting mixture was comigrated. Figure 5 illustrates the results of double-labelling gel electrophoresis of dorsal root ganglia proteins from control and methylmercury-treated rats. On day 5, almost all of protein species were uniformly suppressed within the confidence limits. However, on day 10, a considerable number of components represented variations in the $^{14}C/^{3}H$ ratio over the confidence limits. Somewhat lower suppression of protein synthesis than the mean value was found in the fractions with low molecular weight in the vicinity of 15,000. By contrast, the protein species with high molecular weight of around

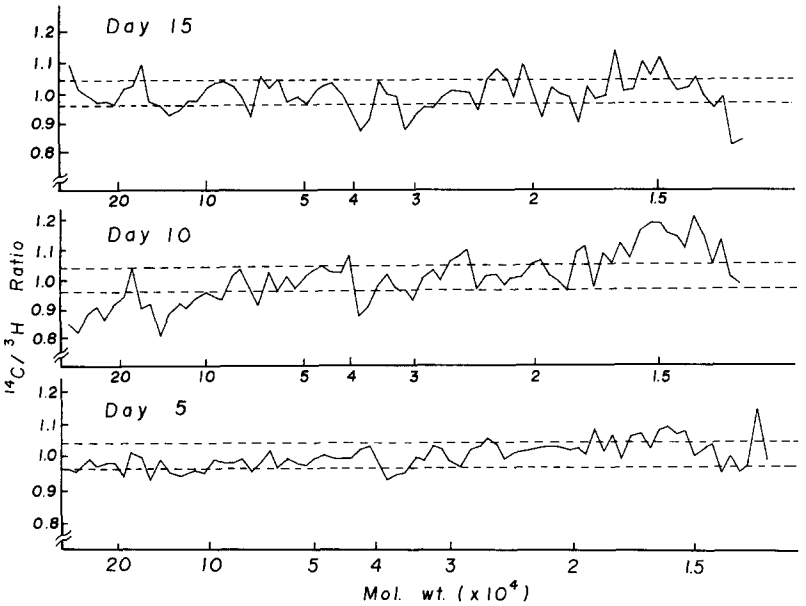

Fig. 5. Comparison of ganglia proteins synthesized in vitro of control and methylmercury-treated rats by double-labelling technique. Ganglia from control and methylmercury-treated rats were incubated in the presence of ^{3}H- and ^{14}C-leucine, respectively, and both were combined and comigrated in sodium dodecylsulfate/polyacrylamide gel. Incubation, electrophoresis, and determination of radioactivity were carried out as described under Materials and Methods. The average $^{14}C/^{3}H$ values for the gels are normalized to 1.0. The standard deviation of $^{14}C/^{3}H$ values in the control study (see Fig. 4) is depicted by broken lines

150,000 and over 200,000 showed rather higher suppression of protein synthesis than the mean value. On day 15, these apparent variations in $^{14}C/^{3}H$ ratio disappeared, but the variations seemed to be slightly large relative to those on day 5. Throughout the experimental period, somewhat higher suppression of protein synthesis than the mean value was observed in protein species of around 39,000 molecular weight.

Discussion

The present study investigated protein synthesis of brain and peripheral nervous tissues in methylmercury-treated rats with improved methods for measuring protein synthesis in vivo (Dunlop et al. 1975; Reith et al. 1978). Our measurements suggest that methylmercury could produce a marked disturbance to protein synthesis activity of dorsal root ganglia at an early stage after the onset of administration of methylmercury, and also that the protein synthesis activity of sciatic nerves and dorsal roots exceeded normal at the symptomatic period. These results have partly agreed with the early findings by Cavanagh and Chen (1971a) who made the measurement of incorporation of a trace amount of

[U-^{14}C] glycine into proteins of peripheral nerves for 3 h in vivo at various times after commencing methylmercury dicyandiamide (8 × 5 mg/kg).

The suppression of protein synthesis activity in the dorsal root ganglia appeared earlier and more markedly as compared with the brain (Fig. 1). These results are consistent with the morphological evidence that the peripheral neuropathy is the most prominent feature in organic mercury-intoxicated rats (Miyakawa et al. 1970; Cavanagh and Chen 1971b; Klein et al. 1972; Chang and Hartman 1972b; Herman et al. 1973; Somjen et al. 1973a; Jacobs et al. 1975) and the spinal ganglion cell is the primary target of methylmercury in the peripheral nervous system (Cavanagh and Chen 1971a; Herman et al. 1973; Somjen et al. 1973a; Jacobs et al. 1975). At early stage after the onset of administration of methylmercury, the most striking ultrastructural changes in these cells consisted of disruption of cytoplasmic protein synthetic apparatus; fragmentation of rough endoplasmic reticulum, loss of membrane-bound ribosomes, disappearance of polysomes, and reduction in total number of ribosomes (Herman et al. 1973; Jacobs et al. 1975).

The mode of disturbance of protein synthesis activity observed in the sciatic nerves and dorsal roots was very different from that in the ventral roots (Fig. 1). This might reflect the ultrastructural observation that in peripheral nerves, pathological changes are selectively found in sensory nerve fibers (Miyakawa et al. 1970; Klein et al. 1972; Chang and Hartmann 1972b). Abnormal stimulation of protein synthesis activity observed in sciatic nerves and dorsal roots on day 15, may be mainly based on the hyperfunction of Schwann's cells which was characterized by swelling of cytoplasm and an increase in endoplasmic reticulum and ribosomes (Miyakawa et al. 1970; Chang and Hartmann 1972b). Klein et al. (1972), with the dosage schedule identical with the present study, described that abnormalities in sciatic nerves and dorsal roots were first apparent on day 6 and the changes progressed and, on day 15, the sciatic nerves showed extensive segments of demyelination, axonal disruption, lipid-filled macrophages, and hypertrophied Schwann's cells and fibroblasts, and that similar changes were visible in dorsal roots, but ventral roots showed only hypertrophy of some Schwann's cells.

Of the nervous system examined, dorsal root ganglia showed the highest accumulation of mercury (Table 4). The difference from the accumulation of mercury in the brain was notably large on day 10. The concentration of mercury in dorsal roots was approximately as much as that in ventral roots. These results are in agreement with those of Somjen et al. (1973). Whether the difference in accumulation of mercury is sufficient to explain the high degree of suppression of protein synthesis activity in dorsal root ganglia, and why the degree of damage affected by mercury is so different between dorsal and ventral roots in spite of similar concentration of mercury in these tissues, are still not clear.

The double labelling studies show that the synthesis of protein species in dorsal root ganglia is suppressed by methylmercury nonuniformly in various apparent molecular size. This trend was especially apparent on day 10, the latent period of methylmercury intoxication. Further characterization of the protein species undergoing the high degree of disturbance of their synthesis remains to be studied.

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