Results of Exposure of Strain C3H Mice to Chloroform.* (20389)

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The following is a report of an accidental exposure of mice of different strains to chloroform, and of later experimental exposures of strain C3H mice to this substance with descriptions of the lesions observed. Late effects of the exposure have been determined and the experiment offers a possible explanation for some puzzling lesions of the kidney previously observed in mice(1). The most remarkable changes were found in this organ.

Accidental exposure. A large number of male mice of strains C3H, $C3H_f$ (strain C3H without the mammary tumor agent), A, and HR in some of the colonies at the National Cancer Institute died in July 1949. In one of the colonies all of the breeding males of strains C3H and $C3H_f$ died and in order to continue the strains it was necessary to use males obtained from litters of animals which had not been weaned or were born later. A smaller proportion of the strain A and strain HR males died than of strain C3H or strain $C3H_f$. No deaths occurred among the females of any of these strains nor among 57 $C3H_f$ males which had received 5-8 mg pellets of 10% stilbestrol in cholesterol several months previously. Mice of strains C57BL, C57BR/cd, C57L, and ST which were kept in the same rooms with the other strains were not affected. The kidneys of the male mice dying at that time were enlarged and pale in color, and the kidneys of those surviving longer developed calcification. The cause of these lesions was not immediately determined but it was thought possible that it was the result of accidental exposure to chloroform. A few days prior to the first death a carboy containing chloroform had been broken on the same floor where the animals were housed but at the other end of the building. Thus, any exposure must have been of low concentration although it may have lasted 24 hours or more.

Experimental exposure. Since more strain C3H males than those of any other strain had died following the accidental exposure, C3H mice were used for the experimental exposure to chloroform. The animals were exposed in an apparatus† which was similar in all essential aspects to that used by Werner, Mitchell, Miller, and von Oettingen(2). Following the methods used by these authors, 3 groups of 6 male and 3 groups of 6 female strain C3H mice 2 months old were exposed to a flow of air containing approximately 5 mg (ranging from 3.38 mg to 5.4 mg) of chloroform per liter of air for one, 2, and 3 hours respectively. (None of the doses administered was sufficient to produce anaesthesia in any of the animals.) In addition 3 groups of 6 male and 3 groups of 6 female strain C3H mice 8 months old were also exposed for one, 2 and 3 hours respectively to doses with the same range. Groups consisting of 22 untreated males and 20 untreated females were maintained as controls. All of the animals were housed in plastic cages and were constantly supplied with Derwood pelleted food and tap water. The animals were observed daily for deaths and were examined at weekly intervals for the presence of tumors or any abnormal condition. Autopsies were performed on all the experimental animals when they died, appeared moribund, or in respect to the females, when mammary gland tumors developed. Seventy-two experimental animals, 17 control males, and 19 control females were examined. Microscopic sections of both kidneys, the liver, spleen, lung, and mammary tumors if present were made routinely. The tissues were fixed in Fekete's modification of Tellyesniczky's fixative (70% ethyl alcohol, 20 parts; formalin, 2 parts; glacial acetic acid, 1 part) and stained with hematoxylin and eosin.

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TABLE I. C3H Mice Exposed to Chloroform.

Conc. of chloroform, mg/l of air	Exposure, hr	Age, mo	Males		Females	
			Interval before death	Kidney lesion*	Interval before death	Kidney lesion
4.5	1	8	5 mo	L†	4 mo	0
			7	L	6	A
			7	L	8	0
4.8	1	8	5 days	E	3	0
			6	I	3	0
			8	I	5	A
5.2	1	2	15 mo	A	8	0
			18	L	10	0
			18	0	11	A
5.4	1	2	17	L	6	0
			17	L	8	0
			17	L	10	0
4.9	2	8	2 days	E	42 days	0
			6	I	49	0
			11	I	2 mo	0
4.6	2	8	1 day	E	37 days	A
			1	E	44	0
			6 days	E	7 mo	A
4.6	2	2	6	I	11	A
			6 mo	L	12	A
			14	L	13	A
4.7	2	2	2 days	E	5	0
			7	I	9	0
			7	E	11	0
5.4	3	8	2	E	49 days	0
			4	E	4 mo	0
			4	E	4	0
3.38	3	8	7	I	2	A
			7	I	3	A
			8	I	3	A
4.4	3	2	14 mo	L	8	0
			15	L	9	0
			17	L	10	0
3.84	3	2	8 days	I	8	A
			11	I	8	0
			11	I	10	0

* See text for description of early, intermediate, and late kidney lesions.
† L = Late; E = Early; I = Intermediate; A = Amyloid.

Results. The observations made after the controlled exposure to chloroform were in agreement with those made following the accidental exposure. Following exposure to air containing approximately 5 mg of chloroform per liter for one, 2, or 3 hours all of the strain C3H male mice showed gross evidence of kidney damage whereas none of the females did. Death occurred as early as one day after exposure in the 8-month-old mice and as early as 2 days after exposure in the 2-month-old group. By 11 days following exposure 15 of the 18 males of the former group and 7 of the 18 males of the latter group had come to autopsy. The remainder of the male animals exposed at 8 months of age lived from 5 to 7 months while the remainder of those exposed at 2 months of age, lived from 14 to 18 months.

TABLE II. Strain C3H Controls.

Sex	Kidney lesion	No. mice	Avg age, mo
♂	0	6	15.5
♂	A*	11	15.4
♀	0	12	12.5
♀	A	7	13.4

* A = Amyloid.

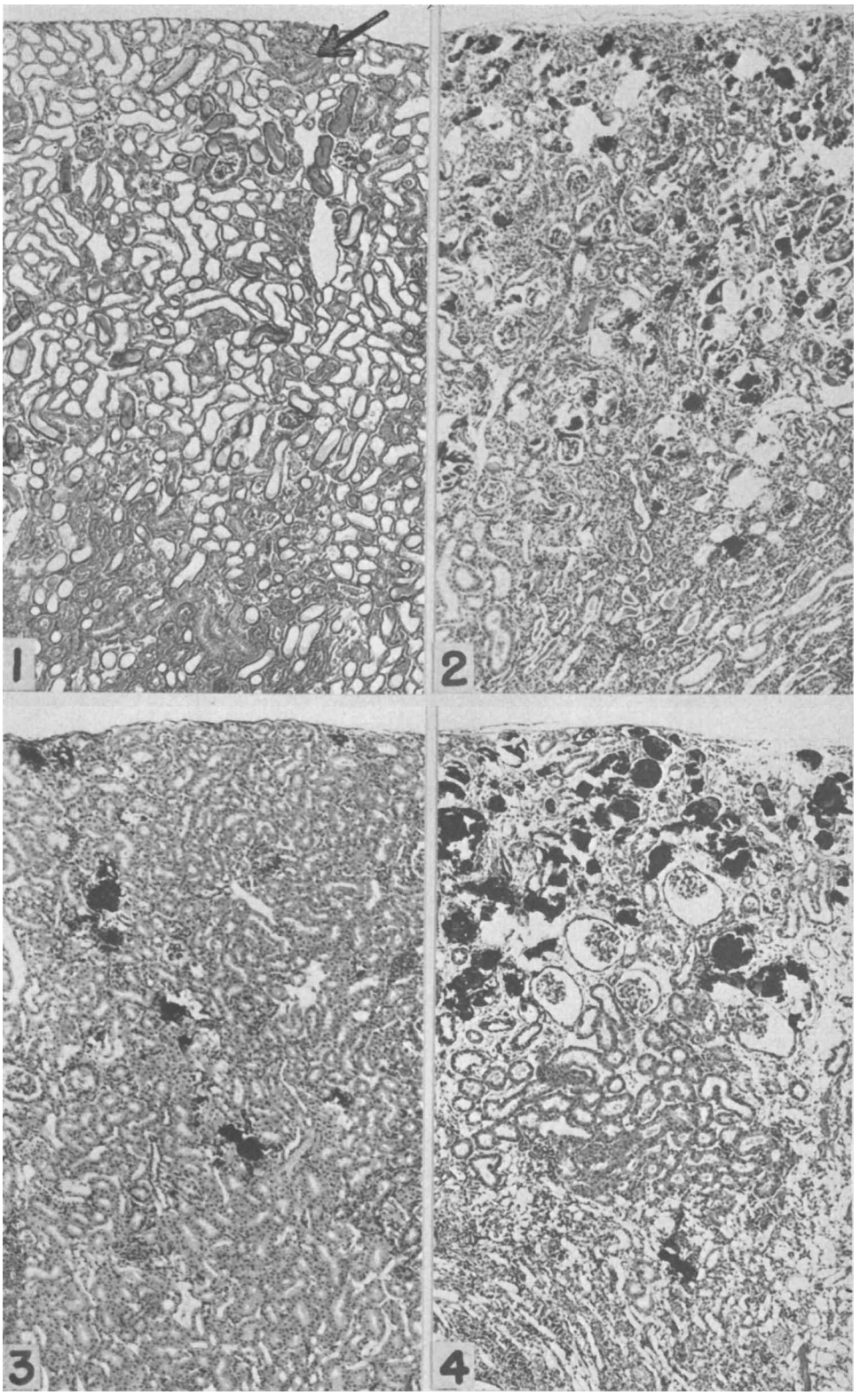
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FIG. 1. Early. C3H ♂, death 1 day after exposure to 4.6 mg chloroform/liter of air for 1 hr. Epithelium of proximal convoluted tubules (outer zone of cortex) and of portions of distal convoluted tubules is generally necrotic. The lumens of these segments of tubules appear dilated. The glomeruli, the cuboidal lining of Bowman's capsule, and the straight portion of the proximal convoluted tubules are relatively unaffected. In a small area (marked by arrow) in the outer zone of cortex, the tubular epithelium is preserved. Occasional hyaline casts are seen. Hematoxylin-eosin. 68 ×.

FIG. 2. Intermediate. C3H ♂, death 8 days after exposure to 3 mg chloroform/liter of air for 3 hr. Necrotic areas are granular and basophilic, suggesting early calcification. Occasionally Bowman's space and tubular lumens appear dilated and the cuboidal lining cells flattened. Hyaline casts are seen in the convoluted and collecting tubules. Hematoxylin-eosin. 68 ×.

FIG. 3. Late. C3H ♂, killed 12 mo after exposure to 4.46 mg chloroform/liter of air for 3 hr. Calcified masses in cortex of a kidney which appears normal otherwise. Hematoxylin-eosin. 68 ×.

FIG. 4. Accidental exposure. HR ♂, 2 mo after exposure. Outer zone of cortex shows many calcified masses and a few surviving tubules. Many glomeruli present, Bowman's spaces greatly dilated, and normal cuboidal capsular epithelium of male is flattened. Tubules of outer zone of medulla (straight portion of proximal convoluted tubules) are dilated and the epithelium is hypertrophied. Hematoxylin-eosin. 68 ×.

An effect of the length of the time of exposure was noted in the males. Some of the animals in each group died shortly after the exposure and some lived for a considerable period of time. However, more of the males which were exposed for 2 or 3 hours died early than did those exposed for one hour. The various degrees of kidney damage were present in some of the mice after either the one-, 2-, or 3-hour exposures. Seven of the females of the group exposed at 8 months of age were killed within one to 2 months following exposure to chloroform because of the development of mammary tumors. The remainder of the experimental females lived from 3 to 13 months. Control males lived to an average age of 15.5 months and control females to an average age of 12.5 months.

Histologic observations. Descriptions of liver damage produced in mice by chloroform are already available(4). In 4 male mice dying within 1 to 6 days after exposures of 4.6 to 5.4 mg of chloroform per liter of air hepatic necrosis similar to the lesion described by Eschenbrenner(4) was found, but any such damage to the liver did not persist in males living for a longer period. No female mice died in the early period and no evidence of liver damage was seen in those dying later. No histologic changes related to chloroform exposure were found in the spleen or lungs of either males or females.

The *kidney* showed the most significant alterations. Histologic sections of the kidney were divided into the following groups: 1) Early group. Male mice dying within the first 5 days after exposure; 2) Intermediate group. Male mice dying from 6 to 11 days after exposure; 3) Late group. Male and female mice surviving for a period of several months; 4) Amyloid group. Unexposed control and exposed male and female mice which showed little or no calcification of the kidney, but had an extreme degree of amyloid infiltration; 5) Normal group. Unexposed control and exposed male and female mice in which the kidneys appeared normal; and 6) Accidental exposure group. Male mice which had been accidentally exposed and survived for a number of months. A characteristic picture for each group can be described. In Table I the experimental animals of the various exposures are classified according to the type of kidney lesion found. The controls are classified in Table II.

Changes in the *early group* where death occurred within 5 days are shown in Fig. 1, and characteristic features are pointed out in the legend. Although not shown in the figure, a short segment of the tubule leading from the glomerulus was also preserved as were the nuclei of the cells of the macula densa although the remaining portion of the tubule was sometimes necrotic. The epithelium of the descending straight limb of the proximal convoluted tubule was generally spared, as was that of Henle's loop and of the collecting tubules. In the *intermediate group,* in which death occurred in from 6 to 11 days, the kidneys showed less extensive damage (Fig. 2). In addition to features noted in the legend mitoses were very infrequent. Characteristic

features of the kidneys of mice in the *late group* that died several months after exposure are shown (Fig. 3). The mice which were in the controlled exposure group showed less extensive alteration than did many of the accidentally exposed mice which lived for a time after exposure (Fig. 4).

Amyloidosis was found in both control and exposed mice in which death occurred late. The kidney lesion with amyloidosis has been described previously(1). The tip of the papilla was often necrotic, and there was amyloid deposition in the glomerular tuft. In some kidneys of the male animals exposed to chloroform both the typical amyloid lesion and scattered patches of calcification were found. The kidneys were *normal* in 12 of the 19 control females, in 6 of the 17 control male mice, and in 24 of the 36 exposed females. No calcification was found in any of the kidneys of the females but amyloidosis was found in 12 instances. Mice of resistant strains (C57BL, C57BR/cd, C57L, and ST) and $C3H_f$ males with stilbestrol pellets all of which survived the accidental exposure and which were autopsied at a later date showed no renal calcification.

Discussion. Susceptibility to exposure to chloroform increased with increase in age of the mice for the strain C3H males exposed to various doses at 8 months of age showed greater response than did those exposed at 2 months of age. Furthermore, young male mice which were not yet weaned at the time of the accidental exposure were not affected. Length of exposure had an effect in that a larger number of animals died within a few days following the two and three hour exposures than following the one hour exposure.

The differences between the responses of the various inbred strains to the accidental exposure to chloroform indicated a genetic difference in response. The susceptible strain A is distantly related to strain C3H, and thus $C3H_f$, in that A and C3H were derived from 2 outcrosses of Bagg's colony of albinos(5). The resistant strains, C57BL, C57BR/cd, and C57L are also related. Strains C57BL and C57BR were developed by inbreeding the black and brown segregants of a cross between mice of a heterogeneous stock and strain C57L arose as a mutation in the C57BR line. As far as is known, neither the susceptible strain HR nor the resistant strain ST are related to any of the other strains mentioned.

The difference in the response of the 2 sexes to an exposure to chloroform in this experiment and in the accidental exposure may be compared with the findings of Eschenbrenner (4), and Eschenbrenner and Miller(3). They observed that in mice receiving oral administration of chloroform necrosis of the convoluted tubules of the kidneys was present in intact males and testosterone-treated castrated males but not in females. Eschenbrenner(4) not only noted the difference in response of male and female mice to chloroform, but also briefly described the early kidney lesion which affected "portions of both proximal and distal convoluted tubules". He noted that the cuboidal cells lining Bowman's capsule were never necrotic.

A similar effect of chloroform has recently been reported by Shubik and Ritchie(6) who observed that male DBA mice housed in a room where animals were sacrificed by chloroforming them, died within 6 days. Large, pale kidneys were noted at autopsy. On microscopic examination, necrosis of the tubules of the kidneys was seen. Females of strain DBA and mice of both sexes of strains A, BALB/c, C3H, and CAF_1 hybrids housed in the same room, were not affected by the exposure to chloroform.

A point of interest in the histopathologic study of the kidney lesions in the present study was the resemblance which was found to previously observed "idiopathic calcification" in the kidneys of mice(1). In the idiopathic lesion a remarkable sex difference was noted; calcification in the kidneys of the males involved the whole outer zone of the cortex, whereas in those of the females it was restricted to a few flecks of calcium at the cortico-medullary junction. In the experimental exposure to chloroform, the kidneys of the females showed no calcification, but in those of the males the calcification was similar to that in the idiopathic type.

The localization of damage to particular segments of the tubule is similar to that described after poisoning with bichloride of

mercury, uranyl nitrate, and potassium dichromate(7). The glomeruli after chloroform exposure appeared to be resistant, and the cells of the macula densa were preserved, even when the rest of the cells lining the tubule at the same level were necrotic. In the animals which survived, it seems probable that a sufficient portion of the kidney remained unaffected to maintain life, since very little evidence of regeneration was found. The fate of the glomeruli after destruction of the tubules is uncertain but many appeared to survive, even though all tubules in an area had undergone necrosis or calcification.

No exact relationship was found between the areas of initial necrosis with later calcification, and the localization of alkaline phosphatase in the tubular epithelium which has been studied in sections from kidneys of normal mice(1). The cuboidal epithelium lining Bowman's space, for example, is heavily blackened when Gomori's technic is used to determine alkaline phosphatase yet almost no necrosis and calcification occurred at this site. This observation supports the studies of Hepler and Simonds(7) in dogs in which phosphatase did not appear to be concerned with calcification in the kidneys.

The alteration produced by chloroform should be useful in studies of kidney damage and repair, as suggested by Eschenbrenner and Miller(3). The sex difference offers an especial opportunity to study further the effect of hormonal alterations upon the injury to the kidney produced by chloroform as observed in the accidentally exposed $C3H_f$ males bearing stilbestrol pellets.

Summary. 1. Exposure of strain C3H mice to air containing approximately 5 mg of chloroform per liter for one, 2, or 3 hours resulted in lesions of the kidneys of all of the males but in none of the females. Histologic details of this lesion were described. 2. The lesion found in the male animals in this experiment was similar to that exhibited by the males of strains C3H, $C3H_f$, A, and HR, some of which died immediately after an accidental exposure to chloroform and some in the months following the exposure. 3. A genetic difference was apparent in that whereas strains C3H, $C3H_f$, A, and HR were susceptible, strains C57BL, C57BR/cd, C57L, and ST were resistant to the accidental exposure.

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Screening of Chicks with Erythromyeloblastic Leukosis for Plasma Activity in Dephosphorylation of Adenosine Triphosphate.* (20390)

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Dephosphorylation of adenosine triphosphate by the blood plasma of chickens diseased with erythromyeloblastic leukosis has been described(1). In qualitative experiments, it

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