Much more information on the nature of action of substances of this kind is required before a proper indication to treatment can be laid down.

Prevention

The usual formulae as to prevention of toxic events apply here, viz. that leaks, overflows, and escape of vapour should be avoided: good ventilation, draughting specially hazardous points, regular medical examination of workers, report by workers of untoward symptoms, detection of susceptible subjects, routine estimation of concentration of vapours at working places are all required. The main problem, as always, is design of plant. The necessity for periodic sampling from reaction vessels requires arrangements for sampling which do not involve the opening of points where escape of fume is inevitable.

The fact that ethylene chlorohydrin appears to be a cumulative poison renders it of the first importance to prevent even low concentrations in the atmosphere: we take the view that no concentration that is chemically determinable should be regarded as satisfactory. Table 1 shows the improvements obtained on one plant when the insidious nature of the poison was recognized as a result of experience in other similar places.

The improvement in the concentrations of ethylene chlorohydrin was very striking and resulted from simple plant measures and not from significant improvement in ventilation (in present circumstances the latter is seldom possible). The target set for ethylene chlorohydrin in the atmosphere is 2 p.p.m. but the attainment of this is likely to be very difficult.

This improvement has been followed by much diminution in the incidence of signs and symptoms among workers; but it is necessary to realize that where ethylene chlorohydrin is a toxic risk, determination of concentrations in the atmosphere must be continued as a routine as long as the process is continued.

Part II.—EXPERIMENTAL

BY

M. W. GOLDBLATT

The cases first recorded by Koelsch (1927) undoubtedly occurred because of the lack of knowledge of the toxic properties of ethylene chlorohydrin, which could have been realized by animal experiment. The very fact that the plant in which it occurred (probably as an intermediate in the manufacture of ethylene glycol) was so efficiently enclosed that no cases had been observed for 20 years, gave a false security which was dispelled only when clinical cases were met with during the utilization of the compound in open conditions. Whether these cases were indeed entirely due to the chlorohydrin it is difficult to say, for admixture with ethylene dichloride and even dichlor-diethyl ether seems

Summary

1. Two cases of death attributed to poisoning by inhalation of ethylene chlorohydrin vapour are described. One case was very acute in its course, the other sub-acute. The causes of death were not clear, but the associated symptoms and some histological evidence suggest that the compound is a violent cerebral and vascular poison. Pathological changes in the lungs, kidneys, and liver were marked.

2. Nine cases of non-fatal poisoning due to inhalation of the vapour are shortly described. The symptoms noted were non-specific and associated with all the main physiological systems in greater or less degree. Indications were obtained that men of poor physical standard are more readily affected than good specimens: women are thought to be more susceptible than men.

3. Some evidence was obtained that the substance is a cumulative poison.

4. It is held that in all processes in which ethylene chlorohydrin is used or appears as an intermediate stage in manufacture, every possible measure must be taken to control the concentration of the vapour in the atmosphere; since no concentration of the compound is considered safe if exposure is a daily occurrence. The diminution in the incidence of cases is soon manifest if even simple precautions are taken.

5. Treatment is purely symptomatic in severe cases. Mild cases in general soon recover but the likelihood of repeated attacks is always to be kept in mind. Some degree of personal idiosyncrasy is suspected.

Our thanks are due to Dr. Charles Cresdee for certain of the clinical data.

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highly probable, since the preparation of pure ethylene chlorohydrin requires considerable care and would probably not be worth doing for crude industrial purposes.

Our cases (see Part I) were certainly exposed to all three compounds, but the amount of the dichlor-diethyl ether was extremely small. We, also, were in a state of false security because of the absence of unfavourable events throughout years of manufacture.

As has already been stated, the relative parts played by the chlorohydrin and the dichloride in the production of the symptoms cannot be stated with certainty.

Toxic effects of ethylene dichloride appear to be very rare, but Browning (1937) refers to a case (reported to the Home Office in 1932) of a worker who, fitting a coil in a glycol plant, complained of vomiting, diarrhoea, giddiness, and drowsiness. We imagine that the attribution of this case only to ethylene dichloride was not justified and that ethylene chlorohydrin was also involved.

Flury and Zernik (1931) state that ethylene dichloride is a narcotic with mild local irritant properties, and that in man inhalation of the vapour produces a burning sensation in the throat, cough, and later vomiting, and go on to say that more severe symptoms than these had not at that time been reported.

For the purpose of experimental study, we have regarded ethylene chlorohydrin as the substance calling for attention and the following experiments deal mainly with it. The closest chemical relatives of ethylene chlorohydrin are ethylene glycol, ethyl and ethylene dichloride (sym-dichloroethane) and ethyl chloride.

In an investigation carried out many years ago, Marshall and Heath (1897) studied the physiological properties of a series of compounds similar to these, viz.,

They worked with a view to elucidating the effects of the introduction of the chlorine atoms into the molecules, and found that whereas the trichloro compound was a powerful hypnotic, the monochloro

compound was hardly narcotic at all. All the chlorinated derivatives produced a fall in temperature, slowing respiration and pulse-rate in rabbits. Administration by the mouth of 1 g. per kg. was more or less rapidly fatal in all cases. Special reference was made to the fact that with the monochloro compounds there was a great delay in the appearance of nervous symptoms in spite of its great solubility. Intravenous injection into anaesthetised rabbits led to a transient fall in blood-pressure, the depressor action increasing with the number of chlorine atoms. The authors held that this effect was in part due to vasodilation rather than to action on the heart muscle. Vasodilation was demonstrated by perfusion of sheep's kidney through the renal artery, and measurement of minute volume delivered from the renal vein. Perfusion with a concentration of 1 in 1,000 produced vasoconstriction in all cases, but at 1 in 5,000 vasodilation was evident and most marked with the trichloro compound.

Perfusion of the isolated frog's heart with the monochloro at 1 in 1,000 had no effect, and at 1 in 100 there was only slight diminution of systole; the dichloro had a more marked effect of the same kind, and the trichloro at 1 in 1,000 depressed the heart and markedly diminished systole, but diastole was complete. In all cases normal activity was re-established on washing the heart through with the normal perfusion fluid.

Toxic effects on voluntary muscle (frog's gastrocnemius) were readily demonstrated and increased with increasing numbers of chlorine atoms.

The authors concluded that these chlorinated compounds act as direct poisons of protoplasm and that this property increases in intensity as the number of chlorine atoms is increased. It is to be observed that as the chlorination is increased, the solubility in water diminishes until the trichloro compound is almost insoluble.

The decrease in solubility is related to the diminishing number of hydroxyl groups. The same progressive diminution in water solubility also obtains in the series of compounds A. until with ethylene dichloride we have relatively almost complete insolubility (1 in 1,000 at 20° C.).

All the compounds B. are liquid, and as to their effects on man, nothing can be said except as to glycerine which is for practicable purposes non-

TABLE 1
LETHAL DOSES

	Ethylene glycol	Ethyl alcohol	Ethylene dichloride	Ethylene chlorohydrin
By mouth	<i>Rat</i> 10-11 g./kg. tolerated	<i>Rat</i> 9-10 g./kg.	?	?
Intraperitoneal	<i>Rat</i> 5-6 g./kg. tolerated 11 g./kg. fatal	<i>Dog</i> 3-5 g./kg.	?	?
Subcutaneous	<i>Mice</i> 2.7 g./kg.	<i>Mice</i> 4-5 g./kg.	?	?
Inhalation	<i>Rat</i> 0.5 mg./l. not fatal (exposure 28 hours in 5 days)	<i>Rat</i> 19-24 mg./l. in 21 hours 85 mg./l. in 4½ hours <i>Guinea-pig</i> 85 mg./l. in 10½ hours	<i>Mice</i> 35 mg./l. in 2 hours 100 mg./l. in ½ hour <i>Guinea-pig</i> 40 mg./l. for 25 min. fatal next day. 240 mg./l. in ½ hour	<i>Guinea-pig</i> 2-3 mg./l 28 hours' exposure dur- ing 2 weeks ; fatal <i>Cat</i> 2-3 mg./l. 3-4 hours every other day for 4 days; fatal

toxic, and indeed enters into the metabolism of the organism by forming glycogen.* Of the series A. all are liquid and all are in greater or less measure toxic. The increased toxicity when the chlorine atom is introduced is very striking.

Exactly comparable figures are difficult to find in the literature, but Table 1, drawn up from Brown-ing's review on industrial organic solvents (1937), gives some idea of the different toxicities in animals.

As regards acute toxicity, these figures indicate, with reservations, that ethylene glycol is less toxic than ethyl alcohol when given to rats by mouth, but apparently more so when injected subcutaneously in mice. The relative toxicities of the vapours are also difficult to conclude upon. For ethylene glycol, experiments on exposure to the vapour have really little significance, but the figures on the effect of the vapours of the remaining substances on the guinea-pig leave no doubt that ethyl alcohol is much less toxic than the two chloro compounds, the more so as the guinea-pig is rather resistant to the action of narcotic vapours.

Müller (1925) gives results on chlorinated hydrocarbons and in particular ethylene dichloride which are readily comprehended. Mice were used and the following results (Table 2) obtained with ethylene dichloride:—

TABLE 2

Weight of mice, grams	Concentration of vapour, mg./litre	Time to side position, mins.	Period of exposure, mins.	Results
15	11.5	—	123	Dead in 1 day
18	23	40	54	Dead in 1 day
15	23	—	70	Dead in apparatus
17.5	57	—	36	Dead in apparatus
20	114	—	15	Moribund next day

The concentrations of vapour used by Müller are too high to give a true picture of the toxicity. The striking characteristic of the effect of ethylene dichloride is that after full narcosis at a relatively low concentration, the animals recover apparently completely but are found dead some hours later, usually next day: the substance is also lethal at concentrations below the narcotic.

No table of results comparable to the above is extant for ethylene chlorohydrin, as far as we know.

EXPERIMENTS

1. Effects of Ethylene Chlorohydrin Vapour on Animals

For this purpose we used a simple apparatus consisting of a gas meter, flow meter, constant dropping apparatus, vaporizing chamber, mixing, and exposure chamber and a suction system. Within reasonable limits of error the concentration of vapour could be calculated for any rate of dropping and flow of air. In general, the flow of air was kept at between 8 and 10 litres per minute and the rate of dropping could be kept steady at from 1 drop per second to 1 drop per minute.

The exposure chamber was a large dessicator suitable for mice, rats, or guinea-pigs. Symptoms were carefully observed during exposures and for several hours after. In many cases the time of death could not be accurately determined as it occurred during the night. In all our experiments the ethylene chlorohydrin and ethylene dichloride were pure.

The following table (Table 3) shows the results of some typical experiments.

The lesser sensitivity of the guinea-pig to the vapour is shown by the fact that exposure for 55 minutes to 0.005 g./litre is not lethal, whereas 0.0039 g./litre for 15 minutes is fatal to 2 out of 3 mice after 2 days and 0.004 g./litre for 30 minutes is fatal to all rats.

The point made by Koelsch that animals do not

TABLE 3

SINGLE EXPOSURES OF ANIMALS TO THE VAPOUR OF ETHYLENE CHLOROHYDRIN

Density of ethylene chlorohydrin liquid: 1.204.

Boiling range: 129.1°–130.3° C.

0.001 g./litre=280 parts per million v/v.

Number of animals		Average weight, grams	Conc. of vapour, g./lit.	Time to side position, mins.	Total exposure, mins.	Results
Mice	3	23	0.001	—	120	Non-lethal
	3	23	0.003	—	60	3/3 dead next day
	3	23	0.0032	—	60	3/3 dead next day
	3	21	0.0039	—	15	2/3 dead after 2 days
	3	21	0.0039	—	120	3/3 dead in 140 to 170 mins.
	3	21	0.0045	—	30	3/3 dead next day
	3	21	0.0052	—	60	3/3 dead in 100 mins. to next day
	3	20	0.007	80	120	3/3 dead in 110–129 mins.
Rats	3	115	0.003	—	15	Non-lethal
	3	120	0.004	—	30	3/3 dead next day
Guinea-pigs	1	450	0.003	—	30	Non-lethal
	1	350	0.003	—	112	Dead next day
	1	450	0.039	—	108	Dead next day
	1	450	0.005	—	55	Non-lethal

* The author has drunk 100 gms. of glycerol in water without toxic effects, other than some temporary irritation of the bowel.

die during exposure is borne out by our findings, although in the case of one mouse exposed to 0·007 g./litre death did occur in the exposure chamber after 110 minutes, whilst the two remaining animals died a very few minutes later. The side position recorded at this last concentration after 80 minutes exposure was really the rolling over of the animals on to their sides as death was imminent and not a manifestation of narcosis.

Since we are interested to determine whether the gross toxicity of ethylene chlorohydrin is greater or less than that of ethylene dichloride, the following results are recorded from experiments performed as in Table 3 for both ethylene chlorohydrin and ethylene dichloride.

For comparisons of this kind it is essential to work at as low concentrations as possible.

Our results have satisfied us that ethylene dichloride vapour is markedly less toxic for these animals than is ethylene chlorohydrin. It is to be noted that narcosis was not produced at any of the concentrations recorded. Narcosis can readily be produced with the dichloride at higher concentrations. Attempts to produce narcosis with ethylene chlorohydrin by using higher concentrations simply kills the animals before a true narcosis can be observed.

Symptoms of Exposure to Ethylene Chlorohydrin Vapour. In general the animals were first obviously discomforted. Later the eyes were closed and obvious depression set in with evidence of nasal irritation. After about an hour's exposure to the higher concentrations there was inco-ordination of movement and the animals became very reactive. A loud noise or smart tapping on the side of the exposure chamber stimulated them to an almost convulsive response, after which they lay on their sides with, in the case of rats and mice, the tail at right angles to the body. A true side position was not attained and none of the animals became narcotized.

On removal from exposure, the general appearance often gave the impression that the animals would survive, but fatalities followed as recorded, preceded in some cases by convulsions. The delay

in the onset of lethal symptoms is quite characteristic and death when it does occur is sudden and appears to be due to respiratory failure.

Macroscopic examination of the organs gave no obvious indication as to the immediate cause of death.

Histological Examination. Rat exposed to 0·010 g./litre for 30 minutes—dead next day.

Lungs.—Areas of collapse but no haemorrhage or oedema.

Kidneys.—Glomeruli normal: marked haemorrhages between the medullary tubules, blood in part haemolysed; convoluted tubules swollen and in part detached; areas of lost structure (Fig. 2. See p. 211).

Guinea-pig exposed to 0·003 g./litre for 112 minutes—dead next day.

Lungs.—Some areas of emphysema; no haemorrhage or oedema.

Kidneys.—Glomeruli normal; large numbers of haemorrhages mainly at junctional areas between cortex and medulla.

Heart, liver, cerebellum, and pons.—No obvious changes.

It thus appears that the main attack of the vapour of ethylene chlorohydrin in these acute cases is upon the kidney and to some extent the lungs.

Effects of Repeated Exposure to Ethylene Chlorohydrin Vapour. Three rats were used and exposed daily as below. After each exposure the urine was collected in a metabolism cage. The average weight of the animals was 115 g. at the outset. Food and drink *ad lib* throughout.

The animals were obviously depressed, lost weight, but ate when in the metabolism cage. The urine collected daily throughout the experiment gave no evidence of renal disease. Repeated exposure at this concentration (average 0·0034) for 15 minutes a day eventually leads to death of all animals. Individual variability is clearly shown in the table.

Histological examination of these animals showed:—

Rat dying on 3rd day: Intensely congested kidney with here and there beginning haemorrhages;

TABLE 4
COMPARISON OF LETHAL EFFECTS OF ETHYLENE CHLOROHYDRIN AND OF ETHYLENE DICHLORIDE (SINGLE EXPOSURES)

Density of ethylene dichloride: 1·25.
Boiling range: 83·5°–84·2° C.
0·001 g./litre=226 parts per million v/v.

ETHYLENE CHLORHYDRIN			ETHYLENE DICHLORIDE				
	Concn. of vapour, g./litre	Time of exposure, mins.	Result		Concn. of vapour, g./litre	Time of exposure, mins.	Result
<i>Mice</i>	0·003 0·004	15 30	Non-lethal Lethal next day		0·004 0·008	120 120	Non-lethal Lethal next day
<i>Rats</i>	0·001 0·003	120 60	Non-lethal Lethal next day		0·002 0·003	120 120	1/3 dead next day Lethal
<i>Guinea-pigs</i>	0·003 0·003	30 112	Non-lethal Lethal next day		0·004 0·006 0·007 0·009 0·017	118 115 117 117 120	Non-lethal Non-lethal Non-lethal Lethal Very ill but recovered

intensely congested liver and small areas of collapse in the lungs. (Fig. 3.)

TABLE 5

REPEATED EXPOSURE OF RATS TO VAPOUR OF ETHYLENE CHLOROHYDRIN

Day	Concn. of vapour, g./lit.	Exposure, mins.	Deaths
1	0.003	15	1st death
2	0.003	15	
3	0.005	15	
4	0.004	15	
5	0.003	15	2nd death — loss in weight 25 g.
6	0.003	15	
7	0.004	15	
8	0.0024	15	
9	0.002	15	3rd death—5 hrs. after last exposure
10	0.004	15	
11	0.004	15	

Rat dying on 6th day: Many small haemorrhages in the kidney but glomeruli normal; cells of convoluted tubules disorganized and many detached; cells swollen and nuclei broken down in many cases. (Fig. 4.)

Liver showed fatty changes; cell outlines lost with large areas of replacement of cells by globules of fat.

Lungs showed collapse, areas of haemorrhage, considerable deposition of pigment but no oedema.

Rat dying on 11th day: Lungs showed areas of rupture of alveoli and of collapse; in many parts the alveolar epithelium was swollen sufficiently to occlude the lumen; some deposition of dark pigment; no haemorrhages, no oedema. The liver was intensely congested with areas of complete degeneration; some formation of pigment in medium sized vessels and marked fatty degeneration. (Fig. 5.)

The kidneys showed normal glomeruli, multiple small haemorrhages, complete disintegration of cells of convoluted tubules, cells of Henle's loops much less affected but cells in parts swollen and detached but with apparently normal nuclei. (Fig. 6.)

It appears clear from these findings that the earliest focus of intense attack is the kidney, starting with small haemorrhages and proceeding to widespread necrosis of the cortical cells; the glomeruli were in all cases strikingly normal in appearance. The second point of attack is the liver with fatty degeneration and later extensive breakdown of the cellular structure. The lungs appear to be affected least severely but some areas of haemorrhage and collapse could be made out.

The absence of signs of kidney damage in the urine of these animals is surprising. Secretion of urine was unimpaired. It would appear that the effect of ethylene chlorohydrin on the kidney in these animals is that of a vascular poison and perhaps only secondarily affecting the cells of the secreting tissue, but the entire absence of attack upon the glomerular vessels is extremely puzzling.

No studies of the effects upon blood and urine constituents have yet been made.

We have no data as yet on the structural changes which may occur in the central nervous system.

2. Intraperitoneal Injections of Ethylene Chlorohydrin

Whilst the main risk attaching to this compound in industry is the toxic effect of the vapour, the fact that evidence of absorption through the skin when the liquid is applied can readily be obtained, renders it of some importance to determine the toxic doses of the liquid. The ready miscibility with water makes injection experiments easy.

The following table (Table 6) shows the effect of intraperitoneal injections in rats.

Two groups of rats were taken (24 and 18), one group starved for 24 hours and the others fed on the stock diet.

TABLE 6

SINGLE INTRAPERITONEAL INJECTION IN RATS

Starved 24 hours		Normally fed	
Dose injected intraperitoneally, mg./100 g.	Deaths	Dose injected intraperitoneally, mg./100 g.	Deaths
4	1/4 next day	5	1/6 next day
5	0/4		
6	4/4 after 3 hours		
7	4/4 (3 after 3 hours—1 next day)		
8	4/4 after 3 hours	10	6/6 (4 after 3 hours—2 next day)
10	4/4 after 3 hours		

Clearly the L.D. 50 is between 5 and 6 mg./100 g. (5.6 mg./100 g.) and the fact of being fed or unfed makes no significant difference. The figures for the starved animals show that the rat can successfully detoxicate between 4 and 5 mg./100 g. as a single dose. If repeated doses are given very much more can be detoxicated, as shown in the following table (Table 7) :—

TABLE 7

REPEATED INTRAPERITONEAL INJECTION IN ONE GROUP OF 6 RATS

Dose, mg./100 g.	No. of daily doses	Total injected, mg./100 g.	Results
1	8	8	No deaths
2	2	4	No deaths
3	4	12	No deaths
5	1	5	4/6 dead

The six rats were able without symptoms or loss in weight to detoxicate 24 mg./100 g., two survived

even 29 mg./100 g. A single dose of 5 mg./100 g. on the 15th day killed 4 out of the 6. These results seem to militate against any idea of cumulative action.

3. Oral Administration of Ethylene Chlorohydrin

One group of 30 rats were given doses varying from 1 mg./100 g. to 6 mg./100 g. in aqueous solution by stomach tube, and a second group of 18 rats doses varying from 5 mg./100 g. to 10 mg./100 g.

TABLE 8

Dose (mouth) mg./100 g.	Deaths	Dose (mouth) mg./100 g.	Deaths
1	0/2	7	2/3
2	0/2	8	2/3
3	0/2	9	3/3
4	1/8	10	3/3
5	0/8 0/3		
6	2/8 1/3		

L.D. 50 approximates 7.2 mg. per 100 g.

Thus, as is perhaps to be expected, the toxicity by mouth is rather less than by the intraperitoneal route.

The symptoms in the case of the animals which later died were delayed in onset and included general prostration, diminished or absent corneal reflex, intermittent spasmodic movements of almost convulsive type, slowed respiration, fall in temperature and death. At no stage could it be said that the animals were narcotized, except as in the ante-mortem state.

We are at pains to insist on this absence of true narcosis which distinguishes the toxic effects of ethylene chlorohydrin from those of ethylene dichloride which produces true narcosis.

The following experiment with a rabbit also shows the absence of true narcosis and the delayed action.

A rabbit of 2.7 kg. weight was injected intravenously with 100 mg./kg. of ethylene chlorohydrin. Three hours after the injection the animal showed signs of increasing inability to keep the head erect, the animal tending to lay it on one side on the floor of the cage. On disturbing the animal by noise or by raising the head there followed intermittent oscillatory movements of the head until it again was laid in the original position. The animal was 'conscious' throughout. Four hours after the injection the body was very cold; the animal gave a convulsive bound and died from failure of respiration. No obvious cause of death could be found on macroscopic examination of the organs.

The absence of true narcotic action may be compared to the finding of Marshall and Heath (*loc. cit.*) with glycerine monochlorohydrin which had no narcotic effect, whilst the trichlorohydrin was a powerful hypnotic.

The difference in narcotic effects between ethylene chlorohydrin and dichloride can be shown in frogs, although, in general, we do not regard frogs as good subjects for this type of experiment. Injection of 0.1 c.c. pure ethylene dichloride into the dorsal sac leads in 3 or 4 minutes to immobility, flaccidity, extremely slow and shallow respiration; in about 6 minutes the corneal reflex disappears; in 15

minutes reflex to acid disappears; in 20 minutes there follows cessation of respiration, fine muscular tremors over the whole body and death.

Injection of 0.1 c.c. ethylene chlorohydrin into the dorsal sac is followed in about 6 minutes by failure of the righting reflex when the frog is placed on its back, the limbs are drawn tightly to the body, but the corneal reflexes are still present; in 10 minutes the animal still makes inco-ordinated efforts to walk and is still able to jump, although weakly; in 17 minutes it is still able to make slow, spasmodic, progressive movements and corneal reflexes are still present; in 34 minutes the corneal reflex had disappeared, but the reflex to acid, though sluggish, was still present, and there was still extremely slow respiration; after 90 minutes the frog was still making stretching movements and what appeared to be attempts to crawl, but at this stage no corneal reflex could be obtained: the passage to death was almost imperceptible.

The dose in both cases was, of course, immensely high, far exceeding the median lethal dose for frogs, and was used to see if narcosis could be obtained. Smaller doses of ethylene chlorohydrin (250 mg. per kilo) produced no apparent symptoms for many hours but are fatal eventually: 50 mg. per kilo are tolerated.

Smooth narcosis of the frog can be obtained with ethylene dichloride in 2 or 3 minutes by simple exposure under a bell-jar to the vapour from cotton wool soaked in the liquid: removal some few minutes after narcosis is established is followed by recovery.

Exposure to ethylene chlorohydrin vapour produces depression and immobility, but even after 30 minutes at a high concentration, true narcosis is not attained: recovery is very slow with protracted inco-ordination and obviously great depression.

Although the depressant action of ethylene chlorohydrin on the nervous system is apparent, this action is different from narcosis, and seems to be complicated in the intact animal by a certain stimulant or irritant element which prevents full establishment of the latter. The depressant action of ethylene chlorohydrin is readily demonstrable on a variety of physiological processes and tissues.

We have referred in the descriptions of clinical cases (see Part I) to the considerable fall of blood-pressure in man which follows the inhalation of the vapour when in considerable concentration. In the following experiments attempts were made to elucidate the mechanism of this depressor action.

4. Effect of Ethylene Chlorohydrin on the Blood-Pressure and Respiration

It must be stated at the outset that we have failed to produce clear evidence in anaesthetized animals (cats and rabbits) that inhalation of the vapour produces a manifest drop in blood-pressure or significant changes in respiratory depth or rhythm. This was the result obtained whether the vapour was inhaled through the nasal passages or through a tracheal cannula, the vapour being inhaled from a duct leading from a continuous evaporating system. Concentrations of 10–15 mg. per litre (2,800–4,200 p.p.m. v/v) were attained and were inhaled for several hours without any fall in blood-pressure or signs of respiratory disturbance.

We proceed, therefore, to examine the effect of intravenous injection. In one experiment the

effect of ethylene chlorohydrin on the blood-pressure of an anaesthetized cat was examined before and after atropinization. A 3-kilogram cat was anaesthetized with ether followed by intravenous chloralose (80 mg. per kilo). Blood-pressure was recorded from the carotid artery and injections made through the femoral vein. Table 9 shows the course of the experiment. Primary and secondary falls in B.P. were observed, the recovery from the primary fall being due to the asphyxial rise which will be demonstrated in a later experiment.

TABLE 9
EFFECT OF INTRAVENOUS ETHYLENE CHLOROHYDRIN ON B.P. OF ANAESTHETIZED CAT

Initial B.P., mm. Hg.	Injection	Fall in B.P. mm. Hg.	
		Primary	Secondary
160	0.2 c.c.	30	68
144	0.3 "	40	46
132	0.3 "	42	44
122	10 mg. atropine sulphate intra-peritoneally	40	
82	0.2 c.c.	10	16
80	0.3 "	8	12
76	0.5 "	16	2
72	1.0 "	42	Death

Fig. 7 shows the effects clearly. The immediate effect of the large doses used is inhibition of the heart with a resultant immediate drop in blood-pressure: this is accompanied by an ephemeral inhibition of respiration with re-establishment of cardiac action and rise in B.P.: the last phase is a slower fall in pressure with a subsequent slower recovery usually to a level lower than that existing before injection. (Fig. 7 (a) and (b).)

After full atropinization the effect of similar doses at suitable intervals is to produce so little effect on blood-pressure (Fig. 7 (c) and (d)), that there seems no alternative but to suppose that the depressor effect is associated with some action on the vagal mechanism. We do not stress the effect on the vagal mechanism since compounds of the type of ethylene chlorohydrin are liable to be responded to in puzzling ways. Very much larger doses (Fig. 7 (e) and (f)), are now required to produce effects comparable with those found before atropine. The fatal dose of 1.0 c.c. was required to produce a fall comparable to that of 0.3 c.c. before atropine: this was almost at once followed by a violent asphyxial rise and death.

5. Effect of Ethylene Chlorohydrin on Vascular Reflexes from Afferent Nerves and Vagal Responses

A cat similarly anaesthetized was prepared for recording B.P. from the carotid, respiration from the trachea, and for electrically stimulating the central end of a cut sciatic nerve.

Fig. 8 (a) shows the effect of 4 seconds stimulation of the central end of the cut sciatic nerve with a simple inductorium (coil distance 7 cm.) and that of the subsequent injection of 0.2 c.c. ethylene chlorohydrin. The rise in blood-pressure from

FIG. 7.—Effect of 0.2 c.c. and 0.3 c.c. ethylene chlorohydrin intravenously on B.P. of cat (chloralose), (a) and (b).

Effect of 0.2, 0.3, 0.5, 1.0 c.c. ethylene chlorohydrin intravenously after full atropinization, (c), (d), (e), (f).

afferent stimuli, the fall in B.P. as described above, and inhibition of respiration are all clearly seen.

Fig. 8 (b) shows the effect of afferent stimuli after the injection of the chlorohydrin when the blood-pressure had become re-established. It is seen that no effect upon the vascular reflex was produced and

FIG. 8.—(a) Effect of 4 sec. stimulation of central end of cut sciatic of cat followed by intravenous injection of 0.2 c.c. ethylene chlorohydrin on B.P. (carotid) and respiration (trachea).

(b) follows (a) and shows that injection of chlorohydrin led to no demonstrable change on result of afferent stimulation. Inhibition of respiration by chlorohydrin is clearly seen.

the subsequent injection of 0.2 c.c. chlorohydrin produced an unaltered effect.

Similarly short stimuli to the left vagus trunk led to the characteristic cardiac inhibition with fall in blood-pressure and rapid recovery after cessation of the stimuli, a few minutes after the intravenous injection of 0.2 c.c. chlorohydrin.

Thus our experiment led us to conclude that:—

- (1) Ethylene chlorohydrin (liquid) inhibits the heart.
- (2) Ethylene chlorohydrin (liquid) inhibits respiration and in fatal doses brings about an asphyxial death.
- (3) Ethylene chlorohydrin does not interfere with the response of the heart to vagal stimulation or with cardio-vascular reflexes from afferent stimulation.

In view of the probable direct action of chlorohydrin on the heart muscle, we tested the effect on the isolated perfused frog's heart.

6. Effect of Ethylene Chlorohydrin and Related Compounds on the Isolated Perfused Frog's Heart

The perfusion of the isolated heart was performed via the sinus using a Syme's cannula and continuously oxygenated Ringer-Locke solution. The arrangement was such that change from the normal perfusion fluid to fluid containing known concentrations of the test substances could be brought about in a few seconds. Fig. 9 shows typical results obtained. The effects may be summarized as follows:—

seems to be mainly on the cardiac muscle. It is to be noted that the concentrations required to produce a marked effect of even partial inhibition are high even for the dichloride and chlorohydrin, and that these compounds are readily washed out of the tissue.

Thus, it is not surprising that the effect of intravenous ethylene chlorohydrin on the blood-pressure of intact animals is transitory, lasting only as long as a high concentration remains in circulation. The fate of the chlorohydrin after removal from the circulation is not known.

7. Effect of Ethylene Chlorohydrin on Smooth Muscle Preparations

(a) Small Intestine of the Rabbit

The longitudinal muscle of the rabbit's jejunum was set up in the usual manner in 100 c.c. oxygenated Tyrode solution and measured amounts of ethylene chlorohydrin added to the bath.

From our observations on the isolated heart it might be expected that any marked effects on the intestinal preparations would be produced only by very considerable concentrations. This proved to be the case.

Fig. 10 shows (a) the effect of 10⁻² (v/v) and (b) the effect of the same concentration after atropinization (atropine sulphate 0.5 × 10⁻⁴) of the same preparation. In all cases, after inhibition, change of the bath fluid was soon followed by re-establishment of rhythm and tone.

A rough comparison was made with the effects

TABLE 10
EFFECT OF ETHYLENE CHLOROHYDRIN AND RELATED COMPOUNDS ON THE FROG'S HEART

Compound	Molar concn.	Effect	Figure 9
Ethylene glycol	0.090	Practically none.	(a)
Ethyl alcohol	0.087	Marked slowing of the heart with diminution of systole. Complete recovery on changing back to normal perfusion fluid	(b), (c)
Ethylene chlorohydrin ..	0.074	Complete stoppage of heart in diastole. On changing back to Ringer-Locke a slowed rhythm followed by original rhythm re-established	(d)
Ethylene chlorohydrin ..	0.0074	Diminution of systole but no significant change in rhythm	(f) (upper tracing)
Ethylene dichloride	0.062	Similar to (d)	(e) The ethylene dichloride was present in the perfusate as extremely fine globules in suspension
Ethylene dichloride	0.0062	Similar to (f) upper tracing	(f) (lower tracing)

We are not concerned further to analyse these effects than to state that—

- (1) the dichloride and the chlorohydrin are very much more depressant to the heart than ethyl alcohol;
- (2) the heart is inhibited in diastole in all cases;
- (3) ethylene glycol in the concentration used has no significant effect upon the isolated heart.

In addition, Fig. 9 (f) indicates that the action is not of the vagal type, since there was no slowing of the rhythm. The effects were exactly similar using the fully atropinized heart. The inhibitory effect

of ethylene dichloride and ethyl alcohol on this preparation. From preliminary tests it became clear that for experiments of this kind with ethylene dichloride, it is essential to disperse the liquid. Exceedingly large doses can be added to the preparation without any effect but, if dispersed, immediate effects follow even very small doses. Table 11 shows effects of dispersed ethylene dichloride.

As to ethyl alcohol, a concentration of 0.5 × 10⁻² leads to rapid increase in tone with no inhibition of rhythm.

Our results show that ethylene dichloride is a

FIG. 9—Effects of ethylene glycol, ethyl alcohol, ethylene chlorohydrin, ethylene dichloride on isolated perfused heart of frog.
(a) Ethylene glycol 0.090 molar.
(b) Ethyl alcohol 0.087 molar.
(c) Recovery from (b) on change of bath.
(d) Ethylene chlorohydrin 0.074 molar showing recovery on changing bath.
(e) Fine suspension ethylene dichloride 0.062 molar, showing recovery on changing bath.
(f) Effect of 0.0074 molar ethylene chlorohydrin (upper tracing) and 0.0062 molar ethylene dichloride (lower tracing).
Time tracing—seconds.

TABLE 11

Concn. v/v.	Molar Concn.	Effect
10 ⁻²	0.125	Immediate and irreversible inhibition
10 ⁻¹	0.000125	Apparent slight immediate increase of tone followed at once by inhibition of rhythm and complete relaxation. Completely reversible

much more powerful inhibitor of the gut than ethylene chlorohydrin, and the effect is independent of the vagal mechanism.

FIG. 10.—Effect of ethylene chlorohydrin on isolated rabbit intestinal muscle.

(b) Rat's Uterus

The inhibitory effect of ethylene chlorohydrin on smooth muscle is readily shown on the slow but fairly regular rhythm of the isolated rat's uterus (O₂-Tyrode). At a concentration of 0.07 molar the period of the regular contractions is slowed some 3 to 4 times: the individual contractions are not greatly diminished and there occurs some slight increase in tone.

It is a matter of considerable interest that such enormous concentrations of ethylene chlorohydrin should be required for these effects on isolated tissues. To speak of this compound as a protoplasmic poison (Koelsch) is, we feel, to be satisfied with a phrase. The ease with which complete recovery of normal activity is attained on merely washing the tissue is not what would be expected from a protoplasmic poison in any ordinary sense of the word.

This striking reversibility even after exposure to very high concentrations of ethylene chlorohydrin requires further elucidation.

8. Effect of Ethylene Chlorohydrin on the Transmission of Nerve Impulse

Clinical evidence indicates that ethylene chlorohydrin probably acts upon nervous tissue. Experimental support is readily obtainable by comparing the effects of electrical stimulation of the sciatic nerve of the frog before and during exposure of a small stretch of nerve to the compound. The ordinary sciatic-gastrocnemius preparation was used and stimulation of the nerve produced through the kymograph. The record of the muscle-twitch was made as usual on a fast-moving kymograph. The nerve-muscle preparation was set up on a paraffin surface with a small cup-like depression, in which fluid could be added or removed at will and in which a short length of the nerve lay between the muscle and the point of stimulation of the nerve. Throughout the experiment the preparation lay undisturbed and weak stimuli were applied at will. The contractions were isotonic.

Fig. 11 shows the effect of exposure of the small stretch of nerve to 1 per cent. ethylene chlorohydrin in Ringer-Lock solution. The lowest tracing shows the normal twitch. After 30 seconds of exposure the response to the same stimulus (coil distance 15 mm.) was already markedly diminished: after 150 seconds there was still further diminution of response, and after 7 minutes no response could be

FIG. 11.—Effect of ethylene chlorohydrin on transmission of nerve impulse in frog sciatic-gastrocnemius preparation—to be read from below up. 1 per cent. ethylene chlorohydrin used. Times given on tracing show periods of immersion until complete blockage of transmission and times after washing until almost complete recovery. Coil distance 15 mm.

obtained. At this point the ethylene chlorohydrin was removed and replaced by successive quantities of Ringer-Locke. Some 9 minutes after this washing process the same stimulus led to a considerable response and 14 minutes later an almost normal response was obtained. It will be observed that the time characters of the twitch are unchanged both during the period of suppression and of recovery of the response. There is thus demonstrated a block of a sufficient number of nerve fibres to prevent the particular stimulus used from producing an effective response of the muscle. This block effect is reversible by simple washing of the nerve with saline.

Similar experiments with 5 per cent. and undiluted chlorohydrin showed an intensification of the effects. But in all cases reversibility could be demonstrated by maintained washing of the nerve with Ringer-Locke solution.

At periods after washing when the initial stimulus

was still ineffective, it could be shown that stronger stimuli produce considerable response. In all cases, however, return to a normal response to the initial stimulus was obtained.

Similar experiments were performed with mammalian nerves *in situ*, e.g. the peripheral end of the cat vagus using the blood-pressure and heart action as the effector organ, the central end of the cut vagus and respiration as the index, and the cervical sympathetic using the pupil as an index. Exactly similar results were obtained, in particular complete reversibility after complete block of a fixed stimulus.

The application of these results to the clinical picture of ethylene chlorohydrin poisoning goes no further, at this stage, than that the compound is capable of exerting a depressant action on nerve tissue and that some of the signs and symptoms may be related to this fact.

The order of magnitude of the nerve-blocking concentrations is not such as could be attained in circumstances of industrial exposure or even by injection into animals without causing rapid death. Nevertheless, it is conceivable that a property of this kind may have great significance when the compound is presented in even relatively very low concentration to the tracts in the brain or cord. The indubitable signs and symptoms of involvement of the nervous system in ethylene chlorohydrin poisoning must, it is suggested, be related to effects not dissimilar from those manifested in these nerve-block experiments.

9. Absorption of Ethylene Chlorohydrin through the Intact Skin

Animal experiments designed to show absorption of a toxic material through the skin should ideally be performed on animals the skins of which resemble in some degree that of man. The horse and the pig are probably the best from this point of view. When, however, one is restricted to the smaller laboratory animals, a good deal of precaution is necessary, both in experiment and in interpretation. Positive results in such experiments give only a qualitative indication. In general we take the view that if a compound is demonstrably absorbed through the skin of mice, rats, or guinea-pigs, then *a fortiori* it will be so absorbed by human skin. But if a negative result is obtained with these animals, it cannot be concluded that this applies also to man.

In the case of ethylene chlorohydrin, the evidence is unequivocal. Koelsch (*loc. cit.*) found it to be absorbed by guinea-pigs' skin. We have found it easy to demonstrate it in mice, but relatively large doses are required when we consider that about 1 mg. per 20 g. mouse is the M.L.D. if injected intraperitoneally.

The following results were obtained with 19 mice: doses varied from 0.03 c.c. to 0.09 c.c. undiluted chlorohydrin and only one dose was applied.

The conditions of diet, temperature, and manner of application to the dehaired area of skin were uniform. On applying the material it quickly spreads over the area and as far as one can make out, the major part of it cannot leave the skin: some will certainly be lost by evaporation. The fact that such enormous doses must be applied to produce death indicates that absorption must be slow enough to permit of excretion or detoxication, or both, of very considerable amounts of the compound.

TABLE 12

EFFECT OF ETHYLENE CHLOROHYDRIN APPLIED TO THE DEHAIRIED SKIN OF MICE

No. of mice	Average weight	No. of drops	Size of drop	Calculated dose/20 g.	Deaths	Time of death
7	25 g.	1	0.03 c.c.	29 mg.	3/7	1 same day 2 next day
6	30 g.	2	0.03 c.c.	48 mg.	6/6	4 same day 2 next day
6	20 g.	3	0.03 c.c.	108 mg.	6/6	Same day (within 3 hours)

In applying these findings to man, all that can be said is that the compound can penetrate skin and that very large doses are required to endanger life. But this should suffice to insist on prevention of all skin contact with the liquid.

There was no evidence of direct skin irritation from the application of the compound.

Summary

1. The following concentrations of ethylene chlorohydrin vapour are lethal:

Rats ... 30 mins. at 0.004 g./lit. (1120 p.p.m. v/v)

Mice ... 60 mins. at 0.003 g./lit.

Guinea-pigs 112 mins. at 0.003 g./lit.

Pathological changes found after such acute exposures are: renal haemorrhages and tubular degeneration, collapse or rupture of pulmonary alveoli.

2. Repeated 15 minute exposures of rats to 0.003 g./lit. lead to death in from 3 to 11 days. Pathological changes were: renal haemorrhages and tubular necrosis, fatty degeneration and necrosis of liver, pulmonary haemorrhages and areas of collapse. In both acute and chronic exposures the renal glomeruli appeared normal.

3. The M.L.D. (rats: intraperitoneal: single injection) of ethylene chlorohydrin is between 5 and 6 mg. per 100 g. Injected repeatedly in doses much less than the M.L.D. very large amounts are tolerated (24 mg. in one group of 6 rats). M.L.D. (rats: by mouth: single dose) is between 7 and 8 mg. per 100 g.

4. Intravenous ethylene chlorohydrin in cats (chloralose) leads to fall in blood-pressure and inhibition of respiration. Vagal action and cardiovascular reflexes are not affected.

5. Ethylene chlorohydrin and ethylene dichloride are inhibitors of the perfused frog's heart. Ethyl alcohol is much less active in this respect and ethylene glycol is inactive in comparable concentrations. Inhibition is reversible in all cases.

6. Ethylene chlorohydrin inhibits both tone and rhythm of smooth muscle (small intestine, uterus): this effect is reversible.

7. Ethylene chlorohydrin (1 per cent.) in contact with nerve leads to complete nerve block. This effect is reversible.

8. Ethylene chlorohydrin is absorbed through the skin of mice and toxic effects follow.

Conclusions

1. Ethylene chlorohydrin is a powerful renal, hepatic, nerve, and vascular poison. It can be absorbed by inhalation, by the gut or through the skin. In respect of the lethal effects of the vapour on small animals, it appears to be more toxic than ethylene dichloride.

In relatively high concentrations it inhibits the heart, respiration, smooth muscle and nerve transmission: all these effects are reversible.

2. In the conditions of manufacture or use of ethylene chlorohydrin, the main hazard is probably inhalation of the vapour, but in certain operations repeated contamination of clothes and skin readily occurs unless adequate precautions are taken.

Such precautions will, in general, be difficult to take unless the toxic properties are adequately realized before the plant is installed.

3. Workers engaged in the manufacture or repeated use of ethylene chlorohydrin should be submitted to regular medical examination. It should be kept in mind that poisoning by this compound is subtle and that irreparable damage may have developed before marked clinical signs are manifest.

Signs and symptoms of cardio-vascular abnormalities of even minor degree, of renal disease, of disturbed hepatic function, of aberrations of nervous function and behaviour, and of gastro-intestinal function must all be sought and routine records kept.

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