Effect of hydrofluoric acid on glucose metabolism of the mouse studied by whole-body autoradiography

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Watanabe, M., Yoshida, Y., Watanabe, M., Shimada, M., Kurimoto, K., and Kihara, T. (1975). *British Journal of Industrial Medicine*, 32, 316-320. **Effect of hydrofluoric acid on glucose metabolism of the mouse studied by whole-body autoradiography.** Distribution of radioactive carbon from [U-¹⁴C]glucose in the mouse poisoned by hydrofluoric acid has been studied by whole-body autoradiography. Under normal conditions, the highest autoradiographic density was found in the Harder's gland, palatine gland, sublingual gland, large intestinal mucosa, and many regions of the central nervous system 30 minutes after intraperitoneal injection of [U-¹⁴C]glucose. On the other hand, after hydrofluoric acid poisoning, it was found that (1) the radioactivity of brain was unchanged throughout all the poisoning; (2) the liver, renal cortex, lung, and blood showed an increase in radioactivity at 180 minutes of poisoning; (3) the abdominal cavity showed a tendency to residual radioactivity with the poisoning; (4) by contrast, Harder's gland, the palatine gland, sublingual gland, and large intestinal mucosa showed a decrease in radioactivity at 180 minutes of poisoning.

Hydrofluoric acid is one of the most interesting of all inorganic fluoride compounds, and it is an intermediate in the manufacture of most other fluoride compounds from fluorspar (Matthews, 1970). Accidents with hydrofluoric acid show a tendency to increase year by year, and it has a highly corrosive and penetrating action in the living body (Haar, 1949; Beck, 1950; Dale and Chir, 1951; Reinhardt *et al.*, 1966).

Although the literature contains several references to the effects of inorganic fluoride on metabolic pathways and on physical activity of cells, the investigation of carbohydrate metabolism has not been extended beyond sodium fluoride (Caruso, Maynard, and Distefano, 1970).

In the present paper, whole-body autoradiography was used to investigate the distribution of radio-

FIG. 1. Experimental method for whole-body autoradiography.

FIG. 2. Whole-body autoradiograph showing distribution of radioactivity in a mouse 30 min after intraperitoneal injection of (U- ^{14}C)glucose under normal conditions. (Note high concentration of radioactivity in palatine gland, large intestine, and brain.)

FIG. 3. Whole-body autoradiograph showing distribution of radioactivity in the mouse 30 min after intraperitoneal injection of (U- ^{14}C)glucose under hydrofluoric acid poisoning. Poisoning time: *A* 30 min; *B* 60 min; *C* 180 min.

active carbon from [U- ^{14}C]glucose in the mouse after hydrofluoric acid poisoning.

Methods

Labelled compound

Glucose labelled uniformly with carbon-14 ([U- ^{14}C]glucose) was received from the Radiochemical Centre, Amersham, England. The specific radioactivity was 1.5 mCi/mg. The radiochemical purity was 99% by paper chromatography; the solvent system used was n-butanol-ethanol-water (52:33:15).

Hydrofluoric acid treatment (HF treatment)

Fifteen adult albino male mice of the d.d. strain weighing about 20 g were used and divided into three groups. Five mice were used for each group. The skin of the mice (part of the right biceps femoris; 1 cm², the hair removed) was painted with 0.02 ml 50% hydrofluoric acid for five minutes; after that, the residual hydrofluoric acid was removed by filter paper.

Whole-body autoradiography

After the above treatment, the groups were injected intraperitoneally with 0.1 ml 0.9% NaCl containing 15 μCi [U- ^{14}C]glucose 30 minutes before the mice were killed. Five adult albino mice of the same sex, strain, and weight were used as controls. These mice were also injected with [U- ^{14}C]glucose in the same way, 30 minutes before they were killed. The relationships between lapse of time after HF treatment and administration of radioactive glucose are shown in Fig. 1.

In this experiment, times of poisoning by hydrofluoric acid were different with each group, but the periods after the administration of radioactive glucose were the same (30 minutes).

The animals were then killed by immersion in dry ice-acetone mixture (about -70°C). The frozen animals were sectioned and autoradiographs prepared according to the method described by Ullberg (1954; 1962). A number of sagittal sections, 20 μ thick, were cut with a Leitz-1300 cold microtome in a cryostat kept at -20°C and dried at the same temperature.

The sections were placed in contact with medical x-ray films (FUJI Photo Co., Japan), and exposed for 10 days in a cool room (4°C), after which the films were developed and fixed. Some sections were stained with haematoxylin and eosin after exposure in order to identify the various tissues. The autoradiographs thus obtained were measured with a microdensitometer equipped with a computer system for correction of film density and radioactivity (Sakura microdensitometer PDM-5, Konishiroku Photo Ind., Co., Japan). This radioactivity is not the absolute radioactivity, but the relative radioactivity. In this experiment, film density measurement utilized ranges for which there is linearity between film density and radioactivity. This instrument has a linear range up to 4 for film density, and so these densities can be analysed statistically.

Measurement of fluoride

The lung, liver, kidney, and brain of mice affected by hydrofluoric acid percutaneously as described above were

removed for analysis. Samples were homogenized with 15 mg calcium oxide as a fixative, and ashed in a muffle oven at 500°C for three hours. Ashed samples were added to 10 mg 60% perchloric acid, 0.5 ml 25% silver perchlorate, and 0.1 g siliceous sand. After that, the samples were distilled for one hour by Willard and Winter's method (1933) for separation of fluoride. These distillates were analysed by a colorimetric quantitative technique using La-Alizarin complexone reagent (Dotite-Alfuson).

Results

Figure 2 shows the whole-body autoradiograph (parasagittal section) after intraperitoneal injection of [U- ^{14}C]glucose under normal conditions. Thirty minutes after administration of [U- ^{14}C]glucose, the highest radioactivity was found in the Harder's gland, palatine gland, sublingual gland, and large intestinal mucosa. A very high level of radioactivity was found in the central nervous system, especially the cerebral neocortex, inferior colliculus, and

FIG. 4. Enlargements of whole-body autoradiograph showing distribution of radioactivity in Harder's gland of mice. *A* under normal conditions, *B* under hydrofluoric acid poisoning (180 min).

TABLE 1

DENSITY OF WHOLE-BODY AUTORADIOGRAPH UNDER NORMAL AND HYDROFLUORIC ACID POISONED CONDITIONS

System	Normal 30 min	30 min	HF poisoning 60 min	180 min
Central nervous system				
1. cerebral neocortex	1.17 ± 0.08	1.17 ± 0.05	1.25 ± 0.07	1.25 ± 0.11
2. cerebellar cortex (glandular layer)	0.95 ± 0.03	0.95 ± 0.04	1.00 ± 0.03	1.04 ± 0.07
3. thalamus	0.97 ± 0.02	1.05 ± 0.05	1.02 ± 0.02	1.20 ± 0.05
4. hypothalamus	0.82 ± 0.06	0.87 ± 0.03	0.90 ± 0.05	0.90 ± 0.05
5. superior colliculus	0.95 ± 0.05	1.05 ± 0.05	1.07 ± 0.07	1.10 ± 0.10
6. inferior colliculus	1.07 ± 0.07	1.12 ± 0.06	1.14 ± 0.07	1.20 ± 0.07
7. base of fourth ventricle	1.12 ± 0.12	1.15 ± 0.07	1.25 ± 0.10	1.32 ± 0.12
Digestive system				
8. palatine gland	1.28 ± 0.04	1.21 ± 0.05	1.22 ± 0.05	0.67 ± 0.03†
9. sublingual gland	1.40 ± 0.04	1.18 ± 0.06	1.20 ± 0.05	0.74 ± 0.02†
10. submaxillary gland	0.61 ± 0.03	0.42 ± 0.03	0.52 ± 0.03	0.30 ± 0.02
11. Harder's gland	1.90 ± 0.07	1.60 ± 0.05	1.50 ± 0.07	0.75 ± 0.04†
12. liver	0.63 ± 0.04	0.72 ± 0.04	0.73 ± 0.03	1.10 ± 0.11*
13. pancreas	0.52 ± 0.02	0.45 ± 0.05	0.46 ± 0.02	0.50 ± 0.03
14. small intestinal mucosa	0.55 ± 0.05	0.57 ± 0.04	0.60 ± 0.05	0.52 ± 0.03
15. large intestinal mucosa	1.30 ± 0.07	1.27 ± 0.06	1.30 ± 0.08	0.70 ± 0.06†
Urogenital system				
16. renal cortex	0.55 ± 0.03	0.52 ± 0.03	0.75 ± 0.06	0.70 ± 0.04*
17. testis	0.27 ± 0.01	0.20 ± 0.05	0.17 ± 0.02	0.15 ± 0.02
Circulatory system				
18. blood (right ventricle)	0.40 ± 0.03	0.45 ± 0.05	0.55 ± 0.05	0.95 ± 0.03*
19. spleen	0.36 ± 0.02	0.33 ± 0.03	0.42 ± 0.03	0.30 ± 0.01
20. myocardium	0.29 ± 0.03	0.32 ± 0.03	0.45 ± 0.03	0.50 ± 0.04
Respiratory system				
21. lung	0.22 ± 0.02	0.30 ± 0.04	0.32 ± 0.05	0.70 ± 0.05*

Five determinations make up the mean values

*Significant increase at $P < 0.05$ compared with normal conditions†Significant decrease at $P < 0.05$ compared with normal conditions

thalamus. The radioactivity in the liver was higher than in the pancreas, small intestinal mucosa, submaxillary gland, and spleen, while, the testis, lung, bone marrow, and thyroid gland exhibited a low radioactivity.

Representative autoradiographs of sections obtained after intraperitoneal injection of [U- 14 C]-glucose into mice previously treated with 50% hydrofluoric acid are shown in Fig. 3.

Representative enlarged autoradiographs of Harder's gland of the mice under normal and hydrofluoric acid poisoning are illustrated in Fig. 4. Table 1 shows the density of the whole-body autoradiographs. Simple t tests were used for the comparisons with the controls. These autoradiographs and Table 1 demonstrate the following:

(1) the liver, renal cortex, lung, and blood showed an increasing radioactivity at 180 minutes for poisoned mice as compared with normal conditions; (2) the radioactivity of the brain was unchanged throughout all the time of poisoning; (3) the abdominal cavity showed a tendency to retain residual radioactivity

with the time of poisoning; (4) by contrast, Harder's gland, the palatine gland, sublingual gland, and large intestinal mucosa showed a decreasing radioactivity at 180 minutes for poisoned mice compared with normal conditions.

Discussion

Hydrofluoric acid has a very high penetrating action for the skin. Therefore, poisoning by hydrofluoric acid is considered to be an acute problem. For this reason, in the present work, mice were injected with radioactive glucose, and killed by immersion in dry ice-acetone mixture 30 minutes later. Before administration of radioactive glucose, the mice had been treated with 0.02 ml 50% hydrofluoric acid at time intervals of 0, 30, and 150 minutes. In these experiments, dead mice were removed. There was a 70% mortality within a week in mice exposed to 50% hydrofluoric acid for five minutes.

Glucose is the most important substance as an energy source, especially in the brain. From our

TABLE 2
CONCENTRATION OF FLUORIDE IN VARIOUS ORGANS OF THE MOUSE

Organs	Normal	HF poisoning		
		30 min	60 min	180 min
Brain	24.7 ± 9.1	24.9 ± 5.5	23.8 ± 5.3	36.6 ± 5.8
Lung	6.0 ± 3.8	31.8 ± 7.4*	53.8 ± 5.6*	61.2 ± 6.4*
Liver	1.9 ± 0.1	6.1 ± 0.5*	9.7 ± 0.5*	12.9 ± 2.6*
Kidney	7.3 ± 1.3	27.0 ± 1.7*	31.0 ± 2.1*	30.1 ± 1.7*

Fluoride concentration $\mu\text{mol/g} \pm \text{SD}$

Five determinations make up the mean values

*Significant increase at $P < 0.01$ compared with normal conditions

results, it appears that after the administration of $[\text{U-}^{14}\text{C}]\text{glucose}$ in hydrofluoric acid poisoning, the amount of radioactivity in the central nervous system was unchanged as compared with normal conditions. This result suggests that the hydrofluoric acid has no effect on brain. These data were in good agreement with those of Ullberg, Appelgren, and Clemmedson (1964) and Ericsson and Ullberg (1958) who showed an especially low fluoride content in the central nervous system.

On the other hand, the abdominal cavity showed a residual radioactivity with the poisoning. This result suggests that the absorbing power from the abdominal cavity to the circulatory system was decreased. The blood, liver, kidney, and lung, however, showed an increasing radioactivity at 180 minutes for poisoned mice. These organs, except for the blood, showed a significant increase of fluoride concentration in the chemical analysis (Table 2). By contrast, a significant decrease in radioactivity was found in Harder's gland, the palatine gland, sublingual gland, and large intestinal mucosa at 180 minutes of poisoning. In other words, all mucous glands showed a decrease in radioactivity.

On histochemical investigation the tissues stained with periodic acid-Schiff and Toluidine blue (Jennings and Florey, 1956). This indicates that these tissues contained large amounts of mucopolysaccharides or muco-proteins.

From these results it is concluded that the possibility of systemic toxicity following cutaneous exposure to hydrofluoric acid should be considered.

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