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90 seconds and to the larger ones, about 280 seconds.

Respiratory effects were more dramatic following exposure to the smaller o-chlorobenzylidene malononitrile particles. None of the six men were able to tolerate the small particles for longer than 30 seconds. During the large particle exposures, four of six men tolerated the cloud for at least 60 seconds.

The results recorded in Table II show that exposure to larger particles definitely prolonged the eye effect. While all six subjects were able to tolerate the exposure, the post-exposure effects on the eyes were quite pronounced in that recovery after exposure to the small particles averaged about 90 seconds and the large, 280 seconds.

The inability of subjects to tolerate the inhalation of small (one micron) particles of o-chlorobenzylidene malononitrile or recover rapidly following inhalation of them is a function of the depth of penetration and deposition in the respiratory tree. Of particles inhaled[6,7,8] those having the size of about one micron have the best chance of going beyond the upper respiratory tract and of being retained deep in the lung. Very few, if any, larger particles ($> 15\ \mu$) will penetrate beyond the upper respiratory tract.

The effect of particle size of o-chlorobenzylidene malononitrile aerosol of eye irritation may be dependent on several factors. The impaction efficiency of small particles on surfaces is of a low order,[9] hence deposition on the eye surface would be rather limited. However, the onset of ocular responses would be rapid due to ease of solubility of small particles in the eye fluids. Larger particles would have a much higher impaction efficiency but the onset of irritation would be delayed somewhat due to the slowness of large particle solubility. Once begun, however, the irritation process would continue for a longer period than that caused by the small particles. The eyes would be cleared in both instances through increased tearing.

When both the eyes and respiratory systems of the subjects were exposed to the irritant the response was predominately respiratory with the small particles and ocular for the large particles. One of six men tolerated the small particles for longer than 60 seconds; five of six did so with the larger particles.

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Medical X-Ray Exposures

The U. S. Public Health Service through its National Health Survey has published a survey report entitled "Volume of X-Ray Visits." The data gathered for this report are based on a continuing household interview survey which shows that on the average there are 48 visits each year for medical x-rays for each 100 persons in the population. In general, persons between the ages 45-64 years reported the highest rate of use of x-ray. One-half of all medical x-ray examinations were made in facilities other than hospitals and doctors' offices. Most examinations were for diagnostic purposes and the chest was the most frequently exposed area. Copies of the report are available from the Superintendent of Documents, Government Printing Office, Washington 25, D. C., at 40 cents per copy.

Effects of Single and Repeated Exposures of Humans and Rats to Vinyl Chloride

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Rats exposed eight hours daily to vinyl chloride at concentrations of 2% for three months and 5% for 19 days exhibited changes in liver and spleen weight and in red and white cell counts. Except in the case of liver, tissue alterations did not accompany these changes. The alterations in liver morphology were within the normal range and were not pathologic in character. Because other facets of the animals' response, such as rate of growth, serum transaminase and hemoglobin, were unchanged, it is suggested that the present threshold limit value of 0.05% need not be lowered.

Introduction

THE TOXICITY of vinyl chloride has been reviewed recently.[1,2,3] Because the threshold limit value of 500 ppm is based on limited data[4] using vinyl chloride less pure than that now obtainable[5] the effects of acute exposure in man and rats and long term exposure in rats was investigated by us in 1959 and is described here. Our data do not support the conclusion of Torkelson, *et al.*[3] that the threshold limit value should be reduced ten-fold; indeed their data indicate a need for further studies prior to any revision of the threshold limit value.

Material

The vinyl chloride monomer was supplied by the Perkins Plant of Solway Process Division, Allied Chemical Corporation, in four 100 lb. cylinders with the characteristics shown in Table I, presumably differing materially from the vinyl chloride used by Torkelson, *et al.*[3] only in the presence of 60 ppm of the inhibitor, phenol. Gas chromatography of the liquid phase indicated the presence of more than 99% vinyl chloride, together with a trace of air and carbon dioxide.

Table I
Analysis of Vinyl Chloride Monomer

Acid	2.2 ppm
Acetylene	0.0
Aldehyde	1.9 ppm
Iron	0.0
Sulfur	0.0
Phenol	60 ppm
Non. Vol.	11 ppm
Water	170 ppm
Color	w.w
Assay	99+%

Methods

Experimental

Desired concentrations were obtained by metering air and vinyl chloride through flowmeters calibrated for these gases and passing the appropriate flows through a 2-liter mixing chamber. The concentration was also continuously monitored by a thermal conductivity meter calibrated for vinyl chloride versus air. The desired concentration of vinyl chloride was maintained with less than 5% deviation. The total gas flow was about 50 liters per minute (lpm) in all but one of the experiments.

Five experiments were conducted: (1) a five-minute exposure of human beings to concentrations of vinyl chloride ranging from 0.0 to 2.0%; (2) an exposure of rats (Sherman strain rats from Rockland Farm, New City, N. Y.) for as long as two hours with concentrations up to 15%; and (3, 4, and 5)

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exposures of rats to concentrations of 8 to 10% for 15 days, 5% for 19 consecutive days and 2% for 92 days.

Pathological

The experimental and control animals were killed by ether inhalation after exposures to 2, 5 and 8 to 10% vinyl chloride were completed. Each animal was autopsied according to standard practice. In addition to the gross examination of all animals, all the livers, kidneys and spleens were examined microscopically and some animals received a complete microscopic examination. Heart, lung, liver, spleen, urinary bladder, testes, prostate, ovary, brain, spinal cord, pituitary, tibia, pancreas, stomach, small and large intestine, adrenals, kidneys, uterus, fallopian tubes, thymus, thyroid, parathyroid, eye, knee joint, bone marrow, skeletal muscle, salivary glands and skin were removed from each animal and preserved in 10% buffered formalin. All tissues were examined grossly both at autopsy and after formalin fixation. Following fixation, representative samples of all tissues were processed according to standard histological procedure and stained with hematoxylin and eosin; separate specimens of liver were stained for fat with the *Flaming Red* technique. The animals receiving such a complete examination of the tissues listed included two males and one female rat that had been exposed 15 times and one female rat exposed ten times to the 8 to 10% level; eight experimental and nine control animals surviving the 5% exposure; and 20 randomly selected rats, equally divided as to experimental and control groups and to sex, exposed to 2% vinyl chloride.

The marked increases in liver weight accompanying the exposures to 2% and 5% of the gas, the decrease in spleen weight in the 2% expsoure and the reported kidney changes in exposure to 500 ppm[3] led us to examine these particular tissues with a blind technique, thus excluding the operation of any bias or prejudice from the judgments. All slides, including duplicates, were randomly numbered so that it was impossible to distinguish, without the code, which had been experimental and which control. The slides of the liver, spleen, and kidney were examined and classified, then recoded and re-examined; in this manner a measure of the consistence and reliability of the pathologist's technique was obtained. This consistency was nearly perfect in the case of liver slides, and only slightly less so for spleen and kidney. The results of these examinations are reported in the appropriate following sections.

Results

Experiment 1

Because the main objective was the determination of the effect of long term exposure to vinyl chloride, the maximum concentration of the gas to which humans might conceivably be exposed without any immediate acute effects was determined; this concentration then became the basis for determining the concentration used during the 92-day exposure of rats.

Three men (26, 35, 50 years; 86, 78, 73 Kg.) and three women (25, 40, 55 years; 64, 52, 61 Kg.) were exposed twice each day, separated by a 6-hour interval, for three successive days to six different concentrations of vinyl chloride: 0.0, 0.4, 0.8, 1.2, 1.6 and 2.0%. The concentrations were presented in a different order to each subject to make it possible to factor out any possible adaptation to either the gas or the experimental situation; the 0.0% concentration was included so that some assessment of suggestibility could be made.

Until its conclusion, the subjects were told neither the effects to expect from the exposure nor the purpose of the experiment; nor was information vouchsafed as to the concentrations that were used at any time. The subjects each sat in a chair separated from the gas mixing equipment by a screen, a simple plastic breathing mask affixed over the face, covering the mouth and nose. The rate of air or air-gas mixture passed through the mask was sufficient (50 lpm) to prevent any dilution effects from the atmosphere. After five minutes of breathing the mixture, the exposure was terminated and the subjects were asked to compare their feelings at this time to the time immediately prior to putting on the mask; no suggestions of any kind were made. The responses of the subjects are summarized in Table II. It is apparent that the maximum concentration causing no effect in any subject lies between 0.8 and 1.2%. From the responses it is evident that vinyl chloride causes clear-cut intoxicating symptoms which can serve as adequate warning signs of its presence.

TABLE II
Responses of Human Subjects to Varying Concentrations of Vinyl Chloride

Per Cent Concentration	Response
0.0	No differences reported by Subjects 1, 2, 4, 5 and 6. Subject 3: "slightly dizzy".
0.4	No differences reported by *all* subjects.
0.8	No differences reported by Subjects 1, 2, 4, 5 and 6. Subject 3: "slightly heady".
1.2	No differences reported by Subjects 1, 3, 4 and 5. Subject 2 unsure, somewhat dizzy in middle of exposure. Subject 6, reeling, swimming head, "just like getting gas".
1.6	No effect reported by Subject 5. All others report various degrees of intoxication with dizziness, lightheadedness, some nausea, dulling of visual and auditory cues; these symptoms disappeared rapidly upon termination of the exposure.
2.0	All subjects reported intoxicating effects, Subject 1 reporting a headache that persisted for 30 minutes. These symptoms appeared earlier in the exposure than at 1.6% and the symptoms were more intense than at 1.6%.

Experiment 2

To gain further insight into the intoxicating effects of vinyl chloride rats were exposed to varying concentrations of vinyl chloride for periods up to two hours. The effluent gas from the mixing chamber, at the desired concentration, was passed through a 10-liter all-glass exposure chamber containing two rats. At a 5% concentration intoxication is moderate but the righting reflex is lost; intoxication is more intense at 6% but the righting reflex is still present. This reflex is lost at a concentration of 7%; the corneal reflex disappears at a concentration of 10%. On removal from the chamber, the animals return to the pre-exposure state rapidly. One animal was sacrificed after exposure to the 10% concentration and showed no visible gross pathology. Exposure to a concentration of 15% resulted in deep anesthesia within five minutes. Effusion of fluid from the mouth preceded respiratory failure in one rat after 42 minutes; autopsy revealed edema and congestion of the lungs. The second rat was maintained under this deep anesthesia for two hours; on removal to air, there was an uneventful and prompt recovery.

Experiment 3

Some notion of the distinctive and specific pathology that might be caused by vinyl chloride was our object in exposing rats to a concentration of 10%.

Thirty-six rats, equally divided as to sex, were divided randomly into an experimental and a control group; the 18 experimental rats were exposed to the gas in a 1100-liter steel chamber. The concentration was initially raised rapidly to the desired level by admitting vinyl chloride without admixture with air until the effluent from the chamber attained the desired level as noted on the thermal conductivity meter. A fan within the chamber, connected by a flexible cable to an electric motor outside of the chamber (thus avoiding the hazard of an explosion), mixed the vinyl chloride with the air within the chamber. Thereafter, the effluent from the 2-liter mixing vessel was admitted to the chamber; to conserve gas, the throughput of this highest concentration was 20 lpm.

The experimental rats were exposed daily from 0830 to 1630 hours while the control animals were exposed from 0000 to 0800 hours. At this concentration, as already noted, rats lose consciousness, regaining it five to ten minutes after removal to air. After two consecutive 8-hour exposures, however, the appearance of the animals suggested that there would be no survivors if this concentration were maintained for the contemplated 15-day period; consequently, beginning with the third exposure, the concentration was reduced to 8%. The test was interrupted for one day after the seventh daily exposure because of a mechanical breakdown. Of the group exposed in this fashion, three female rats died, after the second, fifth and fourteenth exposures; the two animals that died earliest were replaced with substitutes for the remaining exposure period; eight female rats were thus alive at the end of the fifteenth exposure. Female rats exposed 10 and 15 times were autopsied

TABLE III
Classification of Morphologic Changes in Livers

Class	Description
1	No swelling, no vacuoles, sinusoids visible.
2	Slight swelling of cells, only a few faint vacuoles or none, sinusoids visible but compressed.
3	Moderate swelling of cells, most cells with definite fine to medium vacuoles, sinusoids compressed.
4	Marked swelling of cells, large irregular "vacuoles" or clear spaces, compression of sinusoids. Changes focal in distribution.
5	Similar to 4 but changes more widespread and diffuse.

TABLE IV
Classification of Morphologic Changes in Kidneys

Class	Description
1	Cells well preserved, no vacuoles, glomeruli normal.
2	Vacuolization and "pyknosis" of some collecting tubules. Otherwise as 1.
3	As 2 but with more extensive vacuolization and "pyknosis" including proximal collecting tubules in cortex.
4	As 3 but with vacuolization and "pyknosis" extending to convoluted tubules.

at the termination of the exposure; the remaining six rats were autopsied 15 days later, five of these rats having been exposed 15 times and the sixth rat 13 times. The mortality was greater among the male rats: only two males survived 15 exposures, the remaining males, and their replacements, surviving only an average of eight exposures. The two male rats exposed 15 times were autopsied at the termination of the exposure.

There was no weight gain in the initial days of the exposure to this concentration, although after the ninth day some resumption of growth seemed to occur, possibly an indication of the development of tolerance to the effects of the gas as the exposure continued. Upon termination of the exposure, growth resumed promptly at the same rate as the control animals. Both at the 16th and at the 30th days, there appeared to be no differences in liver: body weight ratios between experimental and control rats, although too few livers were weighed at the termination of the experiment to make meaningful comparisons of this ratio.

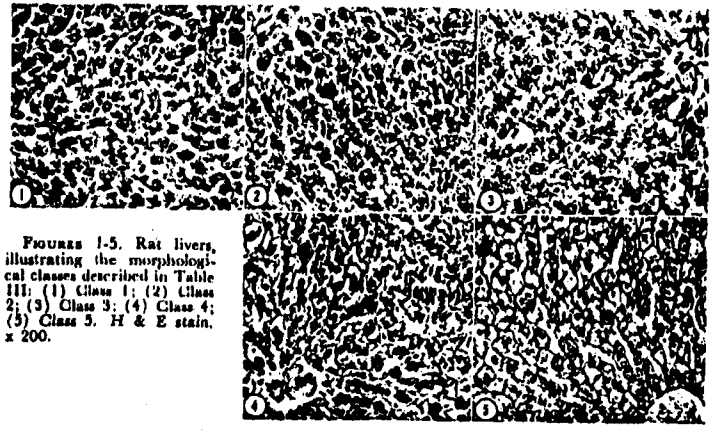
The external appearance, coat and tail, of all the animals was within normal limits. About a third of the animals had parasitic liver cysts. No differences in appearance, color, consistency or degree of congestion were observed between the livers of experimental and control animals. The lungs of three experimental animals sacrificed immediately upon cessation of the exposure had numerous focal fibrinous pleural exudates overlaying nodular yellow-brown parenchymal lesions which had the appearance of regions of acute necrotizing focal pneumonia. Of the six experimental animals sacrificed two weeks after the exposure, the lungs of two revealed a few adherent fibrous pleural adhesions which were interpreted as representing regions of healed pneumonia. All other organs and tissues were within normal limits, with no differences between experimental and control animals.

The experimental animals sacrificed at the termination of the exposure received a complete histologic examination. The lesions in the lungs of the three animals in this group showing gross pleural pathology were seen to be due to acute focal necrotizing pneumonia in varying stages of organization. Some pulmonary edema was present as well as diffuse infiltrates of mononuclear cells in the alveolar septae. Some areas of metaplasia were present in and near the regions of pneumonia and were interpreted as secondary to the pneumonia.

The parasitic liver cysts seen grossly were confirmed microscopically. The liver sections stained for fat revealed individual variation of some degree but no significant deviation from normal. The coded slides of the livers were classed according to the descriptions in Table III while kidney and spleen were judged according to the criteria in Tables IV and V; Figures 1, 2, 3, 4, and 5 are

TABLE V
Classification of Morphologic Changes in Spleen

Class	Description
1	Average size follicles, little or no congestion, balance between lymphocytes, germinal epithelium and interstitial tissue.
2	Obvious congestion, no change in follicles or epithelium, volume of germinal centers less than volume of peripheral lymphocytes.
3	Germinal centers approximately same volume as peripheral lymphocytes, moderate congestion.
4	Increased number of lymphocytes, hyperplasia of germinal centers (greater than volume of peripheral lymphocytes).
5	Active hyperplasia: 2 classes of lymphocytes, (1) a zone immediately around germinal centers containing young lymphocytes surrounded by (2) a peripheral zone of small dark lymphocytes.
6	Lymphocytic hyperplasia extending to include most of the interstitium.



FIGURES 1-5. Rat livers, illustrating the morphological classes described in Table III: (1) Class 1; (2) Class 2; (3) Class 3; (4) Class 4; (5) Class 5. H & E stain, x 200.

illustrative of the class descriptions in Table III. There were no significant differences in the scoring of the groups, although there was a class "5" and no class "3" in the rats killed at the termination of the exposure, whereas there were no livers in class "5" among the control animals. There were no class "5" livers among the experimental rats killed two weeks after the exposure; the slides were evenly divided between scores of 3 and 4.

Kidney slides from the experimental animals were not graded differently than from the controls but all spleen slides from experimental animals received the highest score, differing significantly from the control spleens, although some controls also received such high scores.

Experiment 4

These preliminary tests seemed to indicate that vinyl chloride was an anesthetic gas which might also act as a lung irritant. In order to assess this feature of its action, to highlight significant pathological features and to avoid the potentiality of damage arising from anesthesia alone, five female and five male rats, matched with controls, were exposed for eight hours daily to five per cent vinyl chloride in air for 19 consecutive days. The experimental animals were placed within the chamber at 0830 and removed at 1630 and the control animals from 0000 to 0800 hours. To prevent contamination of food or water, both groups of animals were placed within the chamber in empty cages; hence experimental and control animals were deprived of food and water for eight hours daily. The requisite concentration of the vinyl chloride was attained initially as in Experiment 3, but the chamber was ventilated at 50 lpm.

Although the body weight of the experimental rats decreased initially, this trend was reversed by the fourth exposure, the rate of growth thereafter being no different than the controls. The drop in weight at the start and the subsequent resumption of growth was paralleled by an apparent increasing tolerance to the gas as the exposures continued. At the start, the intoxication of the rats was marked, instability of the hind legs being a prominent feature of the exposure. With each exposure, however, there was an obvious diminution of these symptoms, so that by the fifth or sixth exposure, it was not possible to distinguish any evident symptoms of neurological deficit.

Hemoglobin determinations during the ex-

TABLE VI
Formed Elements of the Blood at Termination of Exposure to 5 and 2% Vinyl Chloride

	RBC ×10⁶/mm³		WBC ×10³/mm³		Lymphocytes %		Neutrophils %	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Control—5% (9)	5.18	0.49	12.67	2.75	89.4	3.7	9.4	3.2
Expt.—5% (8)	5.87	0.75	7.96	1.85	92.3	3.5	6.3	3.1
"p" less than	0.05		0.01		NS		NS	
Control—2% (38)	6.50	0.62	11.34	2.68	84.3	4.80	12.62	4.71
Expt.—2% (29)	6.62	0.62	9.12	3.06	89.7	3.84	8.02	4.94
"p" less than	NS		0.01		0.01		0.01	

posure period revealed no difference between experimental and control groups.

On the twentieth day experimental and control animals were anesthetized with diethyl ether, blood was drawn by cardiac puncture and 1/9 volume of 0.1*M* sodium oxalate was added to the blood. The animals were then killed with an overdose of the anesthetic and autopsied. Measurements of hemoglobin, prothrombin time, hematocrit, red cells, white cells, differential white cells and serum transaminase were performed on the blood drawn. Serum transaminase, hematocrit values and prothrombin times were normal for both groups. Table VI lists the values for some of the formed elements of the blood. Monocytes and eosinophils formed only a small proportion of the white cells; no differences between control and experimental animals occurred. The red cells were somewhat elevated and the white cells lower in the experimental group. Table VII shows that the liver: body weight ratio of the experimental animals was significantly elevated.

All five male experimental animals exposed to 5% vinyl chloride had coats which were somewhat thinner than normal; the tails of these animals were scaly. The three female experimental animals and all the control animals had normal coats and tails. One male experimental animal had fibrous pleural adhesions on the left side; the fibrous nature of these adhesions suggested that the process was several weeks old and probably not related to the exposure. Both experimental and control animals had parasitic liver cysts. No differences in appearance, color, consistency or degree of congestion were noted between the livers of the two groups. The other organs and tissues were within normal limits in their gross appearance, with no differences between experimental and control groups.

TABLE VII
Liver and Spleen Weights of Rats Exposed to Vinyl Chloride

Group	Vapor Conc. (%)	Days on Expt.	Number of Animals	Sex	% of Body Weight			
					Liver		Spleen	
					Mean	S.D.	Mean	S. D.
Control	0	89	14	F	3.65	0.36	0.49	0.160
Expti.	2	92	14	F	4.76[a]	0.50	0.58[a]	0.068
Control	0	89	12	M	3.73	0.19	0.55	0.052
Expti.	2	92	15	M	4.76[a]	0.50	0.58[d]	0.060
Control	0	19	5	F	3.19	0.28		
Expti.	5	19	5	F	3.95[b]	0.51		
Control	0	19	4	M	3.17	0.46		
Expti.	5	19	5	M	3.71[b]	0.49		
Control[c]	0		4	F	4.98	0.20		
Expti.[c]	5-10	See Text	6	F	5.15[b]	0.35		
Control[c]	0		5	M	5.45	0.57		
Expti.[c]	5-10	See Text	4	M	6.09[b]	1.08		

[c]Pastured for two weeks
[a]"p" <0.001
[b]"p" <0.01
[d]"p" <0.05
[d]"p" <0.02
[e]"p" not significant

With the exception of the pleural adhesions in one animal, the microscopic appearance of all the organs and tissues was normal. The gross observation of parasitic liver cystes in all animals was confirmed microscopically. Liver sections from all animals were stained for fat, but none revealed evidence of increased fat nor were there any differences in intracellular fat between the two groups.

Classification of the liver slides for the morphological designations of Table III showed differences between the control and experimental groups. The mean score of the control group was 2.93 and that of the experimental group was 4.56, with only one control animal being graded "4" and no experimental animal being graded less than "4". The differences between the means was thus highly significant, yielding a "p" of less than 0.001.

No differences in kidney or spleen slides between experimental and control animals were noted.

Experiment 5

Because human exposure to vinyl chloride seems unlikely at concentrations of the gas much greater than the concentration causing signs of intoxication, that is, at 1.2 to 1.6%, the long term exposure of rats was conducted at a concentration of 2.0%.

Sixty rats, each weighing about 75 grams, were separated randomly into two groups of 15 males and 15 females and placed in eight separate cages. In the week before the exposure was started, the rats were observed, weighed twice and blood withdrawn for hemoglobin determination. The experimental animals were exposed in the 1100-liter chamber to 2.0% vinyl chloride for eight hours per day (0830 to 1630) on Monday through Friday for a period of three months. The control animals were exposed to 0.0% of the test gas, that is, to a flow of 50 lpm of air, in the same chamber as the experimental group for eight hours per day on the same days of the week. No food or water was present in the cages during either exposure. All rats were weighed at approximately weekly intervals; hemoglobin determinations, from tail blood, were made at monthly intervals. In neither body weight nor in hemoglobin values were there any significant differences between the control and experimental groups.

During the course of the exposure, there were five deaths; of these, four occurred in the control group. No data from these animals are included in any of the tables.

On the 89th day blood was withdrawn from the control animals under anesthesia as previously described (Experiment 4); these animals were then killed with ether and autopsied. A similar procedure was followed on the 92nd day for the experimental animals. The livers and spleens of all animals were weighed prior to fixation in formalin. The mean values of the tissue: body weight ratios are shown in Table VII. The differences in the means were in all instances significant, the livers larger and the spleens smaller in the experimental as compared with the control animals. No significant differences between the groups appeared in the values for hematocrit and prothrombin. The serum transaminase was not determined. Monocytes and eosinophils showed no differences between the groups; values for the other blood elements are shown in Table VI.

The external appearance of all animals was normal. Parasitic liver cysts were present in all animals. There were no differences in appearance, color, consistency or degree of congestion between the livers of the two groups. All other organs and tissues were similarly normal, no differences between the groups being apparent.

All the organs and tissues examined histologically were within normal limits, no path-

ology being evident in either experimental or control animals.

The parasitic liver cysts seen grossly were confirmed microscopically. Liver sections stained for fat revealed normal variation, but no animals had increased intracellular fat nor were there differences between the experimental and control animals. Graded in accord with the morphology in Table III, the liver slides revealed differences between the groups. The mean score of the control group was 1.58, that of the experimental group 3.63. No liver in the control group scored more than 2 and none in the experimental group scored less than 3; one liver scored 5. Because there was no overlap the differences between the means are highly significant.

There were no differences in score for the spleens in the two groups, but the kidney slides of the experimental animals scored significantly less than their controls.

Discussion

The data from the present investigation confirm the acute effects to be expected from various concentrations of vinyl chloride: concentrations below 1%, when exposure is limited to five minutes, cause no observable intoxicating effects; five minutes, however, is shorter than the time necessary to reach an equilibrium level in the circulation; from behavioral observations in the rat, it may be estimated that in five minutes some two-thirds of the equilibrium level is reached; consequently a concentration of 0.6 to 0.7%, if long continued would not produce intoxication. As the concentration rises above this level the intensity of the intoxicating signs increases until at a concentration of 7% the righting reflex is lost; at 10% the corneal reflex disappears and at concentrations of 15% and above respiratory failure takes place. Vinyl chloride thus acts as an anethetic gas, its depressant action increasing with increasing concentration of the gas in the air breathed, the corresponding increasing neurological deficits ending in death at concentrations greater than 15%. The purpose in exposing rats to concentrations causing anesthesia (8 to 10%) was an attempt to produce some singular or characteristic pathology. It cannot be said that this goal was achieved, the results being questionable and uncertain. Lung lesions were certainly present but these could not be ascribed with certainty to any irritant properties of the gas since they might well have arisen from the long-continued anesthesia; there was an apparent regression of these lesions in rats allowed a 14-day recovery period. The presence of pneumonia certainly raises the possibility of an acute toxic effect on lung tissue at these concentrations; however, the pneumonia could just as well be caused by secondary infection during the severe central nervous system and respiratory depression, an interpretation favored somewhat by the diffuse lesions and by the irregular occurrence of the pneumonia.

The kidney and liver changes described by Mastromatteo, *et al.*[3] in rats exposed for 30 minutes to 20, 30 and 40% of vinyl chloride, were not observed here, although 15 repeated 8-hour exposures to an anesthetic concentration is also a relatively severe stimulus. The findings in the lungs agree with the relative lack of effect found by Mastromatteo *et al.* in rats exposed for 30 minutes to 10% vinyl chloride, except that continued exposure, for days, does result in mortality. The relative lack of pathology as the result of 30 minute exposure at 10% found by Mastromatteo *et al.* would seem to support the view that lung lesions found after repeated exposures could well arise from the anesthesia and not from some action peculiar to vinyl chloride. There was no indication at either the 2% or 5% level of any untoward or other effects upon the lung tissue.

As an overall measure of general health, body weight and rate of growth are sensitive indicants. Even in the exposure to a concentration of 5%, which at first produces a marked and severe intoxication, the sharp drop in the weight of male experimental animals seen at the start of the exposure was soon reversed so that by the end of the 19-day exposure there was no difference between experimental and control rats of either sex. Body weight and rate of growth similarly showed no differences between the groups in the 2% exposure. It must be emphasized again that the growth of the rats during the 5% exposure support the observation of the rat's behavior which indicated that there was a rapid development of tolerance to the intoxicating effects of this concentration.

Neither the 2% nor the 5% concentrations caused changes in the prothrombin time, hematocrit or hemoglobin values. At both the 2% and the 5% concentrations, the white cell count was lowered significantly, although still well within the normal range. The increase in red cells, although significantly elevated in the 5% exposure, was not correlated with changes in the hemoglobin content. The increase in concentration of the vinyl chloride is associated with a greater fall in the white cells and a greater (and significant) increase in the red cells at the 5% level. Although lymphocytes and neutrophils are increased, only the change at the 2% concentration unlike the previous cell changes, reaches the statistically significant level. It is difficult to know if these changes have any toxic significance, since no tissue changes were seen upon microscopic examination that would account or be associated with a drop in the white cells or an increase in red cells. The decrease in spleen: body weight ratio is in a direction opposite to that usually associated with a severe drop in white cells; although the white cell count did not suffer a severe drop, the decrease was substantial at the termination of the exposure.

The only finding suggesting a specific toxic action of vinyl chloride is the increase in liver weight on exposure to 5% for 19 days and to 2% over 92 days. The increase in liver weight is not only highly significant statistically but is also substantial, amounting to a 30% increase over the controls. It is unfortunate that no information is available as to whether this increase is reversed on discontinuing the exposure. The increase in liver weight may be interpreted as indicating alterations in water, electrolytes and protein content of the liver parenchyma, but that such changes presage the development of actual histologic lesions is not certain in view of the wide range that the liver: body weight ratio may encompass (Table VII). Rather this increase may signify a non-specific response to metabolic derangements occasioned by mild and moderate intoxication for daily 8-hour periods. From the data obtained here, it is not certain that histopathological change would have occurred had exposures been carried out for longer times.

In the paper by Torkelson *et al.*, histopathological changes in the liver and increased liver:body weight ratios are reported in male rats exposed to 500 ppm vinyl chloride for 4.5 months. That there is no causal relation between the reported histopathology and the increases in liver:body weight ratio is evident from the extensive data gathered by these investigators. Female rats exposed to 500 ppm vinyl chloride for 4.5 months showed no statistically significant increase in liver weight but are reported to have histopathological changes in the liver. Female rats exposed to lower concentrations (100 and 200 ppm) for six months had significantly increased liver:body weight ratios but no pathology. On the other hand, rabbits of both sexes exhibited liver pathology without showing any increase in liver weight after exposure for six months to a concentration of 200 ppm. The authors correctly point out that organ:body weight ratios may well be artefactual, illustrating the point by the significant decrease in kidney weight found in female rats exposed to 50 ppm for six months; such rats exhibited no changes when exposed to higher concentrations. By the same token, but overlooked by the authors, the increased liver:body weight ratio in female rats at 100 and 200 ppm is with equal reason artefact because no statistically significant increase occurred at 500 ppm. Apparently, also, species differences are of importance in the reactivity to vinyl chloride, male guinea pigs suffering a significant *decrease* in their liver:body weight ratio when exposed to 100 ppm for six months.

There are six possible combinations of the presence or absence of pathology and increases, decreases and no change in liver weight. The one combination not observed

by Torkelson, *et al.* is histopathological change associated with a decrease in liver weight. Obviously, if five of the six possible combinations have been observed in a relatively small sample, no causal connection can be said to exist between these two measures.

Changes in the liver:body weight ratio may well have some toxic significance, but if they are unaccompanied by histopathological alterations, increased fat content or serum transaminase changes it is impossible to conclude that taken alone they signify much. Reference to Table VII will show that the liver:body weight ratios of the rats used as controls animals in the 5% exposure were significantly higher than the experimental animals in the 2% exposure. This illustrates well the fact that though control animals are used, unknown and non-specific changes in the environment, time of the year, temperature, diet, etc., may be responsible for changes in organ:body weight ratio without at the same time producing pathological alterations in the organ.

Similar considerations apply to the decrease in spleen weight; here, not only was pathology not observed, but there was no difference in the morphological character at either the 2% or 5% level between the experimental and control groups.

Unlike the interstitial and tubular changes in kidneys of rats exposed to 500 ppm of vinyl chloride reported by Torkelson, *et al.*, was the lack of any pathology in our animals exposed to 2% and 5% and the fact that the only morphological alteration in which a significant difference between control and experimental animals occurred (kidney at 2%) indicated that the control animals were further from "normal". Because it is unreasonable to attach toxic significance to changes associated with a control air exposure, it is our belief that the morphological alterations we have observed should not be interpreted as manifestations of pathology.

The concentrations to which the rats were subjected in these experiments were at least 40 times those used by Torkelson, *et al.* If any reliance is to be placed in a dose-effect relationship, pathology of some considerable degree should have been found in our experiments. Yet only morphological alterations of the character already described and pictured were seen, which, in our knowledge and experience, are of no pathological significance. Whether the explanation resides in a difference between the rat strains used by us and by Torkelson *et al.* or elsewhere is not known, but without additional data it is impossible to resolve the contradiction.

On the basis of the present data, and the seeming unimportance of the liver weight changes seen by Torkelson *et al.*, and without further evidence, a change of the present threshold limit value of 500 ppm seems unwarranted.

Summary

From 5-minute exposures of human subjects to concentrations of vinyl chloride ranging from 0.0 to 2.0%, it is estimated that a prolonged exposure to a level of more than 0.6% is necessary to produce minimum symptoms of intoxication. Rats exposed for up to two hours to higher concentrations exhibited moderate intoxication at 5%, lost their righting reflex at 7% and the corneal reflex at 10%. Respiratory failure occurred at 15%. If the exposure to 10% was long continued (two 8-hour daily exposures), mortality increased; death was apparently caused by a pneumonic process, but it was impossible to decide whether this was the result of a primary action or secondary to the anethesia.

Rats exposed eight hours daily to 5% for 19 days or to 2% over 92 days did not show any lung involvement. These levels had no effects upon growth rate, hemoglobin, hematocrit or prothrombin time. At both concentrations there were increases in the liver:body weight ratio and decreases in the white cells; at 2% the spleen:body weight ratio decreased and at 5% there was an increase in red cells.

No gross or microscopic changes were found in any tissue that was correlated with the changes in the formed elements of the blood. The increases in liver:body weight ratios were associated with morphological alterations in the liver which appeared to have no pathological significance, while no meaningful morphological alterations were evident in either kidneys or spleens.

Because, in the long term exposures, it was impossible to attach any toxic significance to the changes noted or to observe any histopathology, and because other aspects of the animals' reactions, such as growth rate, were unimpaired, the presently accepted threshold limit value of 500 ppm for vinyl chloride, one-fortieth of the concentration tested here, seems to offer an adequate margin of safety for human exposure.

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Sources and Use of Toxicological Information

The New York University Medical Center in cooperation with the American Industrial Hygiene Association will present a course on *Sources and Use of Toxicological Information.* The course will be given October 14-18, 1963, at the Onchiota Conference Center, Sterling Forest, Tuxedo, New York.

The course is designed to acquaint those concerned with advanced methods of securing and interpreting pertinent toxicological information. Application and principles will be stressed rather than details of experimental toxicology. The design of the course eliminates the need for specialized scientific background so that all administrative and technical persons having responsibilities for use of toxicological information can benefit. Guest lecturers who are specialists in selected fields will supplement the staff of N.Y.U. Institute of Industrial Medicine for presentation of the subject material. Areas to be covered are: biological principles involved, sources of toxicological information, applications to in-plant problems, Federal regulations, and requirements of various agencies.

Inquiries and registrations should be directed to New York University Medical Center, Institute of Industrial Medicine, 550 First Avenue, New York 16, New York. The registration fee is $150 with a deposit of $25 required. Accommodations (double occupancy) at the Onchiota Conference Center for the evening (dinner) October 13 through lunch October 18 are available at $21 per day including meals (payable to the Center on departure).

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FIBEROPTIC STOMOSCOPY. C. J. Lightdale, G. Posner, P. Sherlock and S. J. Winawer. Gastroenterology Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, N.Y.

The situation which allowed Beaumont to look into the stomach of St. Martin was unique at the time but has become commonplace with the creation of operative gastrointestinal stomas. Standard gastrointestinal fiberscopes are usually too large in diameter to pass easily through operative stomas. Since the fiberoptic bronchoscope has a much smaller diameter (ACMI 0.56 cm) than the standard duodenoscope (ACMI 1.25 cm), we decided to use the former as a stomoscope. The first 5 patients examined in this manner are presented. All had feeding gastrostomies. Their stomachs could not be examined by the usual endoscopic means because of esophageal obstruction. In the first patient a permanent esophageal narrowing was ruled out when the cardioesophageal junction was entered from the stomach and the esophagus examined. The functional nature of the patient's disorder was suspected but could not be confirmed by standard endoscopic or x-ray techniques. In the second patient the response to radiotherapy and chemotherapy of an adenocarcinoma of the cardia was documented and gastric biopsy, brushings and washings for cytology were obtained. The third patient had a pharyngolaryngectomy for carcinoma and was evaluated for the possibility of a gastric tumor. Stomoscopy and cytology studies were negative. The fourth patient had a stricture of the distal esophagus and was being evaluated for further dilation or surgery. The fifth patient was one year postresection for carcinoma of the cardia who developed a smooth stricture in the anastomotic area. The bronchoscope was passed to the area under fluoroscopic control and no evidence of recurrent tumor was found. Brush cytology was negative. The advantage of using the fiberoptic bronchoscope is that it is available on many thoracic services and could be shared by gastroenterologists. Its flexibility makes it safer than the more rigid instruments that are generally used for this purpose. Areas can be visualized that are blind or cannot be reached by the more rigid instruments.

LAPAROSCOPY IN ALCOHOL-INDUCED LIVER DISEASE

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Percutaneous liver biopsy with the Menghini needle will confirm the diagnosis of fatty liver, but cannot be relied on to reveal the extent of liver disease when it has reached an advanced stage. The investigation is based on 668 laparoscopies and 2080 biopsies performed in the last two years. Laparoscopy was performed on 22 patients out of 281 with fatty liver, and on 49 patients out of 146 with fatty liver and mesenchymal activity. There was alcohol abuse in 78 % of 83 patients with finely nodular cirrhosis (laparoscopy performed in 52 patients) and in 53 % of 83 patients with postnecrotic cirrhosis (laparoscopy in 46 patients). It is much easier to classify the disease by inspecting the liver through the laparoscope than by scrutinising a small biopsy specimen. In 38 % of cases the presence of finely nodular cirrhosis was not appreciated on biopsy. Blind percutaneous biopsy with the Menghini needle gave even worse results. Out of 83 cases of postnecrotic cirrhosis the proportion incorrectly classified was much the same (30 %).

The laparoscopic appearance of acute alcoholic hepatitis, fatty liver (alone and with periportal fibrosis), fatty cirrhosis (alone and with cholestasis), toxic-degenerative cirrhosis and postnecrotic cirrhosis (as an advanced form of alcohol-induced liver damage) are illustrated by characteristic examples. Zieve's syndrome is characterized by the presence of cholestasis, while the most striking feature of alcohol-induced siderosis is the chocolate-brown colour of the liver.

The main advantage of laparoscopy combined with needle biopsy of the liver under direct vision is that it enables the physician to establish the diagnosis with certainty.

POSITIVE ESOPHAGEAL CYTOLOGY WITHOUT DETECTABLE NEOPLASM. H.N. Maimon and A.E. Cocco.

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The early diagnosis of esophageal cancer is the only hope of alleviating an otherwise poor prognosis. We have seen four patients in whom positive cytology specimens were obtained during evaluation for dysphagia. Carefully performed roentgenography, endoscopy and multiple biopsies failed to demonstrate a lesion. The patients were followed with diagnostic procedures for 6 to 30 months before a lesion was defined. One patient was explored and had hiatus hernia repair with no other lesion visualized. All patients eventually had exploration from an abdominal approach with endoscopy from above with discovery of primary esophageal carcinoma in each case. The question of appropriate management of patients who demonstrate positive cytology of the esophagus and negative concomitant diagnostic studies is raised.

HEREDITARY HEMORRHAGIC TELANGIECTASIA (HHT) AND DIFFUSE GASTRIC POLYPOSIS (DGP): VALUE OF ENDOSCOPY IN DIAGNOSIS AND TREATMENT

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A patient with HHT was found to have recurrent upper gastrointestinal bleeding due to DGP, a combination of findings not previously reported. Serial endoscopic examinations and gastrophotography permitted not only an accurate diagnosis as to the source of the bleeding (DGP), but also documented recurrence after initial gastric resection and then a marked progression in the size and number of polypid masses, which ultimately involved the mucosa of the lower esophagus and entire gastric remnant. Sigmoidoscopy, x-rays of the colon and small bowel and liver scan were normal. Selective celiac and mesenteric arteriography revealed no vascular abnormality. A fasting gastric aspirate was negative for malignant cells, and had a PH of 6.7 with 4 meq/liter of acid (volume 14 ml.). Serum gastrin was normal (142 PG per ml.). Immunoglobulins IGA and IGG were low (83 and 260 mgs. %, respectively). Because of the high incidence of malignancy associated with DGP, total gastrectomy was performed; pathologic sections showed diffuse benign polyposis without neoplasm. The patient recovered and has had no evidence of recurrent lesions or anemia. The frequent association of atrophic gastritis and hypochlorhydria with both HHT and DGP is an interesting observation, but an uncertain factor in the pathogenesis of the lesions found in our patient. Endoscopy is the most valuable diagnostic procedure in patients with gastric polyposis and should be performed periodically after sub-total gastric resection; if recurrences develop total gastrectomy is a procedure of choice.

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HAEMANGIO-ENDOTHELIAL SARCOMA OF THE LIVER

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Plates X–XIV

Haemangio-endothelial sarcoma of the liver, also known as haemangio-sarcoma and Kupffer-cell sarcoma, is an extremely rare tumour. Videbaak (1946) reported two peaks in the age distribution of this tumour, one at 8 mth and the other at 49 yr. Blumenfield, Fleming and Johnson (1969) reviewed the literature on juvenile haemangio-endothelioma of the liver and found 37 cases, only five of which were solitary tumours. Ninard (1950, cited by Edmondson, 1958) accepted 44 published cases in adults.

In the present communication we report the three adult cases of haemangio-endothelial sarcoma of the liver that have been seen in this department since 1900. In particular we wish to draw attention to the difficulties that were presented by two needle biopsies in one of these patients, and to an attempt that was made to exclude a possible occupational factor in one case.

Materials and methods

The three cases occurred in two males aged 55 and 51 yr, and in an 81-yr-old female. Tissues collected at necropsy were fixed in 4 per cent. formaldehyde in saline and embedded in paraffin wax. Sections were stained as a routine with haematoxylin and eosin (HE), and by Masson's trichrome method. Selected sections were stained for reticulin (Gordon and Sweets' method) and by the periodic acid-Schiff (PAS) method.

Case reports

Case 1

A male fibre-glass worker aged 55 presented in Feb. 1970 with a 3-mth history of generalised pruritis and increasing mental apathy. He admitted to moderate alcoholic excess, but gave no previous history of jaundice. Clinical examination revealed mild icterus marked facial telangiectasis with a few spider naevi, and early bilateral Dupuytren's contracture. The liver was enlarged and was palpable three finger-breadths below the right costal margin. Liver scan showed a diffuse uptake abnormality compatible with cirrhosis. Liver biopsy was performed. He was treated subsequently with prednisolone, phenergan, amitriptyline and cholestyramine with some transient subjective improvement. However, there was continued objective evidence of progressive hepatic dysfunction, and, when he was readmitted in hepatic precoma in Aug. 1970, a liver scan showed a more severe diffuse reduction in isotope uptake. A further liver biopsy was obtained. There was slow deterioration and death in hepatic failure occurred in Dec. 1970.

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Necropsy. The body was jaundiced, and there were numerous spider naevi. The peritoneal cavity contained 500 ml of free fluid. The liver weighed 2 kg, and showed evident macronodular cirrhosis. On section several cystic areas were present, and in addition there was diffuse, mottled, pale yellow and haemorrhagic tumour. No metastases were found. There was bilateral pulmonary oedema and congestion.

Case 2

A male shipyard worker aged 51 was first admitted in June 1963 with a 3–4-wk history of listlessness, progressive abdominal swelling, ankle oedema and occasional diarrhoea. A clinical diagnosis was made of hepatic cirrhosis with portal hypertension, oesophageal varices and hepatocellular failure. Diuretic therapy effected some improvement in his ascites and oedema, but his general condition deteriorated and he died in Aug. 1963.

Necropsy. The body was jaundiced, and there was gross bilateral ankle oedema, and 1 litre of free fluid in the abdomen. The liver weighed 2·6 kg. The right lobe was largely replaced by an irregular tumour 10 cm in diameter, haemorrhagic and partly necrotic. The left lobe showed a macronodular cirrhosis. Oesophageal varices were present and the spleen was enlarged to 310 g. The lungs showed bilateral lower-lobe oedema. No metastatic tumour was found.

Case 3 (Grimsby General Hospital)

A housewife aged 80 was first referred to hospital in May 1967 because of recent rapid and severe increase in abdominal girth, accompanied by increasingly severe central abdominal pain of approximately 12 months' duration. She was grossly obese, with some peri-umbilical bruising, and an ill-defined abdominal mass was palpable. She was not considered suitable for laparotomy, and died 2 wk after admission.

Necropsy. There was an excess of fluid in the peritoneal cavity. The liver weighed 2·8 kg, and was largely replaced by haemorrhagic, partly necrotic tumour, with some related areas of fibrosis. The lungs showed mild chronic bronchitic changes and bilateral basal bronchopneumonia.

MICROSCOPIC FINDINGS

Case 1

The two biopsies submitted showed similar features, but posed considerable diagnostic difficulty. Macronodular cirrhosis was present and, in addition, a continuing active hepatitic process, characterised by a moderately intense lymphocytic and plasma-cell infiltration of the portal areas and fibrous septa, with erosion of the limiting plates (fig. 1). The most striking feature, however, was a fairly generalised hyperplasia of what appeared to be Kupffer cells. These cells were larger than normal Kupffer cells, and in some areas lined dilated sinusoidal spaces. In other areas small groups of individual hepatocytes were surrounded by the proliferating cells resembling littoral cells (fig. 2). Mitoses were not noted. Although a differential diagnosis of haemangio-endothelioma was considered at this time, the possibility of the changes being reactive, possibly to fibre-glass or some other exogenous agent, was also entertained, particularly since no solid tumorous aggregates were seen. Post-mortem histological examination of the liver clearly established a diagnosis of haemangio-endothelioma. Areas similar in appearance to those shown in figs. 1 and 2 were readily found (fig. 3), but, in addition, there were areas of clearly neoplastic tissue composed of elongated spindle-shaped cells. Mitotic activity was low. Large blood-containing spaces lined by tumour cells were

HAEMANGIO-ENDOTHELIAL SARCOMA OF THE LIVER

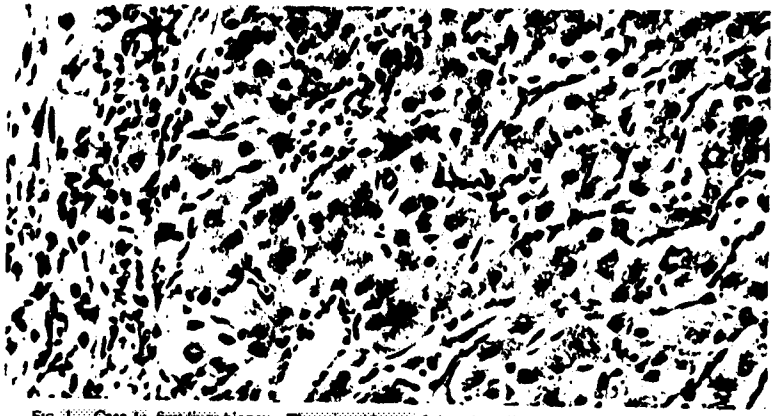

FIG. 1.—Case 1; first liver biopsy. There is evidence of chronic active hepatitis with cirrhosis (left). There is generalised hyperplasia of Kupffer cells. Haematoxylin and eosin (HE). ×250.

FIG. 2.—Case 1; second liver biopsy. Generalised Kupffer-cell hyperplasia is again evident, and a clump of hepatocytes (arrow) is completely surrounded by tumour cells. HE. ×250.

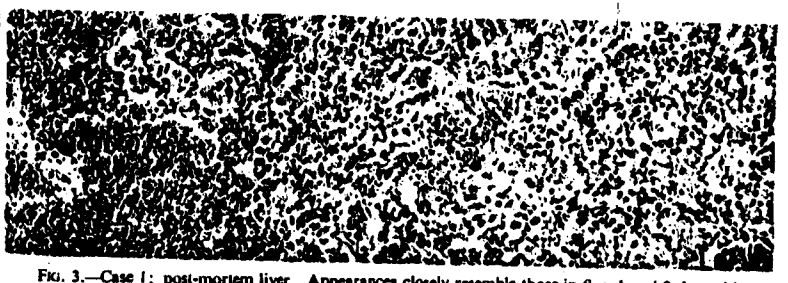

FIG. 3.—Case 1; post-mortem liver. Appearances closely resemble those in figs. 1 and 2, but with additional large vascular spaces and some small aggregates of spindle-shaped tumour cells. HE. ×100.

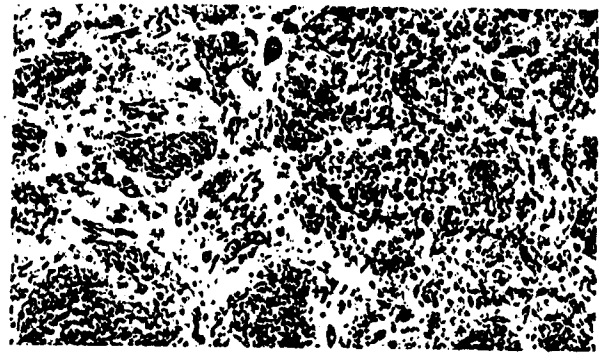

FIG. 4.—Case 1; post-mortem liver. Numerous large tumour-cell-lined vascular spaces have replaced normal hepatic parenchyma. HE. ×100.

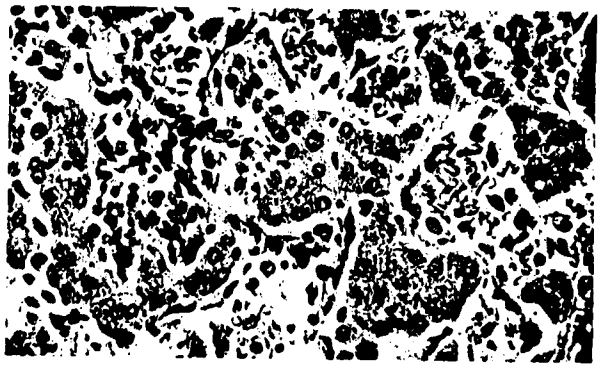

FIG. 5.—Case 1; post-mortem liver. Small tumour-cell-lined vascular spaces are seen; note also the angioformative pattern of the tumour. Foci of extramedullary haemopoiesis are present within some vascular spaces. HE. ×250.

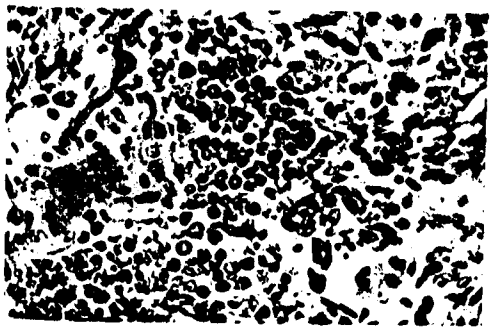

FIG. 6.—Case 1; post-mortem liver. Large focus of haemopoiesis present within central vascular channel. HE. ×250.

HAEMANGIO-ENDOTHELIAL SARCOMA OF THE LIVER

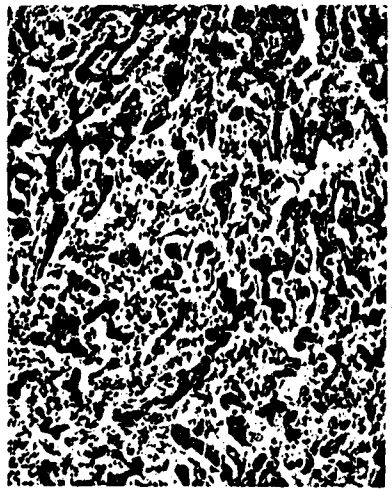

FIG. 7.—Case 2. Diffuse tumour-cell infiltration of the liver with separation of hepatic cords and hepatocytes. HE. ×100.

FIG. 8.—Case 2. High-power view of tumour-cell infiltration of liver. These cells are less intimately related to the hepatocytes than in case 1. The spindle-cell appearance of the tumour cells, pleomorphism, and nuclear hyperchromatism are well marked. HE. ×250.

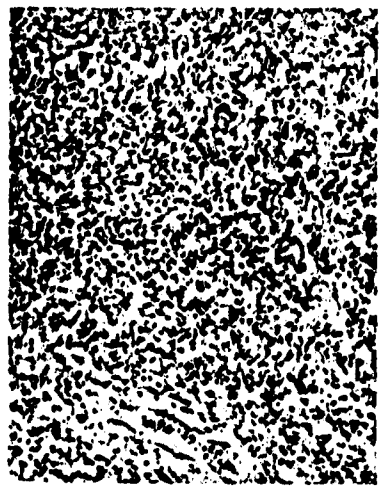

FIG. 9.—Case 2. Area in which the tumour is compact and of spindle-cell composition. HE. ×100.

FIG. 10.—Case 2. High-power view of the more compact area of tumour. The angioformative pattern, however, is fairly well shown. HE. ×250.

HAEMANGIO-ENDOTHELIAL SARCOMA OF THE LIVER

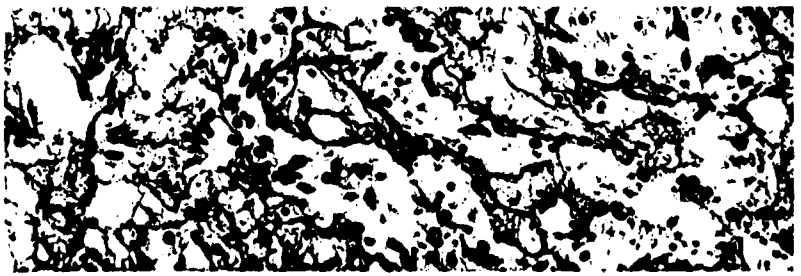

FIG. 11.—Case 2. Reticulin staining shows clearly the angioformative characteristics of the tumour, with numerous focal connexions between tumour-cell-lined vascular channels. Gordon and Sweets' reticulin. ×250.

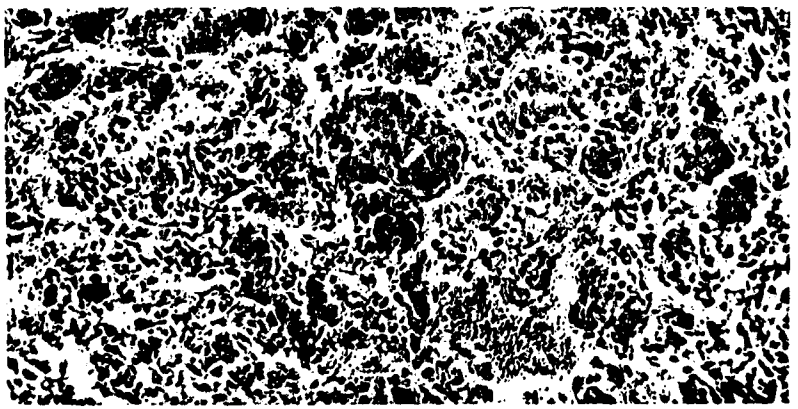

FIG. 12.—Case 3. Areas of compact spindle-shaped cells, numerous vascular spaces and diffuse Kupffer-cell hyperplasia. HE. ×100.

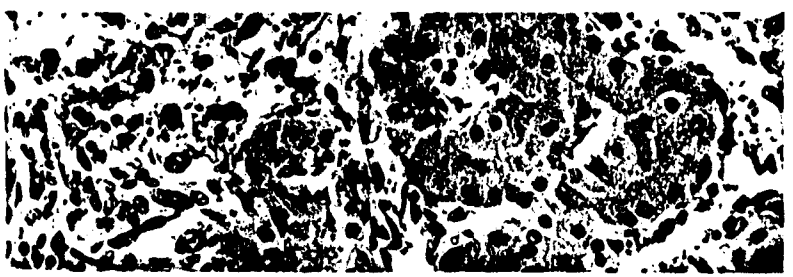

FIG. 13.—Case 3. High-power view to show tumour-cell aggregates (left) and the intimate relation between tumour cells and hepatocytes (right). HE. ×250.

HAEMANGIO-ENDOTHELIAL SARCOMA OF THE LIVER

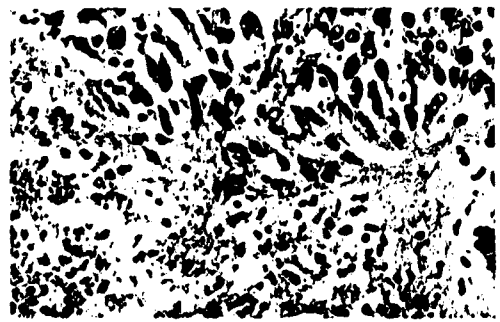

FIG. 14.—Case 3. Hypercellular tumour-cell aggregates totally replacing liver parenchyma. Mitotic figures are seen, and there are extensive areas of necrosis. HE. ×250.

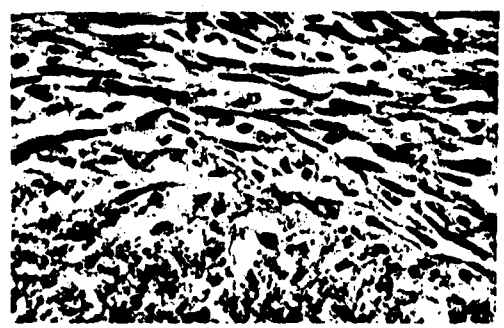

FIG. 15.—Case 3. Anaplastic elongated tumour-spindle cell aggregates with no distinct vascular pattern, and a related area of necrosis. HE. ×250.

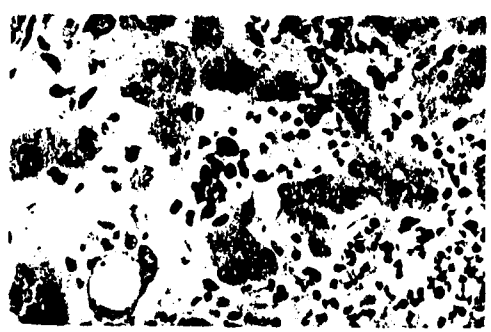

FIG. 16.—Case 3. Focus of extramedullary haemopoiesis intravascularly in centre of field. HE. ×250.

present diffusely throughout the liver (fig. 4). In addition there were smaller tumour-cell aggregates that showed a well-defined angioformative appearance (fig. 5). In some areas the tumour was of a more compact spindle-cell appearance (fig. 6), with moderate cellular pleomorphism and occasional mitotic figures. Foci of extramedullary haemopoiesis were widespread and always appeared within the vascular channels of the tumour (figs. 5 and 6).

Case 2

Diffuse neoplastic infiltration with disruption of hepatocyte cords was also evident in this liver (fig. 7), but the relation between the tumour cells and the hepatocytes was less intimate than in the previous case (fig. 8). There was more marked cellular pleomorphism, both in the infiltrating areas of tumour and in the areas almost entirely composed of tumour cells (figs. 8 and 9). The cells were distinctly spindle-shaped, with many elongated and mainly hyperchromatic nuclei (figs. 9 and 10). Mitotic activity was again low. Reticulin stains, even in the more compact areas of the tumour, clearly demonstrated its angioformative nature (fig. 11); the tumour cells lay within the reticulin framework and lined blood-containing channels. In contrast with the findings in case 1 there were few large vascular channels, and no foci of extramedullary haemopoiesis were found.

Case 3

The intimate relationship between the tumour cells and individual and groups of liver cells was again evident in this case (figs. 12 and 13). There was mild pleomorphism in these areas, but this was considerably less than that seen in more compact areas of the tumour (figs. 14 and 15). In these areas, elongated spindle-shaped cells that showed a moderate degree of mitotic activity formed solid tumour masses in which, both on HE and reticulin staining, no vascular channels were readily seen. Extensive necrosis was present in relation to these hypercellular anaplastic sarcomatous areas (figs. 14 and 15). Transition between the diffuse (fig. 12) and compact areas of tumour (fig. 15) was easily found. Foci of extramedullary haemopoiesis were widespread within this tumour and, as in case 1, these were present within vascular spaces lined by tumour cells (fig. 16).

EXAMINATION OF LIVER FROM CASE 1 FOR THE PRESENCE OF FIBRE-GLASS PARTICLES (J. F.)

A series of techniques were used in an attempt to establish the presence of fibre-glass particles in the liver.

Microscopic examinations

Randomly selected samples of liver were cut with a microtome and mounted in water between a microscope slide and coverslip. They were examined under normal and polarised light at a magnification of ×80. Both transmitted and reflected light were used.

In a variation of the technique a low-power laser beam was used to illuminate

the sample with incident light. Trial experiments had shown that in these circumstances fibre-glass gave a characteristic pattern, and a glass fragment was highly reflecting.

On the basis that glass is highly unreactive, further samples of the liver were digested in concentrated hydrochloric acid and the residue was examined. Other samples were gently pyrolysed to ash and after the ash had been digested in hydrochloric acid, both the ash and the residue were examined.

None of these experiments showed the presence of artefacts that could be attributed to fibre-glass or glass fragments.

Ash content

The abnormal liver and samples of normal liver were dried to constant weight *in vacuo*, and their ash content was determined after pyrolysis. The abnormal liver had a dry-weight ash content of 3·15 per cent. The corresponding figure for the normal liver was 3·78 per cent.

These experiments provided negative results, but they do not totally exclude the presence of fibre-glass particles, although this seems highly unlikely.

Discussion

In the years 1900–1969 inclusive, 120 cases of primary malignant tumours of the liver have been studied *post mortem* at the Western Infirmary, Glasgow (MacSween, unpublished observations). Of these, cases 1 and 2 in the present report are the only instances of haemangio-endothelial sarcoma, an incidence of 1·6 per cent. Hepatic cirrhosis, cryptogenic, and of macronodular pattern, was present in cases 1 and 2. As 501 cases of cirrhosis were examined in the 70 years of review, this represents an incidence of 0·4 per cent. It is thus evident that haemangio-endothelial sarcoma of the liver is an extremely rare tumour in this area, and a very infrequent complication of hepatic cirrhosis.

The pathogenesis of haemangio-endothelial sarcoma of the liver is not clear. In approximately 40 per cent. of those reported in the literature the liver was also the site of cirrhosis, but why such a tumour (or even hepatocellular carcinoma or cholangiocarcinoma) should arise in the cirrhotic liver is not understood. Cases of haemangio-endothelial sarcoma of the liver have been reported after thorium dioxide administration (MacMahon, Murphy and Bates, 1947; Horta, 1953, 1956; Lüdin, 1953; Fruhling, Gros and Batzenschlager, 1955; Tesluk and Nordin, 1955; Wuketich and Mark, 1957; Edmondson, 1958; Looney, 1959, cited by Suckow, Henegar and Baserga, 1961; Rosenbaum, 1959), and in Lüdin's case thorotrast was demonstrated within the tumour cells. Hepatocellular carcinoma and cholangiocarcinoma, also, may develop after thorotrast administration, as well as other tumours in various extrahepatic sites (Suckow *et al.*; Grampa, 1971). Chronic arsenic intoxication also has been implicated in the development of haemangio-endothelial sarcoma of the liver (Roth, 1957, cited by Regelson *et al.*, 1968; Regelson *et al.*). In Roth's series of 82 cases of German vintners exposed to chronic arsenic intoxication from insecticide sprays and dusts, there were 28 cases of hepatic cirrhosis, eight of

which were complicated by haemangio-sarcomas—a considerably higher incidence in cirrhosis than has been reported in any other review. Ross (1932) described a malignant angioformative tumour of the liver and attributed its development to prolonged gamma-ray exposure from a lost radium needle. No particular aetiological factor could be cited in any of the present three cases. In particular the presence of fibre-glass particles could not be demonstrated in the liver in case 1.

The features seen in the two needle biopsies obtained in case 1 presented considerable diagnostic difficulty in interpretation, and in the absence of frankly malignant tumour aggregates, the appearances were described as those of a diffuse Kupffer-cell hyperplasia. Baker, Paget and Davson (1956) drew attention to the presence of hyperplastic Kupffer cells in areas remote from the primary haemangio-endothelioma, and advanced this as evidence in favour of the Kupffer-cell derivation of the tumour type. These field changes were seen in all of the present cases, and it was relatively easy to see a gradual transition from an apparent in-situ Kupffer-cell hyperplasia to frankly malignant tumour-cell aggregates. Hastings-James (1949) considered that the appearances remote from the tumour were caused by intrahepatic infiltrative spread of malignant cells. It seems unlikely, however, that the degree of invasion could be as extensive as we have seen in the present material, as Baker *et al.* also point out. Furthermore, careful examination, particularly in cases 1 and 3 (figs. 2 and 13), showed apparently malignant cells lining hepatic sinusoids and there were no areas in which a double layer of " intact " Kupffer cells and tumour cells could be defined.

Examination of our three tumours leads us to agree with the suggestion that haemangio-endotheliomas may be derived from Kupffer cells, and the angioformative properties of the malignant cells in our cases are therefore not surprising.

Foci of haemopoiesis, present in two of our cases, are quite a common finding (Edmondson). During foetal life, and in conditions of infiltrative marrow replacement, Kupffer-cell differentiation into haemopoietic stem cells is well recognised. The occurrence of extramedullary haemopoiesis in these tumours could thus be interpreted as a further point in favour of their Kupffer-cell histogenesis.

A fairly rapid downhill course was followed in all three patients, and such a clinical pattern of behaviour is well documented (Buntine, Lyall and Renowden, 1971). Metastatic tumour spread did not occur, but in previous reports approximately 50 per cent. have shown metastases, involving lung, portal lymph-nodes, and other abdominal viscera, including occasionally the spleen (Edmondson).

Summary

Three cases of haemangio-endothelial sarcoma of the liver are described. The histological features in these cases are outlined in detail and in particular attention is drawn to the appearances seen in one of these cases on liver

biopsy, appearances that are considered as suggestive evidence of the Kupffer-cell origin of these tumours. The previous literature on this type of tumour is briefly reviewed.

We are grateful to Dr G. Watkinson for permission to publish case 1.

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THE DISTRIBUTION OF RADIOACTIVE LEAD (^{210}Pb) IN THE CEREBELLUM OF DEVELOPING RATS

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PLATES XV–XVII

ALTHOUGH toxic effects of lead have been known from early times, it was not until the eighteenth century that its effects on the nervous system were studied. Since then many investigations have been made on human material.

Pentschew and Garro (1966), who introduced the first experimental model of lead encephalopathy by using newborn rats and poisoning them through their mothers' milk, produced severe oedema and haemorrhage of the brain, mainly in the cerebellum. From their experiments, Pentschew and Garro postulated that lead encephalopathy, like Wernicke's encephalopathy, was due to a chronic metabolic dysoxidosis of the capillaries.

Ule, Kolkmann and Brambring (1967) concluded that the oedema of Wernicke's encephalopathy owed its origin to a hydropic swelling of the neuropil, that developed independent of vascular involvement. This view was further substantiated by the work of Wegener, Kolkmann and Rein (1968) by means of an autoradiographic study using radioactive-labelled serum albumin.

Thomas, Dallenbach and Thomas (1971) in further studies on experimental lead encephalopathy in suckling rats demonstrated degenerative changes in the endothelial cells of numerous cerebellar capillaries. This finding was associated with massive oedema, platelet thrombi and Purkinje-cell changes. Whether the damage to the capillary endothelial cells represented a direct toxic action of lead, or an indirect injury due to a vitamin deficiency or a deficiency of other substances, could not be determined.

In an effort to localise lead in the developing rat cerebellum, we have injected radioactive lead intraperitoneally and studied the cerebellar tissues by means of light-microscope and electron-microscope autoradiography.

MATERIALS AND METHODS

Four Wistar female rats, each with a 1-day-old litter, were used for this experiment. Two litters served as controls and two litters as the experimental groups. One experimental group was given an intraperitoneal injection of 10 μCi and the other one of 5 μCi of radioactive lead (^{210}Pb). Individual rats from each group were killed at 1, 3, 6, 24, 72 and 168 hr after the injection. Before the animals were killed, a heparin anticoagulant (Liquemin) with a short-acting barbiturate as anaesthetic was given intraperitoneally. The vascular system was perfused with 3·5 per cent. glutaraldehyde through the left cardiac ventricle, at

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