WHOLE-BODY AUTORADIOGRAPHIC STUDIES ON THE DISTRIBUTION OF RADIOACTIVE CARBON FROM [U-¹⁴C]GLUCOSE IN CARBON MONOXIDE POISONED MICE

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Changes in the distribution of radioactive carbon from [U-¹⁴C]glucose in mice with carbon monoxide were studied by whole-body autoradiography.

Two periods could be distinguished in the changes of ¹⁴C concentrations in organs affected by CO poisoning with prolonged exposure time up to 6 hrs: the first stage of 30 and 60 min and the second stage of over one hr. In the first stage, a marked decrease in ¹⁴C concentration was shown for the brain, palatine, submaxillary and Harder's gland, while an increase was shown for the myocardium, liver, kidney cortex and blood. In the second stage, however, changes of ¹⁴C concentration in organs were less pronounced and showed for almost all organs a trend rather resembling that of blood.

When radioactive glucose is administered *in vivo*, part is oxidized into CO₂, part is stored as glycogen and part is used to synthesize glucose dependent amino acids and other compounds (7, 38). As to the uptake of injected radioactive glucose in normal and altered organs, there have been investigations by other researchers (1, 4, 6, 7, 8, 9, 22, 24, 25, 26, 27, 28, 32, 33, 36). In our recent investigations using [U-¹⁴C]glucose (28), whole body autoradiography was employed in mice to examine the glucose carbon distribution and its alteration in organs with time. The findings obtained, reflecting to a certain extent intact glucose metabolism in organs and tissues, have shown the necessity to examine further the changes of glucose carbon uptake and its retention in organs on injuring histo-respiration of animals experimentally.

Carbon monoxide, as is generally known, is toxic to animals because of its specific affinity to haemoglobin and inhibitory activity on cytochrome oxidase by binding with the iron of cytochrome oxidase. In the present work, we therefore used carbon monoxide as an agent to block cellular oxidation and cause histotoxic anoxia in animals.

MATERIALS AND METHODS

Labeled compound

Glucose labeled uniformly with carbon-14 ([U-¹⁴C]glucose) was obtained from the Radiochemical Center, 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).

Carbon monoxide inhalation chamber system

The inhalation chamber for animals consisted of two air tight tanks (chamber *A* and chamber *B*), having a capacity of $50 \times 50 \times 30$ cm and $50 \times 50 \times 60$ cm, separated by a tight door. Chamber *B* was filled with air and chamber *A* with carbon monoxide-air mixture. The carbon monoxide concentration in chamber *A*, while the door was open, was adjusted so as to retain 1000 ppm by mixing the air of both chambers thoroughly with an electric fan.

To check the CO concentration we employed a gas chromatographic technique with a Shimadzu model GC-4APTF thermoconductivity gas chromatograph equipped with two glass columns of $1.5 \text{ m} \times 4 \text{ mm}$ I. D. packed with a 5 A molecular sieve. Helium was used as the carrier gas. At checking, 1 ml of the carbon monoxide air in chamber *A* was introduced into the gas chromatograph with a Hamilton's gas tight syringe.

Experiments

Thirty adult albino male mice (d.d. strain, weighing approximately 20 g) were used. Twenty-four mice, the first group, were poisoned with carbon monoxide and the other six, the second group, were kept as a control. The first group was carried into chamber *B* and the tight door opened so that the animals inhaled 1000 ppm carbon monoxide. The experimental group was divided into 4 groups of 6. For each 6, inhalation time was respectively 30 min, 1, 3 and 6 hrs; 30 min prior to completion all were given an intraperitoneal injection of 15 μCi [$\text{U-}^{14}\text{C}$]glucose (in 0.1 ml of 0.9% NaCl) (Fig. 1).

After the completion of inhalation, each animal was frozen in dry-ice acetone. The freezing procedure was the same for the control animals.

Whole-body autoradiography

Sagittal whole-body sections of the frozen animal were cut at $20 \mu\text{m}$ with a

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

hydraulically driven Leitz-1300 microtome in a cryostat (OT/MSH, Bright Instrument Co., England) adjusted to -20°C . In cutting, each section was fixed tightly onto adherent cotton tape and dried (30, 31). Then the sections adhering to the tape were brought into a dark box (4°C) and applied to X-ray film (Medical X-ray film, Fuji Photo Co., Japan). Exposure time of the film in the dark box was 10 days. After exposure they were developed in Rendol (20°C) for 8 min and fixed in Fujifix (20°C) for 10 min. The autoradiographs thus obtained were measured with a microdensitometer (Sakura microdensitometer PDM-5, Konishiroku Photo Ind., Co., Japan). The densities were calibrated against a density scale which was prepared by exposing a radioactive scale against X-ray film for correction of film density and radioactivity (^{14}C concentration). This radioactivity is not the absolute radioactivity, but the relative radioactivity (36). Some sections were stained after exposure with hematoxyline and eosin and used for histological analysis of autoradiographs (34, 35).

Determination of blood glucose

Blood (1 ml) was collected from 24 poisoned and 6 control animals by decapitation, then deproteinized and centrifuged. The supernatant was freeze-dried and the glucose was extracted with pyridine (2 ml). The glucose thus obtained was converted into trimethylsilylether derivatived by mixing with silan's reagents (0.4 ml of hexamethyldisilazane and 0.2 ml of trimethylchlorosilane) according to the method of Sweely *et al.* (29), added to an internal standard solution (β -phenyl-D-glucoside) and agitated for 30 min at 80°C in an oil-bath.

The resulting reaction mixture was introduced directly into a glass column packed with 1% OV-1 and subjected to gas chromatographic analysis.

RESULTS

Fig. 2 is a whole-body autoradiograph of a control animal which was sacrificed 30 min after glucose injection, and Fig. 3 is those of four poisoned animals which inhaled CO for 30 min, 1, 3 and 6 hrs respectively and sacrificed 30 min after glucose injection. Autoradiographic densities of control and poisoned animals are listed in Table 1. A simple t test was applied for a statistical analysis of densitometrical values.

1) *Central nervous system*

In control mice which all survived 30 min after glucose administration, ^{14}C concentration among organs was high for the brain, with a value only lower than that of the mucous glands. In the brain ^{14}C concentration showed regional differences with high values for the cerebral cortex and the base of the fourth ventricle, and following these, a gradient of values was shown in the order of the thalamus, cerebellar cortex (granular layer) and hypothalamus.

In the poisoned animals with 30 and 60 min CO inhalation, a marked decrease in ^{14}C concentration was observed in all divisions examined. But the rate of decrease varied among brain divisions. In animals with 60 min inhalation, the extent of decrease was 35 to 50% and a high rate of decrease of over 45% was observed for the cerebral cortex and base of the fourth ventricle. In the case of 3 hrs CO inhalation, however, ^{14}C concentrations showed a marked increase over 1 hr CO

inhalation in all brain divisions examined, and a high rate of increase of over 50% was observed for the base of the fourth ventricle, cerebellar cortex and cerebral neocortex, presenting respectively nearly identical values to those of the control animals. In the animals with 6 hrs CO inhalation, ^{14}C concentration for each division again decreased, and the rate of decrease varied among brain divisions from 25 to 33% and was highest for the cerebellar cortex and cerebral neocortex.

2) Digestive system

In control animals, a high level of ^{14}C concentration in the digestive organs was observed for the palatine gland, submaxillary gland and mucosa of the large intestine, these belonging to the mucous glands. But the highest concentration was observed for Harder's gland, a special lipid gland located in the orbital cavity (11, 14, 15, 37). In contrast with such high concentration in the mucous glands, the liver, pancreas and mucosa of the small intestine were considerably low, on the average presenting about 44% of the value in the mucosa of the large intestine.

In poisoned mice, however, marked changes of ^{14}C concentration were observed for certain organs. Three patterns of ^{14}C incorporation could be distinguished among organs with prolonged CO inhalation time. The first pattern, a continuous decrease of ^{14}C concentration with CO inhalation times up to 6 hrs, was observed for all mucous glands examined. In these organs ^{14}C concentration at 6 hrs inhalation decreased by over 50%. The greatest decrease of 87% was measured for Harder's gland. The second pattern, shown in the liver and mucosa of the small intestine, was characterized by a continuous increase of ^{14}C concentration at 30 min, 1 and 3 hrs CO inhalation, and at 3 hrs CO inhalation ^{14}C concentrations in the

FIG. 2. Whole-body autoradiograph at 30 min after intraperitoneal injection of $[\text{U-}^{14}\text{C}]$ glucose under normal conditions.

FIG. 3. Whole-body autoradiographs at 30 min after intraperitoneal injection of $[U-^{14}C]$ glucose under CO poisoning. Poisoning time: A 30 min; B 1 hr; C 3 hr; D 6 hr.

TABLE 1. *Density of whole-body autoradiograph under normal and carbon monoxide poisoned conditions*

System	Normal 30 min	CO Poisoning			
		30 min	1 hr	3 hr	6 hr
Central nervous system					
1. cerebral neocortex:	1.17±0.08	0.79±0.06 ↓	0.58±0.04 ↓	0.89±0.05 ↓	0.61±0.06 ↓
2. cerebellar cortex (granular layer)	0.95±0.03	0.80±0.03 ↓	0.62±0.03 ↓	0.99±0.04	0.66±0.01 ↓
3. thalamus	0.97±0.02	0.68±0.04 ↓	0.56±0.01 ↓	0.77±0.03 ↓	0.58±0.03 ↓
4. hypothalamus	0.82±0.06	0.62±0.04 ↓	0.48±0.03 ↓	0.64±0.03 ↓	0.51±0.01 ↓
5. base of fourth ventricle	1.12±0.12	0.82±0.04 ↓	0.62±0.03 ↓	1.05±0.05	0.74±0.04 ↓
Digestive system					
6. palatine gland	1.28±0.04	1.23±0.11	1.00±0.08 ↓	0.30±0.03 ↓	0.55±0.06 ↓
7. submaxillary gland (mucosa)	1.30±0.04	0.90±0.03 ↓	0.75±0.04 ↓	0.57±0.05 ↓	0.55±0.05 ↓
8. Harder's gland	1.90±0.07	0.88±0.03 ↓	0.64±0.06 ↓	0.34±0.04 ↓	0.25±0.05 ↓
9. liver	0.63±0.04	0.68±0.07	0.77±0.07	1.27±0.08 ↑	0.70±0.07
10. pancreas	0.52±0.02	0.41±0.06	0.30±0.02 ↓	0.57±0.03	0.30±0.03 ↓
11. mucosa of small intestine	0.55±0.05	0.64±0.04	0.68±0.05	0.78±0.03 ↑	0.48±0.04
12. mucosa of large intestine	1.30±0.07	1.21±0.08	0.97±0.02 ↓	0.75±0.03 ↓	0.50±0.05 ↓
Urogenital system					
13. kidney (cortex)	0.55±0.03	0.73±0.07	0.60±0.05	0.86±0.01 ↑	0.48±0.04
14. testis	0.27±0.01	0.23±0.02	0.22±0.02	0.35±0.04	0.17±0.04
Circulatory system					
15. blood (right ventricle)	0.40±0.03	0.55±0.06 ↑	0.57±0.07 ↑	0.91±0.04 ↑	0.64±0.05 ↑
16. spleen	0.36±0.02	0.48±0.05 ↑	0.35±0.05	0.41±0.04	0.30±0.02
17. myocardium	0.29±0.03	0.82±0.07 ↑	0.74±0.02 ↑	0.99±0.01 ↑	0.83±0.05 ↑
Respiratory system					
18. lung	0.22±0.02	0.40±0.05 ↑	0.35±0.05 ↑	0.38±0.05 ↑	0.35±0.05 ↑

Six determinations make up the mean values: density±S.D.

↓ : Significant decrease at $p<0.05$ as compared with normal condition.

↑ : Significant increase at $p<0.05$ as compared with normal condition.

liver and mucosa of the small intestine were respectively about 2 times and 1.4 times higher than those of control animals. At 6 hrs CO inhalation, ^{14}C concentrations in these organs again decreased to a level that was not significantly different from that of control animals. The third pattern, rather resembling that of brain, was observed for the pancreas, in which ^{14}C concentration first decreased, then increased, and then decreased again. A gradual decrease of ^{14}C concentration was seen for this organ at 30 min and 1 hr CO inhalation. But at 3 hrs inhalation, it markedly increased and attained a level which is not significantly different from that of normal animals. At 6 hrs inhalation, the concentration in the pancreas again decreased to about 42% lower than the level of normal animals.

3) *Circulatory system*

The myocardium in control animals showed a low ^{14}C concentration of about 22% of the value for the large intestine mucosa. In poisoned animals, however,

TABLE 2. *Blood glucose concentrations (mg/100 ml) of mice under normal and CO poisoned conditions*

Normal	CO Poisoning			
	30 min	1 hr	3 hr	6 hr
110	170	280	150	115

^{14}C concentration in the myocardium for all cases of CO inhalation was markedly raised to over 3 times the values for control animals, and the highest jump of about 4.5 times was recorded for the 3 hrs inhalation cases.

As to the blood, its ^{14}C concentration in the cardinal cavity was also low for control animals, with a value about 69% lower than that for the large intestine mucosa. In poisoned animals, a relative increase of ^{14}C concentration was observed in the blood, which was about 38% and 60% for 30 min and 1 hr inhalation cases, respectively, and the highest rate of over 2 times was measured for the animals of 3 hrs CO inhalation.

^{14}C concentration in the spleen (except 30 min inhalation cases) did not show any significant difference from that of control animals. At 30 min inhalation, however, ^{14}C concentration was about 33% higher than in control animals.

4) *Urogenital system*

^{14}C concentration in the kidney in control animals was not significantly different from that of the mucosa of the small intestine. However, certain differences in ^{14}C concentration could be found between the cortex and medulla, with a relatively high density for the former. In poisoned animals, changes of ^{14}C concentration in this organ could be observed rather more distinctly in the cortex than in the medulla, with increases at 30 min, 1 hr, and 3 hrs inhalation, but a decrease at 6 hrs inhalation. All these changes, however, were almost identical to those in control animals.

5) *Blood glucose*

The blood which was obtained at decapitation of the animals, as measured by gas-chromatography, was significantly higher in glucose content at 30 min and 1 hr CO inhalation than in control animals (Table 2).

But the highest value of over 2.5 times was lowered for animals of 3 hrs and 6 hrs inhalation and an identical level to that of control animals was obtained at 6 hrs inhalation.

DISCUSSION

In the present investigation by whole-body autoradiography in mice, we examined the effects of CO on glucose carbon distribution in mice exposed to CO up to 6 hrs and injected with $[\text{U-}^{14}\text{C}]\text{glucose}$ in the abdominal cavity 30 min prior to the completion of inhalation. By densitometry of autoradiographs we found considerable changes in glucose carbon distribution in affected animals as the exposure time was prolonged up to 6 hrs. According to the changes of ^{14}C concentration in organs we can distinguish two stages of hypoxia. In the first stage, that of 30 and 60 min of CO exposure, we found that the changes of ^{14}C concentration in

organs are acute and exhibit two different trends. A marked decrease in ^{14}C concentration in this stage was shown for the brain and mucous glands such as palatine and submaxillary gland, including Harder's gland which secretes lipid (11, 14, 15, 37). On the other hand, increased ^{14}C concentration was shown for the myocardium, liver, renal cortex and blood. In the second stage, that of over 1 hr of CO exposure, however, changes of ^{14}C concentration in organs were less pronounced and showed for almost all organs a trend rather resembling that of blood, indicating a metabolic adaptation of organs to the histotoxic condition of prolonged CO exposure.

It is generally known that hypoxia in animals produces hyperglycemia (10, 12, 16, 17, 18). In our experiment we observed also a marked increase of glucose content in the blood; its maximum value was attained at 1 hr of CO exposure, presenting a value over two times that of non-exposed mice. In spite of such an acute increase, the rate of increase of labeled glucose concentration in the blood was rather low and its maximum value was attained 2 hrs later than it was attained for the total glucose. This discrepancy between the non-labeled and labeled glucose is thought to be caused mainly by the excessive discharge of non-labeled glucose into the blood during the first hour of CO exposure.

Johlin and Moreland (13) stated that an increase in blood sugar under CO poisoning must result from the conversion of glycogen in the liver.

In the second stage, the patterns of ^{14}C concentration for the blood and liver were very similar, even though the level of concentration for the liver was higher and its changes more acute. These findings suggest for the liver the possibility of acute accumulation of labeled glucose metabolites and their discharge into the blood during CO exposure over 1 hr. Changes of ^{14}C concentration similar to those of the blood in the second stage were moreover observed in many organs such as the liver, pancreas, renal cortex, cerebellar and cerebral cortex, and the base of the fourth ventricle. This indicates that in the second stage many organs are in a condition to regulate the influx-efflux ratio of glucose and its metabolites, probably due to metabolic adaptation of organs to CO hypoxia during CO exposure prolonged over 1 hr. Moreover, there is a possibility of glucose metabolite accumulation in the myocardium also, since the decrease in ^{14}C concentration in this tissue was not so marked as that of the blood in prolonged exposure of over 3 hrs. The spleen and lung showed the most stable ^{14}C concentration in all cases of CO exposure. This indicates that the influx-efflux ratio of glucose and its metabolite in these organs was not affected even though CO exposure was prolonged for over 1 hr. In contrast to the second stage, ^{14}C concentration changes in the first stage, as stated above, were much more acute for many organs examined. An abrupt increase of ^{14}C concentration during 30 min CO exposure was shown for the renal cortex and myocardium, and one relatively low in degree for the spleen and lung. We consider this may be due to the disturbance of capillary barrier function in these organs at the onset of CO exposure. On the other hand, a marked decrease of ^{14}C concentration in the first stage was shown for the brain and mucous glands.

It is generally accepted that the brain uses glucose as its sole energy source for respiration, nutrition, and to maintain nerve function (20). At the same time it has been also behaved that the brain is the organ most sensitive to hypoxia (5, 21, 23). According to Shimada *et al.* (27), ^{14}C concentration of glucose dependent amino

acids in the brain of mice with cyanide-induced hypoxia showed a marked decrease. These results using cyanide poisoned animals seem to suggest a reason why the ^{14}C concentration abruptly decreased in the first stage. The possibility must be considered, as proposed by Miller *et al.* (19), that the glycolytic and TCA cycle intermediates would be utilized in the brain of hypoxial animals as fuel instead of glucose. We also found regional differences in the rate and degree of ^{14}C concentration decrease in the brain in the first stage. A notable decrease at 30 min CO exposure was observed for the cerebral cortex and base of the fourth ventricle. According to the conception of Miller, this might be largely attributed to the regional O_2 consumption which is thought to be different in rate among brain divisions. Besides the brain, a marked decrease of ^{14}C concentration in the first stage was proved for mucous glands and Harder's gland. Here it must be noted that ^{14}C concentration decrease in these glands was, unlike the brain, continuous throughout the second stage, even though the rate of decrease became slower with time. This continuous decrease of ^{14}C concentration in mucous glands is thought probably to be caused by the inhibition of capillary barrier function for glucose, due to the inhibition of mucopolysaccharides synthesis in the secretory cells of these glands. When the ^{14}C concentration for the large and small intestine mucosa in the first stage were compared, we found marked differences in glucose carbon uptake. In the second stage, however, the concentration curves for these two tracts were almost identical. These findings are sufficient to indicate that the glucose metabolism in these tracts may be similarly influenced by CO exposure if we omit the mucous cells which are much more predominant in number in the large intestine mucosa compared with that of the small intestine mucosa and in which the glucose metabolism was seriously inhibited by CO exposure. On viewing the digestive organs in CO exposed mice, the ^{14}C concentration curve in the pancreas was peculiar in its dip at 1 hr CO exposure, rather resembling the pattern of the brain. A similar phenomenon, though less pronounced in degree, was also observed in the testis.

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