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Reactions of Vinyl Chloride with RNA and DNA of Various Mouse Tissues in vivo

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Abstract. The irreversible binding of ^{14}C-vinyl chloride metabolites to RNA and DNA of mouse brain, lung, liver, kidney, spleen, pancreas, and testes after a single i.p. injection has been studied. Hydrolysates of nucleic acids from selected organs were separated on Aminex A6 for quantitation of alkylation products.

Radioactivity in nucleic acids was registered in all of the studied organs with the exception of brain. RNA from spleen, pancreas and liver, and DNA from spleen and liver contained the highest amounts of radioactivity. In nucleic acids from spleen and pancreas, both organs of high metabolic activity, the entire radioactivity was found metabolically incorporated as C_1-fragments. In RNA of kidney and liver, a large part of the radioactivity was also present as incorporated C_1-fragments, but 3,N^4-ethenocytidine (in kidney) as well as 1,N^6-ethenoadenosine and 1,N^6-ethenoadenine (in kidney and liver) were identified as alkylation products. In liver DNA, incorporation of C_1-fragments was insignificant, indicating different interactions of vinyl chloride metabolites (C_1-fragments and alkylating products) with RNA and DNA. The elution profiles of radioactivity in hydrolysates of liver DNA were dominated by an alkylation product of unknown structure (probably a derivative of deoxyguanosine). Possibly, 1,N^6-ethenodeoxyadenosine and 1,N^6-ethenoadenine were also present in liver DNA.

The results are consistent with the ability of vinyl chloride to act as a multipotent carcinogen by alkylation of DNA in several tissues.

Key words: Alkylation – Irreversible binding – Vinyl chloride – 1,N^6-Ethenoadenosine – 1,N^6-Ethenodeoxyadenosine – 1,N^6-Ethenoadenine – 3,N^4-Ethenocytidine

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Introduction

Exposure to vinyl chloride is strongly associated with the development of hepatic angiosarcoma in man (Monson et al. 1974; Creech and Johnson 1974; Block 1974; Falk et al. 1974; Tabershaw and Gaffey 1974; Lloyd 1975; Waxweiler et al. 1976; Berk et al. 1976; Fox and Collier 1977) and experimental animals (Maltoni 1976; Holmberg et al. 1976; Suzuki 1978; Lee et al. 1978). The animal studies have also established the ability of vinyl chloride to induce tumours at other sites such as the lung, kidney, skin, brain, and mammary glands. In addition to hepatic angiosarcoma, an increased frequency of brain and lung tumours has been suggested to be a result of occupational vinyl chloride exposure (Tabershaw and Gaffey 1974; Monson et al. 1974; Berk et al. 1976; Waxweiler et al. 1976).

Studies of the biotransformation of vinyl chloride point to an activation of vinyl chloride to chloroethylene oxide which rearranges spontaneously to chloroacetaldehyde. Both metabolities are electrophilic and reactive towards glutathione and nucleophilic sites of tissue macromolecules (Hefner et al. 1975; Watanabe et al. 1976; Green and Hathway 1977; Guengerich and Watanabe 1979). In accordance with the "somatic mutation theory" of chemical carcinogenesis, vinyl chloride is mutagenic in several test systems (Drevon and Kuroki 1979; Verburgt and Vogel 1977; Greim et al. 1975; Rannug et al. 1974; Bartsch et al. 1975). In *Salmonella typhimurium,* vinyl chloride (after metabolic activation), chloroethylene oxide and chloroacetaldehyde are mutagenic towards strains indicating base-pair substitutions (Rannug et al. 1974; Bartsch et al. 1975; Rannug et al. 1976; Elmore et al. 1976), which is characteristic of compounds covalently bound to DNA. This is confirmed by the demonstration of the ability of vinyl chloride to bind covalently to RNA and DNA in vitro in the presence of rat liver microsomes, NADPH and oxygen (Kappus et al. 1975; Laib and Ottenwälder 1978), by the reactivity of chloroethylene oxide, chloroacetaldehyde and metabolically activated vinyl chloride towards adenosine (Barbin et al. 1975) and by the reaction of chloroacetaldehyde with adenosine and cytidine (Barrio et al. 1972). Laib and Bolt (1977, 1978) have identified 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine as alkylation products of vinyl chloride in rat liver RNA in vivo. Green and Hathway (1978) have established the presence of 3,N^4-ethenodeoxycytidine in liver DNA of rats exposed to vinyl chloride in the drinking water. In addition, the results of Green and Hathway (1978) suggest the presence of small amounts of 1,N^6-ethenodeoxyadenosine. In vivo, vinyl chloride also alkylates the N-7 position of guanine in DNA with the formation of N-7-(2-oxoethyl)guanine (Osterman-Golkar et al. 1977; Laib et al. 1981).

In view of the multipotent nature of vinyl chloride as a carcinogen, it was considered of interest to investigate the irreversible binding of vinyl chloride in vivo to nucleic acids from several tissues of the mouse and, if possible, to study the nature of the binding.

Materials and Methods

Administration of ^{14}C-Vinyl Chloride to Mice. (1,2-^{14}C)-Vinyl chloride (spec. activity 0.48 mCi/mmol, dissolved in peanut oil) of 99% purity by gas radiochromatography was obtained from New England Nuclear, Dreieichenhain, FRG. Male albino NMRI mice (weight 20 g) were administered 25 µCi ^{14}C-vinyl chloride (3.25 mg, 162.5 mg/kg body weight) by a single intraperitoneal injection.

Preparation of Nucleic Acids. Groups of 7–8 mice were killed at 2, 4, 8, 24, and 48 h after the injection. The livers, kidneys, lungs, brains, testes, spleens, and pancreas were dissected and frozen rapidly on solid carbon dioxide. The pooled organs from each group of mice were stored at −75° C until isolation of r-RNA and DNA by the phenol/cresol extraction procedure of Irving and Veazey (1968). The nucleic acids were dried in a stream of nitrogen and stored at −20° C until enzymatic hydrolysis.

Enzymatic Hydrolysis. RNA was dissolved (4 mg/ml) in 0.1 M ammonium acetate pH 6.7 and DNA was dissolved (4 mg/ml) in 0.1 M ammonium acetate/0.01 M magnesium acetate pH 6.7. The solutions were incubated for 4–6 h at 37° C with 0.1 mg/ml ribonuclease A (E.C. 3.1.4.2.2, bovine pancreas, Sigma Chem. Co., St. Louis, Mo, USA) and deoxyribonuclease I (E.C. 3.1.4.5., bovine pancreas, Sigma Chem. Co., St. Louis, USA) respectively. Phosphodiesterase I (E.C. 3.1.4.1, Crotalus adamanteus venom, Sigma Chem. Co., St. Louis, USA) and acid phosphatase (E.C. 3.1.3.2, potato, Sigma Chem. Co., St. Louis, USA) were added to final concentrations of 0.1 U/ml and 3 U/ml respectively and incubation continued for 48 h. The hydrolysates were stored at −75° C until analysis.

Determination of Radioactivity in RNA and DNA. The radioactivity of the hydrolysates was determined by liquid scintillation counting of 50–500 µl of hydrolysate dissolved in 1 ml of Soluene-350 (Packard Instrument Co., Downers Grove, Ill, USA) after the addition of 10 ml of scintillation fluid (4.9 g PPO and 0.1 g dimethyl-POPOP/l toluene). Quench corrections were made using an external standard. The exact RNA and DNA concentrations of the hydrolysates were determined spectrophotometrically with the orcinol method for RNA (Schneider 1957) and the diphenylamine method for DNA (Burton 1956).

Ion Exchange Chromatography. Reference compounds (UV-markers) for the alkylated RNA nucleosides 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine were obtained from Sigma Chem. Co., St. Louis, USA. Since the corresponding deoxy derivatives were not commercially available they were synthesized by the method of Barrio et al. (1972) from chloroacetaldehyde [synthesized according to Gross (1963)] and deoxycytidine or deoxyadenosine (Sigma Chem. Co., St. Louis, USA) respectively. Mass spectrometry of the products gave a mass spectrum for 3,N^4-etheno-deoxycytidine identical to that reported in the literature (Green and Hathway 1978), whereas the mass spectrum for the deoxyadenosine derivative showed only the presence of 1,N^6-ethenoadenine. Since repeated attempts to synthesize 1,N^6-ethenodeoxyadenosine gave the same result, an acid phosphatase hydrolysate (3 U/ml) of a 10 mM solution of 1,N^6-ethenodeoxyadenosine monophosphate (Sigma Chem. Co., St. Louis, USA) in 0.1 M ammonium acetate/0.01 M magnesium acetate pH 6.7 was used as reference for 1,N^6-ethenodeoxyadenosine. However, chromatography of a sample of this hydrolysate on Aminex A6 (see below) gave two peaks in the chromatogram (see below), which by mass spectrometry were shown to correspond to 1,N^6-ethenodeoxyadenosine and 1,N^6-ethenoadenine. Since it was considered possible that alkylation of adenosine in RNA might produce 1,N^6-ethenoadenosine, which might be transformed to 1,N^6-ethenoadenine in vivo [by analogy with what has been assumed to occur in DNA (Green and Hathway 1978)] or during enzymatic hydrolysis, synthesized 1,N^6-ethenoadenine was added to RNA hydrolysates, prior to chromatography, in addition to 3,N^4-ethenocytidine and 1,N^6-ethenoadenosine. During enzymatic hydrolysis, adenosine and deoxyadenosine were completely converted to adenine.

RNA and DNA samples of reasonably high specific activities available in amounts large enough to enable a study of the nature of the irreversible binding were analyzed after addition of 50–200 µl 10 mM solutions of the reference substances by ion exchange chromatography on Aminex A6.

(Bio-Rad Labs., Richmond, Ca, USA) as described by Laib and Bolt (1978). Due to the large amounts of nucleic acids used for each chromatographic run with a risk of poor separation performance, the column length was extended to 50 cm. The UV-absorbance of the eluate at 254 nm was continuously recorded by an Altex Model 150 Biochemical UV Monitor. Fractions of 3.0 ml were collected at a flow rate of 18 ml per hour. Radioactivity in the fractions was determined by liquid scintillation counting after the addition of 18 ml of Instagel (Packard Instrument Co., Downers Grove, Ill, USA). Quench corrections were made using an external standard. More than 90% of the radioactivity in the samples could be recovered in the eluate. The background varied between 18 and 22 dpm (mean value 20 dpm). Due to the relatively low radioactivity content, samples were counted for extended periods of time to ensure a correct determination of the radioactivity.

Uridine (U), guanosine (G), 3,N^4-ethenocytidine (EC), cytidine (C), and 1,N^6-ethenoadenosine (EA) were eluted at 36, 77, 216, 265, and 295 ml respectively with formate buffer pH 4.35 (Laib and Bolt 1978). Thymidine (T), deoxyguanosine (dG), 3,N^4-ethenodeoxycytidine (EdC), deoxycytidine (dC), and 1,N^6-ethenodeoxyadenosine (EdA) were eluted at 39, 102, 275, 323, and 397 ml with formate buffer pH 4.35. Adenine (Ad) and 1,N^6-ethenoadenine (EAd) were eluted when 63 and 87 ml respectively of formate buffer pH 7.0 (Laib and Bolt 1978) had passed through the column.

Results

Registration of Radioactivity in RNA. Radioactivity from ^{14}C-vinyl chloride could be registered in RNA from all of the studied tissues with the exception of brain (Fig. 1). RNA from spleen, pancreas and liver displayed the highest amounts of radioactivity. The spleen and liver RNA showed maximal levels of radioactivity at 4 h after the administration, with a second maximum for liver RNA at 48 h, whereas RNA from the other organs, with the exception of the testes, showed maximal levels at 8 h after the injection of ^{14}C-vinyl chloride. The

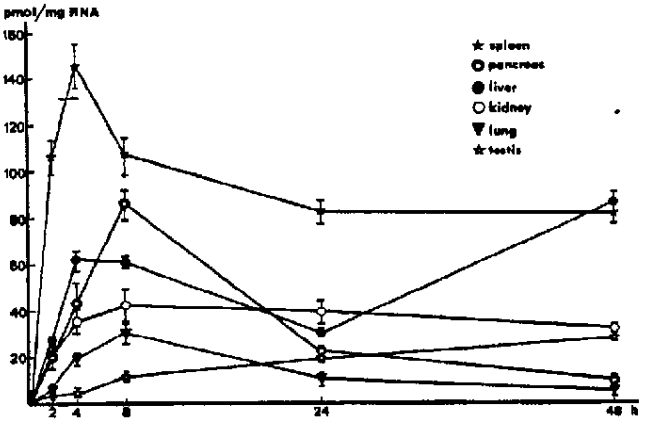

Fig. 1. Radioactivity in RNA (calculated as pmol vinyl chloride bound per mg RNA) of various mouse tissues at different survival times after a single i.p. injection of 25 μCi (3.25 mg) ^{14}C-vinyl chloride. Each value represents the mean of three or four determinations ± SD

radioactivity in testes RNA increased with time throughout the period of study.

Registration of Radioactivity in DNA. Significant amounts of ^{14}C-vinyl chloride radioactivity in DNA could be registered only in the spleen and liver (Fig. 2). No radioactivity was registered in brain DNA. Liquid scintillation counting of DNA samples from kidney, lung, pancreas and testes gave values of 3–9 dpm above the background value (20 dpm) per mg DNA, but the variation between repeated measurements was too large to permit any accurate calculation of the amount of radioactivity. However, it seemed obvious that radioactivity from ^{14}C-vinyl chloride was present in DNA also from these organs.

Ion Exchange Chromatography of RNA Hydrolysates. RNA hydrolysates containing a minimum radioactivity of 200 dpm were analyzed by ion exchange chromatography. Such RNA samples were available from kidney (survival times 8, 24, and 48 h), liver (all survival times), pancreas (survival time 8 h), and spleen (survival times 4 and 8 h).

The radioactivity in all RNA samples from spleen and pancreas was eluted together with the natural nucleosides/bases in RNA (U, G, C, and Ad), indicating that no alkylation of RNA had taken place in these two organs. On chromatography of kidney and liver RNA, a large proportion of the radioactivity in the hydrolysates was also eluted together with natural RNA components. However, in kidney RNA isolated 8 h after the injection of ^{14}C-vinyl chloride, radioactivity was also associated with EC, EA, and EAd (Fig. 3). Twenty-four hours after the administration of ^{14}C-vinyl chloride, radioactivity was still eluted together with EAd, whereas at 48 h all radioactivity could be recovered together with natural RNA nucleosides/bases. The distribution of radioactivity recovered in the eluates on chromatography of kidney RNA between natural RNA components and added reference substances is shown in Table 1. Radioactivity was associated with EA and EAd in liver RNA obtained 24 h after injection of

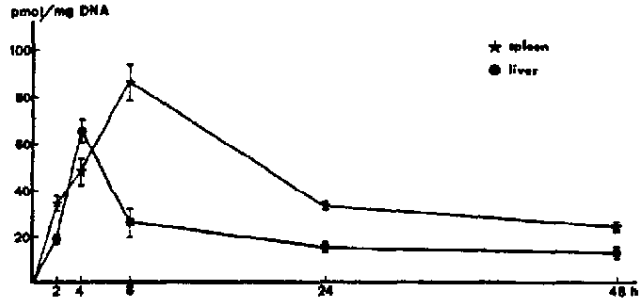

Fig. 2. Radioactivity in DNA (calculated as pmol vinyl chloride bound per mg DNA) from mouse spleen and liver at different survival times after a single i.p. injection of 25 μCi (3.25 mg) ^{14}C-vinyl chloride. Each value represents the mean of three or four determinations ± SD

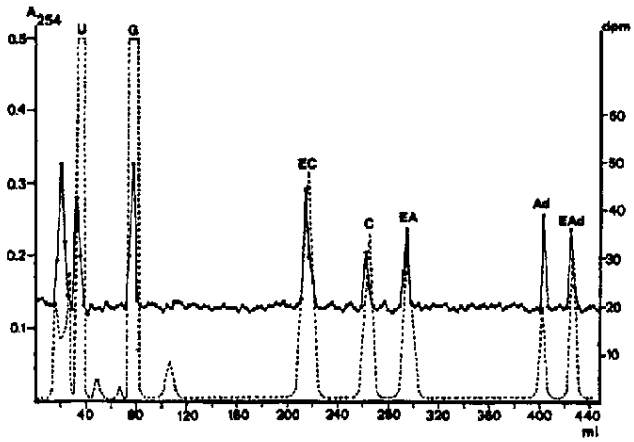

Fig. 3. Aminex A6 ion exchange chromatogram of a hydrolysate of 5.0 mg mouse kidney RNA obtained 8 h after i.p. administration of 25 μCi (3.25 mg) ^{14}C-vinyl chloride. Uridine (U), guanosine (G), cytidine (C), and the added UV-markers 3,N^4-ethenocytidine (EC) and 1,N^6-ethenoadenosine (EA) were eluted with 0.28 M ammonium formate/0.028 M formic acid pH 4.35. Adenine (A) and added 1,N^6-ethenoadenine (EAd) were eluted with 0.4 M ammonium formate/0.04 M formic acid pH 7.0. Absorbance at 254 nm (– – –) was recorded continuously and the radioactivity (●–●) determined in 3.0 ml fractions. The background radioactivity has not been subtracted

Table 1. The distribution of radioactivity in the eluates of Aminex A6 ion exchange chromatograms of hydrolysates of kidney RNA obtained 8, 24, and 48 h after i.p. administration of 25 μCi (3.25 mg) ^{14}C-vinyl chloride to mice

Survival time (h)	Early eluted	% of the recovered radioactivity eluted as						
		U	G	EC	C	EA	Ad	EAd
8	21.9	13.7	12.4	16.7	6.4	9.9	8.2	10.7
24	23.4	31.7	13.1	–	10.3	–	6.9	14.5
48	7.4	18.6	47.9	–	23.3	–	2.8	–

U (uridine); G (guanosine); EC (3,N^4-ethenocytidine); C (cytidine); EA (1,N^6-ethenoadenosine); Ad (adenine); EAd (1,N^6-ethenoadenine)

^{14}C-vinyl chloride (Fig. 4). No radioactivity was found to be eluted together with EC. At all other survival times, all the radioactivity was eluted together with natural RNA components (cf. Table 2). On chromatography of both kidney and liver RNA, relatively large amounts of radioactivity were also eluted in early UV-absorbing fractions corresponding to not completely hydrolyzed RNA fragments (oligonucleotides) (cf. Tables 1 and 2). Early elution of large amounts of radioactivity was not as pronounced on chromatography of spleen and pancreas RNA.

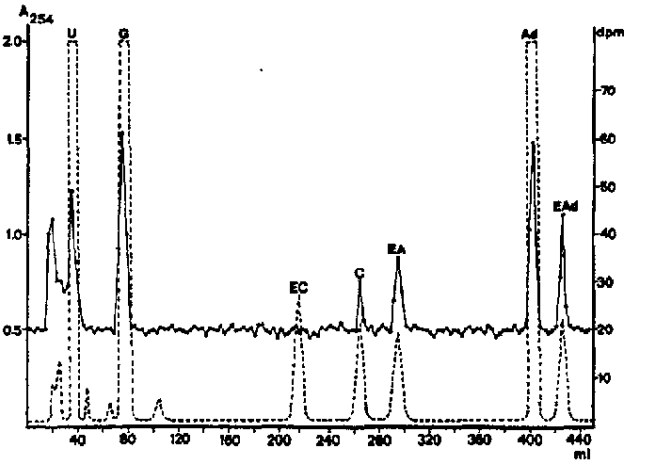

Fig. 4. Ion exchange chromatogram of a hydrolysate of 11.2 mg mouse liver RNA obtained 24 h after i.p. administration of 25 μCi (3.25 mg) ^{14}C-vinyl chloride. For explanation of abbreviations and separation conditions, see legend to Fig. 3. (———) absorbance at 254 nm; (●–●) radioactivity (dpm in each 3.0 ml fraction). The background radioactivity has not been subtracted

Table 2. The distribution of radioactivity in the eluates of Aminex A6 ion exchange chromatograms of hydrolysates of liver RNA obtained 2, 4, 8, 24, and 48 h after i.p. administration of 25 μCi (3.25 mg) ^{14}C-vinyl chloride to mice

Survival time (h)	Early eluted	% of the recovered radioactivity eluted as						
		U	G	EC	C	EA	Ad	EAd
2	10.8	20.1	17.4	–	6.4	–	46.4	–
4	54.9	13.9	12.2	–	–	–	17.9	–
8	47.3	13.6	26.0	–	–	–	13.2	–
24	19.5	16.2	21.1	–	2.7	8.0	20.3	11.0
48	5.4	17.7	34.3	–	–	–	42.5	–

U (uridine); G (guanosine); EC (3,N^4-ethenocytidine); C (cytidine); EA (1,N^6-ethenoadenosine); Ad (adenine); EAd (1,N^6-ethenoadenine)

Ion Exchange Chromatography of DNA Hydrolysates. Hydrolysates of DNA from spleen (survival times 4 and 8 h) and liver (all survival times) were used for ion exchange chromatography.

The radioactivity in spleen DNA was eluted completely together with natural DNA nucleosides/bases. In contrast, elution profiles of hydrolysates of liver DNA were characterized by large amounts of radioactivity eluted in early fractions as well as by a dominant peak of radioactivity eluted at 123 ml with formate buffer pH 4.35. This peak appeared in all chromatograms of liver DNA.

In addition, radioactivity was eluted together with EAd on chromatography of hydrolysates of liver DNA obtained 8 h (Fig. 5) and 24 h after the administration of ^{14}C-vinyl chloride. However, it is possible that at least part of this radioactivity may be due to contamination of DNA with RNA. A small peak of radioactivity (8 dpm above the background value) was also eluted together with EdA on chromatography of liver DNA obtained 8 h after dosing with ^{14}C-vinyl chloride (Fig. 5). Since the optical peak of EdA is distributed over six fractions and the

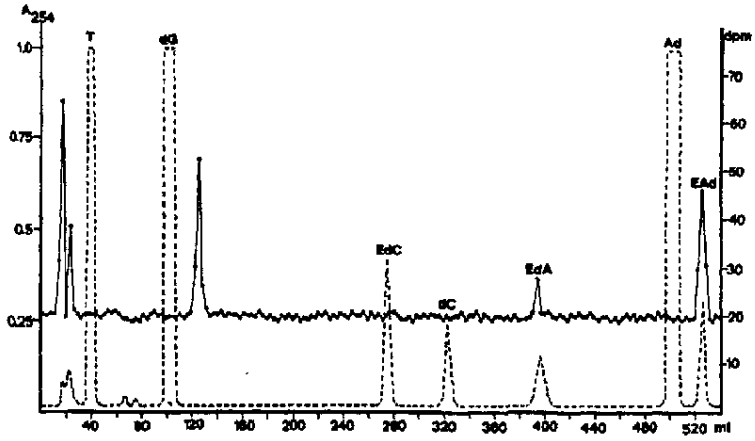

Fig. 5. Aminex A6 ion exchange chromatogram of a hydrolysate of 6.0 mg mouse liver DNA obtained 8 h after i.p. administration of 25 μCi (3.25 mg) ^{14}C-vinyl chloride. Thymidine (T), deoxyguanosine (dG), deoxycytidine (dC), and the added UV-markers 3,N^4-ethenodeoxycytidine (EdC), and 1,N^6-ethenodeoxyadenosine (EdA) were eluted with 0.28 M ammonium formate/0.028 M formic acid pH 4.35. Adenine (Ad) and added 1,N^6-ethenoadenine (EAd) were eluted with 0.4 M ammonium formate/0.04 M formic acid pH 7.0. Absorbance at 254 nm (———) was recorded continuously and the radioactivity (●–●) determined in 3.0 ml fractions. The background radioactivity has not been subtracted

Table 3. The distribution of radioactivity in the eluates of Aminex A6 ion exchange chromatograms of hydrolysates of liver DNA obtained 2, 4, 8, 24, and 48 h after i.p. injection of 25 μCi (3.25 mg) ^{14}C-vinyl chloride to mice

Survival time (h)	Early eluted	% of the recovered radioactivity eluted as							
		T	dG	Unknown	EdC	dC	EdA	Ad	EAd
2	33.3	–	9.9	49.0	–	–	–	7.8	–
4	68.2	–	5.9	20.1	–	3.4	–	2.5	–
8	42.9	–	–	24.7	–	–	4.7	–	27.6
24	4.3	–	–	59.1	–	–	–	19.1	17.4
48	21.4	–	–	78.6	–	–	–	–	–

T (thymidine); dG (deoxyguanosine); EdC (3,N^4-ethenodeoxycytidine); dC (deoxycytidine); EdA (1,N^6-ethenodeoxyadenosine); Ad (adenine); EAd (1,N^6-ethenoadenine)

radioactivity recovered in one fraction only, the radioactivity cannot be definitely associated with EdA. The distribution of radioactivity recovered in the eluates on chromatography of hydrolysates of liver DNA between natural DNA components and added reference substances is shown in Table 3.

Discussion

Experimental animals dosed with ^{14}C-vinyl chloride exhale $^{14}CO_2$ in addition to non-metabolized vinyl chloride (Watanabe et al. 1976; Green and Hathway 1975). Hence, it is obvious that vinyl chloride is partly metabolized via splitting of the C-C bond resulting in the formation of C_1-fragments. These can be used in the tissues for the de novo synthesis of nucleic acid bases, which explains the elution of large amounts of radioactivity together with natural RNA and DNA nucleosides and bases in this study and supports similar observations in other studies of the interaction of vinyl chloride with rat liver RNA in vivo (Laib and Bolt 1977, 1978). This phenomenon complicates the interpretation of the results shown in Figs 1 and 2. The curves of the radioactivity registered in RNA and DNA of various mouse tissues at different intervals after the administration of ^{14}C-vinyl chloride probably reflect to a large extent, particularly in the case of RNA (see below), the metabolic incorporation of C_1-fragments into the nucleic acids rather than the irreversible binding of alkylating vinyl chloride metabolites. The variation in radioactivity content between the organs and with time is most likely due to different rates of RNA and DNA turnover in different tissues. It is thus not surprising that nucleic acids from the spleen and pancreas, two organs characterized by a high metabolic activity, showed the highest specific radioactivities after administration of ^{14}C-vinyl chloride and that the radioactivity could be completely recovered as metabolically incorporated into natural nucleic acid components on chromatography. In contrast, no radioactivity could be registered in nucleic acids isolated from the brain, which is an organ with an extremely slow RNA and DNA turnover. The absence of radioactivity in brain nucleic acids would also argue against a role of vinyl chloride as an initiator of brain tumours, which has been suggested in studies of occupational vinyl chloride exposure (Tabershaw and Gaffey 1974; Monson et al 1974; Berk et al. 1976; Waxweiler et al. 1976). However, the failure to detect radioactivity in brain nucleic acids may also be due to the very low specific radioactivity of the administered ^{14}C-vinyl chloride.

Among the other organs studied in this investigation (liver, kidney, lung, and testes), a proper estimate of the degree of RNA and DNA alkylation versus metabolic incorporation of radioactivity was possible only in kidney RNA and in liver RNA and DNA. It is interesting to note that a comparison between the alkylation/incorporation pattern in liver RNA and DNA (Tables 2 and 3) shows that vinyl chloride metabolites (C_1-fragments and alkylating products) seem to interact in different ways with the two nucleic acids. In liver RNA, metabolic incorporation dominates the elution pattern of radioactivity, whereas in liver DNA the radioactivity is mainly associated with alkylation products. If this difference exists also between RNA and DNA of other organs, the low amounts

of radioactivity registered in DNA from kidney, lung, and testes may reflect alkylation rather than incorporation. In the mouse, vinyl chloride induces both kidney and lung tumours (Maltoni 1976; Holmberg et al. 1976; Suzuki 1978; Lee et al. 1978). In addition to cancer development, alkylation of testicular DNA may affect reproduction. Infante et al. (1976) have observed an increased frequency of spontaneous abortions in wives of vinyl chloride exposed men. However, dominant lethal studies of vinyl chloride in male rats (Short et al. 1977) and mice (Anderson et al. 1976) have been negative. Vinyl chloride alkylates testicular protein (Osterman-Golkar et al. 1977), a finding which has established an alkylating action of vinyl chloride towards macromolecules of the testis.

Although all the radioactivity in RNA isolated from spleen and pancreas and a large part of the radioactivity in liver and kidney RNA was due to the metabolic incorporation of C_1-fragments, it is possible that part of the radioactivity registered in RNA of lung and testes could represent RNA alkylation. Alkylation of RNA, particularly r-RNA, is not considered to be of any major importance for carcinogenesis (Brookes 1977), but its occurrence nevertheless demonstrates the reactivity of vinyl chloride towards nucleic acids in vivo.

The present results confirm the observations of Laib and Bolt (1977, 1978) and Green and Hathway (1978) that vinyl chloride acts as a bifunctional alkylating agent towards (deoxy)adenosine and (deoxy)cytidine in vivo. However, the etheno derivative of cytidine could only be isolated in kidney RNA in contrast to previous studies where EC was identified in liver RNA (Laib and Bolt 1978) and EdC in liver DNA (Green and Hathway 1978) of rats exposed to vinyl chloride. Possibly, the failure to detect EC or EdC in liver nucleic acids in this study could be explained by species differences in alkylation patterns after exposure to vinyl chloride, but it could also depend on the low specific activity of the administered ^{14}C-vinyl chloride, which would seriously decrease the possibilities to detect small amounts of EC or EdC.

The introduction of etheno groups in RNA and DNA bases has been assumed to disturb normal base pairing mechanisms, which may lead to miscoding effects (Zajdela et al. 1980). Recent experiments with chloroacetaldehyde treated DNA-like polymers [poly(dA-dT) and poly(dC-dG)] by Hall et al. (1981) have shown a decreased ability of the polymers to act as templates for DNA polymerase and an increased level of non-complementary nucleotides incorporated during DNA synthesis. Green and Hathway (1978) have interpreted the presence of EAd in liver DNA of rats exposed to vinyl chloride as evidence of increased lability of the glycoside bond in vivo as a result of alkylation. This means a risk of depurination, which constitutes a serious DNA damage since the gaps produced could be filled on replication by various bases resulting in "mispairing". The results of the present study show that EAd is present in RNA (from kidney and liver) and possibly also in DNA (from liver) after administration of vinyl chloride to mice, but it cannot be decided whether EAd was actually formed in vivo or during enzymatic hydrolysis, since the monophosphate of EdA, used as an UV-marker, was transformed into both EdA and EAd during hydrolysis (see Methods above).

Laib and Bolt (1978) have noted that EC persists for a longer period of time than EA in liver RNA of rats after inhalation of vinyl chloride. For this reason, they assigned alkylation of cytidine more importance than alkylation of adenosine for the mutagenic/carcinogenic effects of vinyl chloride. Green and Hathway (1978) found a larger amount of EdC than EdA in liver DNA of rats exposed to vinyl chloride in the drinking water for 2 years. Since EC or EdC could not be identified in this study, with the exception of kidney RNA, these assumptions and observations cannot be supported. Moreover, the EC observed in kidney RNA disappeared relatively rapidly (Table 1). If quantity and persistence are used as criteria of the relative importance of different alkylation products for mutagenicity/carcinogenicity, great interest ought to be focussed on the as yet unidentified alkylation product in liver DNA found in this study. The position of the unidentified peak in the chromatogram (Fig. 5) suggests the presence of an alkylation product of deoxyguanosine. Vinyl chloride has been found to react with deoxyguanosine in vitro (Laib and Ottenwälder 1978) as well as with guanine of mouse liver DNA in vivo with the formation of N-7-(2-oxoethyl)guanine (Osterman-Golkar et al. 1977). Indeed, N-7-(2-oxoethyl)guanine has been identified as the only alkylation product of vinyl chloride in vivo in rat liver DNA (Laib et al. 1981). This is particularly interesting in view of the results of the present study where the elution profiles of liver DNA radioactivity were characterized by 1) a dominating peak of radioactivity probably representing a deoxyguanosine derivative 2) the absence of EdC and 3) the doubtful presence of EdA as well as EAd. N-7 of guanine constitutes a quantitatively important alkylation site in nucleic acids, although its modification by a series of chemicals is considered to be of little importance for carcinogenesis (Singer 1975). However, in spite of the accumulating evidence of a promutagenic effect of the introduction of etheno groups in DNA (Zajdela et al. 1980; Green and Hathway 1978; Hall et al. 1981), alkylation at N-7 in guanine may also have to be considered as important for vinyl chloride induced mutagenesis/carcinogenesis in view of the results of Laib et al. (1981) and the results of this study.

The relatively large amounts of radioactivity eluted early on chromatography of hydrolysates of RNA and DNA from kidney and liver, as compared to hydrolysates of RNA and DNA from spleen and pancreas, may indicate the presence of additional alkylation products in nucleic acids of tissues sensitive to vinyl chloride alkylation.

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