near future an opportunity may be afforded of studying the blubber and milk fats of a seal at various stages during lactation, and also the blubber fat of the calf.

SUMMARY

1. Grey (Atlantic) seal (*Halichoerus grypus*) milk resembles whale milk in that its fat content is of the order of ten times that found in the milks of land animals.

2. The composition of seal milk fat has been investigated: it contains only those acids which occur in its blubber fat.

3. Qualitatively grey seal milk fat resembles that of the blubber oil of the same and of a number of other species, notable differences, however, occurring in the proportions of palmitic and unsaturated acids.

4. Grey seal milk fat is relatively more saturated than that of the blubber oil in contradistinction to that of the blue whale, for which the reverse holds true.

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The Liberation of Chloride Ions from Organic Chloro Compounds by Tissue Extracts

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There have been several reports of the liberation of chloride ions from organic chloro compounds. Liebreich (1869), who introduced chloral hydrate as a hypnotic, stated that this compound was in part excreted as inorganic chloride. This was disputed by von Mering & Musculus (1875), von Mering (1882) and Külz (1882) who found that chloral hydrate was eliminated by man and dogs as urochloalric acid (trichloroethyl- β -glucuronide). Kast (1887) failed to find an increased chloride excretion after administration of chloral hydrate to dogs, although he observed that chloride excretion was considerably increased after chloroform anaesthesia. The increase in man was slight. Willstätter, Straub & Hauptmann (1922) detected only small amounts of urochloalric acid in urine when therapeutic doses of chloral hydrate were given to man or rabbits. They maintained that urochloalric acid was formed when large doses were given, but that chloral was broken down

mainly to inorganic constituents when therapeutic doses were given. The only metabolic product detected in urine after giving trichlorourethane to man, rabbits or dogs was inorganic chloride. Akamatsu & Wasmuth (1923), using rabbits, showed that even after large doses of chloral hydrate no more than half could be accounted for as urochloalric acid. More recently Heppel & Porterfield (1948) claimed the discovery of an enzyme in rat liver, kidney and spleen which liberated halide ions from halogenated aliphatic hydrocarbons.

Since metabolic studies on a number of chloro-compounds were being undertaken in this laboratory it was desirable to confirm the existence of an enzyme such as that claimed by Heppel & Porterfield, especially as these workers did not appear to have considered the possibility of chloride ions being liberated as a result of interaction between chloro compounds and SH compounds. Several instances

of reaction between halogen compounds and SH compounds according to the general scheme

have been reported, e.g. halogenated lachrymators (Dixon, 1950), halogenated acetic acids (Dickens, 1933; Rapkine, 1933; Quastel, 1933; Michaelis & Schubert, 1934), lewisite and 2:3-dimercaptopropanol (Thompson, 1950), formation of benzyl mercapturic acid from benzyl chloride in the rabbit (Stekol, 1939). The work here reported describes a preliminary investigation of the liberation of chloride ions from 29 chloro compounds, and a more detailed study of the effect of SH compounds upon the liberation of chloride ions from three of those compounds.

METHODS

Materials. The chloro compounds were all purchased (British Drug Houses Ltd.). DL-Cysteine hydrochloride and L-cystine (British Drug Houses Ltd. and Light and Co. Ltd.) were used. Glutathione was obtained from Roche Products Ltd. and The Distillers Co. Ltd. A phosphate buffer, pH 6.8 (equal vol. Na_2HPO_4 and NaH_2PO_4 , 0.2M), was used unless otherwise stated. The phosphoric-tungstic acid solution was prepared by dissolving 6 g. $Na_2WO_4 \cdot 2H_2O$ in 1 l. 0.15M- H_3PO_4 .

Extracts. The animal was killed by a sharp blow on the back of the neck and immediately bled. The liver was removed, weighed and ground with acid-washed sand (British Drug Houses Ltd.) and water (1.5 $\times$ the original weight). The resulting brei was centrifuged and the supernatant liquid decanted and used immediately.

Digests. NaCl (55 mg./100 ml.) was dissolved in phosphate buffer to ensure that the concentration of Cl^- in the final digest was not less than 3 mM. Chloro compound, to give the required final concentration (usually 0.024M), was added to a mixture of the buffer (15 ml.) and extract (10 ml.). Three drops of toluene were added as preservative. (Control experiments had shown that, up to 7 hr., toluene had no significant effect upon the liberation of Cl^- .) Chloro compounds insoluble in the mixture were dissolved in ethanol (2.5 ml.) and the volume of buffer reduced to 12.5 ml. As will be seen later, ethanol causes slight inhibition of the liberation of Cl^- from chloral hydrate. The digests were incubated at 37.4° in stoppered flasks. Control solutions were: (a) the digest with the extract replaced by a solution of NaCl (70 mg./100 ml.), and (b) the digest without chloro compound. The first gave a measure of the spontaneous liberation of Cl^- . A control with boiled extract was not used for the reason stated below. The usual concentration of SH compounds was 0.02M and when these were used, control digests without chloro compound were set up to give some indication of the extent of loss of SH-groups due to atmospheric oxidation, since it was impracticable to carry out the digestions, withdrawal of samples and analyses in an atmosphere of N_2 .

Estimation of chloride ions. The method finally adopted was essentially a modification of that described by Van Slyke & Hiller (1946), which was effective with concentrations of Cl^- over 3 mM. Digest (2 ml.) was thoroughly mixed with phosphoric-tungstic solution (5 ml.) and $AgIO_3$ (60 mg.) and centrifuged. The supernatant liquid was

separated, filtered and titrated (2 ml. portions with starch as indicator) against 0.01N- $Na_2S_2O_3$ immediately after addition of 200 mg. NaI. This method gave good results in the absence of tissue extracts, but recovery of added Cl^- tended to be low by up to 5% in the presence of extracts. The complete liberation of one Cl atom from 1 mol. substrate under the above conditions should cause an increase in titre of 8.21 ml. $Na_2S_2O_3$. Calculations have been based upon the assumption that this titre represents 100% liberation of Cl^- . Values significantly greater than 100% thus indicate the liberation of more than one Cl atom/mol. substrate. Under the conditions used, added SH compounds did not interfere, provided that titrations were carried out within 20 min. of the addition of $AgIO_3$. In some experiments the results by this method were confirmed by a polarographic method based upon that of Zimmerman & Layton (1949). For these, trichloroacetic acid solution (5%, w/v) was used in place of the phosphoric-tungstic acid solution.

Estimation of SH content. The method was based on those of King, Baumgartner & Page (1930), Kühnau (1931) and Lucas & King (1932). Digest (2-5 ml. according to SH content) was added to trichloroacetic acid (10%, w/v) to give total vol. 20 ml. The protein precipitated was removed by centrifugation (1 min. at 3000 rev./min.) followed by filtration. Filtrate (10 ml.) was cooled to 0° and mixed with 1 ml. KI (10% w/v) and 3 ml. 0.01N- I_2 . Excess I_2 was titrated with 0.002N- $Na_2S_2O_3$ using starch as indicator. The I_2 consumption was referred to calibration curves from which the SH content was calculated. Calibration curves were prepared from buffered (pH 6.8) NaCl solution of the SH compounds concerned in concentrations up to 0.026M. Glutathione (0.006M) was used for the calibration curve when cystine was added to digests. Confirmation of the presence and disappearance of SH groups was obtained by the nitroprusside test (Hopkins, 1921).

Estimation of urease. The method was essentially that of Marshall (1913), using urease tablets (British Drug Houses Ltd.) as the source of urease.

Estimation of papain. The milk-clotting method of Balls & Hoover (1937) was modified by mixing the enzyme with buffer (pH 4.6) and using fresh cow's milk (10 ml.) in place of the dried-milk solution.

RESULTS

Chloral hydrate and trichloroacetamide

Chloral hydrate (2:2:2-trichloro-1:1-dihydroxy-ethane) and trichloroacetamide were selected as substrates for the initial experiments. The former liberates chloride ions spontaneously, but the latter is stable in solution at pH 6.8 in the absence of liver extract.

Activity of liver extracts of various species. Digests were prepared with extracts of rabbit, guinea pig and rat liver. Owing to the small size, rat-liver extract was made with 2.5 parts water. The digests were incubated for 24 hr., samples being withdrawn at intervals. The results are shown in Table 1, which also shows the extent of the spontaneous liberation of chloride ions from chloral hydrate. Trichloroacetamide digests included ethanol (10%, v/v).

Table 1. *Liberation of chloride ions from chloral hydrate and trichloroacetamide caused by liver extracts*

(Substrates 0.024M, pH 6.8. Results expressed as the percentage of 1 Cl/mol. Ranges are given in parentheses below the average values.)

		Percentage of chloride liberated				
Source of liver extract	No. of samples tested	1 hr.	2 hr.	4 hr.	10 hr.	24 hr.
Chloral hydrate						
Rabbit	19	2.9 (1.0-5.2)	4.7 (2.1-7.3)	7.2 (4.1-9.7)	11.5 (7.2-16.1)	14.9 (9.8-20.7)
Guinea pig	6	2.7 (1.2-4.1)	4.8 (2.3-6.7)	8.0 (4.5-10.1)	13.9 (9.3-16.3)	18.0 (13.0-19.9)
Rat	6	1.1 (0.8-1.4)	1.7 (1.4-2.3)	2.8 (2.3-3.9)	4.9 (3.9-6.9)	6.7 (4.8-9.2)
None	7	0.2 (0.2-0.3)	0.4 (0.3-0.5)	0.8 (0.7-0.9)	1.9 (1.5-2.2)	4.6 (3.5-5.4)
Trichloroacetamide						
Rabbit	6	1.2 (0.9-1.5)	2.4 (1.7-3.0)	4.4 (2.9-5.5)	9.1 (5.2-11.6)	13.1 (7.3-16.8)

Effect of pH. Digests with chloral hydrate were made up with phosphate buffer of pH varying from 6.2 to 8.0. Liberation of chloride ions in the former was small, but an increased liberation at pH 8.0 was largely due to spontaneous decomposition of the chloral hydrate. Results from a typical experiment

with chloral hydrate are shown in Fig. 2 and similar results were obtained with trichloroacetamide in digests including ethanol (10%, v/v). Digests which liberated 8% chloride after 24 hr. with unboiled extract, liberated 6% when the extract had been previously boiled.

Fig. 1. The effect of pH upon the liberation of chloride ions from chloral hydrate (0.024M) by rabbit-liver extract (unbroken lines). Spontaneous liberation of chloride ions in absence of extract shown by broken lines.

are shown in Fig. 1. It is clear that the pH (6.8) selected for routine use is one at which liver extract causes appreciable liberation of chloride ions, whilst the extent of spontaneous decomposition is low.

Effect of boiling. Controls with boiled extracts were not used, since the extracts showed considerable activity even after boiling 1 hr. under reflux. (The coagulum formed on boiling was finely ground before incubation.) The results of a typical experi-

Fig. 2. The effect of boiling (unbroken lines) and of dialysis (broken lines) upon the chloride-liberating activity of rabbit-liver extract. Substrate, chloral hydrate (0.024M) at pH 6.8. Curve A, digest with untreated extract. Curve B, digest with the extract previously boiled 1 hr. Curve C, spontaneous liberation of chloride ions from chloral hydrate. Curve D, digest with another untreated extract. Curve E, digest with this extract previously dialysed 20 hr. against running water.

Effect of dialysis. Extracts were dialysed against running water for 20 hr. and their activity compared with that of the original extract which had been kept at the same temperature. In every case some loss of activity was observed, but the dialysed

extract always retained some activity. The results of an experiment with chloral hydrate are shown in Fig. 2. Similar results were obtained with trichloroacetamide, about half of the chloride-liberating activity of the extract being retained after dialysis.

Effect of ethanol. Since many of the chloro compounds used were sparingly soluble in the absence of ethanol, the effect of the addition of ethanol to chloral hydrate digests was examined. It is clear from Table 2 that the inclusion of ethanol (10% of total vol.) caused some inhibition of the

Table 2. *Liberation of chloride ions from chloral hydrate caused by rabbit-liver extracts with and without the addition of ethanol*

(Chloral hydrate 0.024M, ethanol 10% of total volume, pH 6.8. Results expressed as the percentage of 1 Cl/mol. Ranges are given in parentheses below the average values.)

Digest	No. of samples tested	Percentage of chloride liberated				
		1 hr.	2 hr.	4 hr.	10 hr.	24 hr.
Extract + chloral hydrate	7	3.1 (2.1-4.1)	5.1 (3.8-6.1)	8.1 (6.3-9.2)	13.2 (9.9-16.1)	17.3 (13.6-20.7)
Extract + chloral hydrate + ethanol	8	2.6 (1.1-4.2)	4.2 (2.1-6.5)	6.5 (3.7-9.4)	10.2 (6.3-14.1)	13.0 (9.4-17.9)
Chloral hydrate	7	0.2 (0.2-0.3)	0.4 (0.3-0.5)	0.8 (0.7-0.9)	1.9 (1.5-2.2)	4.6 (3.5-5.4)
Chloral hydrate + ethanol	4	0.2 (all 0.2)	0.4 (0.3-0.5)	0.8 (0.7-0.9)	1.9 (1.7-2.1)	4.6 (4.0-5.0)

Table 3. *Liberation of chloride ions from various chloro compounds by rabbit-liver extracts*

(Substrates normally 0.024M in 10% ethanol, pH 6.8. Results expressed as a percentage of 1 Cl/mol.)

Substrate	Percentage of chloride liberated									
	With extract					Without extract				
	1 hr.	2 hr.	4 hr.	10 hr.	24 hr.	1 hr.	2 hr.	4 hr.	10 hr.	24 hr.
Chloroform	0.6	1.1	2.0	3.9	6.4	0.0	0.1	0.2	0.4	1.2
Benzyl chloride	9.3	14.8	22.0	35.1	52.8	4.6	8.4	14.3	26.2	46.9
Benzotrichloride	23.5	38.1	62.5	120.2	193.1	28.7	46.9	71.9	108.0	159.8
Ethylene dichloride (1:2-Dichloroethane)*	0.0	0.0	0.1	0.3	0.8			None		
1:1:2:2-Tetrachlorethane	0.7	1.3	2.4	5.4	12.6	0.2	0.4	0.8	2.1	5.2
Pentachloroethane	2.0	3.7	6.7	15.1	31.9	1.1	2.1	3.8	7.1	13.0
Hexachloroethane	2.3	4.1	7.0	13.5	23.3			None		
Trichloroethylene	0.0	0.1	0.2	0.5	1.1			None		
Ethylene chlorohydrin (2-Chloroethanol)†	0.3	0.5	0.8	1.4	2.3	0.0	0.1	0.2	0.5	1.2
Trichloroacetic acid‡	1.1	1.8	3.0	4.9	6.9			None		
Trichloroacetamide	1.4	2.8	5.3	11.0	16.8			None		
Chloral hydrate†	4.1	5.9	8.1	12.3	15.0	0.2	0.4	0.8	1.9	4.6
Chloralose	1.1	2.2	4.1	7.9	11.3			None		
ω -Chloroacetophenone	4.5	7.5	13.0	25.1	43.2	0.1	0.2	0.4	1.1	2.5
1:2-Dichloropropane	0.2	0.4	0.6	1.3	2.0	0.0	0.1	0.2	0.5	1.1
1-Chloropropionic acid‡	1.1	1.9	2.9	4.6	6.2	0.1	0.2	0.4	0.9	2.1
2-Chloropropionic acid‡	2.3	3.7	5.6	9.0	11.6	0.4	0.8	1.6	4.1	9.6
1:3-Dichloroacetone†	40.8	52.7	70.0	101.5	127.8	9.9	19.7	40.0	85.6	130.1
Chloretone	0.9	1.6	2.8	6.2	13.3	0.1	0.2	0.4	0.9	2.3
Butyl chloral hydrate	13.1	25.3	36.1	45.9	49.6	0.2	0.4	0.7	1.9	4.5
iso-Amyl chloride§ (1-Chloro-3-methylbutane)	0.1	0.2	0.2	0.3	0.4			None		
Chlorobenzene	0.5	0.6	0.9	1.4	1.9			None		
p-Dichlorobenzene	0.9	1.4	1.9	2.3	2.8			None		
o-Chlorobenzoic acid‡	0.4	0.6	0.8	1.3	1.5			None		
m-Chlorobenzoic acid‡	1.1	1.9	2.5	3.1	3.3			None		
p-Chlorobenzoic acid‡	0.8	1.3	1.7	2.0	2.1			None		
o-Chlorobenzamide	0.3	0.5	0.9	1.7	2.7			None		
m-Chlorobenzamide	0.1	0.2	0.3	0.8	1.6			None		
p-Chlorobenzamide	0.3	0.5	0.9	1.7	2.8			None		

* 0.036M.

† Substrate made up with 1 equiv. NaHCO_3 .

‡ Digests did not contain ethanol

§ 0.072M.

liberation of chloride ions, although it had no effect upon the spontaneous decomposition of chloral hydrate.

Other chloro compounds

Rabbit-liver extract was used in the examination of twenty-seven other chloro compounds. In every experiment a digest with chloral hydrate was used to confirm the activity of the liver extract. Typical results for each of the twenty-nine compounds used

hydrate upon guinea pigs and rats. Six animals were given thirteen injections of chloral hydrate (25 mg.) subcutaneously on alternate days. The animals were then killed and the activity of the livers compared with those of six animals kept as controls. The activity of the livers of the treated animals was slightly less than those of the control animals. In no experiment was increased activity observed. A typical experiment with guinea pigs (350–450 g. body weight) is shown in Table 4.

Table 4. *Effect of previous injection of guinea pigs with chloral hydrate upon the liberation of chloride ions from chloral hydrate caused by extracts of their livers*

(Substrates 0.024M, pH 6.8. Results expressed as percentage of 1 Cl/mol. Ranges are given in parentheses below the average values. There were six animals in each group.)

Source of liver extract	Percentage of chloride liberated				
	1 hr.	2 hr.	4 hr.	10 hr.	24 hr.
Injected animal	2.6 (1.4–4.4)	4.2 (2.6–6.1)	6.5 (4.6–8.7)	11.0 (8.5–13.8)	15.3 (11.7–18.6)
Untreated animals	2.7 (1.2–4.1)	4.8 (2.3–6.7)	8.0 (4.5–10.1)	13.9 (9.3–16.3)	18.0 (13.0–19.9)

are given in Table 3. It is clear that the extent of liberation of chloride ions by liver extract is not directly related to the ease of spontaneous liberation of chloride ions from a compound. Hexachloroethane, trichloroacetamide and chloralose (1-(2':2':2'-trichloro-1'-hydroxyethyl)-1:6-anhydroglucopyranose), yield a considerable percentage of their chlorine as ions with tissue extract but none spontaneously. Some compounds, butylchloral hydrate (2:2:3-trichloro-1:1-dihydroxybutane), ω -chloroacetophenone, chloral hydrate and chloretone (1:1:1-trichloro-2-hydroxy-2-methylpropane), yield small amounts of chloride ions spontaneously but much more in the presence of tissue extract. Other compounds which readily liberate considerable amounts of chloride spontaneously, benzyl chloride, benzotrichloride (ω -trichlorotoluene), and pentachloroethane, liberate still more with tissue extract. 1:3-Dichloroacetone, which liberates more than half its chlorine as ions spontaneously, does not liberate any more after 24 hr. in the presence of liver extract, although the extract accelerates the initial liberation of chloride ions. Several compounds, including those in which chlorine is directly attached to the benzene ring, liberate no significant amount of chloride either spontaneously or with liver extract.

Activity of liver extracts after repeated injection of chloral hydrate

Heppel & Porterfield (1948) observed that the livers of rats which had been repeatedly exposed to an atmosphere containing bromochloromethane caused a greater increase in liberation of bromide ions than the livers of untreated animals. Experiments with this object were carried out with chloral

Experiments with SH compounds

When chloral hydrate is incubated with liver extract there is in addition to the liberation of chloride ions, a loss in the SH content of the digest. This can be seen from the results recorded in Table 5 which gives the liberation of chloride ions and the loss of SH in m-equiv./l. digest. While the differences between the chloride values give the increased liberation of chloride ions due to reaction of chloral hydrate with extract, the differences between SH values are unlikely to give a measure of the SH compounds combining with chloral hydrate since the reaction with chloral is obviously very rapid compared with the reactions (e.g. atmospheric oxidation) causing disappearance of SH from extracts in the absence of chloral hydrate. For example 40 % of the total SH is lost in the first hour when chloral hydrate is present, but there is no detectable loss in the absence of chloral hydrate. It should also be remembered that the method for SH estimation determines only SH soluble in trichloroacetic acid and that any SH groups bound to protein would be precipitated and removed before titration. The results do, however, indicate that the liberation of chloride ions involves a loss in the SH content of the extract.

The effect of the addition of cysteine. It can be seen from the results of a typical experiment in Fig. 3 that cysteine in the absence of liver extract causes liberation of chloride ions and that if liver extract is also present the chloride liberation is much greater than when either extract or cysteine alone is present. Table 6 summarizes the results of some experiments with chloral hydrate and cysteine with

Table 5. *Average liberation of chloride ions from chloral hydrate and the average loss in SH content of rabbit-liver extract when incubated together*

(Chloral hydrate 0.024M, pH 6.8, 37°. Results expressed in m-equiv./l. digest and ranges given in parentheses.)

Digest	No. of samples tested		1 hr.	2 hr.	4 hr.	10 hr.	Initial SH content
Extract + chloral hydrate	16	Cl ⁻ liberated	0.9 (0.5-1.1)	1.4 (0.9-1.8)	2.1 (1.3-2.5)	3.2 (2.3-3.6)	—
Chloral hydrate alone	4	Cl ⁻ liberated	0.0 —	0.1 (0.1)	0.2 (0.1-0.3)	0.5 (0.4-0.7)	—
		Difference	0.9	1.3	1.9	2.7	—
Extract + chloral hydrate	8	SH lost	0.9 (0.1-1.4)	1.2 (0.2-1.8)	1.5 (0.5-2.2)	1.7 (0.9-2.5)	2.3 (1.3-2.9)
Extract alone	8	SH lost	0.0 (+0.4*-0.3)	0.2 (+0.4*-0.5)	0.6 (0.1-0.9)	1.2 (0.6-1.8)	2.3 (1.3-2.9)

* + indicates a gain in SH content.

Fig. 3. The effect of cysteine (0.02M) upon the liberation of chloride ions from chloral hydrate (0.024M) by rabbit-liver extract at pH 6.8. Curve A, digest with chloral hydrate, extract and cysteine. Curve B, digest with chloral hydrate and cysteine. Curve C, digest with chloral hydrate and extract. Curve D, spontaneous liberation of chloride ions from chloral hydrate at pH 6.8.

Table 6. *Average liberation of chloride ions from chloral hydrate and average loss in SH content of cysteine when incubated with and without rabbit-liver extract*

(Chloral hydrate 0.024M, cysteine 0.02M, pH 6.8 at 37°. Results expressed in m-equiv./l. digest and ranges given in parentheses.)

Digest	No. of samples tested		1 hr.	2 hr.	4 hr.	10 hr.	Initial SH content
Cysteine + chloral hydrate	6	Cl ⁻ liberated	0.6 (0.5-0.8)	1.2 (0.9-1.4)	2.2 (1.7-2.5)	4.7 (3.7-5.0)	—
Chloral hydrate alone	4	Cl ⁻ liberated	0.0	0.1 (0.1)	0.2 (0.1-0.3)	0.5 (0.4-0.7)	—
		Difference	0.6	1.1	2.0	4.2	—
Cysteine + chloral hydrate	4	SH lost	1.1 (0.4-1.6)	1.7 (1.0-2.4)	3.4 (2.5-4.0)	8.6 (8.5-9.1)	20.3 (18.9-22.5)
Cysteine alone	4	SH lost	0.5 (0.2-0.6)	1.0 (0.7-1.2)	2.1 (1.6-2.4)	5.5 (4.9-6.2)	20.2 (18.9-22.5)
		Difference	0.5	0.7	1.3	3.1	—
Extract + cysteine + chloral hydrate	3	Cl ⁻ liberated	3.2 (2.8-3.7)	5.8 (5.0-6.3)	9.2 (7.9-10.0)	13.6 (12.6-14.8)	—
		Difference*	3.2	5.7	9.0	13.1	—
Extract + cysteine + chloral hydrate	3	SH lost	3.9 (2.5-4.6)	6.4 (4.5-7.5)	10.0 (7.6-11.8)	15.2 (13.7-16.7)	20.5 (19.6-21.0)
Extract + cysteine	3	SH lost	1.0 (0.7-1.4)	1.9 (1.4-2.7)	3.6 (2.9-4.6)	8.3 (7.6-9.5)	20.5 (19.6-21.0)
		Difference	2.9	4.5	6.4	6.9	—

* By subtracting 'chloral hydrate alone' values (see text).

and without liver extract in which both chloride liberation and SH loss have been determined. Again it is seen that the liberation of chloride ions involves a loss of SH. For the experiments in the presence of extract the values for chloride liberated, corrected for spontaneous liberation of chloride ions (i.e. by subtraction of the 'chloral hydrate alone' values), represent the liberation of chloride due to extract + cysteine. By subtracting the SH lost for 'extract + cysteine' from that for 'extract + cysteine + chloral hydrate', values should be obtained which represent the SH loss from extract + cysteine concerned in the liberation of chloride ions from chloral hydrate. This difference, therefore, should be comparable with the corrected values for chloride liberation. The assumption that the loss of SH due to oxidation (or other unknown reactions) is the same in the presence or absence of chloral hydrate is not, however, justified since oxidation would almost certainly be greater in the absence of chloral when there is no competition with the chloride-SH reaction. The difference values would thus be expected to be low, and it may reasonably be concluded that both with and without liver extract there is a loss of SH corresponding fairly closely with the liberation of chloride ions.

Chloral hydrate and other SH compounds. Experiments similar to those described above were carried out with other SH compounds. From these only the average values (from at least three experiments) corresponding to the 'differences' in Table 6 have been recorded in Table 7 and only 4 hr. values are given. The loss of SH with glutathione and extract was greater than that expected from the chloride ions liberated. With thioglycollate and extract the SH loss was also somewhat greater than the chloride liberation, although there was no loss of SH when chloride ions were liberated in the absence of extract.

Other chloro compounds with cysteine. With butyl chloral hydrate in the presence of extract the loss in SH was approximately equivalent to chloride liberated, but in the absence of extract the SH loss was greater than that equivalent to the chloride liberated (Table 7). With trichloroacetamide, both with and without extract, the SH loss was much less

than would have been expected from the amount of chloride liberated.

Chloral hydrate and cystine. In experiments in which cystine (0.02M) was added to liver extracts with chloral hydrate there was a significant increase in chloride liberation (1.1 m-equiv./l. digest in 4 hr.) over that obtained from extract and chloral hydrate alone (2.1 m-equiv./l.). There was no increase in the absence of extract. When cystine was added to extract without chloral hydrate it was found that there was almost always an increase in SH content (average 0.3 m-equiv./l. in 4 hr.). This increase may explain the effect of cystine upon the activity of liver extract. In the presence of chloral hydrate there was a loss in SH corresponding to 0.7 m-equiv./l.

Fig. 4. The effect of previous boiling (1 hr.) upon the SH content of rabbit-liver extract incubated with and without chloral hydrate (0.024M) at pH 6.8. Each curve represents average results from three experiments. Curve A, untreated extract. Curve B, boiled extract. Curve C, boiled extract with chloral hydrate. Curve D, untreated extract with chloral hydrate.

Effect of boiled liver extract. It has already been stated that considerable liberation of chloride ions from chloral hydrate occurs in the presence of boiled extract. The average results for utilization of SH from three experiments are summarized in Fig. 4.

Table 7. Average liberation of chloride ions from chloro compounds and the corresponding loss in SH content due to incubation with SH compounds with and without rabbit-liver extract

(Chloro compound 0.024M, SH compound 0.02M, pH 6.8, 37°, 4 hr. incubation. Results expressed as m-equiv./l.)

Substrates	Without liver extract		With liver extract	
	Cl ⁻ liberated	SH loss	Cl ⁻ liberated	SH loss
Chloral hydrate with:				
Cysteine	2.0	1.3	9.0	6.4
Glutathione	1.8	1.7	4.5	6.5
Thioglycollate	3.1	0.0	4.0	4.6
Butyl chloral hydrate with cysteine	1.3	1.7	7.3	6.4
Trichloroacetamide with cysteine	2.5	0.5	1.8	0.5

Boiling causes a reduction in the SH content of the extract, but even with boiled extract there is a more rapid disappearance of SH with chloral hydrate than with boiled extract alone.

Effect of chloral hydrate upon the activity of urease. Urease is an SH enzyme and should be inhibited by a substance such as chloral hydrate which reacts with SH groups. Urease was incubated with chloral hydrate for periods up to 3 hr. prior to determination of the urease activity of the digest. The results recorded in Table 8 show that treatment with chloral hydrate causes considerable inhibition of urease.

chloride ions by tissue extracts. These compounds are either aliphatic, or the chlorine is attached to an aliphatic side chain. In no instance was there appreciable liberation of chloride ions from nuclear-substituted aromatic compounds. It is far from certain, however, that the liberation of chloride from the aliphatic compounds is due to an enzyme such as that claimed by Heppel & Porterfield (1948) to liberate halide from some halogenated hydrocarbons. The greater part of the factor responsible is remarkably stable to heat. Only part of the activity of a liver extract is retained after dialysis. It has been shown that chloride ions can readily be liber-

Table 8. *Effect of preliminary incubation with chloral hydrate upon the activity of urease*

(Results expressed as percentage conversion of urea, 0.9 g./l.)

Exp. no.	Time of preliminary incubation (hr.)	Concn. of chloral hydrate (M)	Percentage of urea converted				
			0.5 hr.	1 hr.	1.5 hr.	2 hr.	2.5 hr.
1	0	0	44	72	86	91	91
		0.024	31	41	57	63	66
2	0	0	41	69	85	91	93
		0.096	23	30	33	34	34
3	1	0	40	69	83	—	92
		0.096	11	16	19	—	20
4	2	0	30	52	67	—	82
		0.096	5	7	8	—	9
5	3	0	26	43	54	61	67
		0.096	3	5	6	7	7

Table 9. *Effect of preliminary incubation for 30 min. with chloro compounds upon the clotting power of papain*

Exp. no.	Chloro-compound	Amount of papain (mg./10 ml. milk)						
		20	24	28	30	32	36	40
		Reciprocal of clotting time (min. ⁻¹)						
1	None	0.8	0.9	1.0	1.2	1.5	1.7	—
	Chloral hydrate (0.02M)	0.5	0.7	0.7	0.9	1.0	1.2	—
2	None	—	1.0	1.5	1.6	1.7	2.1	2.2
	Chloral hydrate (0.04M)	—	0.7	0.8	0.9	1.1	1.3	1.5
3	None	—	0.8	0.9	1.2	1.3	1.6	1.8
	Butyl chloral hydrate (0.01M)	—	0.8	0.8	0.9	0.9	1.2	1.5

Effect of chloral hydrate upon the activity of papain. Another SH enzyme, papain, was incubated with chloro-compound for 30 min. previous to determination of activity in clotting of milk. As with urease there was considerable loss of activity. Typical results are shown in Table 9 which records the reciprocal of the time in minutes taken to clot milk using varying amounts of papain.

DISCUSSION

It is clear from the experiments described that a considerable percentage of the chlorine of certain organic chloro compounds may be liberated as

ated from chloral hydrate in the absence of liver extract if cysteine is present, and that in the presence of liver extract the addition of cysteine enhances the liberation of chloride ions. The experimental results suggest a quantitative relationship between the chloral hydrate and the SH groups of cysteine and/or liver extract, although such a relationship was not always clear when other chloro or SH compounds were used. It must, however, be remembered that the method of estimation of SH content did not permit the estimation of SH groups bound to protein, and that SH groups may also be used up in reactions, other than atmospheric oxidation, not involving the chlorine atoms, e.g. by condensation

between SH and aldehyde group (cf. Schubert, 1936). Such reactions could account for a loss in the SH content of the digest greater than that expected from the chloride ions liberated, e.g. from chloral hydrate with glutathione or thioglycolic acid (Table 7). It seems probable that the action of liver extract in liberating chloride from chloro compounds is for the most part not enzymic but due to chemical reaction between the chloro compound and SH compounds in the liver extract. If there is a chloride-liberating enzyme it can only play a very small part in the reactions which have been studied. The fact that the addition of the disulphide cystine causes some enhancement of liberation of chloride ions in the presence of liver extract suggests that the extract can reduce disulphide compounds and so enable them to react with chloro compounds.

SUMMARY

1. The effect of incubation with tissue extracts upon the liberation of chloride ions from twenty-nine organic chloro compounds has been examined.

2. Some compounds, including those in which the chlorine is directly attached to the benzene ring, do not liberate appreciable amounts of chloride. Some

aliphatic chloro compounds which liberate little chloride, e.g. chloral hydrate, butyl chloral hydrate, or none, e.g. trichloroacetamide, hexachloroethane, spontaneously in aqueous solution, liberate considerable amounts in the presence of liver extract; others which liberate considerable amounts of chloride spontaneously, e.g. benzotrichloride, benzyl chloride, liberate even more in the presence of liver extract.

3. The loss in SH content of the extract always observed when chloride ions are liberated suggests that the reaction is largely one between the chlorine of the chloro compound and the SH of SH compounds.

4. The liberation of chloride ions is enhanced by the addition of SH compounds both in the presence and absence of liver extracts.

5. Previous boiling does not greatly diminish the chloride-liberating activity of liver extracts.

6. It is concluded that the liberation of chloride ions in the presence of liver extract is mainly a reaction between chloro compound and SH compounds which is not enzymic.

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