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Pathologic Changes in Rats and Dogs from Two-Year Feeding of Sodium Arsenite or Sodium Arsenate[1]

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Arsenical compounds, both organic and inorganic, are important as pesticides and feed additives. This paper reports one of the relatively few long-term feeding studies of arsenical compounds that have been carried out in animals, and one in which an unusual lesion of the common bile duct was produced in the rat.

Although a large number of toxicologic studies of all types have been made on arsenical compounds, they have been chiefly short-term studies, especially those in animals. The ramifications and extent of the problem have been reviewed by Vallee *et al.* (1960). For man, long-term exposures included industrial and medicinal uses, with their accompanying question of carcinogenesis. Animal experiments specifically directed to carcinogenesis have been recently discussed by Baroni *et al.* (1963), Hueper and Payne (1962), and Vallee *et al.* (1960). All conclude that attempts to produce arsenical cancer in animals have shown essentially negative results. Another negative study, appearing too late for inclusion in the paper by Baroni *et al.* (1963) is that of Boutwell (1963).

A few chronic feeding experiments with both inorganic and organic arsenical compounds have been reported. In 1935, the Division of Pharmacology of the Food and Drug Administration began short-term and long-term dog- and rat-feeding studies with certain inorganic compounds of lead and arsenic, pursuant to recommendations of an expert committee called by the U.S. Department of Agriculture to advise on tolerances for lead and arsenic in food consumed by man. Five dogs were fed 107.5 ppm or 26.8 ppm As (as As_2O_3) for 180–356 days. No effects were observed during life, and the dogs were actually regarded as controls for others receiving lead compounds (Calvery *et al.*, 1938; Table 2, dogs 19, 20, 3, 4, and 26). The experiments were not completed, and histopathologic examinations (if any were made) were not published. Rats were fed as much as 215 ppm of As as As_2O_3 for about 11 months (Morris *et al.*, 1938). This was, however, a reproduction study, and histopathologic examination was not reported. Prior to the Food and Drug Administration studies, Coulson *et al.* (1935) fed rats 13.7 ppm As in the form of As_2O_3 or shrimp arsenic (naturally occurring) for 1 year without evidence of histologic injury to the liver, spleen, or kidney.

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In a study to determine whether the lead or the arsenic radical was principally responsible for the toxicity of lead arsenate, Fairhall and Miller (1941) fed lead arsenate, lead carbonate, and calcium arsenate to rats for 2 years. The amounts of lead and arsenic in the latter two compounds were equal to those in the lead arsenate. The exact arsenic level of the test diets was not given but may be calculated from various figures in the paper to have been in the range of 200–250 ppm. The authors concluded from mortality rates that calcium arsenate was the most toxic, lead arsenate (even with the added lead) less so, and lead carbonate the least toxic. Histopathologically, the rats on calcium arsenate differed from the controls in having excess hemosiderin in the spleen, swollen cells, and a brown iron-free nonbirefringent granular pigment in the renal convoluted tubules, and hyaline casts in the collecting tubules. Certain other lesser differences also occurred.

Douglass and Blendermann (1961) studied the effect of arsenicals on the pyruvic oxidase system. After feeding 250 ppm of inorganic pentavalent or trivalent arsenic for up to 6 months, they reported that "As^{III} feeding causes a significant depression of growth, and after four months a striking pathological condition of the bile ducts" based on gross observation. No description of the condition was given, and no further publication beyond the abstract was made. We later examined these rats microscopically, and found that the common bile duct lesion was similar to that observed in the rat studies reported here. An abstract of our present study has appeared (Byron *et al.*, 1965).

Of the organic arsenical feed additives, arsanilic acid has probably been the subject of the greatest amount of animal study. The long-term studies of Frost *et al.* (1962) include the feeding of arsanilic acid (34.5% As) to chickens at up to 0.02% of the diet and to pigs at 0.01% for approximately 4 years, and to rats at up to 0.1% for 2 years. Histopathologic examination showed no deleterious effects. Prier *et al.* (1963) fed 3-nitro-4-hydroxyphenylarsonic acid (28.5% As) at 100 ppm in the diet to dogs and mice, and at 200 ppm to rats, all for 2 years. Histopathologic examination in all three experiments showed no deleterious effect from the arsenical.

METHODS

Rats. For either sodium arsenite[2] ($NaAsO_2$) or sodium arsenate[2] ($NA_2HAsO_4 \cdot 7\ H_2O$), 300 weanling Osborne-Mendel rats from our own colony, divided into six groups of 50 each, each group evenly divided by sex, were started on a 2-year feeding study at As levels for sodium arsenite of 250, 125, 62.5, 31.25, 15.63, or 0 (control) ppm and for sodium arsenate of 400, 250, 125, 62.5, 31.25, or 0 (control) ppm mixed in a commercial laboratory diet.[3] Mean starting weights of the male groups were 44–46 g and of the females 43–45 g. The rats were individually caged. Body weight records were made weekly. Hematologic examinations (hemoglobin, hematocrit, and total and differential leukocyte counts) were made on 10 males and 10 females from each group of

[2] These were reagent-grade chemicals manufactured by Matheson, Coleman and Bell, Norwood, Ohio.

[3] Purina Laboratory Chow, Ralston Purina Company, St. Louis, Missouri.

50 rats, after approximately 3, 11, 17, and 22 months of the test period. All rats dying during the experiment, except four markedly autolyzed, were necropsied. During the 104th week of the feeding tests (107 weeks of age) survivors were killed and necropsied, and organ weights were recorded for heart, liver, spleen, kidneys, and testes. Viscera, pituitary, gross lesions, and one hind leg from each rat were fixed in formalin and submitted for histopathologic study. Hematoxylin-eosin-stained paraffin sections were prepared from lung, heart, liver, common bile duct, spleen, kidney, adrenal, pituitary, testis and prostate (or uterus and ovary), pancreas, stomach, intestine (3 levels), mesenteric lymph nodes, thyroid, parathyroid, urinary bladder, bone, and bone marrow from six male and six female survivors from the control groups in both instances, and from eight males and four females which completed the 2-year study in the 250-ppm sodium arsenite group. In the group on the high dose (400 ppm) of sodium arsenate, only nine rats survived, so two nonsurviving females and one male were selected on the basis of length of time on the test feed and state of tissue preservation to total the desired number of 12 for detailed histologic examination. Additionally, in all groups, all tumors were sectioned, along with the liver, kidney, and spleen if a female, and liver, kidney, and testis if a male. A total of approximately 1600 pieces of rat tissue were sectioned.

Dogs. Forty-eight beagle dogs approximately 6 months old, distributed into eight groups of six each and evenly divided by sex, were fed either sodium arsenite or sodium arsenate at As levels of 125, 50, 25, and 5 ppm mixed in a commercial laboratory diet[3] for 2 years. A similar control group of six for each arsenical in each instance received the same basal diet untreated. Body weight records were kept. Hematologic studies were made during the pretest period, at intervals during the first year, and at 1 week prior to termination of the experiments. At the death of any dog, or at the conclusion of the experiment, autopsies were done. Microscopic examination included detailed sectioning in the 125-ppm, 50-ppm, and control groups, of liver, kidney, heart, adrenal, brain (4 levels), spleen, stomach, intestine (3 levels), mesenteric lymph node, gall bladder, pancreas, urinary bladder, thyroid, skeletal muscle, bone, and testis and prostate (or uterus and ovary). At 25 and 5 ppm, liver, kidney, adrenal, and bone were sectioned. Sections were of the same type as for the rats; in addition, paraffin sections from Zenker-fixed portions of liver, kidney, and adrenal were made in the majority of instances, and frozen sections from liver and kidney were stained with Oil Red O in all cases. Over 1000 pieces of dog tissue were sectioned. For all except two dogs, bone marrow smears were prepared and stained by the Wright-Giemsa method.

RESULTS

Rats

At the end of the first year, there was increased mortality (Tables 1–4) in the high-dose group with each compound compared to controls and to a lesser extent with the next two lower levels of sodium arsenate. During the second year, some leveling-off took place. On the high level of sodium arsenite there were 12 survi-

vors, with 23 at the next level and 19–22 in the remaining groups, including controls. Arsenate survivors numbered 9 and 13, respectively, at the two highest levels, and 17–19 in the remaining groups, including controls. There was no sex difference in mortality. Thus, only the highest (250 ppm) level of As as sodium arsenite lessened survival, whereas with sodium arsenate both the highest level of 400 ppm As and the 250-ppm As level affected survival.

At the highest level of each compound, and for both sexes, *weights* (Tables

TABLE 1

WEIGHT AND MORTALITY OF MALE RATS FED SODIUM ARSENITE

Arsenic in diet (ppm)	Duration of experiment (weeks)	Mean weight ± SE (g)	Number dead in period	Cumulative mortality
Control	12	394.3 ± 6.74	0	0
	27	477.4 ± 10.68	0	0
	52	514.8 ± 20.82	2	2
	78	536.4 ± 7.25	3	5
	104	482.0 ± 21.07	8	13
15.63	12	401.8 ± 7.79	0	0
	27	483.0 ± 10.73	0	0
	52	525.7 ± 12.28	1	1
	78	531.9 ± 15.30	1	2
	104	485.1 ± 19.70	8	10
31.25	12	407.8 ± 7.16	0	0
	27	498.9 ± 9.40	0	0
	52	542.2 ± 12.82	3	3
	78	556.1 ± 15.18	2	5
	104	487.7 ± 17.02	8	13
62.5[a]	12	377.8 ± 6.28	1	1
	27	477.4 ± 7.54	0	1
	52	510.0 ± 13.72	3	4
	78	520.6 ± 11.05	2	6
	104	472.8 ± 12.01	10	16
125	12	357.8 ± 6.69	0	0
	27	448.0 ± 9.03	0	0
	52	469.5 ± 16.81	3	3
	78	486.3 ± 16.98	6	9
	104	427.9 ± 17.24	5	14
250	12	232.7 ± 6.34	1	1
	27	330.2 ± 8.71	1	2
	52	412.2 ± 13.14	5	7
	78	435.6 ± 17.89	3	10
	104	406.3 ± 20.29	7	17

[a] This group contained 26 animals at the start. All other groups contained 25 animals at the start.

1–4) of the animals as compared to the controls were depressed throughout the experiment. At the second highest level of each compound, weights of the males were slightly lower and of the females moderately lower than those of the controls, throughout the experiments. At the middle level of each compound, weights of the females were slightly less, of the males on arsenate very slightly less, and of the males on arsenite essentially identical, compared with the controls.

At the highest dosage of sodium arsenite, *hematologic study* showed a slight

TABLE 2

WEIGHT AND MORTALITY OF FEMALE RATS FED SODIUM ARSENITE

Arsenic in diet (ppm)	Duration of experiment (weeks)	Mean weight ± SE (g)	Number dead in period	Cumulative mortality
Control	12	245.8 ± 5.60	0	0
	27	295.3 ± 6.51	0	0
	52	318.5 ± 8.82	2	2
	78	351.8 ± 13.14	8	10
	104	350.9 ± 26.36	6	16
15.63	12	236.1 ± 4.06	0	0
	27	291.4 ± 7.03	1	1
	52	328.6 ± 9.99	3	4
	78	357.3 ± 15.63	5	9
	104	337.0 ± 28.75	9	18
31.25	12	234.8 ± 3.59	0	0
	27	287.2 ± 5.30	0	0
	52	327.6 ± 8.95	2	2
	78	336.4 ± 16.62	6	8
	104	322.4 ± 21.38	10	18
62.5[a]	12	213.1 ± 4.05	0	0
	27	267.3 ± 4.89	0	0
	52	303.9 ± 7.84	2	2
	78	323.1 ± 11.36	1	3
	104	280.4 ± 14.43	11	14
125	12	199.6 ± 5.12	0	0
	27	242.9 ± 6.80	0	0
	52	276.0 ± 9.25	0	0
	78	289.7 ± 11.06	1	1
	104	268.4 ± 12.49	12	13
250	12	164.9 ± 4.35	0	0
	27	207.6 ± 4.55	2	2
	52	226.3 ± 7.33	5	7
	78	249.8 ± 8.94	7	14
	104	237.8 ± 46.07	7	21

[a] This group contained 24 animals at the start. All other groups contained 25 animals at the start.

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decrease in hemoglobin in the 3- and 11-month counts, and a slight decrease in the hematocrit value at 11 months in the females only. The leukocyte count was slightly elevated in both sexes throughout, and moderately so terminally. The differential counts showed nothing of note. There were no effects at lower dosages. With sodium arsenate, the only effect was a slight elevation of the leukocyte count in the females on the highest dosage throughout the 2 years, and in the males for the first year.

No distinct differences in ratio of *organ weight* to body weight were noted.

ARSENITE

ber dead period	Cumulative mortality
0	0
0	0
2	2
8	10
6	16
0	0
1	1
3	4
5	9
9	18
0	0
0	0
2	2
6	8
10	18
0	0
0	0
2	2
1	3
11	14
0	0
0	0
0	0
1	1
12	13
0	0
2	2
5	7
7	14
7	21

contained 25 animals at the

TABLE 3

WEIGHT AND MORTALITY OF MALE RATS FED SODIUM ARSENATE

Arsenic in diet[a] (ppm)	Duration of experiment (weeks)	Mean weight ± SE (g)	Number dead in period	Cumulative mortality
Control	12	409.7 ± 9.31	0	0
	27	518.4 ± 10.86	0	0
	52	550.0 ± 15.11	0	0
	78	580.4 ± 18.22	5	5
	104	489.0 ± 28.31	10	15
31.25	12	411.2 ± 9.59	0	0
	27	528.5 ± 14.70	2	2
	52	557.8 ± 19.76	0	2
	78	562.1 ± 25.60	5	7
	104	440.7 ± 33.97	11	18
62.5	12	401.0 ± 7.56	0	0
	27	508.6 ± 9.66	0	0
	52	540.5 ± 12.39	1	1
	78	574.1 ± 16.15	3	4
	104	497.4 ± 28.50	10	14
125	12	375.5 ± 6.00	0	0
	27	491.4 ± 9.24	0	0
	52	542.4 ± 18.19	5	5
	78	520.4 ± 36.67	2	7
	104	465.2 ± 26.99	9[b]	15
250	12	348.5 ± 6.09	0	0
	27	463.1 ± 10.25	1	1
	52	528.4 ± 16.18	2	3
	78	541.5 ± 23.27	7	10
	104	454.4 ± 27.09	7	17
400	12	245.0 ± 9.49	1	1
	27	365.8 ± 10.91	2	3
	52	420.9 ± 14.21	5	8
	78	443.9 ± 19.85	6	14
	104	414.6 ± 30.44	6	20

[a] There were 25 animals in each group at the start.

[b] One rat was killed accidentally after 29 weeks.

Gross pathology. In the two highest dosage groups of both compounds there was a striking enlargement of the common bile duct (Table 5). With sodium arsenite, 45 of 49 common bile ducts in the 250-ppm As group were enlarged, and at 125 ppm the incidence was 7 of 50. With sodium arsenate, in the 400-ppm As group, 42 of 48 common bile ducts were enlarged, and 25 of 50 were enlarged in the 250-ppm group. Enlargement of the ducts was infrequent or absent in the lower dose and control groups with either compound. The ducts were

TABLE 4

WEIGHT AND MORTALITY OF FEMALE RATS FED SODIUM ARSENATE

Arsenic in diet[a] (ppm)	Duration of experiment (weeks)	Mean weight ± SE (g)	Number dead in period	Cumulative mortality
Control	12	238.5 ± 6.58	0	0
	27	292.7 ± 6.01	0	0
	52	338.9 ± 8.01	1	1
	78	382.5 ± 13.46	3	4
	104	395.4 ± 24.40	13	17
31.25	12	236.2 ± 4.18	0	0
	27	283.5 ± 6.95	0	0
	52	325.5 ± 8.72	1	1
	78	352.4 ± 12.26	5	6
	104	320.5 ± 21.71	7	13
62.5	12	230.5 ± 3.63	0	0
	27	286.8 ± 5.61	0	0
	52	336.1 ± 7.85	0	0
	78	365.0 ± 14.04	3	3
	104	345.8 ± 28.81	15	18
125	12	223.7 ± 4.12	0	0
	27	258.4 ± 7.17	1	1
	52	313.6 ± 10.09	1	2
	78	321.8 ± 15.67	5	7
	104	310.6 ± 25.40	11	18
250	12	205.4 ± 2.81	0	0
	27	250.0 ± 4.54	0	0
	52	285.0 ± 7.49	4	4
	78	285.9 ± 19.45	6	10
	104	294.0 ± 63.51	10	20
400	12	167.0 ± 4.84	1	1
	27	215.6 ± 4.77	0	1
	52	240.2 ± 7.71	4	5
	78	269.6 ± 8.98	3	8
	104	246.0 ± 13.62	13	21

[a] There were 25 animals in each group at the start.

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ARSENATE

ber dead period	Cumulative mortality
0	0
0	0
1	1
3	4
13	17
0	0
0	0
1	1
5	6
7	13
0	0
0	0
0	0
3	3
15	18
0	0
1	1
1	2
5	7
11	18
0	0
0	0
4	4
6	10
10	20
1	1
0	1
4	5
3	8
13	21

arbitrarily graded for degree of enlargement on the basis of increase in diameter, as stated in Table 5. The maximum normal diameter (Figs. 1 and 4) was considered to be 1 mm. Examples of grade 4 enlargement are shown in Figs. 2 and 3. In the 250-ppm sodium arsenite group, the largest common duct measured 4 cm in diameter. The largest duct in the 400-ppm sodium arsenate group measured 2.6 cm in diameter. Many of the enlarged ducts were filled or partially filled with material varying in color from gray to brown to green, and varying in consistency from fluid to semisolid to solid, often containing small granules, some of which were gold-colored (especially in the rats fed sodium arsenate). Although unrelated to arsenical feeding, pneumonia, nephritis, and the usual tumors (Table 6), similar to the conditions we commonly see in our older Osborne-Mendel rats, occurred fairly uniformly among the control rats and those treated with each compound.

TABLE 5

TOTAL NUMBER OF ENLARGED BILE DUCTS AND DEGREE OF ENLARGEMENT IN RATS FED SODIUM ARSENITE AND SODIUM ARSENATE FOR UP TO TWO YEARS

Arsenic in diet (ppm)	Total number	Grade 1[a]	Grade 2[b]	Grade 3[c]	Grade 4[d]
		Arsenic added as sodium arsenite			
Control	0	—	—	—	—
15.63	1	1	—	—	—
31.25	0	—	—	—	—
62.5	1	1	—	—	—
125	7	4	2	1	—
250	45	5	15	1	24
		Arsenic added as sodium arsenate			
Control	1	—	—	1	—
31.25	3	2	—	—	1
62.5	0	—	—	—	—
125	0	—	—	—	—
250	25	10	8	3	4
400	42	7	12	10	13

[a] Diameter greater than 1 mm up to and including 3 mm.
[b] Diameter greater than 3 mm up to and including 5 mm.
[c] Diameter greater than 5 mm up to and including 7 mm.
[d] Diameter greater than 7 mm.

Microscopic pathology. The chief microscopic lesion was thickening of the wall of the enlarged common bile duct to approximately 1 mm, compared to a control thickness of 0.1 mm. The increased thickness was due primarily to slight to moderate fibrosis, sometimes accompanied by infiltration with inflammatory cells including neutrophils, eosinophils, lymphocytes, and macrophages. Slight to moderate hyperplasia of glandular components generally accompanied these changes (Fig. 5). In a few instances in which inflammation of the bile duct wall was rather extensive, granulomatous lesions 2–3 mm in diameter projected

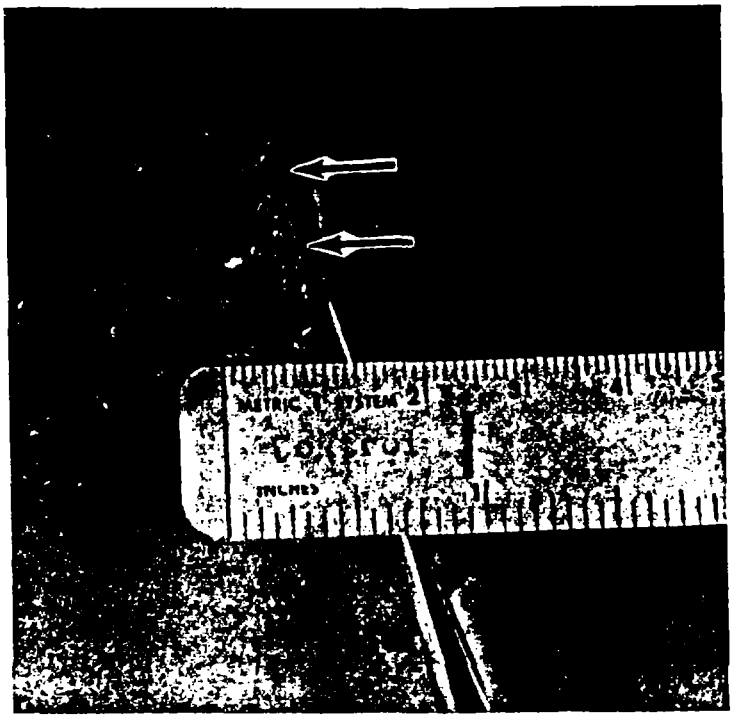

FIG. 1. Viscera of a male, control rat, 85 weeks of age, showing normal appearance of the common bile duct (arrows). Magnification: × 1.3.

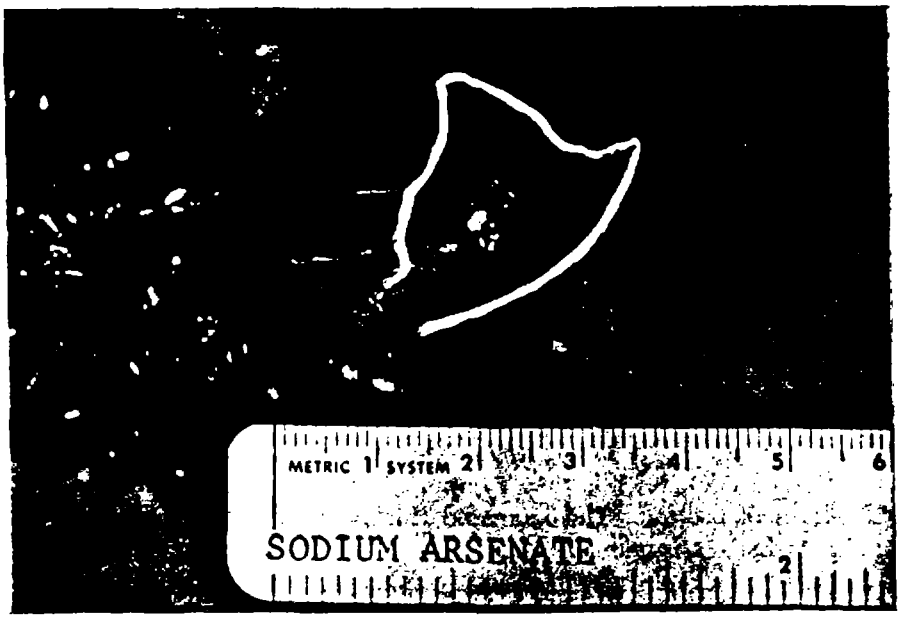

FIG. 2. Viscera of a female rat, 52 weeks of age, that had received 400 ppm As as sodium arsenate in the diet since weaning. The common bile duct (outlined in white) is markedly enlarged. Magnification: × 1.4.

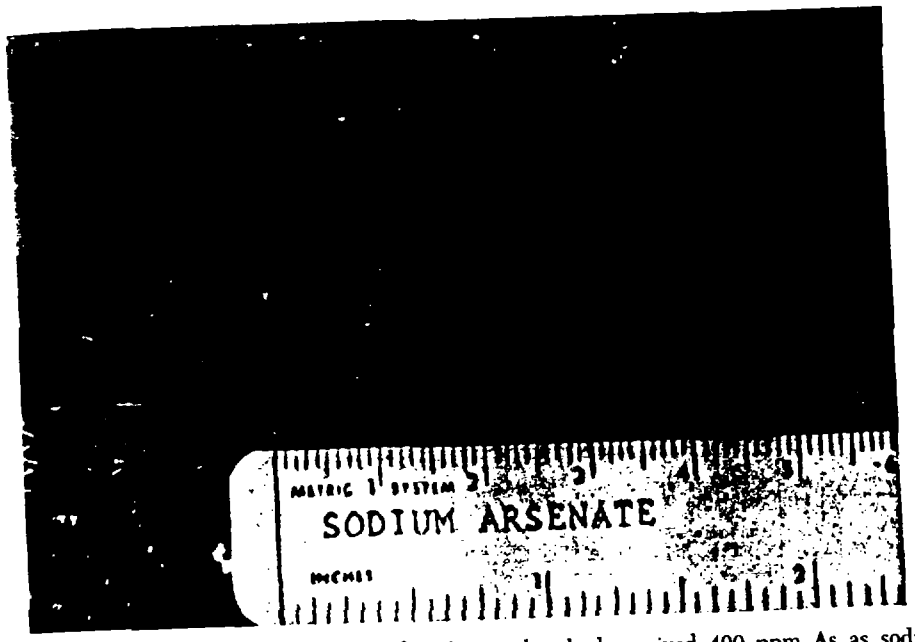

FIG. 3. Viscera of a male rat, 38 weeks of age, that had received 400 ppm As as sodium arsenate in the diet since weaning. The enlarged common bile duct is cut transversely to show the dark contents and thickened wall. Magnification: × 1.4.

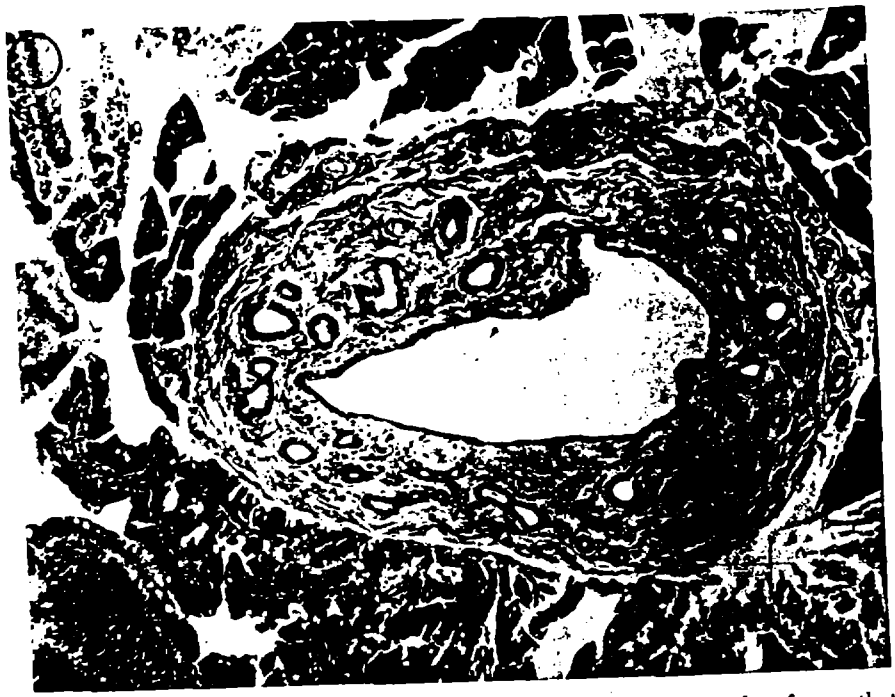

FIG. 4. *Cross section of* the common bile duct of a male rat, 107 weeks of age, that had received 250 ppm As as sodium arsenite in the diet since weaning. The duct is of normal diameter. Magnification: × 90.

normal appearance of the

received 400 ppm As as
duct (outlined in white)

TABLE 6

INCIDENCE AND TYPES OF TUMORS IN RATS FED SODIUM ARSENITE AND SODIUM ARSENATE FOR UP TO TWO YEARS

Type of tumor	Dietary level of arsenic added as sodium arsenite (ppm)						Dietary level of arsenic added as sodium arsenate (ppm)					
	250	125	62.5	31.25	15.63	0	400	250	125	62.5	31.25	0
Mammary adenocarcinoma	0	3	8	5	3	4	1	4	2	5	10	6
Mammary fibroadenoma	2	5	10	7	6	6	1	4	5	5	12	11
Pituitary adenocarcinoma	0	0	1	3	1	0	0	2	3	0	4	1
Pituitary adenoma	1	5	8	4	1	6	0	2	2	4	4	1
Lymphosarcoma, thoracic	0	2	2	2	2	1	1	0	1	1	0	0
Lymphosarcoma, abdominal	0	1	0	0	1	1	0	0	0	0	0	0
Fibrosarcoma, subcutaneous	0	0	1	0	0	0	0	0	2	0	0	0
Fibrosarcoma, abdominal	0	0	0	0	0	0	0	0	0	0	1	0
Fibroma, subcutaneous	0	0	0	0	1	0	0	1	1	0	0	0
Fibroma, uterus	0	0	0	0	0	0	0	0	0	0	0	1
Endometrial sarcoma, uterus	0	1	2	0	1	0	1	0	0	0	0	0
Epidermoid carcinoma, head region	0	0	0	0	0	1	0	0	0	1	0	0
Adenocarcinoma, skin	0	0	0	1	0	0	0	0	0	0	0	0
Adenocarcinoma, adrenal	0	0	0	0	1	0	0	0	0	0	0	0
Adenocarcinoma, kidney	0	0	0	0	0	0	2	1	2	0	0	0
Liposarcoma, kidney	0	0	0	0	0	0	0	0	0	1	2	0
Embryonal nephroma	0	0	1	0	0	0	0	0	0	0	0	0
Osteosarcoma, lung	0	0	0	1	0	0	0	0	0	0	0	0
Leiomyosarcoma, ileum	1	0	0	0	0	0	0	0	0	0	0	0
Hepatic cell carcinoma	0	0	0	0	0	0	0	1	0	0	0	0
Mesothelioma, malignant	0	0	0	0	0	1	0	0	0	0	0	0
Granulosa cell tumor, ovary	0	0	0	0	1	0	0	0	0	0	0	0
Interstitial cell tumor, testis	1	0	0	0	1	0	0	1	0	1	1	2
Thyroid adenoma	1	0	0	0	0	0	0	0	0	0	0	1
Lipoma, mesentery	0	0	0	0	1	0	0	0	0	0	0	0
Lymphatic leukemia	0	0	0	0	0	0	0	0	0	0	0	1
Animals with any tumor	6	15	18	15	14	16	6	13	16	15	21	17
Animals with malignant tumor	1	7	15	9	8	8	5	6	10	8	14	8

Leiomyosarcoma, ileum	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Hepatic cell carcinoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mesothelioma, malignant	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Granulosa cell tumor, ovary	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	1	2	2
Interstitial cell tumor, testis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Thyroid adenoma	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipoma, mesentery	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Lymphatic leukemia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Animals with any tumor	6	15*	18	15	14	16	6	13	16	15	21	17						
Animals with malignant tumor	1	7	15	9	8	8	5	6	10	8	14	8						

FIG. 5. Cross section of the wall of the common bile duct of a male rat, 107 weeks of age, that had received 400 ppm As as sodium arsenate in the diet since weaning. The wall of the duct is thickened, and shows fibrosis, epithelial hyperplasia, and infiltration with inflammatory cells. Magnification: × 90.

into the lumen; no indication of tumor was observed in these lesions. The content of the enlarged ducts varied; it included necrotic tissue debris, proteinaceous material, dark brown or gold-colored material, some of which was birefringent (Fig. 6), and occasional small foci of calcification. Other treatment-related histopathology included some proliferation of small intrahepatic bile ducts in the high-dose group with either compound, and variation in hepatic cell size in the two higher-dose sodium arsenite groups.

A small amount of nonbirefringent, golden-brown pigmented material, appearing as small globules or granules, was observed within tubular epithelial cells and sometimes in the lumen of tubules of the kidney cortex of rats from either experiment. Some of the pigment was iron positive and some was not. Traces of this material were seen in control groups and in the three lower-dose groups with either compound. A slight increase compared to controls was obvious only in the two higher-dose groups with either compound. The occurrence in the 400-ppm and 250-ppm sodium arsenate groups of small simple cysts lined with flat epithelium in the kidney cortices in five and seven rats, respectively, was considered possibly related to treatment; other groups had 0 or 1.

Dogs

Of the high-dose sodium arsenite group of dogs, four were found dead after 3–9 months and one after 19 months, and one was found moribund and was

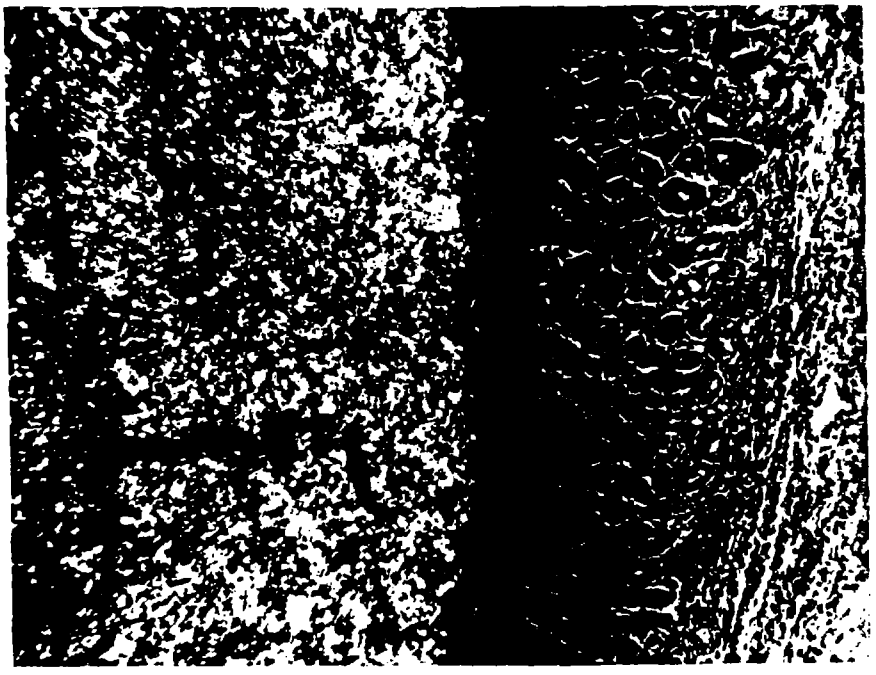

FIG. 6. Cross section of the thickened wall of the common bile duct of a male rat, 107 weeks of age, that had received 250 ppm As as sodium arsenite in the diet since weaning. The photograph was made with polarized light to show the birefringent material in the lumen of the duct. Magnification: × 90.

killed after 8 months of the experiment. Except for anorexia and listlessness, nothing special was noted clinically. All these high-dose dogs showed a weight loss of 44–61% of their starting weight. The remaining groups finished the 2-year experiment, except for one female on 5 ppm and one female control, which died after 3 and 20.5 months, respectively, and both of which showed the same pronounced weight loss as in the high-dose group. Hematologic study showed the development of slight to moderate anemia in the high-dose group. The surviving treated dogs (50 ppm and less) could not be said to be different from the surviving controls. At necropsy, the nonsurviving dogs all showed moderate or marked emaciation, and from the high-dose group one had definite and one possible bleeding into the gut. In the survivors there were no noteworthy abnormalities. The *microscopic changes* in the nonsurviving dogs were essentially those of inanition, including atrophy of spleen, bone marrow, testis, and skeletal muscle. No source for the bleeding mentioned above was found. In addition to the changes of inanition, very small to moderate amounts of hemosiderin attributable to arsenite, were present in hepatic macrophages. In the surviving animals, hepatic hemosiderin was not present, and no changes elsewhere could be attributed to arsenite.

No systemic effects related to treatment were reported for sodium arsenate except for weight depression and mild anemia at the high level. Two animals

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died during the test from unrelated causes, and the death of a female on 125 ppm after 13.5 months was considered to be caused by arsenic. The remaining dogs finished the 2-year experiment and were necropsied at the end of the test. No gross pathology was reported except for weight loss at the high level, and traumatic brain hemorrhage in one dog. The only microscopic finding related to treatment (except for organ atrophies with marked weight loss) was the presence of a granular, brownish, iron-positive, nonbirefringent pigment in the liver macrophages; it was present in all dogs on high doses, but was seen only randomly below this level and was noted in one control animal.

DISCUSSION

Barnes and Magee (1958) briefly reviewed the excretion of various chemicals via the bile, and the biliary system lesions produced by some of them. They illustrated and described in detail the enlargement and inflammation of the common bile duct of the rat after a small number of oral doses of dibutyltin dichloride. While the lesion they reported was produced much more acutely than the one we produced, the basic similarity is evident. They comment that although many foreign chemicals are excreted in the bile, the comparative infrequency of biliary tract damage is surprising. Barnes and Stoner (1958) in a 6-month feeding of dibutyltin dichloride to rats found that 20 ppm in the diet did not produce common bile duct damage but that 50 ppm did. Hill *et al.* (1954) stated that feeding of 0.6% of 1,2,5,6-dibenzofluorene for 30 weeks of a 60-week experiment caused cystic dilatation of the extrahepatic bile ducts, but gave no further description.

A considerable degree of enlargement of the common bile duct of the rat has been produced without the addition of "foreign" chemicals or drugs to the diet by the use of suboptimal diets deficient in nutrients, particularly in vitamin A (Hamre, 1950; Gillman *et al.*, 1954).

In the Food and Drug Administration's experience with the feeding of a large number of chemicals to rats, the only compound other than arsenicals which produced common bile duct enlargement of any consequence was sorbitan monolaurate, fed at 15–25% of the diet for 23 weeks. This was described and illustrated by Fitzhugh *et al.* (1960). These ducts were up to 2 cm in diameter, and contained only a fluid, in contrast to those described in the present paper.

SUMMARY

Feeding of sodium arsenite or sodium arsenate to weanling Osborne-Mendel rats in 2-year experiments resulted in marked enlargement of the common bile duct in a large proportion of the animals on the highest dosage of each compound, namely, 250 and 400 ppm As in the diet, respectively. These levels also caused pronounced weight depression and decreased survival.

At the next lower dosages, 125 ppm As as arsenite and 250 ppm As as arsenate in the diet, enlargement of the common bile duct was also present, but was less pronounced. Survival was slightly reduced with the arsenate, and both compounds caused reduced weight, particularly in the females.

Levels of 62.5, 31.25, or 15.63 ppm As as arsenite and 125, 62.5, or 31.25 ppm As as arsenate did not cause common bile duct enlargement and did not affect survival. Weight

was slightly reduced in both sexes with 125 ppm As as arsenate, and in females only with 62.5 ppm As as arsenite.

No carcinogenic effect of the two arsenicals in these experiments could be detected.

Histologically, the enlarged common bile ducts showed hyperplasia of the glandular elements, focal necrosis, and fibrosis. Intrahepatic bile ducts were little affected.

Young beagle dogs in a 2-year experiment were fed either of the arsenicals at As levels of 125, 50, 25, or 5 ppm in the diet. None of the six on the high level of arsenite survived 2 years; all lost much weight during the feeding period. Only one of six dogs on the high level of arsenate died, and this one dog lost much weight; the survivors showed some weight loss. Gross and microscopic changes in the nonsurviving dogs were essentially those of inanition. Dogs on levels of 50 ppm or less of As as either compound did not differ from the controls.

For a given degree of effect in either species (dog survival and weight loss, rat survival and weight depression, and rat common bile duct enlargement), more arsenic in arsenate than in arsenite form was required.

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