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AUTORADIOGRAPHIC WHOLE BODY STUDIES OF ^{14}C-NITROGEN MUSTARD IN NORMAL AND TUMOUR-BEARING MICE

by

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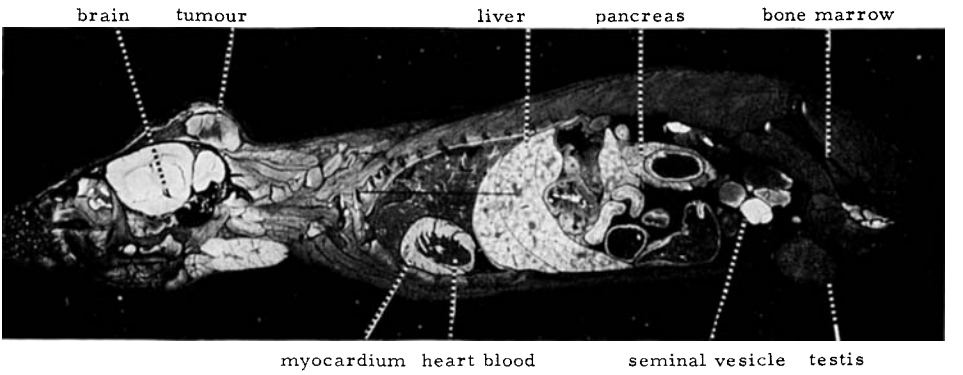
In an earlier investigation (CLEMEDSON et coll. 1963), the distribution of sulphur mustard in mice was studied by whole body autoradiography. It was found that the compound was fairly uniformly distributed, with accumulation mainly in excretory organs.

Since the nitrogen mustards have certain properties in common with the sulphur mustards we considered it of interest to study the distribution of a ^{14}C-labelled nitrogen mustard, methyl-dichlorethylamine, using the same autoradiographic technique. The distribution of this compound in animals has previously been studied by measurements on dissected organs (see SMITH et coll. 1958, MANDEL 1959, and BROWN 1963), but it could be expected that the use of the whole body autoradiographic technique would give additional information, especially concerning the distribution in organs not previously studied by the dissection technique.

As nitrogen mustards have found therapeutic application in the treatment of neoplastic diseases, a series of tumour-bearing mice was also included in the present investigation.

Submitted for publication 9 June 1965.



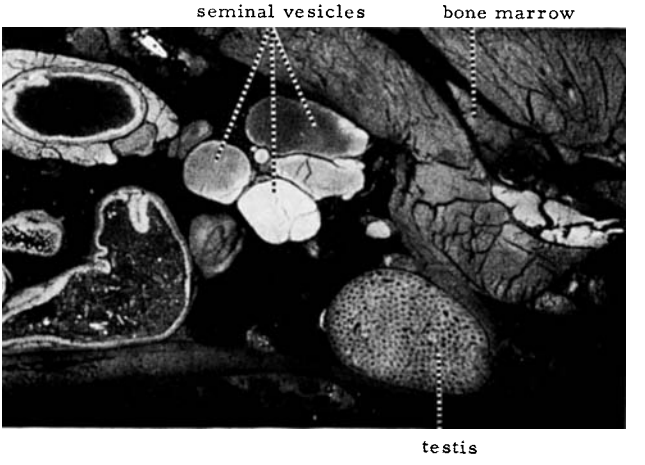


Fig. 1. Autoradiograms of male mouse. *Upper:* At 5 min after intravenous injection of ^{14}C-nitrogen mustard. White areas correspond to high radioactivity; the blood is rapidly cleared of labelled substance. Highest uptake is seen in brain and seminal vesicles; fairly high concentration in liver, salivary gland, myocardium, and in the transplanted tumour. *To the right:* Detail of upper view. Very high activity in the epithelium (but not in contents) of the seminal vesicles; the testicular tubular walls show an intermediate concentration, while the tubular lumina are void of radioactive substance.

Methods. The nitrogen-^{14}C-mustard gas compound, methyl-dichlorethyl-amine with a total activity of 105μCi, and a specific activity of 1 mCi/mM was obtained from CEA-CEN, Centre d'études de l'énergie nucléaire, Belgium, and was dissolved in 2 ml isotonic saline solution.

Ten adult male CBA mice, weighing about 30 g, were used for the experiments. One group of five animals had been tranplanted with small pieces of ^{90}Sr-induced osteosarcomas subcutaneously in the dorsal neck region (NILSSON 1962). The tumours had been used for transplantation in several generations of animals and had lost their original content of ^{90}Sr. In one month, the neoplasms had reached a diameter of about 1.5 to 2 cm.

The animals were injected intravenously into a tail vein, each animal receiving 0.1 ml of the mustard solution, corresponding to 1 mg mustard or

5.3 μCi. This high dose, which is lethal to mice (STERNBERG et coll. 1958) was used in order to obtain autoradiograms within a reasonable length of time. All animals survived for the relatively short observation times (up to one hour) used in this study. The animals were sacrificed under ether anesthesia at various intervals by immersion in a mixture of solid carbon dioxide and hexane (about — 70° C). The survival times in each group were 1 min, 5, 10, 20 and 60 minutes.

The frozen animals were sectioned and autoradiograms prepared according to the method described by ULLBERG (1954, 1958). Sagittal sections, 20 μ thick, through the whole animal were cut in a refrigerated room (— 10° C) at different levels and were dried at the same temperature. The sections were pressed against Structurix (Gevaert) X-ray film. After an exposure time of 80 days the autoradiograms were developed, and some of the sections were stained while kept on the tape.

Results

There was a rapid fall of the activity in the blood, and already 1 min after the injection several soft tissues showed a higher concentration of ^{14}C than the blood. This is in agreement with the observations of NADKARNI et coll. (1956). The highest concentrations after 5 min were found in the kidney, central nervous system, nasal mucosa, salivary glands and myocardium (Fig. 1). Very few changes in this distribution picture could be seen at the longer survival times studied (Figs 3 and 4).

Urinary system. Immediately after the injection the kidneys showed the highest radioactivity in the body, and one hour after the injection the kidneys still showed the highest activity. After 5 min the urinary bladder showed a high ^{14}C-concentration which persisted throughout the whole experiment.

Central nervous system. One minute after the injection the activity was very high in the central nervous system with the gray matter dominating. Plexus choroideus showed an intense uptake of ^{14}C-mustard and so did the ganglia (Fig. 3). The distribution pattern was the same one hour after the injection.

Eyes. A high level of ^{14}C was seen in the retina and the optic nerve at all the times studied. A still higher level could be noticed in the pigment layer of the choroid (Fig. 2, a and b). It was most marked from 1 to 10 min after injection and then disappeared. A slight uptake was seen in the periphery of the lens (Fig. 2a).

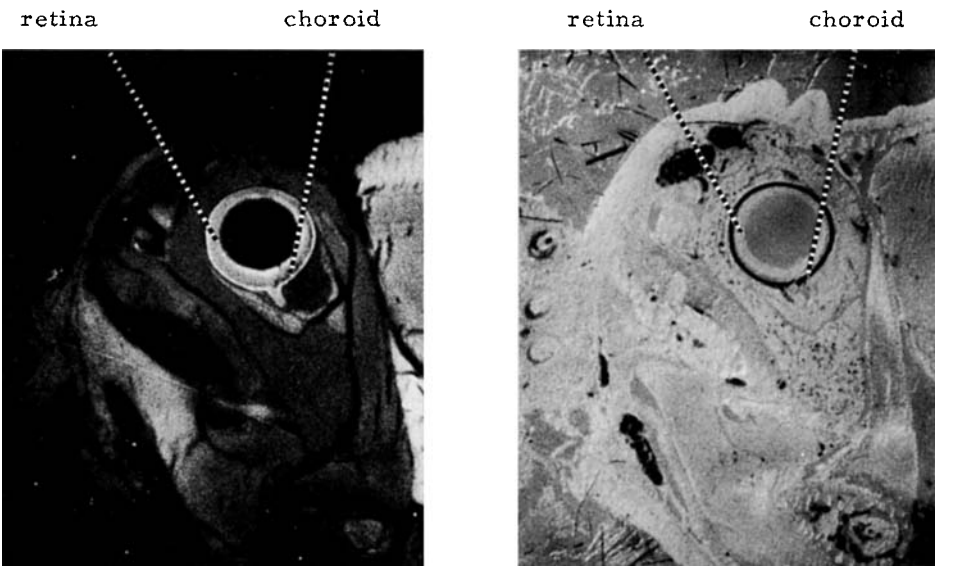


Fig. 2. a) Detail of whole body autoradiogram showing eye of mouse 10 min after injection of ^{14}C-nitrogen mustard. Highest activity is seen in the choroid; high concentration also in the retina. b) Section corresponding to (a).

Digestive system. The salivary glands showed relatively strong accumulation already 1 min after the injection. This could still be observed after 1 hour. Some radioactivity was seen in the gastric mucosa and content from 5 to 60 min.

The liver showed a relatively high uptake 1 min after injection and after 5 min the excretion through the bile was apparent. A biliary excretion of metabolites from nitrogen mustard has previously been demonstrated by TRAMS (1958).

The radioactivity in the pancreas increased gradually from 1 min to 60 min. The intestinal mucosa showed an intermediate level of activity throughout the experiment. In the mice killed after 1 hour rather high activity could be detected in the intestinal content.

Circulatory system. The myocardium, at all the times studied, showed a higher uptake than the skeletal muscles. The larger arteries (e.g. aorta and the pulmonary artery) were very prominent.

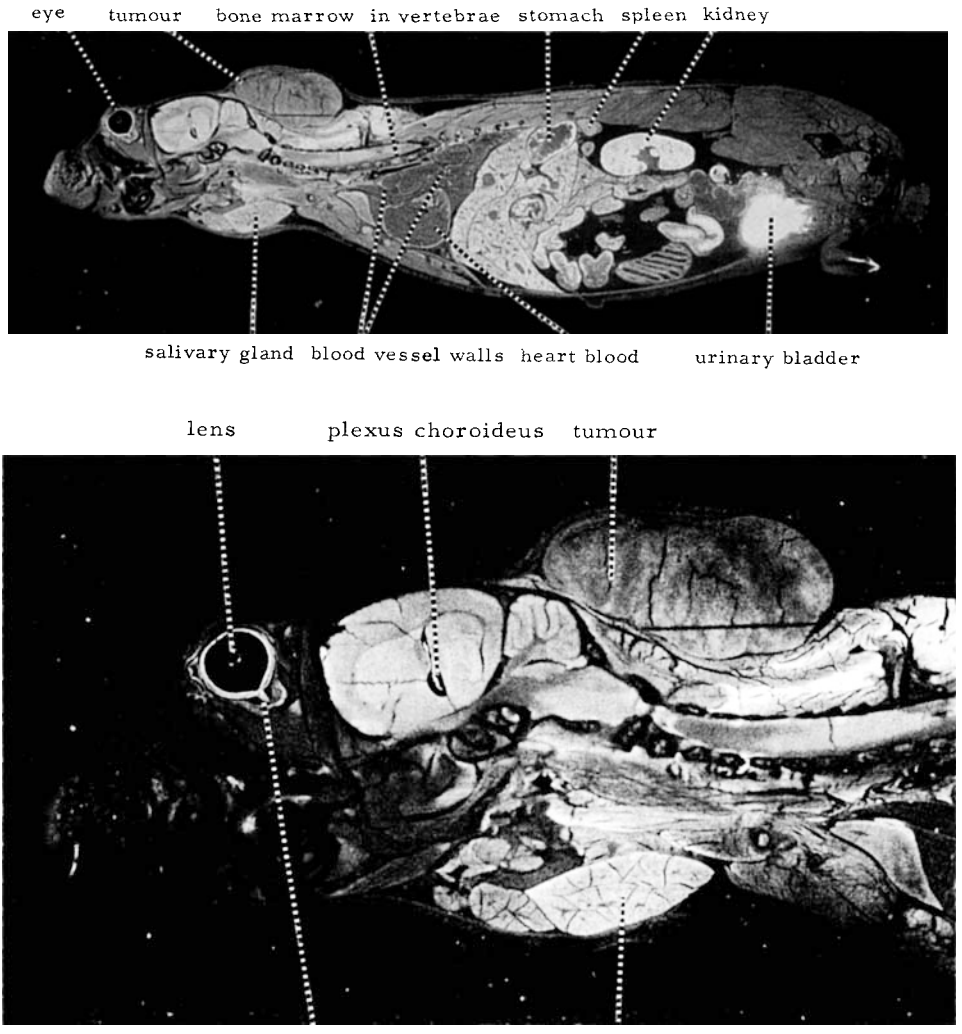


Fig. 3. Autoradiograms of male mouse. *Upper:* At 20 min after injection. High concentration in eye, brain, kidney and urinary bladder; bone marrow, spleen and thymus show intermediate uptake. *Lower:* Detail of upper view. High concentration in retina and periphery of the lens; activity in the choroid layer is still high. In the brain the highest activity is seen in the choroid plexus; the grey matter has slightly higher activity than the white.

Respiratory system. An intense accumulation was seen in the nasal region already at 1 min and the isotope concentration persisted throughout the observation period (Fig. 5).

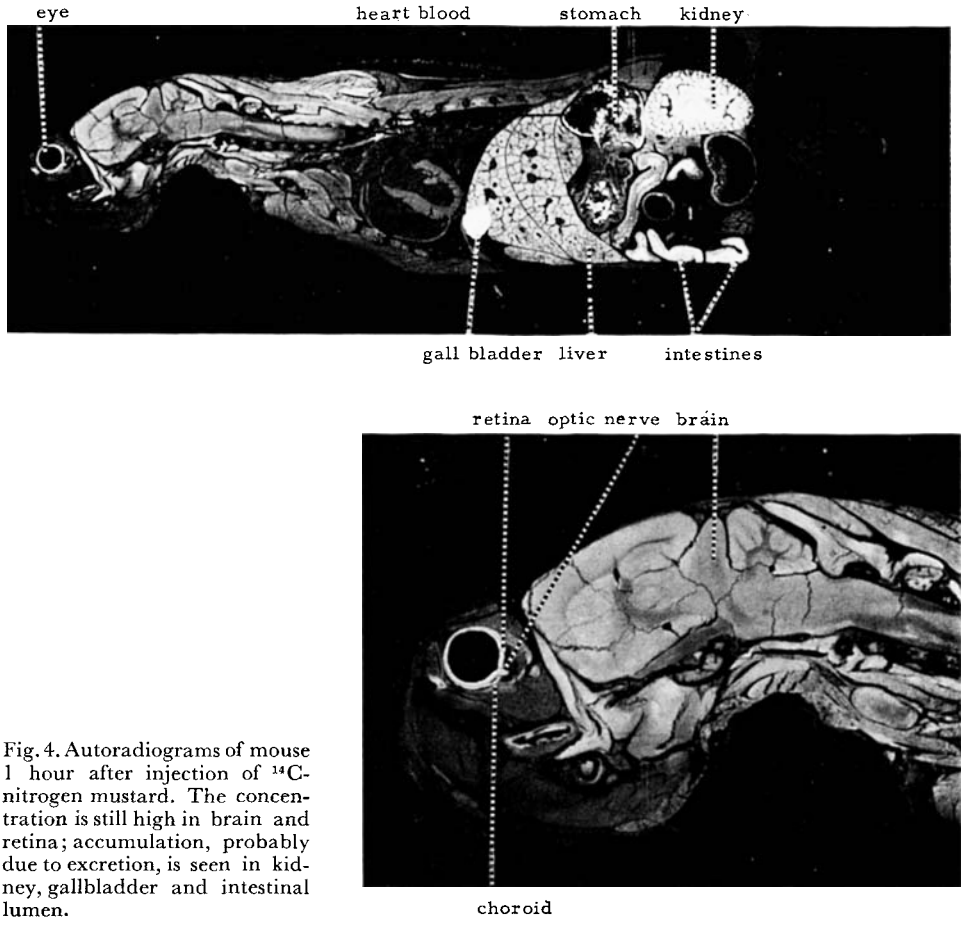


Fig. 4. Autoradiograms of mouse 1 hour after injection of ^{14}C-nitrogen mustard. The concentration is still high in brain and retina; accumulation, probably due to excretion, is seen in kidney, gallbladder and intestinal lumen.

The lung parenchyma did not show any uptake while the bronchi had an intermediate activity. The pleural fluid showed some activity.

The *bone marrow* showed an intermediate isotope concentration.

Endocrine organs. The thyroid showed a relatively high uptake of ^{14}C. Five and ten minutes after the injection, thymus showed a rather high concentration which then declined slightly. The adrenal showed a moderate uptake both in the cortex and medulla. In the testes a very distinct pattern of the tubular walls could be seen. The epididymis showed a still more marked accumulation. The seminal vesicles showed a rather high activity in their glandular parts while the secretion was rather free from ^{14}C (Fig. 1).

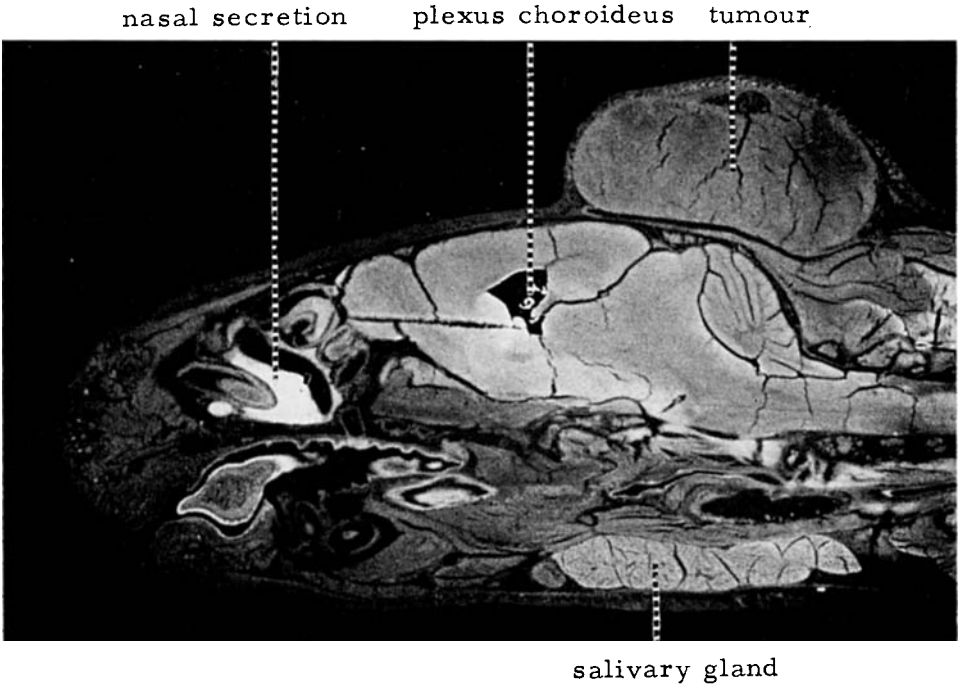


Fig. 5. Autoradiogram of head of mouse 1 hour after injection of ^{14}C-nitrogen mustard. Strong accumulation in nasal mucosa.

Brown fat. A high isotope-concentration could be seen in the brown fat.

Tumour. During all the times studied the transplanted tumour had an uneven, partially high isotope concentration. The areas of high uptake seemed to correspond to tumour tissue with viable cells while the uptake was absent in necrotic parts.

Discussion

When the distribution pattern of the ^{14}C-nitrogen mustard is compared to that of ^{35}S-sulphur mustard previously reported (CLEMEDSON et coll. 1963) it is apparent that the radioactivity left the tissues much more slowly in the nitrogen than in the sulphur mustard experiment.

Another remarkable difference is the much stronger tendency for ^{14}C-nitrogen mustard to accumulate in the central nervous system and also in the choroid and the retina of the eye. The accumulation in the brain may be

related to the neurotoxic symptoms observed after high doses of nitrogen mustard (PHILIPS 1950, STERNBERG et coll. 1958). With respect to the strong but transient deposit in the choroid it can be mentioned that a similar accumulation in the choroid was not observed in the autoradiographic study of sulphur mustard (CLEMEDSON et coll. 1963) but has been found for another tertiary amine and some N-substituted phenothiazines (POTTS 1962). It thus seems as if the affinity to the choroid may be ascribed to the tertiary nitrogen group.

Both the ^{35}S-sulphur and the ^{14}C-nitrogen mustard had a strong tendency to accumulate in the nasal region; the fact that this was apparent already 1 min after injection indicates that the unmetabolized compound has an affinity to some structure in the nasal mucosa. If this is of any practical consequence is not known, as any injurious effects on the nasal mucosa of parenterally administered mustards have apparently not been reported in the literature. This point deserves further attention.

The uptake of ^{14}C-nitrogen mustard in thyroid and in blood vessel walls was remarkable. A specific uptake in the thyroid was noticed also for the ^{35}S-sulphur mustard.

With respect to the cytostatic properties of nitrogen mustard it may be noted that organs with a rapid cell formation such as bone marrow, lymphoid tissues, testicular tubuli and the growing parts of the tumour all showed an intermediate to fairly high concentration. A clear relation between rate of cell formation and affinity to the labelled compound could, however, not be established.

Apparently, the uptake of labelled nitrogen mustard in tumours has not been previously studied but other alkylating agents such as triethylenephosphoramide show a moderate uptake in tumour tissue (SMITH et coll. 1958).

The results from the present investigation on ^{14}C-nitrogen mustard agree to a large extent with the previous findings by SKIPPER et coll. (1951) on the distribution of the compound in mice as studied by determinations of radioactivity in dissected organs. However, certain discrepancies are found for instance with respect to the uptake in brain and in bone marrow. SKIPPER et coll. thus found a low concentration of ^{14}C in the brain, and a very low concentration in bone marrow, whereas in the present work a high uptake was found in brain and a moderate uptake in bone marrow. These discrepancies may be due to the lower dose of nitrogen mustard and the longer observation times (6 and 24 hours) used by SKIPPER and his co-workers. It may be added that the results obtained in the present investigation pertaining to brain and bone marrow are supported by recent work by others. Thus MAHALEY et coll. (1961) found a high uptake of labelled nitrogen mustard in certain regions of the brain in dogs, and MELLETT & WOODS

(1960) have demonstrated by a fluorometric technique a relatively high concentration of unchanged nitrogen mustard in the bone marrow of dogs after intravenous injection of the compound.

Finally it should be pointed out that a considerable fraction of the isotope localized by autoradiography (and by measurements on dissected organs) may be present, not as the parent compound but as metabolites. For a discussion of the metabolism of the nitrogen mustard, see reviews by SMITH et coll. 1958, MANDEL 1959, and BROWN 1963.

SUMMARY

The ^{14}-C-labelled nitrogen mustard, methyl-dichlorethylamine, was given by intravenous injection to mice, and the distribution at various times was studied by whole body autoradiography. An accumulation of the radioactive substance was found in the excretory organs and in the central nervous system, the choroid and retina of the eye, the nasal mucosa, blood vessel walls, the thyroid and the growing parts of transplanted tumours.

ZUSAMMENFASSUNG

Mit ^{14}C gezeichnetes Nitrogen-Mustard, Methyl-dichloräthylamin, wurde Mäusen i.v. injiziert und die Verteilung zu verschiedenen Zeitpunkten mittels Ganzkörperradiographie studiert. Es wurde eine Anhäufung der radioaktiven Substanz in folgenden Organen gefunden: in den Exkretionsorganen, im ZNS, in der Choroidea und Retina des Auges, in der Nasenschleimhaut, den Wänden der Blutgefässe, der Schilddrüse und den wachsenden Teilen von transplantierten Tumoren.

RÉSUMÉ

Une ypérite azoteé marquée au ^{14}C, la méthyl-dichloréthylamine, a été administrée par voie intraveineuse à des souris, et sa distribution a été étudiée à divers moments par autoradiographie de tout le corps. On a observé une accumulation de la substance radioactive dans les organes excréteurs et dans le système nerveux central, la choroïde et la rétine de l'oeil, la muqueuse nasale, les parois des vaisseaux sanguins, la thyroïde et les parties en croissances des tumeurs transplantées.

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