DISTRIBUTION OF ACRYLAMIDE IN MOUSE AFTER REPEATED DOSES

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ABSTRACT

The distribution of radioactive acrylamide was studied in mice by both radioautography and quantitative measurement of radioactivity in tissues. Mice treated with non-radioactive acrylamide were injected intraperitoneally with radioactive acrylamide one or four times at various times. Radioactive acrylamide was found accumulated in the blood, spleen, liver, kidney, gut epithelium, salivary glands and the distal part of the sciatic nerve. The radioactivity in the tissues of the central nervous system was about one tenth of that in the blood, and that in the distal part of the sciatic nerve was 2.5 and 7.2 times as high as that in the tissues of the central nervous system at one and four weeks after the administration of radioactive acrylamide, respectively. No difference in radioactivity was observed between the tissues of mice dosed with radioactive acrylamide in the first week and those dosed in the fourth week, except for the brain tissues where the radioactivity in the group dosed in the last week was significantly lower than in that dosed in the first week. The distal part of the sciatic nerve seems to have a stronger affinity to acrylamide than the other nervous tissues.

Monomeric acrylamide ($CH_2 = CH - CONH_2$) is well known to produce peripheral neuropathy including the ataxia of legs in human beings and of the hind limbs of various experimental animals[4,5,8]. In order to elucidate the pathogenesis of this neuropathy it seems neccessary to know the distribution of this compound in the body, especially in the nervous tissues.

There have been experimental studies on the binding of acrylamide with protein sulphydryls in tissues and the time course of the distribution in tissues after a single dose[3,6]. Furthermore, Hashimoto and Aldridge have shown that rats become more sensitive to acrylamide after the administration of N--hydroxymethylacrylamide which is less active to produce neuropathy but reacts with sulphydryls in tissues as acrylamide. There is a possibility that acrylamide reacts with both sites irrelevant and relevant to the production of the lesion.

The present study of its distribution in the tissues of mice after various dose schedules was made with radioactive acrylamide using whole-body radioautography and liquid scintillation counting.

MATERIAL AND METHODS

Male mice of ICR strain weighing 18–20 g were divided into four groups consisting of three to seven animals each. They were injected intraperitoneally with non-radioactive or radioactive acrylamide in saline (0.4% w/v) twice weekly (Monday and Thursday) for one to four weeks with a dose of 90 mg/kg body weight. The animals were fed with a commercial cube diet (Oriental Co.) and given water ad libitum during the experiment. Radioactive ^{14}C acrylamide (350 μCi/mmol) was purchased from the Japanese Atomic Research Institute.

Male mice were injected intraperitoneally with either non-radioactive or radioactive acrylamide twice a week for one or four weeks (each dose 90 mg/kg body weight). They were divided into four groups and administered acrylamide as follows:

Group 1: acrylamide was given for a week, first radioactive then non-radioactive.

Group 2: acrylamide was given during four weeks; only the first injection was radioactive, while the subsequent seven were non-radioactive.

Group 3: acrylamide was given during four weeks; only the first injection of the fourth week was radioactive.

Group 4: acrylamide was given during four weeks; every first injection in a week was radioactive and the second was non-radioactive.

For the whole body radioautography, mice in Group 1, 2 and 3 were killed in an ether jar on the fourth day after the last administration of acrylamide. They were then buried in a 2% carboxymethylcellulose (CMC) paste in water in a plastic box and frozen in an acetone-dry ice freezer. Longitudinal 15 μm thick sections were prepared by a cryostat (Bright Co.) on adhesive tapes (Scotch No. 810). After freeze-drying, the sections were covered with a polyester film 4 μm in thickness (Diafoil; Mistubishi-jushi Co.), fit tightly against the X-ray film (Fuji RX-200) and left for one to two months at 4 °C.

For the quantitative determination of ^{14}C acrylamide in tissues, mice in all groups were anesthetized with ether on the fourth day after the last administration of acrylamide. The animals were then perfused with a physiological saline solution, and the cerebral cortex and medulla, cerebellum, spinal cord, sciatic nerves, liver, kidneys, spleen and gastrocnemius were dissected. The blood was withdrawn from the heart before the perfusion. The spinal cord was cut into three parts, i.e., upper, middle and lower. The sciatic nerve was divided into two parts, proximal and distal, in the inguinal region. After weighing, radioactivities in the tissues were determined by means of a liquid scintillation counter (Packerd Tri-carb 3380) after burning the samples in a sample oxidizer (Packerd 305).

RESULTS

Symptoms of poisoning

Two to three weeks after starting to inject them with acrylamide, the animals began to show symptoms of poisoning such as general weakness, ataxic gait and staining of hair with excreta due to incontinence. Maximal intensity of

the symptoms was observed in the fourth week, and when the administration of acrylamide was continued, the animals died from general weakness.

Whole body radioautography

Similar labelling patterns with ^{14}C acrylamide were obtained from sections of animals in Groups 1 and 3 in which the animals were given radioactive acrylamide only once, one week before they were sacrificed. Blood, liver, spleen and kidneys were labelled most heavily, followed by the bone marrow, gut epithelium and salivary glands (Fig. 1, 2 and 3). The brain and spinal cord were
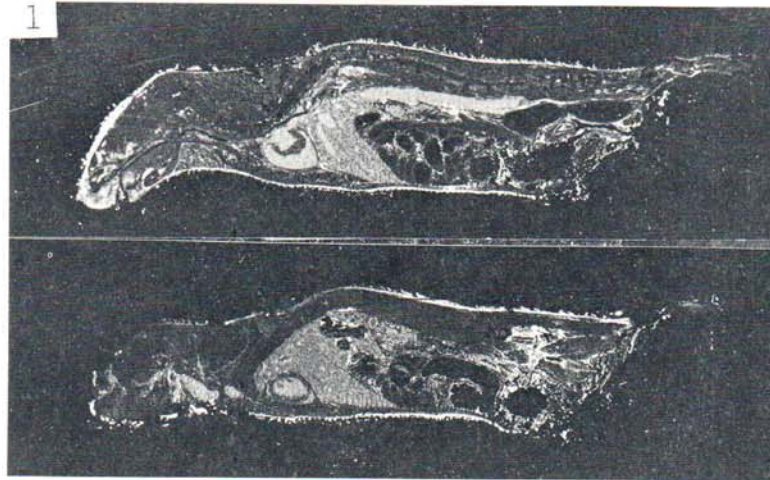

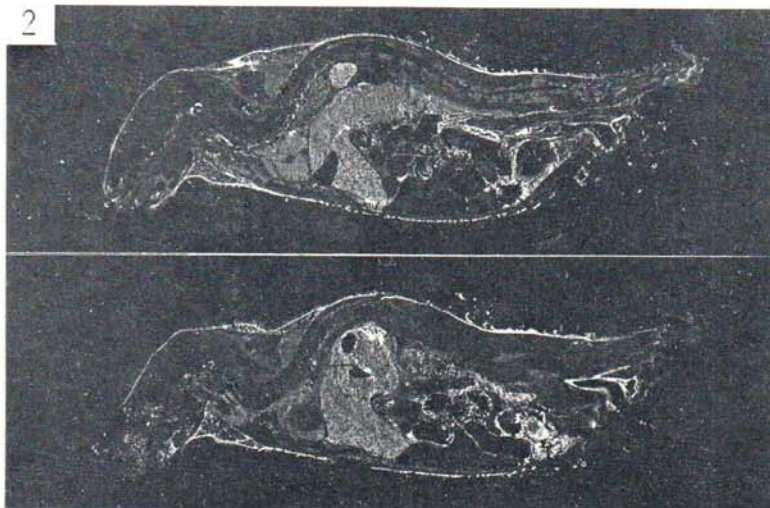FIG. 1 – Whole body radioautograms of mice from Group 1 dosed with ^{14}C acrylamide.

FIG. 2 – Whole body radioautograms of mice from Group 2 dosed with ^{14}C acrylamide.

slightly more labelled than the background level. The distal part of the sciatic nerve was as highly labelled as the blood (Fig. 4), while the proximal part of the nerve and the spinal ganglions were only slightly labelled, similarly to the central nervous tissues (Fig. 5). The liver showed a homogeneous labelling, while in the kidneys heavily labelled granular spots were seen in the cortex. In the spleen heavy labelling was seen in the red marrow but not in the white marrow.
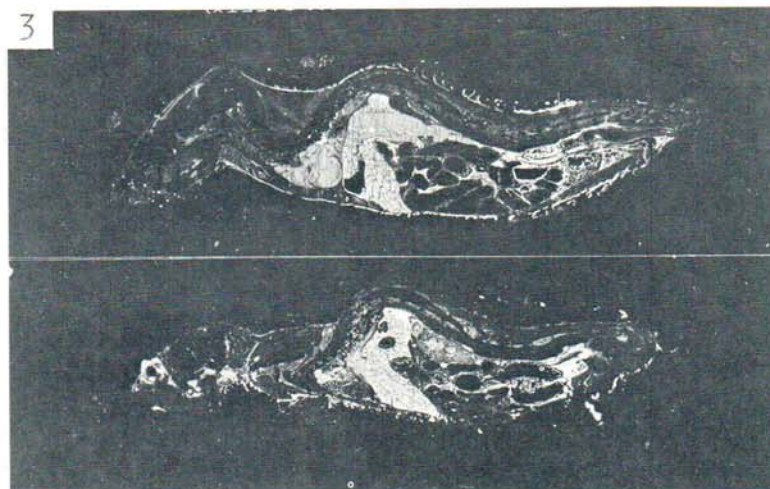

FIG. 3 — Whole body radioautograms of mice from Group 3 dosed with ^{14}C acrylamide.

FIG. 4 — Radioautograms of the sciatic nerve of mice from Group 3.

In animals of Group 2, the labelling of the blood was much less than that of Groups 1 and 3, but in the liver, kidney, spleen and gut epithelium the labelling was still heavy. With decreased labelling in the blood the heterogenecity of labelling in tissues such as the kidneys and spleen became more evident in Group 2 (Fig. 6 and 7).
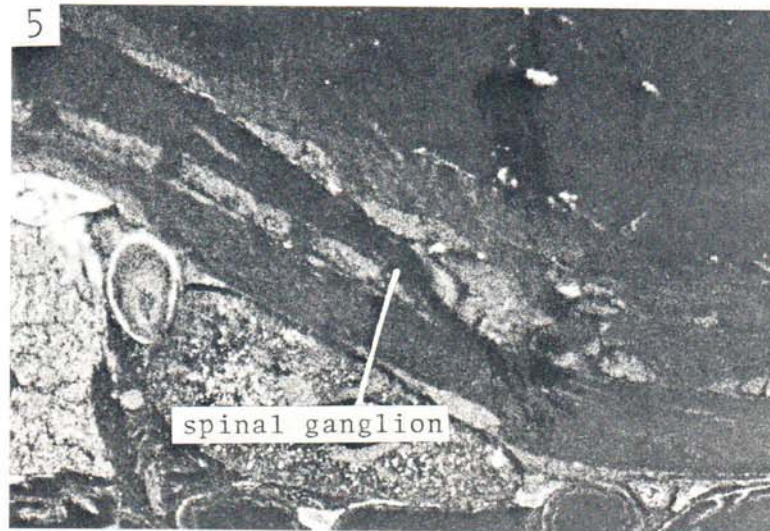

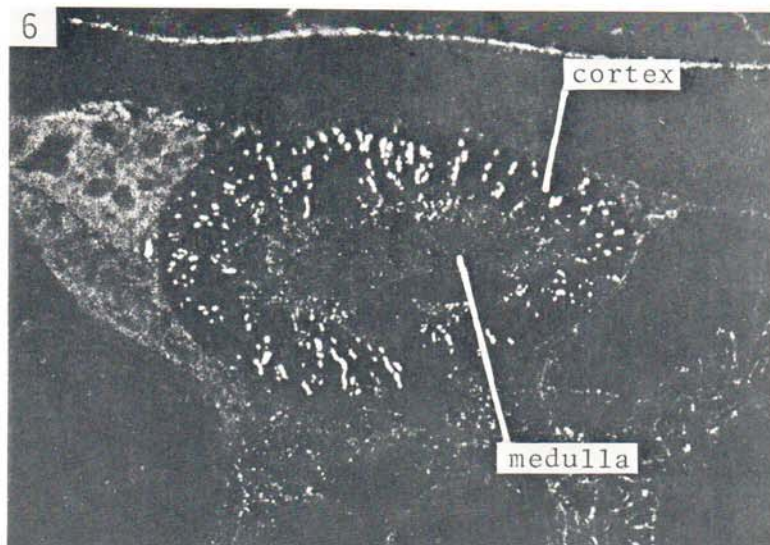FIG. 5 – Radioautograms of the spinal ganglion of mice from Group 3.

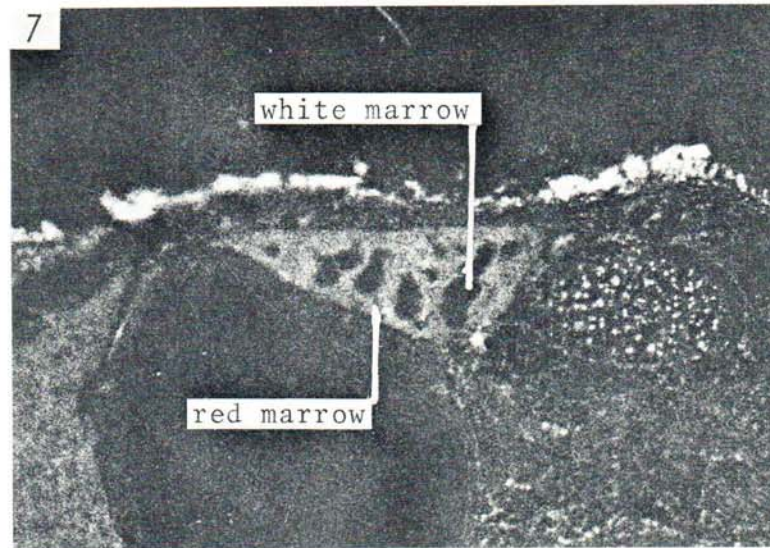FIG. 6 – Radioautogram of the kidney of mice from Group 2.

FIG. 7 – Radioautogram of the spleen of mice from Group 2.

Quantitative determination of radioactivity in tissues

The distribution of ^{14}C acrylamide in thirteen tissues of animals in four groups is summarized in Table 1.

In Group 1, the highest radioactivity was observed in the blood and spleen, followed by the liver and kidneys where the activity was about half of that in the blood. In the central nervous system counts in the different parts were similar and about one tenth of those in the blood. In the sciatic nerve, radioactivity in the distal part was twice that in the proximal part and about three times that in the central nervous tissues. The gastrocnemius showed about the same radioactivity as the distal part of the sciatic nerve.

In Group 2, in which the animals were given radioactive acrylamide once in the first week and then non-radioactive acrylamide seven times, the radioactivity in the blood was about one third of that in Group 1, while the activity in the liver and kidney was approximately the same. In the spleen, the activity was twice that in Group 1. Counts in the central nervous system were about one third of those in Group 1, in the muscle and the proximal part of sciatic nerve about one half, and in the distal part almost the same.

In Group 3, the radioactivity in tissues was similar to that in Group 1 as seen in the whole-body autoradiography and quantitative radioactivity determination. However, the radioactivity in the brain tissues was significantly lower than that in Group 1 $(0.001 < P < 0.01)$.

In Group 4, in which the animals were given radioactive acrylamide once a week for four weeks, the count in the spleen was the highest of all, followed by the liver and kidneys where the activity was about two to three times higher than

TABLE 1

Quantitative determination of ^{14}C acrylamide in mouse tissues. Each value represents mean ± S.D. Figures in parentheses are numbers of rats.

Organ		Radioactivity DPM/10 mg tissue			
		Group 1	Group 2	Group 3	Group 4
Blood		1 145.0 ± 443.4(4)	400.7 ± 67.6(3)	1 144.2 ± 353.6(6)	980.8 ± 120.2(5)
Cerebrum	cortex	128.0 ± 15.9(7)	27.5 ± 3.2(3)**	102.5 ± 11.7(7)*	122.5 ± 41.7(6)
	medulla	126.9 ± 15.3(7)	33.6 ± 6.8(3)**	99.7 ± 6.5(7)*	131.4 ± 44.8(6)
Cerebellum		121.0 ± 17.2(7)	31.6 ± 3.9(3)**	93.9 ± 10.0(7)*	116.3 ± 40.2(6)
Spinal cord	upper	108.7 ± 15.3(7)	34.4 ± 3.5(3)**	89.7 ± 14.7(7)	137.9 ± 46.2(6)
	middle	99.9 ± 18.9(7)	34.2 ± 2.3(3)**	84.0 ± 9.2(7)	127.7 ± 31.5(6)
	lower	99.5 ± 15.7(7)	33.0 ± 3.8(3)**	84.1 ± 4.2(5)	130.6 ± 36.3(6)
Sciatic nerve	proximal	138.4 ± 16.8(6)	74.9 ± 10.8(3)*	138.4 ± 53.3(6)	209.7 ± 77.9(6)
	distal	287.7 ± 32.0(6)	233.3 ± 43.0(3)	304.0 ± 77.4(6)	513.3 ± 221.0(6)*
Liver		471.3 ± 129.8(6)	581.8 ± 95.0(3)	647.8 ± 112.0(6)	1 343.8 ± 258.1(4)**
Kidney		544.8 ± 139.7(6)	444.1 ± 32.5(3)	578.4 ± 66.6(6)	979.4 ± 93.7(4)*
Spleen		954.4 ± 328.6(6)	2 197.9 ± 796.2(3)	1 499.0 ± 186.5(6)	2 694.7 ± 278.2(4)*
Muscle		222.7 ± 46.1(4)	122.1 ± 23.1(3)	162.0 ± 25.6(6)	284.1 ± 41.1(4)

*$0.001 < p < 0.01$
**$p < 0.001$

that in Group 1. The nervous system and muscle showed counts 1.0 to 1.5 times as high in Group 1, except that the distal part of the sciatic nerve showed an activity which was twice higher than that in Group 1.

The ratios of the radioactivity in the distal part of the sciatic nerve to that in the central nervous tissues were 2.5, 7.2, 3.3 and 4.0 in Groups 1, 2, 3 and 4, respectively.

DISCUSSION

Monomeric acrylamide combines with cysteine sulphydryls in tissue protein to produce S-carboxyethyl-cysteine after hydrolysis[6] and leads to a rapid fall of the concentration of glutathione in liver and its recovery within one day after dose[3]. In the present study, the distribution of radioactive acrylamide in tissues was determined in four different administration schedules. The concentration of acrylamide in the blood, spleen, liver and kidneys was always higher than that in other tissues. In the blood, however, acrylamide seemed to decrease faster than in other tissues, except in the central nervous tissues as shown in Group 2. The acrylamide which disappeared from the blood might have entered the other tissues such as the spleen, liver and kidneys to maintain its concentration in these tissues for a considerable time as shown in Group 2. The fact that the activities in these tissues in Group 3 were not lower than in Group 1 may suggest that these tissues have a rather high capacity of keeping acrylamide.

Fullerton and Barnes[5] have shown a degeneration of the axis cylinder and myelin sheaths in peripheral nerves which is predominant in the distal part of the longest fiber, and a cumulative effect of acrylamide after doses given at various intervals. In the present study, the radioactivity in the distal part of the sciatic nerve was always higher and decreased much more slowly than that in the other parts of the nervous tissues. Furthermore, while the amount of acrylamide in the central nervous tissues of pre-treated animals was lower than in those of non-pre--treated animals, that in the distal part of the sciatic nerve was the same both in pre-treated and non-pre-treated animals. These evidences indicate that acrylamide may have a special affinity to certain components in the distal part of the sciatic nerve. Hashimoto and Ando[7] have shown in rats fed for four weeks on a diet containing acrylamide (500 ppm) that the incorporation of radioactive amino acid into the spinal cord as well as into the sciatic nerve *in vitro* is significantly increased after four weeks of dosing. The accumulation of acrylamide may itself not have any direct bearing on the acceleration of amino acid incorporation into nervous proteins but may be related with lesions in these tissues which might cause the acceleration.

The results obtained by radioautography showed that acrylamide accumulates in the bone marrow, gut epithelium and salivary glands. These tissues are known to be the sites of rapid cell proliferation. Pleasure, Mishler and Engel[9] and Bradly and Williams[2] have found evidence of an interruption of the axonal transport of ^3H leucine in the peripheral nerves of cats suffering from acrylamide neuropathy. Both axonal flow and the migration of chromosomes during cell division are believed to be dependent on an intact microtubular

system. Abe, Haga and Kurokawa[1] have demonstrated that the rapid transport of labelled protein within the frog sensory fibre in the sciatic nerve becomes blocked by locally injected methyl mercury. In addition, they produced more direct evidence that methyl mercury depolymerises cerebral microtubules *in vitro*, interacting with sulphydryl groups in microtubule protein (tublin). The fact that acrylamide tends to accumulate in both the sciatic nerve and the mitotic tissues suggests that acrylamide, like methyl mercury, might have certain effects on microtubules.

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