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COMPARATIVE CHEMICAL AND HISTOLOGICAL EXAMINATIONS OF AORTAS FOR CALCIUM CONTENT *

SERIES I

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In 1929 and 1930 we made a series of parallel chemical and histological tests for the metallic calcium content of human aortas. Experience soon taught us that single samples from the aortas of young persons gave uniform analyses and were satisfactory both for chemical and for microchemical examination, but that aortas from persons over 40 years of age, those that had undergone sclerotic changes, varied greatly from area to area so that no single sample was representative of the whole. We then took three samples from each aorta and arbitrarily chose segments from the arch, the thoracic portion and the abdominal portion. The chemical work and the histological examinations were done in separate laboratory departments and almost 50 aortas had been examined before comparisons were attempted. The results appeared to be beyond interpretation and the work was temporarily abandoned.

After reading Wells' [1] discussion of calcification of the aorta in Cowdry's "Arteriosclerosis," in which he said that "no one appears to have tried to correlate the chemical calcium content and the microscopic findings in the same aortas," we resumed the work.

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Nature of the Materials Examined

The series included 52 aortas from persons varying in age from 2 months to 78 years. At autopsy the thoracic and abdominal viscera were removed, including the heart which was severed from the aorta just above the aortic valve cusps. The inominate, left carotid, left subclavian and iliac branches were then ligated and cut off with very short stumps. The aorta was opened longitudinally and a gross description made. The vessel was then removed and all fat and adventitial adhesions stripped away to the media. Chemical samples, 10 gm. when available, and pieces for microscopic study were then taken respectively from the arch, the thoracic and the abdominal portions, as already stated.

Chemical Examination

The calcium of the aorta was estimated by the method of Corley and Denis.[2] All samples for analysis were weighed with an error of not more than 0.5 per cent before appreciable evaporation had taken place. The opened aorta was flattened and the samples were then taken from about the sites of the histological specimens, as nearly as possible, equally from above and below these points.

The use of fresh material rather than the dried residue as a basis for the analysis simplified the procedure. At the same time it yielded figures that are more reasonably to be compared with microscopic findings since it avoids any concern with the relative rates of deposition of calcium and other tissue constituents.

The samples were placed in the tubes with the sodium hydroxide, sealed with tin-foil and saved until a sufficient number had been accumulated before heating under pressure. Corley and Denis recommend grinding the tissue. The omission of this step in no way changed the character of the solution. The tissue was completely disintegrated.

After digestion the solutions were acidified, diluted to a known volume and filtered through Jena fritted glass filters, thereby avoiding contamination from filter paper. In many instances there were stones present in the digestion tubes. These were dissolved and added to the solutions before filtration.

Duplicate aliquots of the filtered solutions were precipitated with

oxalate for the actual calcium determination and the procedure followed exactly as described. Blank determinations were made at intervals and were uniformly found to be too small to be significant. They were neglected in the calculation. The results were stated in mg. per cent of fresh tissue.

Numerous determinations of the composition of the calcifications have been made and are recorded in the literature. The minerals are deposited in proportions similar to those found in bone. Schönheimer [3] has recently made such a comparison. For our purpose the estimation of the calcium alone sufficed. The significance of the phosphorus determination would be complicated by the presence of varying and often large quantities of phosphatides.

Histological Technique

Six pieces were taken from each aorta, duplicate pieces from each of the three segments being fixed in Zenker's fluid containing 5 per cent acetic acid and in 10 per cent neutral formalin.

The Zenker material was embedded in paraffin and cut in the usual way. The sections were stained with Mallory's phloxine-methylene blue stain, Weigert's elastic stain and Mallory's phosphotungstic acid hematoxylin. Zenker-fixed tissues were preferable for inflammatory changes and degenerations but were useless for estimating calcium content because the acetic acid removed the alkaline staining substances.

These stains were useful in comparing the sections with others removed at autopsy over a period of years. We had sometimes made the mistake of calling blue stained materials calcium but this practice was found to be in error, save in the instances of heavy calcium plates and areas of true bony metaplasia. None of them is a stain for calcium.

Formalin-fixed material * was cut with the freezing microtome and was examined both for fatty changes and for calcium contents with Sudan III and hematoxylin, and for calcium deposits with

* Formalin was neutralized with magnesium oxide for the general routine. Comparative tests were made by fixing pieces of infant's aorta after the routine method and in formalin kept over calcium carbonate. The specimens were washed in distilled water and stained with alizarin. Both sets were negative for absorbed calcium. After several months the amount of stainable calcium in atheromatous aortas kept in formalin was greatly decreased. Formalin becomes acid on standing and we interpreted the decrease as an evidence of acid decalcification.

Roehl's hematoxylin, von Kossa's silver method and Cameron's alizarin stain. All calcium stains were made in duplicate to exclude artifacts and extraneous precipitates and were mounted in glycerine jelly.

The Sudan III and hematoxylin stain was used to show, when possible, the relation between the various fatty changes as manifested by red or orange stained fat granules, crystals and globules, and the metallic salt deposits which included calcium. Macallum[4] in 1897, and later Wells[5] and Cameron,[6] showed that hematoxylin is not a specific stain for calcium but that it stains iron and chromium salts as well. While almost any of the alkaline salts may take the blue stain, iron is the most important of the group that may be mistaken for calcium.

In spite of the study of a great many preparations, we were unable to establish a consistent relation between the types of fatty change and the location of the metallic deposits. A few instances were seen where blue amorphous deposits were interspersed with fatty granules or globules. Other areas were found where large calcified plaques were surrounded by visible fat. Yet it was far from uncommon to find cysts with fat contents in which no calcium salts were stained and calcium and iron salt deposits with nothing in the way of visible fat.

Roehl's Hematoxylin Stain for Calcium[4]*:* This method has been recommended for the removal of the iron deposits, leaving calcium and one or two less important salts to be stained.

Frozen sections were washed thoroughly in distilled water, transferred to a 3 per cent solution of oxalic acid for ½ hour, washed and stained for about 1 hour in a ripened (3 months old) solution of hematoxylin in 50 per cent alcohol, and mounted in glycerine jelly.

We had little success with this method. The calcium plaques stained rather lightly. Some of the segments that gave high calcium values by chemical analysis were entirely negative in the stained sections. It was useful in showing that the salts which could be demonstrated occupied the same sites as the salts demonstrated with alum hematoxylin and von Kossa's stain.

Von Kossa's Silver Stain: This stain formerly was used as the routine method for the demonstration of calcium salts. Many modifications have been recommended and we tried several of them. Our best results were obtained in the following way:

Frozen sections were washed in distilled water and transferred to freshly prepared 10 per cent silver nitrate in open dishes. They were then placed in direct sunlight, out of doors and without the interposition of window glass for from ½ to 1 hour, depending on the season of the year. They were then washed in distilled water for at least ½ hour and counterstained lightly in phloxine and mounted in glycerine jelly. In the wintertime we exposed the section to an arc light instead of the sun, but the reaction was less intense.

Cameron [6] tested the action of silver nitrate on various salts in gelatin and reported that von Kossa's stain blackened calcium phosphates, carbonate, oxalate and oleate, also the salts of barium, strontium (ferric) iron, copper and magnesium.

Of all methods tested by us the von Kossa stain was, in spite of its non-specific action, the best index of the amount of calcium to be obtained by chemical analysis.

Alizarin: The amounts of calcium brought out with alizarin were relatively small and there was constantly a discrepancy between the amounts of stained calcium and that found by quantitative chemical analysis. When present the reactions were distinct and clear-cut.

By a series of experiments in which known quantities of salts were injected, Cameron [6] concluded that alizarin is differential for calcium with the exception of strontium, and that only freshly deposited salts of these metals are brought out in tissues.

Microscopic incineration was applied to several frozen sections and specimens were obtained that showed the location of the salts in the arterial walls. The test is not differential and discloses no more than von Kossa's stain.

Summary of Technical Methods: None of the methods shows all of the calcium salts present. Von Kossa's silver method most nearly paralleled the chemical analyses, in spite of the fact that it brings out ferric and other salts as well as calcium. Both Roehl's hematoxylin and alizarin fell far short of indicating the amount of calcium actually present.

Comparison of Chemical and Microchemical Results

For the purpose of comparing the amounts of calcium found chemically with the amounts that could be shown by microchemical means in the various types of arteriosclerosis of the aorta, a protocol

was prepared for each case. These data included the name, age, color and sex of the patient, the clinical diagnosis, the serological reactions with reference to lues, and in parallel columns the diagnosis for each type of aortic lesion, the relative amounts of calcium shown in the sections, and the number of milligrams of metallic calcium per 100 gm. of wet aorta, found chemically, for each respective segment of each aorta. Seven apparently normal aortas from young persons gave an average of 22.8 mg. of calcium per 100 gm. of wet aorta.

The first step was to adopt a satisfactory nomenclature to be applied uniformly throughout the series.

Nomenclature

For some years it has been our practice to use Klotz'[7] classification of arteriosclerosis. However, Klotz' classification was devised for general arteriosclerosis and does not provide for some of the differentiations that seemed to us to be of advantage in our work. With only the lesions of the aorta in mind we adopted a descriptive terminology of our own as given below.

Aortic Arteriosclerosis

(A) Intimal lesions
- (1) Fatty streaking
- (2) Nodular thickening (without cysts)
- (3) Atheroma
  - (*a*) Grumous cysts
  - (*b*) Crystalline cysts
  - (*c*) Ulcerated cysts
- (4) Visible calcification
  - (*a*) Diffuse
  - (*b*) Scaly plaques
  - (*c*) Heavy plates

(B) Medial lesions
- (1) Fatty changes
- (2) Cystic degeneration
- (3) Calcification
  - (*a*) Diffuse
  - (*b*) Plaque formation

(C) Combined intimal and medial sclerosis

(D) Aortitis
- (*a*) Exudative
- (*b*) Degenerative
- (*c*) Proliferative

} { Acute, Rheumatic, Luetic

(*A*) *Intimal Lesions*

Fatty streaking was applied to the superficial intimal yellowish discolorations where the microscopic picture showed the fat to be intracellular or in pockets where the foam cell partitions had disappeared: six segments showing fatty streaking in persons under 40 years of age gave an average of 77.2 mg. of calcium and were all negative to calcium stains.

Nodular thickenings included the intimal nodular proliferations prior to cyst formation: ten segments averaged 168 mg.

Atheroma was used in the original sense of Haller (Klotz[8]) and limited to cystic changes in the intima. We included all cystic changes from early liquefaction necrosis to the typical cysts filled with "free fats, lipoids and cholesterol compounds." Some of the contents were amorphous or grumous, while others were crystalline and contained varying amounts of stainable calcium: fifteen segments averaged 298 mg.

Visible calcification of the intima was manifested in several degrees of intensity. Calcium occurred in diffuse granules in intimal nodules; in the form of very fine superficial scales just beneath the intimal surface; as definite plaques of varying size and thickness, some of which were firmly fixed while others were ulcerated; and as actual heavy plates which tore the tissues on removal and which occasionally had a supporting structure of metaplastic bone.

Owing to the way in which the samples were collected it was not possible to separate the intimal calcification histologically from the medial lesions. None of the segments could be interpreted as fairly representing an entire aorta.

(*B*) *Medial Lesions*

Changes in the aortas were rarely limited to the media, although instances were found where the medial lesions were predominant at the levels examined: thirteen such segments averaged 345 mg. of calcium and all gave positive silver stains.

The earliest changes observed were brought out by the positive Sudan III staining of the smooth muscle cells. Calcium granules were sometimes found in these degenerated muscle cells and sticking to the elastic fibrils between them.

Cysts were found in the medias of some aortas unassociated with those in the intima. Their contents varied from grumous to crystalline: eight segments showing ulcerated crystalline cysts averaged 770 mg. of calcium.

Calcium salts in stainable forms were manifested in three degrees of intensity: as sparsely distributed granules clinging to the elastic tissue fibrils; as bands varying in width from narrow streaks lying just beneath the internal elastic lamina to broad, dense, unbroken zones involving more than half of the medial thickness; and as concentrated masses in plaque form.

(*C*) *Combined Intimal and Medial Plates*

In the more advanced examples of arteriosclerosis practically every aorta presented combinations of any or all of the lesions mentioned above, and they were rarely limited sharply to the intimal or medial layers but extended from one to the other. Separate lesions of the intima and media were sometimes found near each other, but without apparent relation. Thirteen segments containing combined intimal and medial plates averaged 1009 mg. of calcium.

(*D*) *Aortitis*

Several examples of aortitis were encountered: these varied from acute leukocytic exudates in the intima, as seen in a case of septicemia, to widespread degenerations of the muscle cells and elastic tissue accompanied by proliferation of connective tissue and vascular invasions from the adventitia. The latter were examples of luetic and rheumatic aortitis. In all cases in this group there was considerable overlapping of exudative, degenerative and proliferative processes. Three of the cases presented numerous calcium plates in addition to aortitis. One such segment reached 957 mg. of calcium. As a rule the examples of aortitis gave lower calcium values than the average for the age group in which they occurred. The average calcium value for 10 cases of aortitis was 337 mg.

The calcium content in relation to types of lesions as shown above was inconsistent. Not only did similar lesions vary in the amounts recovered chemically but there were wide variations between similar lesions from patients of different ages and in the segments from vari-

ous levels of the same aorta. Since the results seemed to depend largely on the ages of the patients, we tabulated the amounts obtained chemically according to decades into eight groups.

Group I. Children Under 10 Years of Age

The first group included 6 aortas from children under the age of 10 years (Table I). Microchemical methods failed to demonstrate calcium in any aortas of this group. Three were grossly and microscopically negative. A fourth presented simple fatty streaking. Two were thickened and inelastic. The outstanding one of the group was from a child with rickets which yielded 114 mg. of calcium by chemical analysis.

Table I

Group I. Birth to 10 Years

Autopsy No.	Age	Sex	Color	Average*	Gross examination	Cause of death
				mg.		
A-29-85	5 mo.	F	B	35.0	Degenerative aortitis	Congenital lues
A-29-89	1 yr.	M	W	37.0	Fatty streaks	Hydrocephalus
A-29-90	1 yr.	M	W	12.5	Negative	Acute infection
A-29-101	2 mo.	F	B	114.0	Thickening in gross	Rickets and diphtheria
A-30-20	19 mo.	F	W	25.0	Negative	Polyglandular dystrophy
A-30-27	10 yr.	M	W	16.3	Negative	Auto accident

40 = Average of 6 — Birth to 10 years

* The figures in this column represent metallic calcium in mg. per 100 gm. of wet aorta.

Groups II and III. 11 to 21 Years, and 21 to 30 Years

Groups II and III included 3 cases between the ages of 10 and 20 years, and 3 between 20 and 30. As shown in the tables, 4 aortas were negative in the gross and 2 presented simple fatty streaking. No calcium was shown by microchemical methods in any of them. With the exception of the 25 year old patient with chronic tuberculosis, the calcium content was fairly uniform.

Group IV. 31 to 40 Years

In this group there were 8 aortas that presented a variety of pathological changes. Three showed nothing more in the gross than fatty intimal streaking. In these the microchemical tests were negative

and an average for all nine segments gave a chemical analysis of 95 mg. of metallic calcium. Two presented nodular intimal thickenings which were negative to all microchemical tests except silver, the silver reaction being brown rather than black. Three presented very definite aortitis, and of these 2 were characteristic of lues. The aorta from case A–29–86 was dilated and thickened in the first portion. Microscopically the elastic and muscular layers were replaced by connective tissue. The microchemical reactions were

TABLE II

Group II. 11 to 20 Years

Autopsy No.	Age	Sex	Color	Arch *	Thoracic*	Abdominal *	Average *	Gross examination	Cause of death
	yrs.			*mg.*	*mg.*	*mg.*	*mg.*		
A–29–72	17	F	W	25.6	..	..	25.6	Negative	Tuberculosis
A–29–80	18	F	W	..	28.4	29.0	28.7	Negative	Endocarditis
A–29–100	13	F	W	..	14.0	..	14.0	Negative	Paratyphoid fever

22.8 = Average of 3 — 11 to 20 years

TABLE III

Group III. 21 to 30 Years

Autopsy No.	Age	Sex	Color	Arch *	Thoracic *	Abdominal *	Average *	Gross examination	Cause of death
	yrs.			*mg.*	*mg.*	*mg.*	*mg.*		
A–29–89	22	M	W	43.0	40.0	29.0	37.3	Negative	Pneumonia
A–29–102	28	M	W	69.0	56.0	50.0	58.3	Fatty streaks	Septicemia
A–30–12	25	M	W	62.0	60.0	72.0	64.6	Fatty streaks	Tuberculosis

53.4 = Average of 3 — 21 to 30 years

* The figures in this column represent metallic calcium in mg. per 100 gm. of wet aorta.

negative and the metallic calcium low. In the thoracic segment two processes were found—aortitis and cystic atheroma. Microscopically the cysts were filled with crystalline material and calcium granules. The von Kossa stain brought out a moderately heavy granular deposit in the media. The calcium content by chemical analysis, 853 mg., appeared to be out of proportion and greatly in excess of the other changes (see Table IV). In this age group the thoracic segments gave the highest calcium values.

TABLE IV

Group IV. 31 to 40 Years

Autopsy No.	Age	Sex	Color	Arch †	Thoracic †	Abdominal †	Average †	Gross examination	Cause of death
	yrs.			*mg.*	*mg.*	*mg.*	*mg.*		
A-29-86	38	F	W	90.0	853.0	131.0	358.0	Atheromatous aortitis	Lues and alcoholic cirrhosis
A-29-92	38	F	W	118.0	253.0	166.0	179.0	Aortitis	Streptococcus cellulitis
A-29-94	31	F	W	108.0	103.0	61.0	90.6	Fatty streaks	Pneumonia
A-30-25	38	M	W	*	258.0	*	258.0	Luetic aortitis	Cerebral hemorrhage
A-30-33	36	M	W	209.0	133.0	178.0	173.3	Moderate thickening	Pneumonia
A-30-34	37	M	W	88.0	124.0	98.0	103.3	Fatty streaks	Pneumonia
A-30-38	35	M	W	130.0	101.0	63.0	98.0	Fatty streaks	Acute alcoholism
S-34-193	35	M	W	66.0	100.0	77.0	81.0	Nodules	Pneumonia
				115.5	240.6	110.5	167.6 = Averages of 8 — 31 to 40 years.		

* Segments lost in autoclave.
† The figures in this column represent metallic calcium in mg. per 100 gm. of wet aorta.

Group V. 41 to 50 Years

Seven of the 11 cases in this group had an average age of 42 years and an average calcium content by analysis of 214 mg. They presented little contrast in calcium content from segment to segment (see Table V). In the remaining 4 cases the mean age was 48 years and the average calcium content 270 mg. Microchemically calcium was demonstrated in the aortas from cases A–29–74, A–29–103, A–30–2, A–30–24 and A–30–37. In case A–29–96, where the picture was that of luetic aortitis and the average calcium content was 374 mg., no calcium was shown microchemically by any method. This apparently inconsistent result indicates that the quantity of calcium present is independent of its visibility. In this group the highest calcium values were in the abdominal segments.

Group VI. 51 to 60 Years

All of the aortas in this group presented arteriosclerotic changes and calcium was stained in all except cases A–29–76 and A–30–6. The amounts of calcium obtained varied more widely than in any other age group. The lowest amount was recovered from case A–29–76, where the average for the three segments was 153.6 mg. This patient was a man 50 years of age with typical luetic aortitis, who died from a cerebral hemorrhage. Three cases A–29–97, A–29–106 and A–30–8 presented widespread combined lesions including atheromatous cysts, diffuse medial calcification and isolated calcium plaques or plates. All averaged over 1100 mg. of metallic calcium. In the first sections the abdominal segment from case A–30–8 showed 2312 mg. of calcium and only atheromatous cysts with granular calcium and crystalline material. As it was the only instance of excessively high calcium without plates, the original formalin-fixed material was re-examined and definite plates found. Two of the 10 cases were 60 years of age and barely escaped the 61 to 70 year group. These cases averaged well over 1100 mg. of calcium. In this group the highest average calcium content was found in the abdominal segment.

Group VII. 61 to 70 Years, 1 Case 78 Years of Age

The calcium content of the respective aortas in this group was higher than in the group between 51 and 60 years of age, but the

TABLE V

Group V. 41 to 50 Years

Autopsy No.	Age	Sex	Color	Arch †	Thoracic †	Abdominal †	Average †	Gross examination	Cause of death
	yrs.			*mg.*	*mg.*	*mg.*	*mg.*		
A-29-71	42	M	W	257.0	178.0	*	217.0	Aortitis	Gangrenous lung
A-29-74	50	M	W	241.0	440.0	795.0	492.0	Atheromatous cysts	Septicemia
A-29-83	42	M	W	139.0	192.0	137.0	156.0	Nodular thickening	Carcinoma
A-29-96	42	M	B	330.0	413.0	380.0	374.0	Aortitis	Lues, pneumonia
A-29-103	47	M	W	215.0	150.0	106.0	157.0	Atheromatous cysts	Exophthalmic goiter
A-30-2	43	M	W	201.0	209.0	324.0	245.0	Intimal plaque	Lung abscess
A-30-14	48	M	W	130.0	219.0	110.0	153.0	Aortitis	Arthritis, pneumonia
A-30-24	43	M	W	314.0	324.0	242.0	293.0	Combined intimal and medial cysts	Cardio-renal
A-30-28	41	M	B	84.0	66.0	55.0	68.0	Nodular thickening	Gangrenous lung
A-30-37	47	M	B	*	250.0	307.0	278.0	Atheroma and aortitis	Pneumonia
A-30-39	41	M	B	153.0	104.0	145.0	134.0	Negative	Pneumonia
				206.0	231.4	260.0	233.4	= Averages of 11 — 41 to 50 years.	

* Segment lost.
† The figures in this column represent metallic calcium in mg. per 100 gm. of wet aorta.

TABLE VI

Group VI. 51 to 60 Years

Autopsy No.	Age	Sex	Color	Arch*	Thoracic*	Abdominal*	Average*	Gross examination	Cause of death
	yrs.			*mg.*	*mg.*	*mg.*	*mg.*		
A–29–76	50+	M	W	114.0	121.0	226.0	153.6	Cyst and aortitis	Cerebral hemorrhage
A–29–78	54	M	W	182.0	213.0	923.0	439.3	Atheromatous cysts	Alcoholic cirrhosis
A–29–97	60	M	W	1148.0	1175.0	1067.0	1130.0	Medial plaques	Chronic vegetative endocarditis
A–29–106	60	M	W	351.0	1265.0	1926.0	1180.0	Atheromatous cysts	Chronic cardio-renal
A–30–5	58	M	W	475.0	355.0	418.0	416.0	Luetic aortitis	Chronic cardio-renal
A–30–6	55	F	W	493.0	193.0	466.0	384.0	Atheromatous cysts	Hodgkin's disease
A–30–8	57	M	W	508.0	718.0	2312.0	1179.0	Atheromatous cysts	Septicemia
A–30–15	53	M	W	396.0	399.0	320.0	372.0	Nodular thickening	Sarcoma
A–30–31	51	M	W	180.0	512.0	71.0	254.0	Atheromatous cysts	Septicemia
A–30–32	50+	M	W	111.0	130.0	957.0	399.3	Atheroma and plates	Lues
				395.8	508.1	868.6	590.8	= Averages of 10 — 51 to 60 years.	

* The figures in this column represent metallic calcium in mg. per 100 gm. of wet aorta.

TABLE VII

Group VII. 61 to 70 Years, Including 1 Case, Age 78 Years

Autopsy No.	Age	Sex	Color	Arch*	Thoracic*	Abdominal*	Average*	Gross examination	Cause of death
	yrs.			*mg.*	*mg.*	*mg.*	*mg.*		
A-29-81	63	M	W	313.0	133.0	420.0	289.0	Atheromatous cysts	Pneumonia
A-29-95	66	M	W	314.0	405.0	694.0	471.0	Atheromatous cysts, aortitis	Sarcoma (lues)
A-29-99	70	M	W	919.0	1644.0	930.0	1164.0	Ulceration, plate	Cerebral hemorrhage
A-30-7	66	M	W	523.0	492.0	1348.0	788.0	Atheromatous cysts	Cirrhosis
A-30-16	78	M	W	1135.0	716.0	1144.0	998.0	Atheromatous cysts and plates	Nephritis and diabetes
A-30-19	62	M	W	121.0	263.0	983.0	456.0	Ulceration, plaques	Pneumonia
A-30-21	67	M	W	426.0	340.0	1068.0	611.0	Atheromatous cysts and plates	Pneumonia
A-30-26	67	M	W	223.0	368.0	316.0	302.0	Nodular intima	Accident
S-34-190	61	M	W	288.0	200.0	512.0	333.3	Atheromatous cysts and plaques	Cardio-renal
S-34-191	61	F	W	1050.0	420.0	800.0	757.0	Diffuse medial and plaques	Burns
S-34-192	64	M	W	392.0	160.0	520.0	357.0	Luetic aortitis	Cardio-renal
				518.5	467.3	794.0	593.2	= Averages of 11 — 61 to 70 years; and 1 aorta from a patient 78 years of age.	

* The figures in this column represent metallic calcium in mg. per 100 gm. of wet aorta.

total average was not as high because there were 3 aortas with unusually high calcium content in Group VI. Practically all of the segments presented combined atheromatous cysts and diffuse medial disease with plaques and plates. All of the aortas contained stainable calcium. Again, the heaviest calcium deposits were found in the abdominal segments just above the bifurcation.

There was 1 aorta from a patient over 70 years of age in the series. It presented advanced arteriosclerosis with both intimal and medial changes and is tabulated in Group VII.

Discussion

A summary of the results by decades is shown in Table VIII. It is apparent that changes occur in the aortas during the course of life which lead to an accumulation of calcium, the total amounts of which exceed that of the bodily tissues generally. The affinity of the tissues of the aorta in advanced years for calcium is greater than that of the circulating blood.

A number of theories have been suggested to explain this excess of calcium. None has been altogether satisfactory, but since there are so many and varied histological changes in the vessel it is probable that multiple factors are responsible. Wells [1, 9] called attention to the fact that living and dead colloids behave similarly; fresh gels contain much water and, as age increases, the capacity to retain it decreases and eventually the gel becomes granular. The elastin in the aortic walls very likely behaves in this way, and he further compared the process to the gradual hardening of rubber tubing. He cited hyaline cartilage as another example of a colloid that loses its elasticity and takes up calcium. Gazert [10] reported that sclerosis and calcium increased as the proportion of nitrogen decreased. Klotz [11] explained the increase in calcium in atheromatous cysts as a calcium replacement of soluble salts in the soaps formed during the degeneration of fatty substances. Wells,[1, 5, 9] Schönheimer,[3] and others, have raised many objections to Klotz' views. It is certain that we recovered large amounts of masked calcium and demonstrated calcium deposits in the aortas of our series where no previous fatty changes could be brought out.

Whatever the nature of the calcification process of aortas, the ratio of calcium phosphate to calcium carbonate appears to be

similar to that of bone. This has been shown by Barillé,[12] in 1910, and by Schönheimer,[3] in 1928. The generally accepted ratio is given as calcium phosphate 88 to 90 per cent, carbonate 10 to 12 per cent, and magnesium salt 1 to 1.5 per cent.

Calcium Content According to Type of Lesion

The results of our efforts to correlate the calcium recovered chemically with the types of lesion were unsatisfactory. It was soon apparent that moderate intimal lesions existed without an increase

TABLE VIII

Average Calcium Content by Decades per 100 gm. of Wet Aorta

Decades	No. cases examined	No. positive microscopically *	High †	Low †	Average †
yrs.			*mg.*	*mg.*	*mg.*
Birth to 10	6	0	114.0	12.5	40.0
11 to 20	3	0	28.7	14.0	22.8
21 to 30	3	0	64.6	37.3	53.4
31 to 40	8	1	358.0	81.0	167.6
41 to 50	11	5	492.0	68.0	233.4
51 to 60	10	8	1180.0	153.6	590.8
61 to 70	10	10	1164.0	289.0	552.7
71 to 80	1	1	998.0	998.0	998.0

* Includes all cases where Roehl's hematoxylin, alizarin and von Kossa's stains were positive.
† The high and low calcium columns show the high and low calcium contents obtained by averaging three segments from the same aorta, not the amount found in the highest or lowest segment analyzed. The average in the last column represents all of the aortas for each age group.

in calcium, chemically, and that concentrated deposits in the nature of plaques and plates gave the highest amounts. Of eleven examples of luetic aortitis only two contained an excess of calcium, while the average content was lower than that for the age group in which it occurred.

We realized that there are additional factors responsible for our failure to obtain comparable figures for similar lesions. Some of these factors we know are beyond our control while others we think may be brought out by more carefully controlled data and these will be discussed in a later paper.

CONCLUSIONS

Fifty-two aortas from patients between the ages of 2 months and 78 years were analyzed chemically for metallic calcium and the

results compared with the microchemical tests for calcium salts on the same aortas with the conclusion that:

(1) None of the microchemical tests recommended for visible calcium gives more than a vague idea of the amount that can be recovered from the same aorta chemically. Von Kossa's silver method is not a specific stain for calcium, but it is the most satisfactory microscopic indicator of the comparative amounts of calcium deposited in sclerotic lesions.

(2) Calcium deposits were brought out microchemically in only 1 of 20 aortas from cases under 40 years of age, in 75 per cent of aortas from cases over 40 years of age, and in 100 per cent of those over 60 years.

(3) As age advances there is a consistent increase in the calcium content of aortas in excess of that of the bodily tissues generally.

(4) Mild intimal lesions may occur without increased calcium by chemical analysis.

(5) When the segments of all aortas from cases over 40 years of age are averaged, the heaviest calcium deposits are found in the abdominal portions.

(6) In advanced aortic arteriosclerosis there is constantly found to be an overlapping of "type lesions," such as atheromatous cysts, diffuse medial calcification and plaque deposits. In this of all groups of lesions, the greatest inconsistencies between chemical and microscopic results occur.

(7) In very advanced sclerotic lesions, characterized by heavy plate-like calcium deposits, the chemical analyses yield the highest calcium values and the amounts are most nearly consistent, when any 2 given sclerotic aortas are compared.

(8) If the results obtained by chemical analysis are to be correlated with calcium, which can be shown microscopically in sclerotic aortas, additional knowledge is required.

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