

U.S. GEOLOGICAL SURVEY
Reston, VA 22092

Memorandum

Date 6/28/77

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AUTHDR(S): R. W. White, J. R. Tkach, and J. J. Connor

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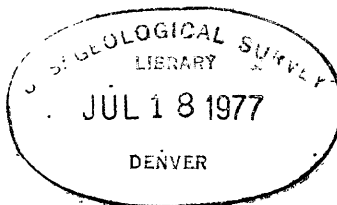
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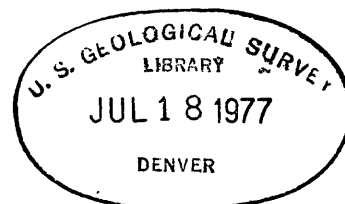


UNITED STATES DEPARTMENT OF THE INTERIOR
GEOLOGICAL SURVEY

COMPOSITION OF GALLSTONES FROM THE DENVER, COLORADO AREA

By

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Survey



Open-File Report 77-570
1977

This report is preliminary and has not been
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JUL 22 1977

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COMPOSITION OF GALLSTONES FROM THE DENVER, COLORADO, AREA
by R. W. White, J. R. Tkach, and J. J. Connor

Introduction

A fairly large body of literature describes the origin and incidence of cholelithiasis (gallstones) around the world. The most relevant factors in stone formation seem to be family history, sex (females have greater incidence than males), and pregnancy (Winch and others, 1969), although diet and geographic location have also been investigated. For example, Hills (1970) concluded that an increase in stones among immigrant women (from southern Europe) in Melbourne, Australia was due, in part, to a change in diet to more fat and less carbohydrates. Sarles and others (1969) noted that northern India had an incidence seven times that in southern India and remarked that more fat is eaten in the north. Also, a difference in the kind of stone has been noted worldwide. Nakayama and Miyaki (1970) stated that whereas the cholesterol stone is predominant in the United States, the bile-pigment stone predominates among the Hong Kong Chinese, and both kinds appear with about equal frequency among the Japanese. The intermediate status of Japan might reflect the change from an eastern to a western economic (environmental) orientation. Hwang (1970) found an association between opium addiction and cholelithiasis in adult male Chinese in Singapore.

One of the most interesting geographically oriented studies was that by Popov (1968), who found two neighboring rural districts of Kazakhstan having similar ethnic and cultural makeup which showed large differences in the numbers of reported cases of cholelithiasis and cholecystitis and large differences in the composition of the drinking water. It is unclear from Popov's data precisely what "cases" refers to, but the magnitude of the reported values is such that they probably represent new diagnoses per 10,000 population per year. Presuming the reporting procedure to be identical in the two districts, the five times greater number of cases in the one area relative to the other certainly must be regarded as significant. The district with the higher incidence had drinking water significantly higher in total dissolved solids, chloride, and sulfate.

The work presented here was undertaken in hopes of assessing the effect, if any, of water source on gallstone composition. Much of the greater Denver area is supplied with drinking water from two distinct sources, which have been shown to contrast in both trace-element composition and hardness (fig. 1). Barnett and others (1969) found, in the summer of 1966, that raw water at the Moffat Treatment Plant generally had half the hardness of raw water at the Marston Plant and contained less Al, Ba, B, Li, Mo, and Sr, but more Fe and Zn. The sources for the Moffat Plant are the Upper Fraser and Williams Fork Rivers (fig. 2). The sources for the Marston/Kassler Plants are the South Platte River and its tributaries and Dillon Reservoir (Snake River, Blue River, and Tenmile Creek).

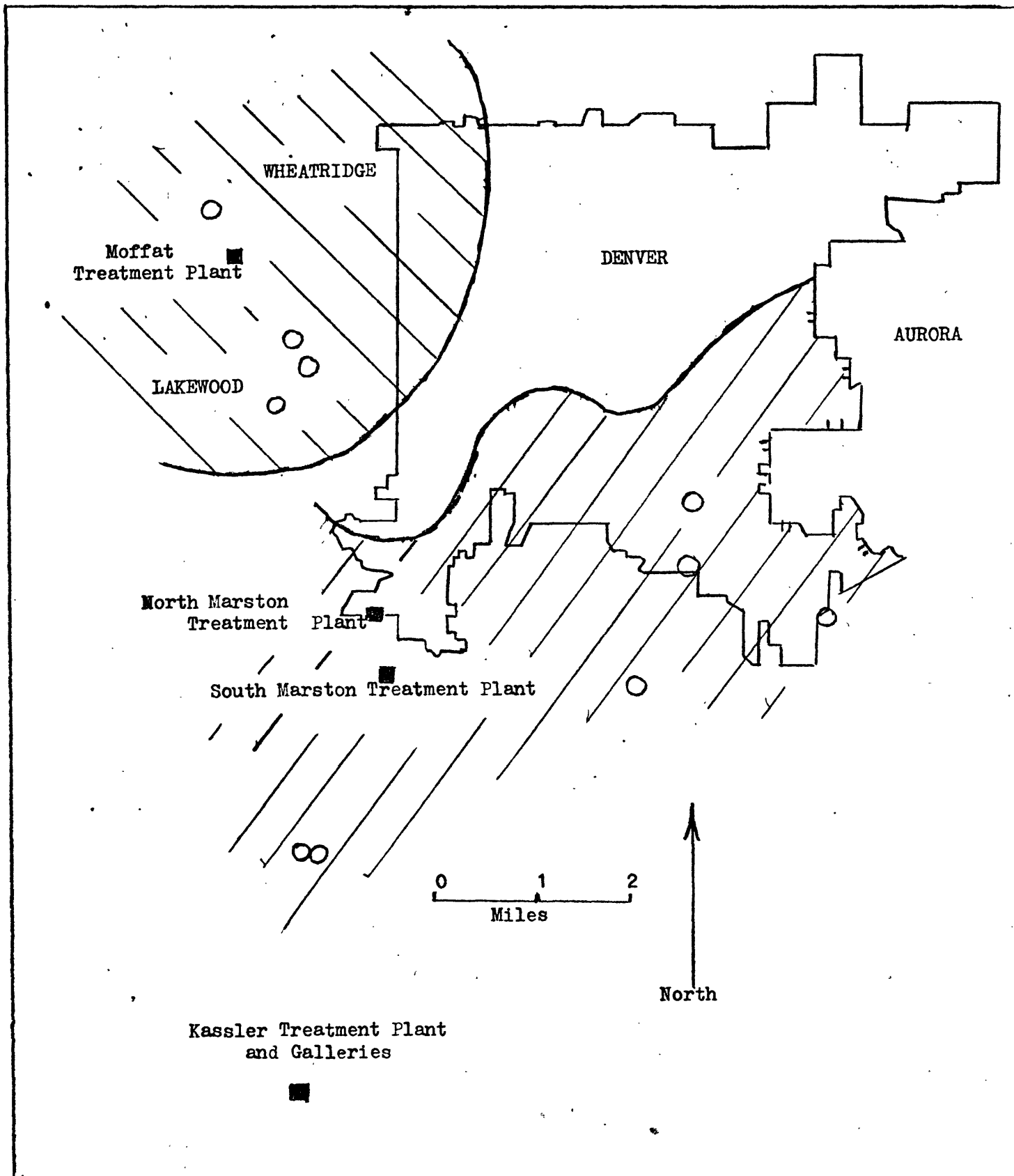


Figure 1. Map showing location of water treatment plants (squares), areas served by treatment plants (hachured lines), and residence of patients (circles).

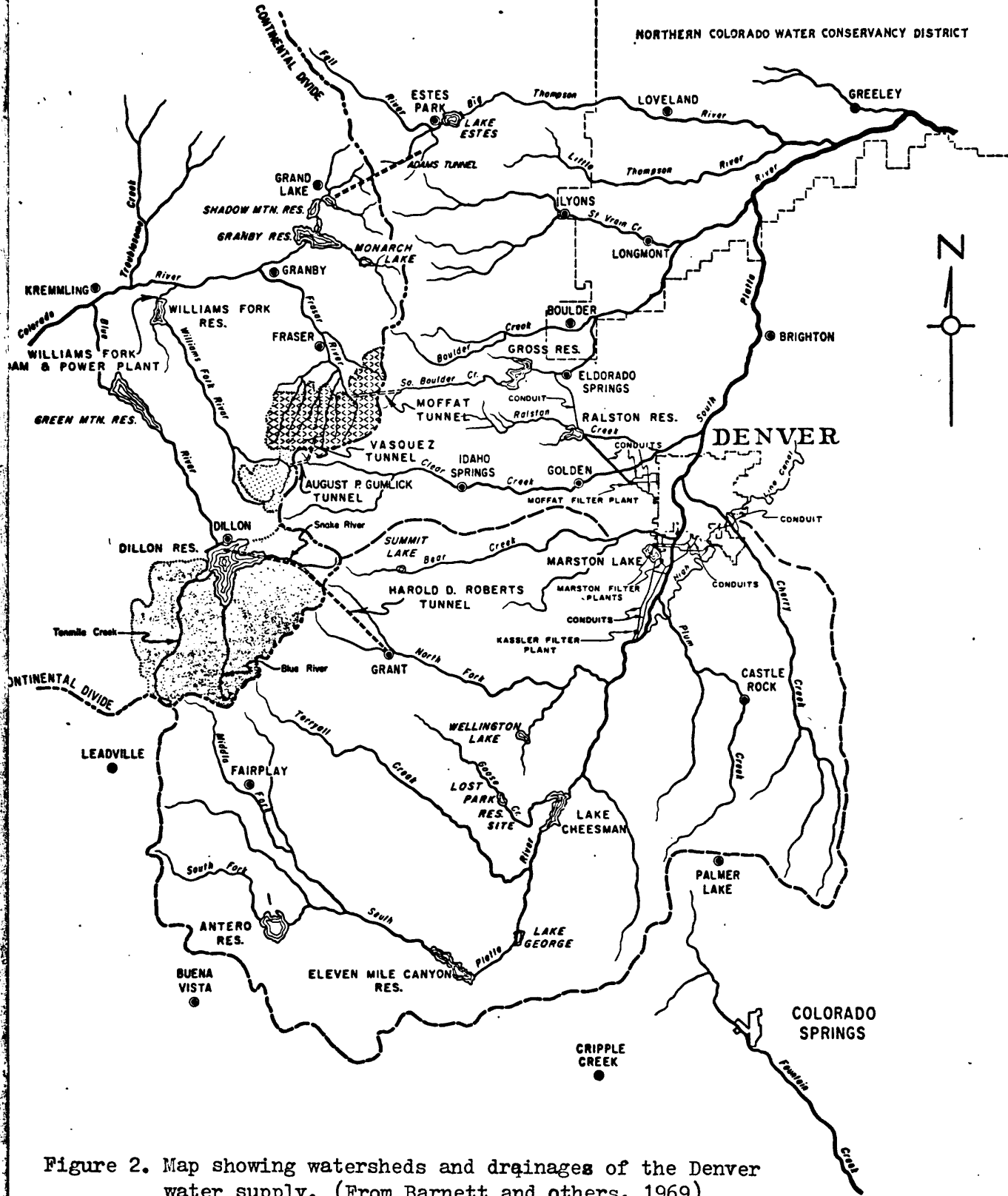





Figure 2. Map showing watersheds and drainages of the Denver water supply. (From Barnett and others, 1969)

- Continental Divide
- South Platte Water Shed Boundary
-  Blue River Diversion Project
-  Fraser River Diversion Project
-  Williams Fork Diversion Project

0 5 10 20
APPROXIMATE SCALE IN MILES

Treatments at the Marston Plant include addition of aluminum sulfate, ammonium sulfate, chlorine, and copper sulfate. Treatments at the Moffat Plant include addition of these four chemicals plus calcium oxide and sodium silico-fluoride.

All of the analytical work performed on the gallstones cited in this report was done in laboratories of the U.S. Geological Survey in Denver, Colorado.

Collection of gallstones

Gallstones were collected after surgical removal at three hospitals in Denver over a three-month period during the spring of 1972. The stones were placed in formaldehyde for one-half to four hours, then rinsed with water, air-dried, and sealed in plastic bags. A total of 75 sets of stones was collected; these ranged in weight from 0.1 g to 3.5 g per set. One sample was studied initially in order to determine the amount of material necessary for analysis, which proved to be about 2 g. Questionnaires were then sent to the 50 patients whose stones had weighed 1.9 g or more. The main purpose of the questionnaire was to establish the living places of each patient prior to the time that he or she developed symptoms of gallstones or that gallstones were diagnosed. Thirty-three patients, or 66 percent, returned the questionnaires, and 10 of the patients had lived within the two contrasting water districts of Denver for at least four years prior to the onset of symptoms or to the diagnosis of cholelithiasis. All 10 patients are Caucasian and all but one (using water from the Moffat Plant) are of northern European extraction.

Mineralogy of gallstones

The mineralogy of the stones was determined by X-ray powder diffractometry. Each set of stones was sampled by selecting one or more whole small stones, or a sector of a large stone. For a few laminated stones, the outer lamina and the central part were examined separately to determine the cause of differences in color or texture. The powdered samples were packed into aluminum holders, and the analyses were run using a Norelco diffractometer and recorded on a paper chart. This method records the low-angle peaks of the organic compounds in gallstones. It is rapid and is easily adapted to semiquantitative determinations of percentage compositions of mixtures of crystalline materials.

A total of 43 sets of stones was examined in this manner, and the following crystalline phases were identified: cholesterol monohydrate, anhydrous cholesterol, vaterite, calcite, aragonite, calcium palmitate, apatite, and whitlockite. One stone was completely amorphous to X-rays, and it is apparent that amorphous material is present in differing amounts in many of the stones, especially in those which yield darkly colored powders

on crushing. The color of gallstones is not a reliable indication of the content of bilirubin, which is the major amorphous component. However, the color of powdered stones (known to mineralogists as the streak) does darken with increasing content of noncrystalline material and may be useful for a rapid qualitative estimate of bilirubin content.

Cholesterol is the dominant constituent in most of the stones studied. After it has been powdered for X-ray diffraction study, cholesterol monohydrate rapidly loses water and inverts to anhydrous cholesterol, if stored in an atmosphere of low humidity. On 15 stones, the dehydration was followed by repeated recording of the diffractogram from the same preparation at intervals as short as 30 minutes. In most of these, the inversion was rather abrupt and was noted by a decrease in intensity of the 16.8-, 5.9-, and 3.8-A peaks of the monohydrate and an increase in intensity of the 5.7-, 5.2-, 5.1-, and 4.9-A peaks, plus several minor peaks of anhydrous cholesterol. In two stones, however, the dehydration took place by way of an intermediate diffractogram very similar to that of anhydrous cholesterol, except for lower relative intensity and a slight shift to lower spacing of the 5.7-A peak and a decidedly lower intensity of the 4.9-A peak. Continued drying of both of these powders led to a pattern identical to that of anhydrous cholesterol; but one, from a transparent crystalline cholesterol stone, required drying in a dessicator at room temperature to achieve full dehydration. The intermediate material has a behavior and diffractogram similar to that of cholesterol II of Sutor and Wooley (1971), but the resolution of the diffractometer method is not adequate for certain identification as cholesterol II.

The dehydration studies reported above served the additional purpose of demonstrating which of the diffraction peaks of cholesterol were least affected by dehydration. It was found that the height and shape of the peak near 5.2-A for anhydrous cholesterol was relatively stable after air drying of the powder overnight. Consequently, the height of that peak, less the background, was used as a measure of the content of crystalline cholesterol in the dehydrated powders. The maximum height was obtained from a stone inferred to be 100 percent cholesterol (coarse, transparent, colorless crystals that crush to a white powder), and the cholesterol contents of other stones which yielded lower heights for the 5.2-A peak were assumed to be linearly related. The precision of measurement of the peak height, as determined by repeat measurements on eight stones, was ± 2.5 percent, but the accuracy of determinations of cholesterol content by this method is not known. Factors which are necessary for accurate determinations include uniform granularity of the powders, absence of preferred orientation of grains, uniform dehydration, uniform matrix, uniform crystallinity, and accurate sampling of stones which may or may not be homogeneous. Available evidence indicates that all of these factors can vary in gallstones and are difficult to estimate or control. Consequently, no great accuracy can be claimed for the results, which have been rounded to the nearest 10 percent cholesterol.

Chemistry of gallstones

The properties of 13 sets of gallstones, grouped according to the water supply, sex, and age of the patient, are given in table 1. Both chemical and nonchemical properties measured in these stones tend to be highly variable with the exception of Al in stone ash, and, perhaps, Ba and Sr in stone ash. Because the low variability observed in these metals exceeds only slightly the expected error of the analytical method, they will not be evaluated further. Ca, Na, K, Cu, Fe, Mg, and Mn were measured by both atomic absorption and emission spectrographic methods, but the former method proved generally insensitive for Fe and Mn and the latter method was generally insensitive to Ca, Na, and K. Comparisons of Cu and Mg between the two methods are not good, reflecting difficulties in analysis. An extensive search of the literature has revealed that 25 metals or semi-metals have been identified in gallstones to date (fig. 3).

Medians and ranges in parts per million (ppm) dry weight of stones and as micrograms of total mass are given in table 2. The chemical variability expressed in these summaries is exceptionally large for many trace-element properties. The highest recorded values for Ag, Cu, and Sr, for example, exceeded the lowest, respectively, by more than a factor of 1,000.

Much of this large variability reflects extremely high or extremely low element concentrations in the stones. The stones of patient 2509 (a male) have a high mineral content--16 percent ash and 8.42 percent Ca, which is equivalent to 21 percent CaCO_3 . This might account for the elevated concentrations in dry weight of Mg, Mn, Cu, and Pb, all of which can rather readily fit into a carbonate crystal lattice. The stones of patient 4142 (also a male) appear to be even more carbonatic (30 percent ash, 9.35 percent Ca), but appear to be elevated in few other elements. The stones of patient 3438 (a female) are low in mineral content, but contain nearly as much K, Fe, and Zn as do the stones of patient 2509.

Results

A visual comparison of stone chemistry based on the two identified water sources is shown in figures 4 and 5. Figure 4 is based on parts per million of element in dry weight, and figure 5 is based on total micrograms of element in the stones. Again, the extreme variation among individuals is apparent. This variation is so large compared to any observed group differences that it has effectively obscured such differences, assuming that they are present.

Similarly, analysis of variance (based on logarithms of metal concentration) failed to demonstrate any significant differences based on these two water sources at the 0.05 probability level (table 3). Although manganese in dry weight exhibits the strongest effect ($F = 2.47$), this difference cannot be defended on any statistical basis, and we conclude that these data are too few to support the thesis that water source can control gallstone chemistry.

Table 1.--Properties of gallstones collected from patients in the Greater Denver area
%, percent; ppm, parts per million; n.d., not determined; leaders (--) indicate no data. Analysts: Claude Huffman, Jr., Leon Bradley, Raymond Havens, and Richard White

Measurement	Hoflat Treatment Plant				Marston/Kessler Treatment Plants				Other or Unknown	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
	2509	3449	3918	3384	3300	3764	5077	399-159	4279	4490
Patients' age, yrs	57	61	35	57	42	52	29	36	46	52
Residence time, yrs ^{1/}	26	8	4	14	11	8	5	9	8	27
Stones, as received:										
Weight, gms	5.4	6.3	2.5	4.7	5.8	26.2	3.3	8.0	8.0	4.0
Diameter, mm	7-15	18	8-10	5-8	3-20	5-30 ^{2/}	18	6-12 ^{2/}	2-10 ^{2/}	1.5-4 ^{2/}
Color of powder	Creamy, ochre yellow	Yellow	White	Bright yellow	Pale, ochre yellow	Ochre yellow	Pale, creamy yellow	Pale yellow	Mustard yellow	Leamon yellow
Mineralogy	Aragonite Vaterite	--	Calcite?	--	--	--	Aragonite Vaterite	--	Vaterite	Vaterite Aragonite
Cholesterol, %	40	90	> 20	90	90	80	80	60	60	60
Ash, %	16.	.18	.18	.41	.20	.56	.07	1.3	4.6	5.0
Ca, % (A) ^{3/}	8.42	.037	.010	.083	.035	.075	.014	.21	1.32	2.62
Na, ppm (A)	2,500	360	500	770	470	1,300	250	810	650	780
K, ppm (A)	170	30	80	40	50	80	30	60	50	50
Cu, ppm (A)	140	14	10	16	13	47	< 5	5	11	20
Fe, ppm (A)	112	< 10	< 10	< 10	< 10	< 10	< 10	< 10	11	12
Mg, ppm (A)	970	33	82	58	42	47	< 20	48	160	190
Mn, ppm (A)	80	< 5	< 5	< 5	< 5	< 5	< 5	18	20	27
Zn, ppm (A)	25	2	3	2	3	< 2	2	3	4	8
Stones,ashed:										
Fe, ppm (S) ^{3/}	700 (110) ^{4/}	1,500 (2.7)	700 (1.3)	500 (2.1)	700 (1.4)	700 (3.9)	700 (.49)	500 (6.5)	300 (14)	300 (15)
Mg, % (S)	.2 (0.032)	1.5 (.0027)	.5 (.0009)	1 (.0041)	1 (.002)	.7 (.0039)	1 (.0007)	.5 (.0065)	.3 (.014)	.2 (.01)
Ca, % (S)	> 10 (> 1.6)	≧ 10 (> .018)	3 (.0054)	> 10 (> .041)	> 10 (> .02)	> 10 (> .056)	7 (.0049)	> 10 (> .13)	> 10 (> .46)	≧ 10 (> .5)
Ti, ppm (S)	< 2 (< .32)	20 (.036)	< 10 (< .018)	5 (.021)	50 (.10)	< 5 (< .028)	50 (.035)	< 5 (< .065)	< 2 (< .092)	< 5 (< .25)
Mn, ppm (S)	100 (.16)	300 (.56)	150 (.27)	200 (.82)	100 (.20)	150 (.84)	300 (.21)	1,500 (20)	300 (14)	300 (15)
Ag, ppm (S)	30 (4.8)	15 (.027)	< 1 (< .0018)	2 (.0082)	5 (.01)	30 (.17)	< 1 (< .0007)	1.5 (.02)	1 (.046)	7 (.35)
Ba, ppm (S)	7 (1.1)	15 (.027)	10 (.018)	15 (.062)	10 (.02)	5 (< .028)	20 (.014)	7 (.091)	15 (.69)	7 (.35)
Bi, ppm (S)	15 (2.4)	< 20 (< .036)	< 20 (< .036)	< 20 (< .082)	< 20 (< .04)	< 20 (< .11)	< 20 (< .014)	< 20 (< .24)	< 10 (< .46)	< 20 (< 1)
Ce, ppm (S)	1 (.16)	15 (.027)	150 (.27)	70 (.29)	15 (.03)	30 (.17)	70 (.049)	15 (.2)	< 1 (< .046)	2 (.1)
Cu, ppm (S)	7,000 (1,100)	5,000 (9.0)	70 (.13)	2,000 (8.2)	3,000 (6.0)	7,000 (39)	700 (.49)	200 (2.6)	300 (14)	700 (35)
Pb, ppm (S)	300 (48)	200 (.36)	< 50 (< .09)	30 (.12)	30 (.06)	150 (.84)	< 100 (< .07)	30 (.39)	< 10 (< .46)	30 (1.5)
Si, ppm (S)	150 (24)	70 (.13)	30 (.054)	70 (.29)	70 (.14)	50 (.28)	< 50 (< .035)	150 (2)	100 (4.6)	70 (3.5)
Al, ppm (S)	700 (110)	700 (1.3)	1,500 (2.7)	700 (2.9)	700 (1.4)	500 (2.8)	200 (.14)	700 (9.1)	700 (32)	500 (25)
Na, % (S)	1.5 (.24)	-- (--)	-- (--)	-- (--)	-- (--)	-- (--)	-- (--)	-- (--)	3 (.14)	3 (.15)
K, % (S)	< .7 (< .11)	3 (.0054)	3 (.0054)	1.5 (.0062)	1.5 (.003)	3 (.017)	< 1.5 (< .0011)	< 1.5 (< .07)	< .7 (< .032)	< 1.5 (< .075)
P, % (S)	< .2 (< .032)	.7 (.0013)	10 (.018)	3 (.012)	3 (.006)	3 (.017)	< 2 (< .0014)	5 (.065)	7 (.32)	3 (.15)

1/ Up to date of surgery in 1972.

2/ Distinctly bimodal.

3/ A, atomic absorption analysis; S, emission spectrographic analysis; I^{1/}, measured at < 3 ppm in 12 samples as received; SI, measured at < 20 ppm in four samples of

ash. Additional elements in ash of 12 samples measured below limits of analytical detection were (detection limits in parentheses): As (< 2,000 ppm), Au (< 50 ppm),

Be (< 3 ppm), Cd (< 100 ppm), Co (< 30 ppm), La (< 100 ppm), Mo (< 7 ppm), Nb (< 20 ppm), Ni (< 50 ppm), Pd (< 5 ppm), Sb (< 500 ppm), Se (< 10 ppm), Sn (< 20 ppm), Te (< 5,000 ppm),

U (< 1,000 ppm), V (< 15 ppm), W (< 200 ppm), Y (< 20 ppm), Zn (< 700 ppm), Zr (< 20 ppm), Ce (< 500 ppm), Ga (< 10 ppm), Ge (< 20 ppm), Hf (< 200 ppm), In (< 20 ppm),

Li (< 200 ppm), Re (< 100 ppm), Ta (< 1,000 ppm), Th (< 500 ppm), Tl (< 100 ppm), and Yb (< 2 ppm).

4/ Number in parentheses is ppm in dry weight of stones.

PERIOD.

	Ia	IIa	IIIb	IVb	Vb	VIb	VIIb	VIIIb	IXb	Xb	XIb	IIa	IIIa	IVa	Va	VIa	VIIa									
1	H																									
2	Li Be																B		C	N	O	F				
3	Na Mg																Al		Si	P	S	Cl				
4	K	Ca	Sc	Ti	V	Cr Mn Fe Co Ni Cu Zn								Ga	Ge	As	Se	Br								
5	Rb	Sr	Y	Zr	Nb	Mo Ti Ru Rh Pd Ag								Cd	In	Sn	Sb	Te	I							
6	Cs	Ba	La	Hf	Ta	W								Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At		
7	Fr Ra Ac																									

Figure 3. Periodic table of the elements showing metals and semi-metals found in galstones (boxed).

Table 2.--Summary statistics of gallstone chemistry

[Md, median; L, lowest observed value; U, highest observed value; A, analyzed by atomic absorption; S, analyzed by emission spectroscopy; leaders (--) indicate no data]

Measurement	Data in parts per million (dry weight)			Data in micrograms of constituents (total weight)		
	Md	L	U	Md	L	U
Ash	7,700.0	700.0	300,000.0	--	--	--
Ca	2,100	100	94,000	17,000.0	250.0	460,000.0
Na	770	250	2,500	2,900	830	34,000
K	60	30	170	200	99	3,200
Cu	13	< 5	140	75	< 17	3,800
Mg	58	< 20	970	260	< 66	9,500
Zn	3	< 2	25	< 52	< 52	630
Fe, S	5.2	.49	110	35	1.6	3,800
Mg, S	60	7	320	220	23	1,700
Mn, S	.84	.16	35	22	.68	160
Ag, S	.027	< .0007	4.8	< .29	< .0023	26
Ba, S	.091	< .028	1.1	< .73	< .73	7.3
Cr, S	< .30	< .30	< .30	< .57	< .087	4.4
Cu, S	8.6	.13	1,100	37	.32	6,100
Pb, S	< 6.0	< 6.0	48	< 11	< 11	260
Sr, S	1.2	< .035	45	7.3	< .12	130
Al, S	< 7.7	< 7.7	150	< 240	< 240	610
P, S	< .06	< .0014	> .23	< 4,900	< 46	> 4,400

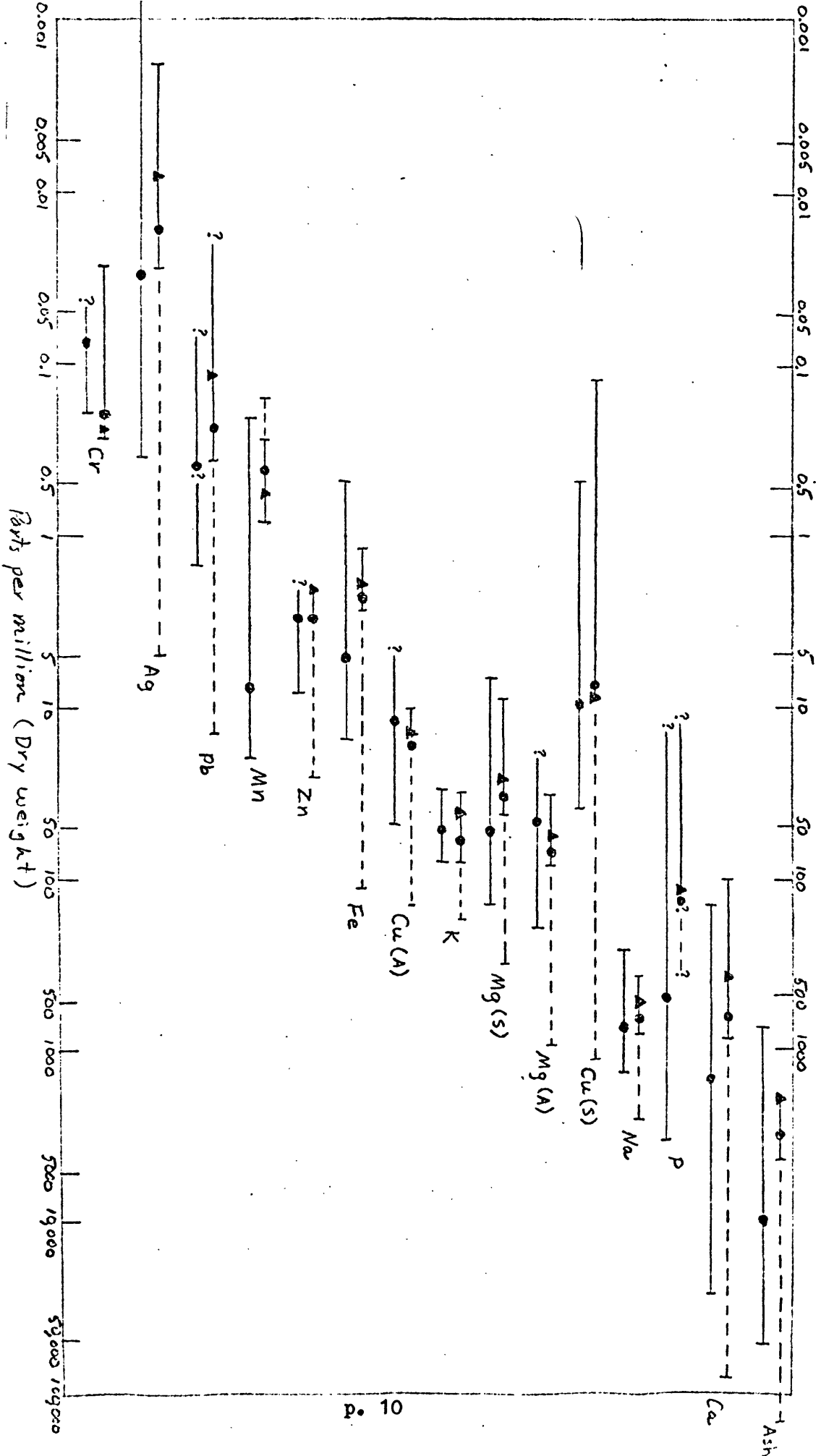


Figure 4. Medians (solid dots) and ranges in element composition of gallstones grouped according to water source. Upper line of each pair represents Moffat water. Dashed part of line includes sample of unusual composition (patient 2509); Solid triangle is median if unusual sample is excluded. Lower line represents Kasler/Warstone water. Where an element was analyzed by two methods, they are denoted by A (atomic absorption) and S (emission spectroscopy),

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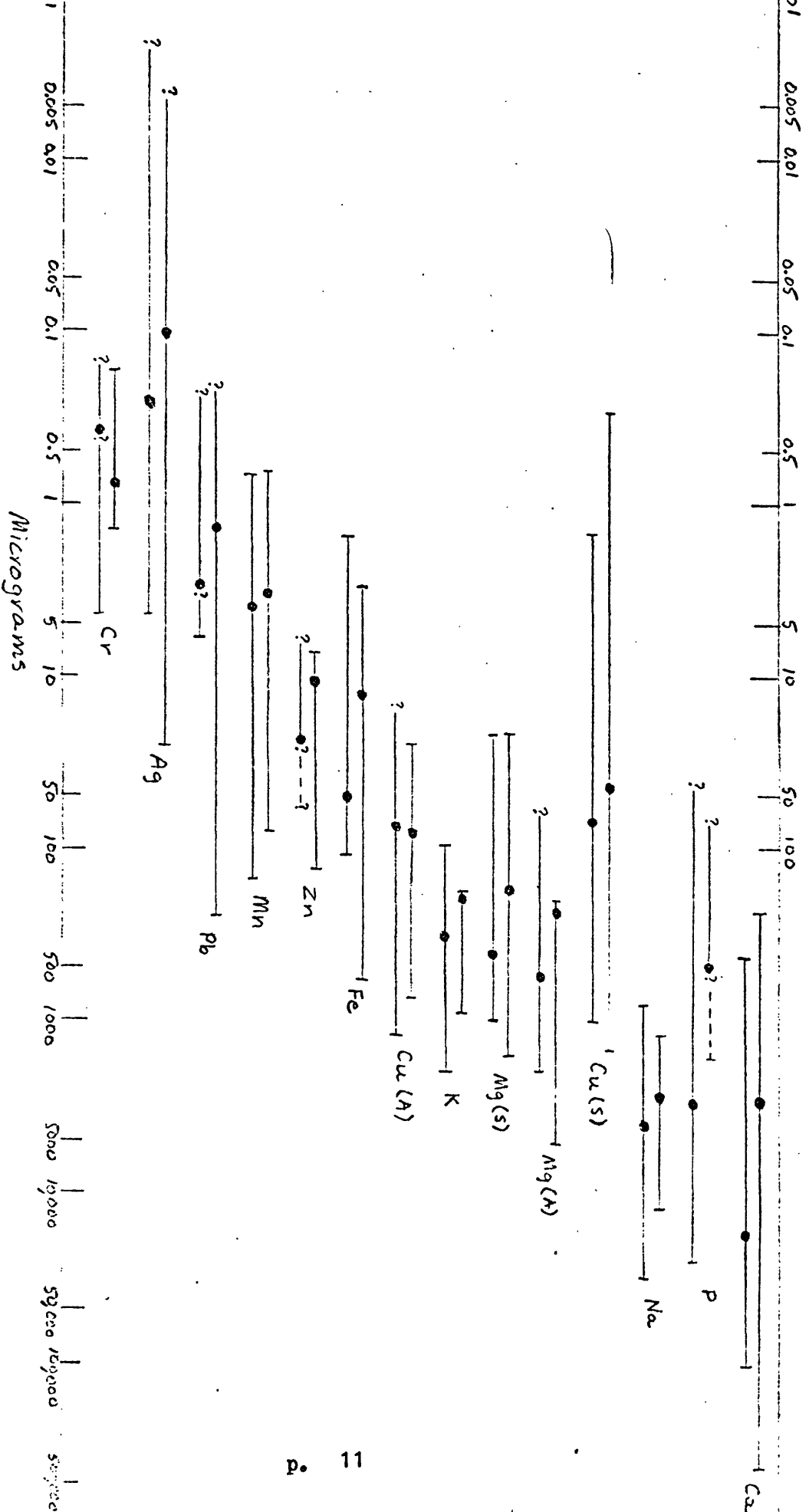


Figure 5.

Medians (solid dots) and ranges in total element mass in gallstones grouped according to water source. Upper line of each pair represents Moffat water. Lower line represents Kassler/Marston water. Where an element was analyzed by two methods, they are denoted by A (atomic absorption) and S (emission spectroscopy), respectively.

Table 3.--Analysis of variance of gallstone chemistry

[S_B^2 , estimated logarithmic variance component reflecting differences between gallstone groups based on water source; S_W^2 , estimated logarithmic variance component reflecting differences among individuals within groups; F values tested with one and eight degrees of freedom except as noted; A, analyzed by atomic absorption; S, analyzed by emission spectroscopy; leaders (--) indicate no data]

	Data in parts per million (dry weight)			Data in micrograms of constituent (total weight)		
	S_B^2	S_E^2	F	S_B^2	S_E^2	F
Ash	< 0.001	0.670	< 1	--	--	--
Ca	< .001	1.11	< 1	< 0.001	1.26	< 1
Na	< .001	.088	< 1	< .001	.250	< 1
K	< .001	.054	< 1	< .001	.167	< 1
Cu	.007	.215	1.16	< .001	.415	< 1
Mg	< .001	.271	< 1	< .001	.368	< 1
Zn	< .001	.149	< 1 ^{1/}	< .001	.191	< 1 ^{1/}
Fe, S	< .001	.513	< 1	< .001	.700	< 1
Mg, S	< .001	.484	< 1	< .001	.309	< 1
Mn, S	.185	.603	2.47	< .001	.939	< 1
Ag, S	< .001	1.57	< 1	< .001	1.95	< 1
Cr, S	.001	.168	1.03	< .001	.267	< 1
Cu, S	< .001	1.31	< 1	< .001	1.71	< 1
Pb, S	< .001	1.01	< 1	< .001	1.07	< 1
P, S	< .001	.737	< 1 ^{2/}	.129	.855	1.60 ^{2/}

^{1/} F value tested with four and five degrees of freedom.

^{2/} F value tested with three and six degrees of freedom.

A question related to the thesis of this work can be approached through these data, however. Clearly, the large scatter in stone chemistry prohibits "seeing" any potential differences due to water source. If small differences, in fact, exist, how many sets of gallstones would be required in order to identify them? Looked at another way, this question focuses on the minimal value of n_r , in the following equation, that would result in a statistically significant F-value:

$$F = 1 + n_r (S_B^2 / S_E^2) \quad .$$

In this equation, n_r is the number of patients residing in either area that are needed for a satisfactory test, and S_B^2 and S_E^2 are taken from table 3. (The theoretical development of this equation is described in Miesch, 1976.) For example, S_B^2 and S_E^2 for manganese in dry weight are 0.185 and 0.463, respectively. If n_r is taken as 10, an F value of 3.07 results, which barely exceeds the critical values of F for 1 and 18 degrees of freedom at the 80-percent confidence level ($F_{0.12, 1, 18} = 3.01$). The magnitude of the sampling problem in this particular study is apparent when the generally low estimates of S_B^2 (table 3) are considered.

The analysis of variance quantifies the magnitude of the sampling problem in this particular study very well. Individual variation in gallstone chemistry is so extreme (at least in the Denver area) that future work along these lines must be based on an extraordinary sampling effort involving at least dozens of patients.

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