Studies on Inorganic Constituents in Biological Materials.—On the Inorganic Constituents in Human Stones

By Naoichi OHTA

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Human stones, or calculi, are mainly formed in the liver, gall-bladder, biliary duct, pancreas, kidney, urethra, bladder, etc. in consequence of the isolation and coagulation of their respective humours caused by various reasons. Although many studies on calculi have been undertaken hitherto, most of them are dealing with etiological or clinical problems, and with all those studies no perfect explanation in the field has yet been made. Concerning the chemical composition of calculi, a long time has passed since their major constituents were made known, but there are only a few studies that deal with the inorganic constituents of calculi systematically. Recently, owing to developments in the instrumental method of analysis, many elements have been detected in calculi, and discussions on the significance of those elements have become active. However, all the analyses whcih have ever been made are limited almost entirely to qualitative research, or even if quantitative, to only a few constituents.

Regarding the formation of calculi as a special chemical phenomenon, the author considers that on the one hand it is important to determine etiologically the contents of inorganic constituents in calculi which are formed in various organs and their respective humours of a human body and to reveal the status of distribution of those constituents in those materials, and that on the other hand it is a matter of interest to clarify the cycle and function of elements in biological materials. The author[1] has reported his studies on each of the constituents of human stones several times up to this time. In this paper, he will report (1) the results of

1) N. Ohta, *J. Chem. Soc. Japan, Pure Chem. Sec.*, **74**, 506 (1953) ; **76**, 590, 1235 (1955) ; **78**, 7, 13, 1033, 1038, 1043 (1957).

quantitative analyses of various human stones and of the humours in which the stones were formed, determining calcium, magnesium, phosphorus, sodium and potassium as major inorganic constituents and iron, copper, zinc, lead and manganese as heavy metal constituents, (2) the distribution of those constituents in various human stones and in their respective humours, and, (3) the mutual relation existing among those constituents.

Experimental

Materials.—The human stones used for this study were 25 gallstones (since three of them were divided into the internal and the external portions, the total number of analyses was 28), 10 urinary calculi and two pancreatic calculi, all of them being (kindly) providedly by the First National Hospital of Tokyo. The characters of these materials have been described in the author's earlier reports[1]. The samples of related humours provided for the analyses were 10 of gall-bladder biles which had been collected at the time of the autopsies at Tokyo Medical Examiner Office, 12 of normal human blood and 12 of normal human urine.

Methods.—Samples were analysed after conversion of their organic matters into ashes with redistilled fuming nitric acid and 60 % perchloric acid.

Calcium and magnesium were determined by the complexometric titration method[2]. The sum of calcium and magnesium was titrated with disodium salt ethylendiamintetra-acetic acid solution using Eriochrom Black T as an indicator, while the calcium was determined indirectly by determining magnesium after precipitation of calcium as oxalate. On the analysis of samples rich in phosphate like urinary calculi, the cation exchanger, Dowex 50, was used for preliminary separation of the phosphoric acid radical. This method was also applied to the determination of lead and zinc in the samples rich in phosphate.

Sodium and potassium were determined by the flamephotometric method[3], using Perkin-Elmer Model-52C flamephotometer, acetylene being used as fuel.

Phosphorus was determined by molybdenum blue method modified by Ikeda[4].

Iron was determined by nitroso-R salt method modified by Dean and Lady[5].

Copper, zinc and lead were determined according to Sandell's dithizone method[6].

Manganese was determined by permanganate method[7].

Of the elements described above, last five were determined by Beckmann Model D. U. Quartz Spectrophotometer.

Results

Analytical results for gallstone, normal gallbladder bile, normal human blood, urinary calculus, normal human urine and pancreatic calculus were shown in Table I, II, III, IV, V and VI, respectively. In these tables, the values of the major and the heavy metal constituents were shown in the mg. per g. (ml.) and p. p. m. unit, respectively, of undried samples. "*m*" is an average value calculated statistically at the 95 % confidence interval.

Discussion

1. Major Constituents.—*(1) Gallstone.*—The chemical composition of a gallstone varies in accordance with the difference among human races and regions. The greater part of a gallstone consists of organic materials[8,9], such as cholesterin, bilirubin and fatty acid, and the content of inorganic constituents is very small, as shown in the ash content in Table I.

Although various gallstones have been classified clinically according to the content of above-mentioned organic constituents in them, most stones are either a mixture or a layer of several kinds (this shows the change of the circumstance in which the sedimentations were made), so that it is difficult to analyse a slight quantity of inorganic constituents after separating these components individually. Accordingly, the materials used in this study were divided into three groups, such as mineral stone, cholesterinpigment-calcium stone and cholesterin stone, and each sample of the gallstone was analysed as it was.

A cholesterin stone consists mainly of cholesterin (soluble in ether), and contains a slight amount of ash and bilirubin. A cholesterinpigment-calcium stone contains a considerable amount of bilirubin (soluble in chloroform) and calcium besides cholesterin. A mineral stone is scarcely soluble in chloroform and ether. It is either hard and contains a considerable amount of ash, or it has a black vitreous appearance.

The content of major constituents in various gallstones varies considerably according to these three kinds of stone

2) G. Schwarzenbach and H. Ackermann, Helv. Chim. Acta, **30**, 1798 (1947).

3) C. E. Bills, F. G. McDonald, W. Niedermier and M. C. Schwartz, *Anal. Chem.*, **21**, 1076 (1949).

4) N. Ikeda, *J. Chem. Soc. Japan, Pure Chem. sec.*, **72**, 23 (1951).

5) J. A. Dean and J. H. Lady, *Anal. Chem.*, **25**, 947 (1953).

6) E. B. Snandell, "Colorimetric Determination of Traces of Metals", Interscience Publisher, Inc., New York, (1950), pp. 295, 616, 388.

7) E. B. Sandell, ibid., p.433.

8) M. Nishimura, *J. Biochem.*, **28**, 265 (1938).

9) K. Kodama, "Clinical Biochemistry", Nanzando, Tokyo, (1951), p. 117.

TABLE I

INORGANIC CONSTITUENTS IN HUMAN GALLSTONES

Species	Sample	Ash, %	Ca mg./g.	Mg mg./g.	Na mg./g.	K mg./g.	P mg./g.	Fe p.p.m.	Cu p.p.m.	Zn p.p.m.	Pb p.p.m.	Mn p.p.m.
Mineral stone	G-4004	6.83	28.70	3.05	1.02	0.26	6.52	860	1,794	43	134	53
	G-4011	7.48	36.00	3.11	0.86	0.20	5.00	1,170	1,435	146	81	157
	G-4011 B	12.57	31.82	4.37	—	—	21.30	2,290	10,400	385	84	277
	G-4012	9.12	29.24	4.52	1.10	0.19	10.60	598	8,705	1,043	97	140
	G-4017	15.56	70.40	21.00	0.77	0.17	4.20	250	1,665	108	47	98
	G-5007	8.45	39.20	9.41	0.93	0.21	4.01	—	2,107	168	33	—
	G-5012	8.75	34.00	10.70	1.34	0.21	5.65	411	1,355	423	45	66
	G-5017	32.96	132.	16.10	1.69	0.51	48.50	629	420	550	173	654
	G-5019	10.01	46.44	4.95	2.30	0.49	7.25	—	3,585	238	—	124
	G-5019 A	8.26	35.31	3.71	—	—	9.32	68	1,382	279	43	83
	G-5019 B	9.08	45.84	3.90	—	—	5.28	373	2,440	574	95	118
	G-5021	54.79	285.	35.52	0.31	0.09	28.10	287	548	159	51	540
	G-5022	36.89	173.	18.15	4.37	0.76	23.30	1,520	7,345	329	296	3,850
	G-8002	5.73	21.87	4.32	—	—	4.82	282	2,660	216	192	107
	Average	16.18	72.06	10.20	1.47	0.31	13.13	728	3,274	333	105	482
Cholesterin-pigment-calcium stone	G-1003	10.24	53.94	3.62	1.21	0.18	7.86	102	598	172	21	32
	G-1005	5.37	28.54	2.60	0.10	0.04	3.37	30	70	40	18	6
	G-4011 A	6.95	34.92	3.27	—	—	4.77	61	281	116	46	33
	G-5009	8.59	43.58	4.65	1.55	0.27	4.92	85	253	243	55	248
	G-5010	1.84	9.06	0.89	0.18	0.04	1.42	93	87	9	55	48
	G-5013	9.30	48.34	4.15	0.91	0.18	6.86	163	1,052	140	83	153
	G-5014	7.32	39.20	3.38	0.65	0.15	4.42	278	185	50	168	186
	G-5018	2.97	12.25	1.18	0.40	0.16	2.45	79	229	50	29	49
	G-8004 A	1.33	5.37	0.42	—	—	1.82	71	255	149	51	55
	G-8004 B	2.95	14.90	0.87	—	—	1.78	277	858	451	148	94
	Average	5.68	29.01	2.50	0.71	0.15	3.97	124	387	142	67	90
Cholesterin stone	G-4015	0.06	(0.03)	—	0.07	0.02	0.12	15	65	17	24	11
	G-4016	0.04	(0.02)	—	0.07	0.02	0.04	31	35	58	28	6
	G-5001	0.08	(0.03)	—	0.15	0.08	0.14	13	35	67	24	11
	G-5015	0.04	(0.02)	—	0.06	0.02	0.06	29	17	79	—	10
	G-8001	0.03	—	—	—	—	—	16	27	12	23	10
	G-8003	0.44	0.94	0.13	—	—	0.94	133	80	105	79	27
	Average	0.12	0.21	0.13	0.09	0.04	0.26	40	43	56	36	13
m.		6.25 ±1.74	31.18 ±12.04	5.52 ±2.36	0.78 ±0.30	0.17 ±0.06	6.30 ±2.71	293 ±147	871 ±383	185 ± 61	71 ±20	121 ± 59

TABLE II

INORGANIC CONSTITUENTS IN POST-MORTEM GALLBLADDER BILE

Samples	Collected volume, ml.	Ca mg./ml.	Mg mg./ml.	Na mg./ml.	K mg./ml.	P mg./ml.	Fe p.p.m.	Cu p.p.m.	Zn p.p.m.	Pb p.p.m.	Mn p.p.m.
No. 1	10.0	1.55	0.24	4.3	1.4	2.52	20.3	26.8	15.0	0.15	1.11
2	22.0	0.36	0.06	3.2	1.0	0.50	9.1	3.2	5.6	0.30	0.53
3	5.0	0.68	0.12	6.5	2.4	1.03	97.4	3.5	38.2	0.60	3.10
4	7.5	0.70	0.12	4.9	1.7	1.19	25.8	9.2	10.0	0.40	4.20
5	7.0	0.98	0.15	6.4	2.0	1.66	45.0	20.5	4.3	0.32	1.46
6	14.5	0.27	0.04	4.3	1.4	0.42	33.3	7.3	10.2	0.15	0.96
7	16.5	0.30	0.05	4.1	1.3	0.58	10.8	2.6	4.6	0.10	0.83
8	8.0	1.02	0.18	6.6	1.5	1.56	22.0	12.0	9.4	0.62	0.27
9	7.0	1.05	0.14	6.3	1.6	1.33	34.4	12.3	13.3	0.36	0.90
10	12.0	0.44	0.06	4.5	1.7	0.67	30.1	11.2	12.5	0.51	0.18
m.	11.0	0.74 ±0.30	0.12 ±0.05	5.1 ±0.9	1.6 ±0.3	1.15 ±0.47	25.6 ± 8.8	10.9 ± 5.5	9.4 ± 3.0	0.35 ±0.13	1.35 ±0.93

TABLE III

INORGANIC CONSTITUENTS IN NORMAL HUMAN BLOOD

Sample	Ca mg./ml.	Mg mg./ml.	Na mg./ml.	K mg./ml.	P mg./ml.	Fe p.p.m.	Cu p.p.m.	Zn p.p.m.	Pb p.p.m.	Mn p.p.m.
No. 1	0.127	0.053	2.18	2.26	0.20	530	1.31	10.9	0.28	0.45
2	0.133	0.055	2.50	2.20	0.20	482	1.26	8.44	0.25	0.36
3	0.114	0.038	2.09	1.62	0.16	480	1.15	7.79	0.29	0.37
4	0.083	0.032	1.81	1.43	0.10	390	0.89	5.50	0.22	0.28
5	0.105	0.043	2.36	1.96	0.18	488	1.10	6.50	0.30	0.25
6	0.130	0.053	2.40	2.08	0.23	542	1.28	7.82	0.36	0.46
7	0.111	0.045	2.47	1.81	0.18	474	1.13	8.60	0.27	0.27
8	0.103	0.045	2.33	1.90	0.16	469	1.17	7.97	0.25	0.30
9	0.087	0.037	2.00	1.99	0.16	498	1.24	8.06	0.34	0.32
10	0.094	0.040	2.34	2.05	0.22	522	1.22	8.25	0.33	0.48
11	0.090	0.037	2.34	2.01	0.16	477	1.19	7.21	0.30	0.45
12	0.082	0.036	2.15	2.09	0.15	470	1.15	7.11	0.32	0.39
m.	0.105	0.043	2.25	1.95	0.18	494	1.20	7.57	0.29	0.37
	±0.012	±0.005	±0.13	±0.15	±0.02	± 17	±0.04	±0.62	±0.03	±0.05

TABLE IV

INORGANIC CONSTITUENTS IN HUMAN URINARY CALCULI

Species	Sample	Ash, %	Ca mg./g.	Mg mg./g.	Na mg./g.	K mg./g.	P mg./g.	Fe p.p.m.	Cu p.p.m.	Zn p.p.m.	Pb p.p.m.	Mn p.p.m.
Bladder	U-1001	17.09	52	7.0	4.30	8.14	30.5	270	4.8	345	36	1.0
calculus	U-5009	24.12	137	7.2	2.55	5.41	9.9	144	26.8	239	20	4.5
Renal	U-5002	58.16	286	12.7	4.57	0.52	63.3	256	17.8	913	43	4.1
calculus	U-5005	57.02	271	12.0	4.76	2.00	69.2	198	7.8	577	26	1.3
	U-5006	63.29	264	9.7	3.66	1.90	94.3	340	14.7	578	37	4.1
	U-5007	38.58	150	8.3	7.65	4.90	61.3	118	26.5	673	31	3.0
Urethral	U-5001	78.28	450	18.2	7.19	0.92	45.8	199	6.3	705	58	0.7
calculus	U-5003	51.67	233	3.8	4.59	1.41	70.5	286	8.7	343	19	1.0
	U-5004	37.37	254	1.5	1.69	0.98	3.3	172	16.9	561	22	1.7
	U-5008	62.61	184	31.4	4.70	2.71	120.	166	6.7	615	43	1.8
m.		48.82	228	11.2	4.57	2.89	56.8	215	13.7	555	34	2.3
		±13.66	± 77	± 6.1	±1.30	±1.77	±25.8	± 50	± 6.3	±142	± 9	±1.0

TABLE V

INORGANIC CONSTITUENTS IN NORMAL HUMAN URINE

Sample No.	Vol. ml./day	Average concentration mg./ml.					Sample No.	Vol. ml./day	Average concentration p.p.m.				
		Ca	Mg	Na	K	P			Fe	Cu	Zn	Pb	Mn
1	1480	0.116	0.052	3.84	1.14	0.64	7	1050	1.83	0.15	1.40	0.045	<0.01
2	2010	0.098	0.051	4.79	1.10	0.70	8	900	2.08	0.25	1.54	0.120	<0.01
3	1600	0.099	0.034	4.19	0.92	0.53	9	1000	2.40	0.22	1.45	0.080	<0.01
4	2000	0.077	0.035	3.20	0.76	0.55	10	1750	1.07	0.08	1.22	0.020	<0.01
5	1550	0.152	0.057	4.93	1.00	0.67	11	1200	1.66	0.14	1.82	0.052	<0.01
6	1630	0.127	0.049	1.74	0.51	0.73	12	1000	2.25	0.23	1.69	0.088	<0.01
Average		0.112	0.046	3.78	0.91	0.64	Average		1.88	0.18	1.52	0.068	<0.01

TABLE VI

INORGANIC CONSTITUENTS IN HUMAN PANCREATIC CALCULI

Sample	Ash, %	Ca mg./g.	Mg mg./g.	Na mg./g.	K mg./g.	P mg./g.	Fe p.p.m.	Cu p.p.m.	Zn p.p.m.	Pb p.p.m.	Mn p.p.m.
P-1001	55.06	382	1.8	0.22	0.09	0.74	75.0	11.	282	136	0.3
P-4002	64.45	440	3.7	1.49	0.68	2.30	52.4	19.	345	112	0.5
Average	59.76	411	2.8	0.86	0.39	1.52	63.7	15.	314	124	0.4

as shown in Table I. The ratios of average content of ash between mineral, cholesterinpigment-calcium and cholesterin stones are 135 : 47 : 1, while the mutual ratios between each of the major constituents do not vary so much as those in ash content. As for the value (weight proportion) of Ca/Mg, that of a cholesterin-pigment-calcium stone (12.0) is greater than that of a mineral stone (7.7) or of a cholesterin stone (7.2). As for the value of (Ca+Mg)/P, there is no significant difference between that of a mineral stone (7.5) and that of a cholesterinpigment-calcium stone (7.6), but the value of a cholesterin stone (0.5) is far smaller than the values of the other two. If calcium and magnesium that present (in the stones) as phosphates, the values of Ca/P and Mg/P would be 1.94 and 1.18, respectively. Accordingly, the value of (Ca+Mg)/P may be regarded as a standard for measuring the state of alkaline-earth metals. Epprecht et al.[10] reported in their experiment using X-ray that calcium in a gallstone was present as a bilirubinate, while Cameron et al.[11] reported the presence of calcium carbonate in a gallstone. As for the value of Na/K, there is no significant difference between that of a mineral stone (4.7) and that of a cholesterinpigment-calcium stone (4.5), while that of a cholesterin stone (3.0) shows that the proportion of potassium is greater than in the other two.

According to the result of an examination of relations existing among major constituents of a gallstone (as a whole), there are positive correlations between calcium and magnesium, sodium and potassium, phosphorus and sodium, and phosphorus and potassium, respectively, with 5 % degree of significance.

(*2*) *Bile and Blood.*—Bile is a complexed colloidal solution containing bilirubine which is a decomposition product of hemoglobin. There are two kinds of bile, hepatic and gallbladder bile, the latter being concentrated 7 to 10 times as densely as the former[12]. A gallstone is nothing but the product of condensed bile constituents. Therefore, it is not only interesting to research the relation between inorganic constituents of bile and blood and those of gallstones in order to understand the cycle of elements in biological materials, but also it is important to clarify the mechanism of the formation of a gallstone.

The relationship between the formation of a gallstone and inorganic constituents mainly in regard to alkali and alkaline-earth metals has been studied previously, and there are several reports on the metabolism of those materials in humours obtained from experiments on animals[13–17].

When the content of potassium is taken as a standard, the ratio of existence of each of the constituents in blood, bile and gallstones are given in Table VII.

TABLE VII

	Ca	Mg	P	Na	K
Blood	0.054	0.022	0.092	1.2	1.0
Bile............	0.46	0.075	0.72	3.2	1.0
Gallstone ...	183.	31.	37.	4.6	1.0

That is to say, gallstones are rich in alkaline-earth metals, especially in calcium and phosphorus, while blood and bile are rich in alkali metals.

When the content of each element in blood is taken as a standard, the ratios of the average content of each of the constituents in blood, bile and a gallstone are given in Table VIII (the figures shown in brackets indicate that the content of each of those constituents in bile is regarded as a standard).

TABLE VIII

Blood	Bile	Gallstone
Ca ......1.	7.0 (1.)	297. (42.)
Mg......1.	2.8 (1.)	128. (46.)
P ......1.	6.4 (1.)	35. (5.5)
Na ......1.	2.3 (1.)	0.35 (0.15)
K ......1.	0.82(1.)	0.087(0.11)

We see then that calcium, magnesium and phosphorus increase according to the process of blood—bile—gallstone, while potassium decreases in the same process. Sodium increases in the process of turning from blood to bile, but the content of the element in a gallstone is less than that in blood. When the differences among solubilities of various salts composed of these metals, PO_4^{3-} and organic matters in a gallstone, and the relation between

10) W. Epprecht, H. Rosenmund and H. R. Schinz, *Fortshr. Gebiete Röntogenstrahlen*, **79**, 1 (1953) ; *C. A.*, **47**, 11458 (1953).

11) A. T. Cameron, F. D. White and S. Meltzer, *Can. Med. Assoc. J.*, **39**, 441 (1938) : *C. A.*, **33**, 1811 (1939).

12) K. Kodama, "Clinical Biochemistry", Nanzando, Tokyo, (1951), p. 114.

13) R. Ohta, *J. Gastroenterology*, **2**, 1201 (1927).

14) T. Maruno, ibid., **6**, 642 (1931).

15) Y. Tachikawa, ibid., **10**, 1349, 1361, 1375 (1935).

16) T. Hukuda and Y. Tachikawa, ibid., **11**, 763 (1936).

17) R. Kono, *Jap. Arch. Internal Med.*, **1**, No. 2, 44, 49 (1954).

these metal ions and the stability[18] of the colloidal state of bile are considered, it is obvious that the abnormality of the metabolism of these major constituents has a strict connection with the formation of a gallstone.

All the values of Ca/Mg, (Ca+Mg)/P and Na/K increase according to the order of blood (2.4±0.1, 0.88±0.10 and 1.2±0.1), bile (6.4±0.5, 0.75±0.06 and 3.4±0.4) and gallstone (9.0±1.3, 5.8±1.3 and 4.3±0.6), because both the rate of increase of calcium and the rate of decrease of potassium are high.

(*3*) *Urinary Calculus and Urine*.—The history of research of a urinary calculus is long. Much literature on this subjects has been published since the end of the eighteenth century. According to the results of all the crystallographic studies using X-rays[19-23] and polarized microscope[24-29], chemical analysis[30-32] and infrared spectroscopic studies[33] ever made, it has been clarified that the urinary calculus contains one or more of the following compounds: $Ca_3(PO_4)_2$, $CaHPO_4 \cdot 2H_2O$, $Ca_{10}(PO_4)_6(OH)_2$, $Ca_{10}(PO_4CO_3OH)_6(OH)_2$, $CaC_2O_4 \cdot H_2O$, $CaC_2O_4 \cdot 2H_2O$, $Mg_3(PO_4)_2$, $MgNH_4PO_4 \cdot 6H_2O$, carbonate, uric acid, NH_4 or Na acid urate, cystine, and xanthine.

Table IV gives the calculi assorted according to the parts where they had been formed, but the samples were so few that it was difficult to compare one with another. According to the result of analysis, it was ascertained that all of these calculi consisted of a large quantity of phosphate and oxalate of calcium and of a small quantity of magnesium compounds. According to Epprecht et al.[22], the genesis of $MgNH_4PO_4 \cdot 6H_2O$ in Europeans is more frequent than in Orientals.

As a whole, the ash content of urinary calculi is greater than that of gallstones, but there is no significant difference between them, so far as the mutual ratio of major constituents is concerned, except that the magnesium content is less. Therefore, regarding the values of (Ca+Mg)/P and Na/K, there is no significant difference between urinary calculi (5.1±3.4 and 3.0±2.1) and gallstones with the exception that Ca/Mg in urinary calculi (23.2±12.3) is greater than that in gallstones.

Urine is usually in a persaturate state against its solute, which may be crystallized to a urinary calculus owing to various reasons. Therefore, the relation between the formation of a urinary calculus and the colloidal character of urine has been observed for a long time[34-38].

The concentrations of calcium, magnesium, sodium, potassium and phosphorus in urinary calculus are 2036, 265, 1.2, 3.2 and 89 times, respectively, as much as those in urine. When the content of potassium is taken as a standard, the ratio of existence of each of the constituents in urinary calculus and urine are given in Table IX.

TABLE IX

	Ca	Mg	Na	K	P
Urinary calculus	78.8	3.9	1.6	1.0	19.7
Urine	0.12	0.05	4.2	1.0	0.7

Thus urinary calculi are rich in alkaline-earth metals and phosphorus, while urine is rich in alkali metals.

The Values of Ca/Mg and (Ca+Mg)/P increase according to the process of urine (2.4 and 0.25)—urinary calculus (there are, however, a great differences between urinary calculi and gallstones in regard to the enrichment of calcium and magnesium), but there is no significant difference between them in regard to the value of Na/K (urine is 4.1).

(*4*) *Pancreatic Calculus*.—Both of the two examples have a greater amount of

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ashes than in other sorts of human stone, and they are almost entirely composed of calcium carbonate. They contain only a small quantity of phosphates. Butturini and Meduri[39] reported similar results.

For this reason, the ratios of existence among those major constituents are (as K=1); Ca : Mg : Na : K : P=1054 : 7.2 : 2.2 : 1 : 3.9 and the mutual ratios are : Ca/Mg=166. (Ca+Mg)/P=35.6.

These values differ considerably from those of urinary calculi and gallstones.

2. Heavy Metal Constituents.—(*1*) *Gallstone, Bile and Blood.*—Heavy metal constituents, such as copper, iron, zinc and manganese are contained in gallstones, especially in mineral stones, much greater in quantity than in any other biological materials. Therefore, in several examples the amount of these metals even exceeds that of alkali metals which are regarded as major constituents.

Although the presence of a large quantity of the heavy metals in gallstones was pointed out long ago[40], most of the successive researches[18,41–45] have been limited merely to the detection of trace elements. The quantitative analysis of the heavy metals has not been made, what with the scantiness of samples and what with the incompleteness of the micro analytical method. Accordingly, reports on the quantitative analysis of heavy metals are very rare (copper was determined quantitatively by Newell[46] and Telfer[47], and lead, by Anderson[48]).

A gallstone was reported to be formed experimentally as a result of continuous feeding of heavy metals, such as copper[49,50], iron[51] and manganese[51], to animals, though physiological hindrances were caused by doing so. Yuta[18] reported that he ascertained the establishment of Hardy-Schulz's law on addition of various electrolytes to dialyzed bile, and also that, in this case, heavy metal ions, even if very small in quantity, took part in accerelating the coagulation of bile constituents by alkaline-earth metals. On the one hand these facts indicate the significance of heavy metals in the formation of gallstones. On the other hand Telfer[47] denies the importance of copper in chololithiasis. However, the significance of heavy metals in a gallstone will be ascertained from their behavior in the process of the formation of a gallstone. Table I shows that the contents of these heavy metals decrease in order of mineral stone, cholesterinpigment-calcium stone and cholesterin stone. The average values of heavy metals of all samples of gallstones are arranged as follows:

Cu>Fe>Zn>Mn>Pb

However, there are some differences among the values of various groups of stones as follows:

Mineral stone......
Cu>Fe>Mn>Zn>Pb

Cholesterinpigment-calcium stone......
Cu>Zn>Fe>Mn>Pb

Cholesterin stone......
Zn>Cu>Fe>Pb>Mn

When the content of each constituent in blood is taken as a standard, the ratios of the average contents in blood, bile and a gallstone are given in Table X (the figures shown in brackets indicate that the content of each of those constituents in bile is regarded as a standard).

TABLE X

	Blood	Bile	Gallstone
Fe	......1.	0.052 (1)	0.59 (11)
Cu	......1.	9.1 (1)	726. (80)
Zn	......1.	1.2 (1)	24. (20)
Pb	......1.	1.2 (1)	245. (203)
Mn	......1.	3.6 (1)	327. (90)

That is, copper, zinc, lead and manganese are concentrated according to the process of the formation of a gallstone. A large quantity of iron is contained in blood as a constituent of hemoglobin, but its large proportion would be lost when hemoglobin decomposes to bilirubine and moves into bile.

The results of this study are insufficient to explain how these heavy metals take part in the formation of a gallstone, and for this purpose further studies should be made to reveal the content of these metals in the bile of chololithiasic patients, and the status of presence of these metals in

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blood, bile and a gallstone (Epprecht et al.[22] ascertained the presence of copper calcium-bilirubinate in gallstone by means of X-ray analysis). But their significance can not be overlooked when the above result is considered together with those of pathological and colloidal studies. There is a positive correlation among the heavy metals at the 95% confidence interval, except the combination of iron and zinc.

(*2*) *Urinary Calculus and Urine.*—Studies of heavy metals in a urinary calculus have been undertaken mainly by means of qualitative spectrographic analyses[34,41,44,52–54]. Yokoyama[55] reported on the renal calculi alimentarily formed in animals by the use of copper and calcium. According to Ishibashi's report[56], zinc, lead, iron and tin in the crystallization of uric and oxalic acid were promoters. Yoshikawa[34] reported that greater quantities of copper, lead, tin, manganese and iron were contained in diseased urine than in normal urine.

The order of average values of heavy metal contents in urinary calculus and urine are arranged as follows:

Urinary calculus...Zn > Fe > Pb > Cu > Mn
Urine......................Fe > Zn > Cu > Pb > Mn

The concentrations of iron, copper, zinc, lead and manganese in urinary calculus are 114, 76, 365, 500 and 230 times, respectively, as much as those in urine. Zinc is the most abundant in urinary calculus just as copper is in a gallstone, and is considerably concentrated. These facts suggest the similar function of copper and zinc in the formation of gallstone and urinary calculus, respectively. Parsons[57] reported that $Zn_3(PO_4)_2 \cdot 4H_2O$ was found in urinary calculus by means of X-ray analysis. Although the content of lead in a urinary calculus or a gallstone is not always abundant compared with the other constituents, the rate of its concentration is the highest both in cases of urinary calculus (from urine) and gallstone (from bile). In a living body, lead is usually accumulated in the bone in which calcium salts are more abundant than in any other parts of the body. These results indicate the strict connection between lead and the formation of calculi which mainly consist of calcium salt, as suggested by Gerlach[41].

There are positive correlations between manganese and copper and between lead and zinc in a urinary calculus at the 95% confidence interval.

(*3*) *Pancreatic Calculus.*—Two reports were made by Schairer[44] and by Kobayashi et al.[58] in regard to the spectrographic analysis of the pancreatic calculus, but the determination of heavy metal constituents in them has not been made as yet.

Table VI indicates that a relatively greater quantity of contents of zinc and lead exists and that iron, copper and manganese are contained but in a very small quantity. Especially, zinc is the most abundant of all, and it is a matter of interest that this relates to the fact that *Insulin*[59]—which is well known as an organic compound that contains zinc in it—exists in the pancreas.

Both of the samples were composed mainly of calcium carbonate, while zinc is contained in *Carbonic anhydrase*[60] as a constituent, and these facts suggest the existence of some relation between zinc and the formation of a pancreatic calculus.

3. Distribution of Inorganic Constituents between the Internal and the External Portion of a Gallstone.—From the standpoint of mechanical structure, gallstones are classified into three types, radial, layer and a combination of the two.

Table XI shows the distribution of inorganic constituents in the internal and the external portions of three samples of gallstones which are typical ones having layer structures. The sample G–4011 consists of mineral stone (internal portion) and cholesterinpigment-calcium stone (external portion). Both the internal and the external portion of samples G–5019 and G–8004 consist of mineral stone and cholesterinpigment-calcium stone respectively. These results indicate, with a few exceptions in the case of calcium and phosphorus, that the internal portion is richer in inorganic constituents, especially heavy metals, than the external portion.

These layer structures demonstrate that the sedimentation circumstance is changed

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TABLE XI

DISTRIBUTION OF INORGANIC CONSTITUENTS BETWEEN THE INTERNAL AND THE EXTERNAL PORTION OF HUMAN GALLSTONES

Sample		Ash, %	Ca mg./g.	Mg mg./g.	P mg./g.	Fe p.p.m.	Cu p.p.m.	Zn p.p.m.	Pb p.p.m.	Mn p.p.m.
G-4011	Internal portion	12.57	31.82	4.37	21.30	2,290	10,400	385	84	277
	External portion	6.95	34.92	3.27	4.77	61	281	116	46	33
G-5019	Internal portion	9.08	45.84	3.90	5.28	373	2,440	574	95	118
	External portion	8.26	35.31	3.71	9.32	68	1,382	279	43	83
G-8004	Internal portion	2.95	14.90	0.87	1.78	277	858	451	148	94
	External portion	1.33	5.37	0.42	1.82	71	255	149	51	55

at the time of the formation of a gallstone, and especially that the character of the internal portion has a direct connection with the reason why a calculus is formed. Although it is not easy to draw out a conclusion from the results of only three examples, from the above mentioned standpoint, it is interesting that the inorganic constituents, especially heavy metals, are abundant in the internal portion of a gallstone.

Summary

Quantitative analyses of major constituents (calcium, magnesium, phosphorus, sodium and potassium) and heavy metal constituents (iron, copper, zinc, lead and manganese) were made on human stones (gallstone, urinary and pancreatic calculus) and their respective humours (blood, bile and urine). Then the distribution of these elements and the mutual relations present among them were discussed.

(1) According to the process of formation of gallstone (blood→bile→gallstone), the concentration of calcium, magnesium and phosphorus increases, while that of sodium and potassium decreases in case of major constituents, On the other hand, the concentration of heavy metal constituents, copper, zinc, lead and manganese increases. The concentration of iron decreases in the process of blood→bile and then increases again in the process of bile →a gallstone, whilst copper is extremely concentrated throughout these processes.

(2) In the process of urine→urinary calculus, each of major constituents increases; calcium, magnesium and phosphorus are extraordinarily concentrated, and heavy metal constituents also increase zinc is especially the most abundant of all. The correlation between lead and the calcification phenomenon in biological materials is observed, for lead indicates the highest concentration ratio in every case of gallstones (203 times that of bile) and urinary calculi (500 times that of urine).

(3) Pancreatic calculi differ greatly from the other stones, as their major constituents are calcium carbonate and they contain but little of the other constituents. Among heavy metals, zinc and lead are contained in a relatively high quantity; it seems that special connections exist between zinc and *Insulin* and between zinc and *Carbonic anhydrase*.

(4) The internal portion of a gallstone that is of layer structure contains a greater quantity of heavy metals than the external portion.

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Department of Chemistry, Faculty of Science, Tokyo Metropolitan University, Setagaya-ku Tokyo