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Effect of Subacute Low Level Dietary Sodium Arsenite on Dogs[1]

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Effect of Subacute Low Level Dietary Sodium Arsenite on Dogs. NEIGER, R. D., AND OSWEILER, G. D. (1989). *Fundam. Appl. Toxicol.* 13, 439–451. Thirty female beagle dogs, 7 to 8 months old, were assigned to five groups. Control, low dosage, medium dosage, high dosage, and pair-fed groups were offered 0, 1, 2, 4, and 0 mg of sodium arsenite per killigram of body weight per day (mg/kg/day), respectively, in their feed. On Day 59, the dosage was doubled for the rest of the experiment, which ended on Day 183. Nominal dosages of 4 and 8 mg/kg/day caused a significant decrease in feed consumption. The initial decreased feed consumption was followed by increased intake over time. Nominal dosages of 4 and 8 mg/kg/day caused a significant decrease in body weight. Body weight loss of high dosage and pair fed groups were not significantly different. Serum aspartate aminotransferase was elevated in dogs exposed to 4 and 8 mg/kg/day of sodium arsenite. Serum alanine aminotransferase was elevated in dogs exposed to 2, 4, and 8 mg/kg/day. No gross or light microscopic lesions were present in the liver of any group. This study shows that dietary sodium arsenite causes a dose-dependent decrease of feed consumption and body weight. Weight loss is caused by decreased feed consumption, not by the direct effect of the sodium arsenite. © 1989 Society of Toxicology.

Arsenic is ubiquitous and is present in rock, soil, water, and living organisms. Therefore, it is impossible for animals to avoid exposure. In addition to natural sources, the frequent use of arsenicals as herbicides and insecticidal baits creates the potential for accidental exposure to higher concentrations of arsenic. Past experimentation in dogs did not use extensive clinical pathological examination to detect subtle and/or subclinical effects of dietary arsenic (Calvery *et al.*, 1938; Byron *et al.*, 1967).

High dosage of arsenic results in dramatic acute clinical signs and lesions (Sullivan and Gruen, 1985; Osweiler *et al.*, 1985; NAS, 1977). Information about chronic effects, primarily from human epidemiological studies, associate arsenic with degenerative, inflammatory, and neoplastic changes of the skin, respiratory system, liver, cardiovascular system, blood, lymphatic system, nervous system, and reproductive system (Pershagen, 1983).

It is well established that inorganic arsenicals affect the function of a large number of enzymes within many metabolic systems. Replacement of PO_4 with AsO_4 in important biochemical compounds such as ATP has also been documented (Squibb and Fowler, 1983). Therefore, it may be assumed all organ systems in the body could be affected by arsenic.

Numerous controlled subchronic and chronic inorganic arsenic studies have been done with rats and a lesser number with mice (Byron *et al.*, 1967; Schroeder and Balassa,

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1967; Schroeder *et al.*, 1968; Kiyono *et al.*, 1974; Fowler *et al.*, 1977; Fowler and Woods, 1979). Inorganic arsenic adversely affects the weight or weight gain of rats and mice. This information must be weighed with care since rats metabolize arsenic differently than other species studied (Ducoff *et al.*, 1948; Lanz *et al.*, 1950; Klaassen, 1974).

In limited studies, inorganic arsenic also adversely affected the weight of pigs and dogs (Byron *et al.*, 1967; Morrison and Chavez, 1983). Dogs chronically fed 0.5 or 2.0 mg of arsenic per killigram of body weight daily had no adverse effects (Calvery *et al.*, 1938). The arsenic was in the form of finely powdered arsenic trioxide. In another study, dogs given feed containing 125 ppm of arsenic as sodium arsenate or sodium arsenite had significant weight loss (Byron *et al.*, 1967). Weight loss was more severe in the group fed arsenite. Dogs exposed to 50 ppm of arsenic of either compound were not affected.

Feed or water is rejected by animals when it is contaminated by a high enough concentration of inorganic arsenic (Fowler and Woods, 1979; Morrison and Chavez, 1983). Therefore, feed rejection is probably the cause of the weight loss described above.

Arsenic has long been associated with liver damage (Soffer *et al.*, 1937; Von Glahn *et al.*, 1938; Finner and Calvery, 1939). There have been a few controlled studies of the effects of chronic low level arsenic exposure on dogs (Calvery *et al.*, 1938; Byron *et al.*, 1967). Significant inanition was produced at 125 μg/gm dietary arsenic as sodium arsenite or sodium arsenate (Byron *et al.*, 1967). However, no gross or light microscopic liver changes were noted.

Sodium arsenite was used in this study. It is one of the most toxic forms of inorganic arsenic because it is water soluble and trivalent (Squibb and Fowler, 1983).

The purpose of this study was to determine clinical and subclinical effects of low level dietary sodium arsenite in dogs. This was accomplished by measuring a wide variety of clinical and clinical pathological parameters. Gross and microscopic evaluation of livers was also done.

MATERIALS AND METHODS

Thirty female beagle dogs, 7 to 8 month old, were randomly assigned to five groups. Control, low dosage, medium dosage, and high dosage groups were offered 0, 1, 2, or 4 mg of sodium arsenite ($NaAsO_2$) per kilogram of body weight in the feed daily. After 58 days, the dosage of all groups was doubled for the rest of the experiment. To account for substantial feed refusal in principals, a pair-fed group (inanition control) was fed the amount of feed, without sodium arsenite, that the high dosage group consumed on a percentage of body weight basis. Pairings were by group, not individual animals, and were readjusted weekly to mimic intake of the high dosage group.

Each dog of the control, low dosage, medium dosage, and high dosage groups was offered daily 2.75% of its body weight of dry Purina High Pro Dog Chow (Ralston Purina Co., St. Louis, MO) for the first 2 weeks, then 3% for the rest of the experiment. Feed samples were quantitated for arsenic by hydride atomic absorption spectroscopy (303 Perkin-Elmer Atomic Absorption Spectrometer, Perkin-Elmer MHS-10 Hydride System, Perkin-Elmer Co., Norwark, CT) according to methods previously described (Hyde *et al.*, 1977). Feed contained less than 0.1 ppm arsenic. Stainless-steel feed and water bowls were used. Dry feed was mixed with an equal weight of water containing the appropriate dose of sodium arsenite dissolved in it. The feed was weighed and mixed with arsenic every morning. Feed and water were mixed thoroughly to ensure the even distribution of arsenic in feed. The amount of water added wet the feed evenly, and no excess water was left free of the feed. The mixture was offered to the dog for 6 to 8 hr. Each night the unconsumed feed was weighed and recorded. The dogs had free choice tap water at all times. Tap water contained less than 0.1 ppm of arsenic.

The dogs were housed individually in stainless-steel cages (Shor-Line, Shroer Manufacturing Co., Kansas City, MO). Each unit consisted of two cages, one above the other. The assignment of dogs to cages was random, except the bottom dog was always from the same group as the top dog. The dogs were observed at least three times a day, and the cages were cleaned daily.

Body weights were determined and the amount of feed and arsenic was adjusted weekly. To decrease the effect of variation of individual body weights, the weekly body weight of a dog was divided by the initial body weight of that dog. The fraction of the initial body weight (FIBW) of each animal was used in the analysis of data. Therefore, the FIBW of all dogs on Week 1 was 1.

The experiment was divided into three phases (I, IIa, and IIb) on the basis of time and groups for analysis of the data generated. Phase I involved all groups from Week 1 through Week 8. In phase I, nominal dosages (intended dosage if dog consumed all feed offered to it) of sodium arsenite for control, low dosage, medium dosage, high dosage, and pair fed groups were 0, 1, 2, 4, and 0 mg per kilogram of body weight per day (mg/kg/day), respectively. Phase IIa consisted of all groups from Weeks 9 through 17 at nominal dosages double that of phase I (0, 2, 4, 8, 0 mg/kg/day). At the end of phase IIa, high dosage and pair-fed groups were terminated when the high dosage group had lost approximately 20% of their original body weight. Phase IIb consisted of control, low dosage, and medium dosage groups from Weeks 9 to 26 at 0, 2, and 4 mg/kg/day, respectively. In all phases, the actual arsenic dosages were calculated daily by multiplying the percentage of offered feed consumed by the appropriate nominal dosage. At the feeding rate of 3% of body weight per day, the phase I dosages were equivalent to 33.4, 66.7, and 133.4 μg of sodium arsenite per gram of dry feed for low, medium, and high dosage groups, respectively.

The pair-fed group was started on the experiment 20 weeks after the other groups. Therefore, data for a certain experimental week were on the average collected 20 weeks later, real time, than data for other groups. An average age difference of 20 weeks existed between pair-fed group dogs and dogs of other groups.

Periodic blood, serum, and urine samples were taken from all dogs and analyzed for a wide range of parameters. Control, low dosage, and medium dosage groups were sampled on Weeks 2, 7, 13, 19, and 26. The high dosage group was sampled on Weeks 2, 7, 13, and 19. Due to the early termination of two dogs of the high dosage group, there were two animals sampled on Week 15 and the other four on Week 19. Because of extenuating circumstances, the pair-fed group was not sampled on the same weeks as the high dosage group but rather on Weeks 3, 6, 11, and 17. Urine was collected by transabdominal cystocentesis and every attempt was not successful due to empty bladders. At times it was 2 days before an attempt was successful so samples from different dogs were secured within a period of 2 to 3 days. Serum samples were taken at the same time urine samples were secured.

The following determinations were made on blood: packed cell volume (PCV) (Autocrit Ultra 3, Clay Adams, Becton Dickinson and Co., Parsippany, NJ), total (WBC) (ZBI Coulter Counter, Coulter Electronics, Inc., Hialeah, FL) and differential (DIFF) white blood cell counts (Ames Hemotech Slide Stainer, Ames Division, Miles Laboratories, Inc., Elkhart, IN), hemoglobin (HGB) (Coulter Hemoglobinometer, Coulter Electronics, Inc.), total plasma protein (TPP), and fibrinogen (FIB) (TPP and FIB were measured on a standard T-S Meter, American Optical Co., Buffalo, NY). The difference between refractometer readings of heated (56°C for 3 min) and unheated plasma was calculated as the FIB content. In addition, partial thromboplastin time (PTT) and prothrombin time (PT) were measured in control and high dosage groups after 1, 6, and 12 weeks on trial (Hyland Clotek Fibrometer, Hyland Diagnostics Division, Cooper Diagnostics, Malvern, PA).

The serum was analyzed for urea nitrogen (BUN) (S.V.R. BUN Test Kit, Calbiochem-Behring, La Jolla, CA), total bilirubin (TB), direct bilirubin (DB) (Serosonic Bilirubin Test Kit, Mallinckrodt Inc. St Louis, MO), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) (S.V.R. AST, ALT, and ALP Test Kits, Calbiochem-Behring), and albumin (ALB) (BCG reaction, Pierce reagents, Pierce Chemical Co., Rockford, IL). Additional blood was collected in sodium fluoride oxalate for the evaluation of plasma glucose (GLU) (S.V.R. GLU Test Kit, Calbiochem-Behring). All photometric tests were run on a chemistry autoanalyzer (Rotochem IIa: American Instrument Co., Silver Spring, MD).

Urine was checked for glucose, blood, ketones (acetoacetic acid), bilirubin, urobilinogen, protein, and pH (Multistix, Ames Division, Miles Laboratories, Inc., Elkhart, IN). Urine ALP was evaluated with the method noted above. Urine ALP to urine creatinine ratios were calculated to eliminate the effect of urine concentration from the data analysis. The specific gravity of every urine sample was measured with a standard T-S Meter (American Optical Co.). Urine osmolality was also measured (Osmette A Osmometer, Precision Systems, Natick, MA).

Concentrations of Na, K, Cl, P, and creatinine (CRT) were determined in the urine and serum of every animal for every test period. Serum Ca determinations were also done. Na and K were measured with flame photometry (343 Flame Photometer: Instrumentation Laboratory, Inc., Lexington, MA) and Cl by AgCl precipitation (Corning Model 920M Chloride Meter: Corning Scientific Instruments, Medfield, MA). Ca and P determinations were made on a chemistry autoanalyzer (Rotochem IIa: American Instrument Co.) using the Worthington inorganic phosphorus reagent set (Worthington Diagnostic Systems, Inc., Freehold, NJ) and the cresolphthalon complexone–diethylamine reaction with Ca. Gil-Chem reagents (Ciba Corning Diagnostics, Oberlin, OH) were used in creatinine determinations to produce a picrate–creatinine color-producing complex which was measured by a spectrophotometer (Gilford 103 Spectrophotometer, Ciba Corning Diagnostics). This method removes pseudocreatinine from the determination.

Simultaneous serum and urine values were used to calculate the percentage of clearance ratios of Na, K, Cl, and P. The percentage clearance ratio is defined by the following formula:

$$\text{PCR of } X = [(X_{ur}/X_{sr})/(CR_{ur}/CR_{sr})] \times 100,$$

where X_{ur} is the concentration of substance X in the

urine, X_{sr} is the concentration of substance X in the serum, CR_{ur} is the concentration of creatinine in the urine, and CR_{sr} is the concentration of creatinine in the serum. Dogs secrete a small amount of creatinine from the renal proximal tubules. Creatinine secretion is greater in males and its clearance overestimates the glomerular filtration rate (Robinson *et al.*, 1974). For this reason females were used.

Livers of all dogs were evaluated grossly and with the light microscope. Dogs were anesthetized with pentobarbital. A small (5–6 cm^3) specimen was collected from deep in the right medial lobe of the liver and was immersed in 10% neutral-buffered formalin. Animals were then euthanized with an overdose of pentobarbital and a complete postmortem was performed. The liver was examined grossly and weighed after the nonhepatic tissue was removed.

The liver for light microscopic examination was taken from 10% neutral-buffered formalin, processed by standard paraffin techniques (AFIP, 1960), sectioned at 5 μm, and stained with hematoxylin and eosin. To evaluate glycogen content, two paraffin sections from each dog of control, medium dosage, high dosage, and pair-fed groups were made. One section from each dog was stained with periodic acid–Schiff (PAS). The other section was treated with diastase and then stained with PAS. To evaluate lipid content, frozen sections of formalin-fixed tissue of each dog in the control and high dosage groups were stained with oil red O. All light microscopic evaluation was done without knowledge of the treatment group. Slides evaluated for glycogen and fat content were graded on a scale of 1 to 10 in ascending order as the content increased.

The experiment utilized a complete random design to compare groups with different treatments. Individual dogs were the experimental units. Analysis of variance was done using the computer-based Statistical Analysis Systems (SAS Institute Inc., Cary, NC). General Linear Models Procedure (Proc GLM) was used to analyze FIBW, feed consumed, and arsenic consumed. Classes were group, week of test, and individual animal. Linear regression lines and slopes were obtained from Proc GLM analysis. It was recognized that the week factor was a repeated measure and hence the significance levels of week and group by week interaction effects may have been exaggerated. However, we took account of this effect by using conservative degrees of freedom when determining the probability of greater F values. Least significant differences (LSD) were calculated from the experimental error of dogs within groups (Snedecor and Cochran, 1967).

The blood, serum, and urine values for phases I and IIa were analyzed via the dog means for that phase without consideration of time since there were only two samples taken per dog in each phase. This was not enough for a valid analysis of the time factor. Proc ANOVA (SAS) was used when data were balanced but GLM was used when data were unbalanced. Phase IIb data were analyzed with consideration of the week by group interaction and the analysis was similar to that for FIBW.

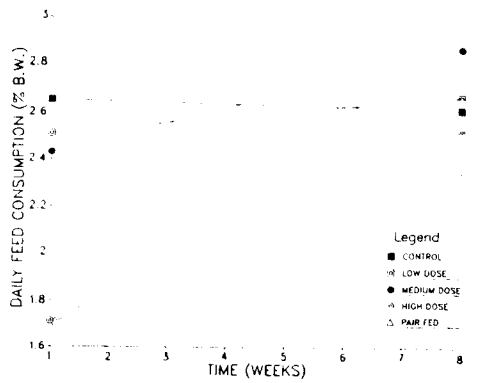

FIG. 1. Linear regression of daily feed consumption as the percentage of body weight over time in phase I. Nominal dosages of sodium arsenite were 0, 1, 2, 4, and 0 mg/kg/day for control, low, medium, high, and pair-fed groups, respectively. Medium and high dose group slopes were significantly higher than that of controls ($p < 0.05$ and $p < 0.01$, respectively).

RESULTS

Pair-fed group feed consumption data were not analyzed with the other groups because it was not independent of high dosage group data. A separate analysis of high dosage and pair-fed groups showed no statistical difference in the feed consumption.

The high dosage group feed intake decreased significantly in Week 1 relative to the week prior to arsenic exposure. The high dosage group Week 1 intake was statistically less than that of control and low dosage groups ($p < 0.05$). By Week 4, there was no significant difference among the groups. The other groups feed intake did not decrease relative to the control group. The linear regression of phase I feed consumption (Fig. 1) demonstrated that the medium dosage group ($p < 0.05$) and high dosage group ($p < 0.01$) had significantly larger slopes than control and

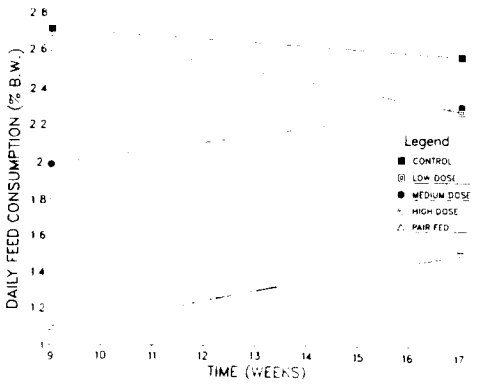

FIG. 2. Linear regression of daily feed consumption as the percentage of body weight over time in phase IIa. Nominal dosages of sodium arsenite were 0, 2, 4, 8, and 0 mg/kg/day for control, low, medium, high, and pair-fed groups, respectively. Slopes for medium dose, high dose, and pair-fed groups are significantly higher ($p < 0.01$) and the slope of the low dose group was lower ($p < 0.05$) than that of controls.

low dosage groups. The high dosage group slope was greater than the medium dosage group slope ($p < 0.01$).

In phase IIa, Weeks 9 through 17, the dosage was doubled for all groups. This was done to better study feed rejection and compensation seen in phase I. Both medium and high dosage groups had decreases in feed intake from Week 8 to 9. However, during Week 9, the medium dosage group feed consumption was not significantly less than the controls ($p < 0.05$). High dosage group feed consumption was significantly less than control, low dosage ($p < 0.01$), and medium dosage groups ($p < 0.05$). By Week 17, the consumption in medium and high dosage groups increased. However, high dosage group intake was still significantly less than the control group ($p < 0.05$). The linear regression analysis of phase IIa data (Fig. 2) showed that all three arsenic-exposed groups had statistically different ($p < 0.01$) feed intake slopes than controls. Medium and high dosage groups had a positive slope and the low dosage group had a negative slope relative to the control group.

The high dosage group was terminated on Week 17. Control, low dosage, and medium dosage groups were continued through Week 26 (phase IIb). The medium dosage group had reduced feed intake in Week 9 (not statistically significant), but feed intake increased and approached the level of controls and low dosage groups by Week 26. The linear regression slope of the medium dosage group (Fig. 3) was significantly greater ($p < 0.01$) than the slope for control and low dosage groups.

According to the experimental design, arsenic consumption was determined by the feed intake. The daily arsenic intake averaged over the weeks was almost always significantly different among groups (largest $p < 0.05$). The exceptions are between low and medium dosage groups in Week 10 and between medium and high dosage groups in Weeks 9, 10, 11, 12, 16, and 17. The average sodium arsenite consumption over all of phase I was 0.0, 0.88, 1.80, 2.88, and 0.0 mg/kg/day for control, low dosage, medium dosage, high dosage, and pair-fed groups, respectively. These overall averages are statistically different from each other ($p < 0.01$). The arsenic consumption from Week 1 to 8 was rel-

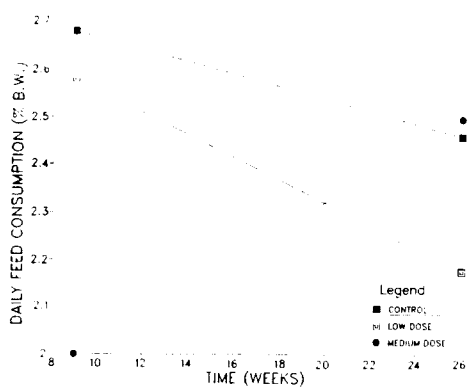

FIG. 3. Linear regression of daily feed consumption as the percentage of body weight over time in phase IIb. Nominal dosages of sodium arsenite were 0, 2, and 4 mg/kg/day for control, low, and medium dose groups, respectively. The medium dose group slope is significantly higher than slopes for low dose and control groups ($p < 0.01$).

atively constant for low and medium dosage groups. The high dosage group sodium arsenite daily intake gradually increased through phase I. This is due to increased feed consumption, as can be seen in Fig. 1.

In phase IIa, the overall average daily sodium arsenite consumption was 0.0, 1.66, 2.86, 3.74, and 0.0 mg/kg/day for control, low dosage, medium dosage, high dosage, and pair-fed groups, respectively. The overall averages are statistically different ($p < 0.01$). Linear regression showed significant increases in medium and high dosage groups arsenic intake from Week 9 to 17 ($p < 0.05$ and $p < 0.01$, respectively). Low dosage group intake ($p < 0.05$) decreased over the same period. In Week 11, medium and high dosage groups consumed similar amounts of arsenite (2.77 and 2.83 mg/kg/day, respectively) because of severe feed rejection by the high dosage group.

Control, low dosage, and medium dosage group average daily sodium arsenite consumption for phase IIb was 0.0, 1.59, and 3.00 mg/kg/day, respectively. The average arsenic intake amoung groups for phase IIb was statistically different ($p < 0.01$). Arsenic intake decreased for the low dosage group and increased for the medium dosage group from Week 9 to 26. The linear regression analysis of the low dosage group decrease and medium dosage group increase was significant ($p < 0.01$).

Sodium arsenite caused a dose-dependent decrease in the FIBW. FIBW of the high dosage group decreased from Week 0 to Week 3. From Week 3 to Week 8, the FIBW of the high dosage group leveled off and slightly increased. The linear regression of FIBW over the first 8 weeks resulted in negative slopes for the high dosage and pair-fed groups that were statistically similar and significantly less than those of other groups ($p < 0.01$). Control, low dosage, and medium dosage groups had gradual weight gains over the same period that were statistically different from each other (Fig. 4).

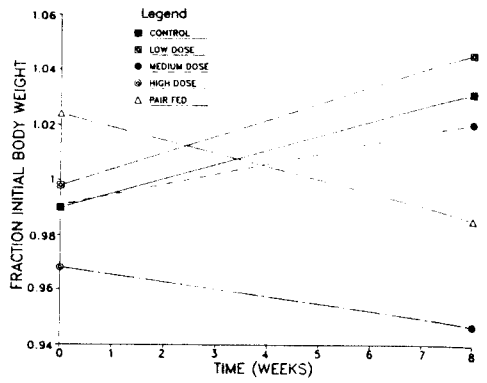

FIG. 4. Linear regression of the fraction of initial body weight over time in phase I. Nominal dosages of sodium arsenite were 0, 1, 2, 4, and 0 mg/kg/day for control, low, medium, high, and pair-fed groups, respectively. High and pair-fed group slopes were statistically similar and significantly lower than that of the other groups ($p < 0.01$).

FIBW of the pair-fed group increased during Week 1 to 1.050 then approximately paralleled the weekly FIBW of the high dosage group for the rest of phase I and IIa. This gave the pair-fed group a linear regression line higher than but almost parallel to that of the high dosage group (Figs. 4 and 5).

In phase IIa, control and low dosage groups continued to gain weight. Hence, the linear regressions of FIBW for these groups had positive slopes (Fig. 5). The medium dosage group gradually lost weight and the linear regression demonstrated a slightly negative slope. High dosage and pair-fed groups lost weight faster than in phase I and had linear regression lines statistically parallel and with negative slopes. The regression slopes of medium dosage, high dosage, and pair-fed groups were statistically less than those of control and low dosage groups ($p < 0.01$). Slopes of high dosage and pair-fed groups were significantly less than the slope of the medium dosage group ($p < 0.01$).

In phase IIb, the medium dosage group gradually lost weight for the first half and then remained stable. This resulted in a linear regression line with a slightly negative slope

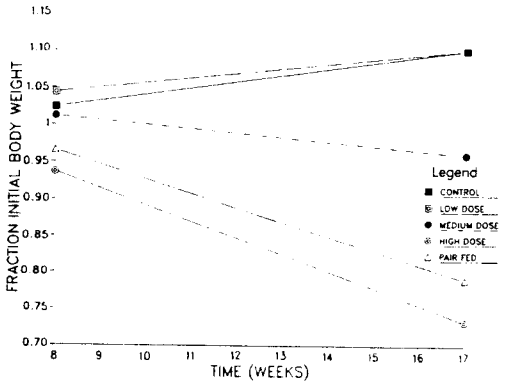

FIG. 5. Linear regression of the fraction of initial body weight over time in phase IIa. Nominal dosages of sodium arsenite were 0, 2, 4, 8, and 0 mg/kg/day for control, low, medium, high, and pair-fed groups, respectively. High and pair-fed group slopes were statistically similar and significantly lower than that of other groups ($p < 0.01$). The medium dose group slope was significantly lower than slopes of low dose and control groups ($p < 0.01$).

(Fig. 6). Both control and low dosage groups gradually gained weight over phase IIb, hence both had positively sloped linear regression lines. The low dosage group gained less than the control group and had a significantly ($p < 0.01$) smaller slope. The slope for the medium dosage group is statistically less than those for either control or low dosage groups ($p < 0.01$).

All group mean blood values were within the normal range. Normal ranges used in this paper were defined by Duncan and Prasse (1986). Blood values other than FIB of treated groups were not significantly different from controls. FIB was significantly ($p = 0.05$) elevated relative to controls in high dosage and pair-fed groups in phase IIa (133, 192, and 258 mg/dl, respectively). The pair-fed group elevation was significantly higher than that of the high dosage group ($p = 0.05$).

Mean PT and PTT combined values for control and high dosage dogs were 8.6 and 16.5 sec, respectively. There was no statistically significant difference between these groups ($p > 0.05$).

All group mean serum values except ALT were within the normal range. No differences in serum creatinine, glucose, total bilirubin, direct bilirubin, and alkaline phosphatase were present among groups. Group differences were present in ALB, BUN, AST, and ALT levels. Phase IIa BUN levels of pair-fed animals were depressed relative to controls ($p = 0.01$). Serum ALB was elevated in medium dosage, high dosage, and pair-fed groups in phase I ($p = 0.03$). In phase IIa, high dosage and pair-fed groups had depressed serum ALB ($p = 0.03$). In all phases, AST and ALT were elevated in one or more treated groups (Table 1). Serum AST values were significantly elevated in high dosage dogs in phases I and IIa ($p < 0.05$) and in medium dosage dogs in phases IIa and IIb ($p < 0.01$). Serum ALT values were significantly elevated in low dosage dogs in phase IIb ($p < 0.05$), medium dosage dogs in phase IIb ($p < 0.01$), and high dosage dogs in phases I and IIa ($p < 0.01$). Pretreatment mean values for ALT and AST were 24 and 19 IU/liter, respectively. There was no statistically significant difference between pretreatment values of control and treated groups.

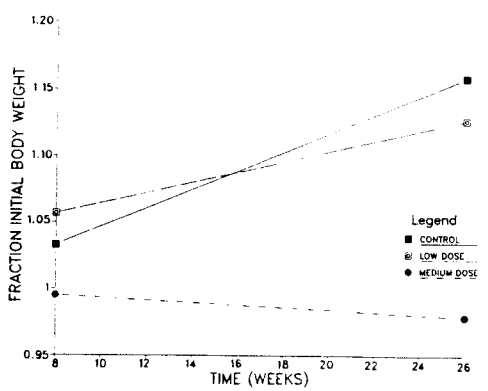

FIG. 6. Linear regression of the fraction of initial body weight over time in phase IIb. Nominal dosages of sodium arsenite were 0, 2, and 4 mg/kg/day for control, low, and medium dose groups, respectively. The slopes of the low dose group and medium dose group are significantly lower than that of controls ($p < 0.01$). The slope of the medium dose group is significantly lower than that of the low dose group ($p < 0.01$).

TABLE 1

MEAN SERUM AST[a] AND ALT[b] VALUES[c] OF DOGS EXPOSED TO DIETARY SODIUM ARSENITE IN PHASES I, IIa, AND IIb

	AST			ALT		
Group	I	IIa	IIb	I	IIa	IIb
Control	18	17	16	29	24	23
Low dosage	21	19	20	37	42	44*
Medium dosage	22	29**	29**	27	42	45**
High dosage	26*	26*		98**	84**	
Pair fed	16	15		23	22	

[a] Aspartate aminotransferase.
[b] Alanine aminotransferase.
[c] Given in IU/liter.
* Statistically different than controls ($p < 0.05$).
** Statistically different than controls ($p < 0.01$).

Urine blood, ketones, urobilinogen, protein, pH, specific gravity, and osmolality were within the normal range and no significant differences were present among groups. Urine bilirubin for the pair-fed group was significantly elevated in phase I ($p = 0.01$). In this period, each dog was sampled twice. Three of six pair-fed dogs in this period had one sample which tested +1 or +2 and the other tested 0. All other dogs of all groups tested 0 on both samples.

Urine glucose was elevated in pair-fed dogs during phase IIa. As was the case for urine bilirubin in phase I, three of the six pair-fed dogs had one of two urine samples test 0.1 g/dl and the other 0. All other dogs of other groups tested 0 in both samples.

Urine ALP of the low dosage group was significantly elevated relative to controls in phase IIa and IIb ($p = 0.01$ and $p < 0.05$, respectively). No other group in any phase varied significantly from controls. The analysis of urine ALP expressed as raw data or urine ALP/urine CRT showed that the low dosage group deviated from controls. Mean urine ALP to CRT ratios for control and low dosage groups for phase IIa were 1.2 and 2 IU/g and for phase IIb were 1.1 and 1.6 IU/g, respectively.

No significant difference in serum Cl was present among groups. Serum Na for low and medium dosage groups in phase I was increased significantly over the control group ($p < 0.05$). Phase I serum Ca was increased in the medium dosage group ($p < 0.05$) and was decreased in the pair-fed group ($p < 0.01$) relative to controls. Phase IIa serum Ca of high dosage and pair-fed dogs was significantly lower than that of control dogs ($p < 0.01$ and $p < 0.05$, respectively). Mean serum Ca for medium dosage was also lower than controls but not significantly. In phase IIb, the medium dosage group had lower serum Ca than the control group ($p < 0.01$). Serum P values of pair-fed dogs in phases I and IIa were lower than those of control dogs ($p < 0.01$). Low dosage and medium dosage groups in phase IIb had higher serum K than the controls ($p < 0.01$). Mean serum K values were 4.2, 4.5, and 4.5 for control, low dosage, and medium dosage dogs, respectively. The LSD was 0.29 ($p = 0.01$).

PCRs for Na, K, P, and Cl were not significantly different among groups in any of the three phases.

The phase IIb analysis of time by group interaction showed no significant treatment effect in any of the parameters measured.

None of the livers had gross lesions at postmortem examination. The liver weight per body weight of the medium dosage group was significantly different from that of controls. The mean liver weight per body weight for control, low dosage, medium dosage, high dosage, and pair-fed groups was 29, 27, 33, 31, and 29 g/kg, respectively. The least significant difference among groups was 3.8 ($p = 0.05$). Therefore, only the medium dosage group differed significantly from the control group.

Light microscopy revealed no difference among groups. No lesions were found in any group on the evaluation of hematoxylin and eosin-stained sections. The hepatocyte glycogen content was abundant and was not significantly different among control, medium dosage, high dosage, and pair-fed groups. The hepatocyte lipid content was limited and was not different between control and high dosage groups.

DISCUSSION

Inorganic arsenic in the feed caused a dose-dependent rejection of feed. Four milligrams per kilogram of body weight per day of sodium arsenite in the daily ration caused medium and high dosage groups to have decreases in feed intake the first week of phases IIa and I, respectively. The high dosage group decrease was significant ($p < 0.05$) and the medium dosage group was nearly significant ($p > 0.05$). Eight milligrams per kilogram of body weight per day caused the high dosage group to have a greater decrease ($p < 0.01$) in intake than when it was first exposed to 4 mg/kg/day. All three decreases in feed intake were followed by a significant ($p < 0.01$) increase of intake depicted by linear regression analysis (Figs. 1 and 2).

The feed intake of control and low dosage groups was not significantly different in phases I and IIb (Figs. 1 and 3). In phase IIa (Fig. 2), the linear regression slopes of these groups were significantly different ($p < 0.05$). When these data were reanalyzed with additional data in phase IIb, there was no statistical difference. This supports the conclusion that exposure to 1 or 2 mg/kg/day of sodium arsenite does not have a significant effect on feed intake. In phase I, the medium dosage group (2 mg/kg body wt/day) had a significant feed intake increase over time relative to controls (Fig. 1). The medium dosage group Week 8 feed consumption was greater (statistically not significant) than that of the controls. This would suggest that arsenic at this concentration stimulates feed intake. However, the low dosage group exposed to 2 mg/kg/day in phase IIb did not exhibit the same change (Fig. 3).

Dogs tolerated nominal dosages of 1 and 2 mg/kg/day of sodium arsenite (Figs. 1 and 3). It appears that after a certain arsenic concentration is reached, the arsenic controls feed intake. When the high dosage group exposure to arsenic was increased from 4 to 8 mg/kg/day, the absolute amount of arsenic consumed was not markedly increased for 6 weeks. This is because when arsenite concentrations were doubled for the high dosage group, the feed intake was voluntarily reduced by half. Midway through phase IIa this trend reversed and the high dosage group started to gradually increase its intake.

The linear regression analysis of FIBW over time demonstrates a significant ($p < 0.01$) decrease in body weight when dogs are exposed to 4 or 8 mg/kg/day of sodium arsenite (Figs. 4–6). Control and low dosage groups gained weight over the same period. Weight loss has been demonstrated previously in pigs, mice, rats, and dogs exposed to inorganic arsenic (Calvery *et al.*, 1938; Byron *et al.*, 1967; Fowler and Woods, 1979; Morrison and Chavez, 1983).

Regression line slopes for FIBW of high dosage and pair-fed groups were statistically the same. This demonstrates that decreased feed intake, not the direct effect of arsenic, caused the weight decrease. In Fig. 4, the re-

gression line for the pair-fed group is higher than the high dosage group. This was caused by the unexplained increase of FIBW for the pair-fed group in Week 1.

Hairless mice given 50 mg/liter of arsenic trioxide in the drinking water had an initial weight loss, but compensated over the following 15 weeks and achieved final weights similar to the controls (Bencko and Symon, 1969). Dogs in this study did not demonstrate this weight compensation. However, dogs offered 4 mg/kg/day had an increased feed intake after an initial decrease and if the study had been longer the decreased weights may have increased toward normal or control values.

Gradual feed intake increasing after the initial rejection of feed containing 4 or 8 mg/kg/day of sodium arsenite suggests that adaptation was occurring (Figs. 1–3). Adaptation to arsenic does occur and has been experimentally shown to increase the arsenic LD_{50} in mice chronically exposed to arsenic trioxide (Bencko and Symon, 1969). This might be accomplished by decreased absorption or by increased excretion of ingested arsenic.

If arsenic-induced nausea was the cause of feed rejection, it is possible that the dogs got so hungry that appetitive drive became the stronger behavioral stimulus. Feed aversion induced by illness in rats is weakened by increased hunger (Peters and Reich, 1973).

FIB in phase IIa was elevated in both high dosage and pair-fed groups. Therefore, arsenic can be ruled out as the cause. This increase was not above what is considered high normal. A major common factor in these groups was limited feed intake. It is not apparent how reduced feed intake could cause the increase. Normal TPP values for the same time period eliminate dehydration as a cause. Inflammation is a common cause of fibrinogen elevation. However, neither group had a differential leukocyte count that suggested inflammation.

Elevated urine bilirubin was not an arsenic effect since no treated group had elevated levels. Because the pair-fed group was tested later than the others and animals that had positive values were negative on other sampling dates and since there was no evidence of elevated serum bilirubin, we concluded that the elevation was not significant. For the same reasons the same conclusion was made regarding the pair-fed group urine glucose elevation.

BUN of pair-fed animals in phase IIa was lower than other groups but was within normal limits. In the same time period, serum creatinine was not significantly different between groups, indicating that renal function was not the cause of the depression. Diminished protein intake can cause decreased BUN (Finco, 1980). Inadequate caloric intake causes increased protein catabolism and increased BUN (Finco, 1980; Duncan and Prasse, 1986). Considering these facts, a possible scenario is that pair-fed dogs were given a limited feed intake, therefore protein was limited and BUN decreased. However, high dosage dogs feed intake was the same as pair-fed dogs but their BUN did not decrease. High dosage dogs were 20 weeks younger and had considerably less fat reserves than the pair-fed dogs going into the trial. Therefore, high dosage dogs had to catabolize structural protein for energy. Hence, factors that caused decreased BUN and increased BUN may have neutralized each other. It is clear that arsenic had no direct effect on BUN levels.

All serum albumin values were within normal limits, however, significant differences were found among groups. In phase I, the serum albumin elevation was significant in medium dosage, high dosage, and pair-fed groups relative to controls. Kaneko (1980) states that true overproduction of albumin has not been known to occur in any animal. Since elevated values are still within the normal range, we conclude that the difference is random variation. In phase IIa, high dosage and pair-fed groups had severely limited feed intake and a significant decrease in serum albumin. Dietary malnutrition causes serum

albumin depression (Duncan and Prasse, 1986). Since the pair-fed group was not given arsenic, decreased serum albumin is not a direct effect of arsenic.

Urine ALP to CRT ratio elevations in the low dosage group was peculiar to that group and was not an arsenic effect. This conclusion was reached because no other treated group had elevated values and a low dosage group pretrial urine sample had an elevated ratio (mean = 4 IU/g).

Liver leakage enzymes AST and ALT were significantly elevated in the serum of dogs treated with sodium arsenite. ALT values for the high dosage group were above the upper normal limit of 66 IU (Table 1). ALT is specific for the liver in dogs and is found normally free in the cytosol. AST is present in the cytosol and mitochondria of many tissues (Cornelius, 1980). These enzymes are found in high concentrations within the normal cell. Serum levels increase when plasma membranes are altered and allow enzyme leakage (Duncan and Prasse, 1986).

The probable causes of elevated serum enzymes include altered hepatocyte plasma membrane permeability or hepatic enzyme induction. We conclude that the former is the most probable. This is supported by the fact that arsenic has an inhibitory effect on a multitude of enzymes, many of which could ultimately cause dysfunction of the plasma membrane (Webb, 1966). Serum AST and ALT increases were low grade and consistent. Since AST and ALT have short serum half-lives (1–3 days), this indicates that hepatocyte damage was persistent (Duncan and Prasse, 1986). Canine exposure to sodium arsenite in the field causes an elevation of ALT (Evinger and Blakemore, 1984). However, serum AST was reduced in rats exposed to 50 mg/kg of arsenic as dietary arsenate (Mahaffey *et al.*, 1981). This discrepancy may be due to the fact that rats and dogs metabolize arsenic differently (Vahter, 1983). Rats accumulate arsenic in the blood with a half-life of 60 to 90 days compared to a half-life of hours in the dog. This high blood arsenic in the rat may have interfered with the measurement of serum enzymes.

Increased serum Na of low and medium dosage groups in phase I was not repeated by these or other groups in the rest of the experiment. This indicates that treatment was not the cause. Increased values were well within the normal range. The reason for these changes was not determined.

The increase of serum K in phase IIb of low and medium dosage groups was not repeated by these or other groups in the rest of the experiment. Increased serum K values were well within the normal range. It is doubtful that this was a treatment effect since no treated group in phases I or IIa had this change. However, the treatment effect cannot be ruled out. If it was a treatment effect, the pathophysiological mechanism for it is unknown.

The decrease of serum P in pair-fed dogs was probably due to their age. Young animals have higher serum P values than adults (Duncan and Prasse, 1986). Pair-fed dogs were 20 weeks older than the other dogs.

Phase I serum Ca elevation in medium dosage dogs and the depression in pair-fed dogs are unexplained. However, the mean serum Ca of control and low dosage dogs decreased in each sequential phase (11.4, 11.2, and 11.0 mg/dl in phases I, IIa, and IIb, respectively). Therefore, the fact that pair-fed dogs were 20 weeks older than the other dogs may explain why they had lower serum Ca values.

Serum Ca depression in high dosage and pair-fed groups of phase IIa and the medium dosage group in phase IIb could have been caused by decreased serum albumin (Duncan and Prasse, 1986). The medium group mean serum albumin of 3.7 g/dl was not statistically lower but was considerably lower than that of the control group (4.5 g/dl).

The increase of the medium dosage group liver weight per body weight was not paralleled by any other group, leaving the cause of

the increase undetermined. High dosage and pair-fed groups had highly significant weight loss and no significant change in the liver weight to body weight ratio. Therefore, the liver weight loss was proportional to the body weight loss. The decrease of liver reserves is expected in chronically starved animals (Kelly, 1985).

Starvation depletes liver stores of carbohydrates (Cheville, 1983; Kelly, 1985). In this study, liver glycogen was highly conserved in all groups evaluated. The pair-fed group dogs may have been better able to conserve the liver glycogen because they were older and weighed more. However, the high dosage group was very thin at termination and the presence of normal amounts of liver glycogen was unexpected. It is possible the glycogen was conserved in the high dosage group because of the blocking of its entry into the citric acid cycle by inactivation of pyruvate dehydrogenase by the arsenic. Another explanation would be if the high dosage and pair-fed dogs converted primarily to lipid and ketone energy metabolism. This would allow what little glucose was taken in daily to be stored as glycogen.

In conclusion, dietary sodium arsenite causes a dose-dependent decrease of feed consumption and body weight. Weight loss was due to decreased feed consumption, not a direct effect of arsenic. Elevated serum ALT and AST suggests that sodium arsenite causes altered hepatocyte plasma membrane permeability. Serum enzyme changes were not accompanied by microscopic liver changes.

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