Fluoride and Calcium Levels in the Aorta

Among soft tissue organs which store fluoride (F^-), the aorta contains the highest levels[1,2]. Calcifications of arteries of the Mönckeberg type have been reported in relatively young persons afflicted with skeletal fluorosis from endemic areas[3-5]. It was, therefore, of interest to determine whether or not there is a systematic correlation of F^- levels with those of calcium (Ca^{++}) in the aorta.

Methods. Aorta tissues were selected at random from 23 autopsies, without reference to sex, age or cause of death, on persons who died in 3 Detroit hospitals[6] of various diseases. In 16 cases F^- and Ca^{++} determinations were made on grossly calcified tissue and compared with those in adjacent tissue which showed no gross evidence of calcification. In 7 additional cases only F^- levels were determined in both grossly calcified and less calcified tissue.

The tissues were collected in plastic containers with an alkaline F^--free formalin solution. From each aorta specimen all grossly calcified pieces were separated and placed together, as were pieces of the less calcified areas. Both sets of samples were finely chopped until they were homogeneous.

F^- was separated by the double distillation method of WILLARD and WINTER[7] and titrated by the WILLIAMS[8] procedure as modified by SMITH and GARDNER[9]. Two determinations were made on each sample[10].

For the Ca^{++} determination, another weighed portion of the same samples was ashed in platinum crucibles at 575 °C for 16 h. The ash was then taken up in the minimum quantity of 0.1 *M* HCl and the Ca^{++} separated from orthophosphate and heavy metals through the use of gelatinous hydrolysis product of Zr^{4+}[11]. The Ca^{++} was then determined by a complexometric titration with EDTA with calcon as the indicator[12,13].

Results. Ca^{++} levels: In all instances, the grossly calcified tissues contained moderately more Ca^{++} than the

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Table I

Case No. and date	Name, age and sex	Cause of death	Fluoride (ppm) Less calcified	Fluoride (ppm) Calcified	Calcium (mg/g) Less calcified	Calcium (mg/g) Calcified	Ca/F molar ratio Less calcified	Ca/F molar ratio Calcified
1 30.I.60	D.T., 32, M	Coronary thrombosis	2.0	4.8	5.1	8.4	1220	835
2 24.V.63	F.A., 65, M	Carcinoma, stomach	20.3	59.0	13.3	161.0	310	1300
3 6.VIII.63	J.L., 63, M	Carcinoma, esophagus	14.3	165.1	2.8	3.9	92	11
4 6.VII.63	V.D., 61, M	Carcinoma, kidneys	17.6	49.5	1.1	5.4	30	52
5 15.I.64	L.K., 82, F	Carcinoma, bladder	6.0	19.6	2.9	7.4	230	180
6 15.I.64	S.E.R., 90, M	Adenocarcinoma, lung	6.7	16.7	4.8	5.7	340	162
7 15.I.64	C.F., 82, F	Arterioscl. hrt. dis., coronary occlusion	5.5	36.2	8.3	17.2	715	226
8 25.I.64	G.S., 49, F	Brain tumor	4.1	14.6	2.6	2.8	298	91
9 25.I.64	L.W., 74, F	Hypertensive hrt. dis., carcinoma, breast	7.4	27.9	3.5	57.8	224	985
10 24.I.64	J.E., 55, M	Occlusion carotid artery, cerebral infarction	4.5	11.2	2.3	6.1	243	258
11 7.III.64	W.M.B., 56, F	Carcinoma, cervix	nil	5.8	8.8	22.4	–	1840
12 7.III.64	J.J., 70, M	Coronary occlusion	nil	4.6	4.1	11.0	–	1140
13 7.III.64	J.D., 50, F	Rheumatic hrt. dis.	nil	nil	6.6	14.2	–	–
14 7.III.64	J.J. Jr., 47, M	Coronary occlusion	3.2	6.3	2.9	11.9	430	895
15 7.III.64	F.A., 70, M	Coronary occlusion	nil	4.1	1.6	2.3	–	266
16 9.VII.64	16 h, M	Sclerosis of arteries	9.4	59.3	8.6	20.0	435	160
17 15.IX.59	G.M., 75, F	Cerebral hemorrhage		158.0				
18 15.IX.59	P.McC., 76, M	Obstructive emphysema		71.5				
19 6.IX.61	R.P., 39, F	Bronchial asthma		6.8				
20 3.XII.62	W.D., 42, M	Pulmonary edema	0.2					
21 15.IV.63	H.B., 67, M	Leukemia		87.8				
22 6.VIII.63	J.B., 51, M	Congenital heart	7.7	77.9				
23 6.VIII.63	H.S., 68, F	Acute enteritis, acute renal failure, chronic pancreatitis	5.6	8.9				

less calcified tissue, except in case 8, a 49-year-old woman with a brain tumor (Table I). Here the Ca^{++} levels of both specimens were relatively low and about equal, namely 2.4 and 2.8 mg/g (2400 and 2800 ppm). The highest Ca^{++} level in the calcified group was 161 mg/g in case 2, the lowest 2.3 mg/g in case 15.

F^- levels: Fluoride content of the aorta varied widely from person to person. In 4 out of the 'less calcified' tissues, the F^- levels were nil. Only one (case 13), of the 'grossly calcified' specimens showed a F^- level of 0. Here the Ca^{++} content was 14.2 mg/g. The highest F^- content of 165 ppm was encountered in a 'grossly calcified' specimen (case 3).

Ca^{++}/F^- ratio: The Ca^{++}/F^- mole ratio varied non-systematically from 11–1820 between the 'grossly calcified' and the 'less calcified' tissues. There was no consistent proportion of Ca^{++} to F^- in either group of aorta samples.

Additional data. Aorta/bone fluoride ratio: Bones are known to store more F^- than other tissues. A possible correlation between aorta F^- and bone F^- was explored by evaluating statistically the detailed data from another study. In 1960, CALL et al.[2] for F^- analyzed 60 aortas (Table II). Their F^- levels showed a remarkably wide range, from 0.3 in a 69-year-old person to 258 ppm in an 80-year-old person, with a mean of 30.0 ppm. The F^- content of dry fat-free bones ranged from 40 ppm to 2025 ppm with a mean of 557 ppm. Bone Ca^{++} levels were more consistent. They ranged from 14.0–29.5%. Statistical evaluation of these data detected no correlation between aorta F^- and bone F^-[14].

Age and F^-: In the present study, as well as in that of CALL et al.[2], there was a strong numerical correlation between age and aorta F^-.

Table II. CALL et al.'s[2] data

No.	Age	Fluoride ppm Aorta	Fluoride ppm Bone	No.	Age	Fluoride ppm Aorta	Fluoride ppm Bone
1	71	1.3	600	31	17	8.1	130
2	51	3.4	390	32	71	86.0	1340
3	61	8.3	342	33	82	16.4	556
4	85	11.2	698	34	29	12.0	340
5	53	5.8	492	35	25	3.5	240
6	66	2.5	320	36	53	3.6	226
7	80	17.8	482	37	78	125.5	463
8	42	4.0	360	38	66	1.0	542
9	42	2.7	470	39	38	21.2	435
10	60	16.9	634	40	55	2.0	347
11	45	21.1	815	41	50	15.6	284
12	16	0.7	82	42	77	0.6	702
13	56	1.0	242	43	67	17.0	422
14	64	8.0	1370	44	55	10.1	382
15	74	16.0	310	45	49	9.7	386
16	55	5.4	312	46	55	9.0	562
17	79	12.0	408	47	72	14.2	660
18	66	5.5	302	48	25	10.0	198
19	55	12.0	622	49	78	118.0	345
20	45	2.1	793	50	88	45.0	973
21	73	79.0	256	51	84	25.1	803
22	47	7.9	474	52	75	58.3	1290
23	80	21.0	554	53	79	31.9	935
24	50	0.8	776	54	32	1.8	220
25	69	0.3	340	55	61	20.0	673
26	61	1.6	240	56	79	45.0	440
27	77	2.0	382	57	70	131.0	707
28	85	57.0	483	58	77	28.0	624
29	26	15.1	220	59	70	143.0	430
30	35	10.9	260	60	79	10.0	1007

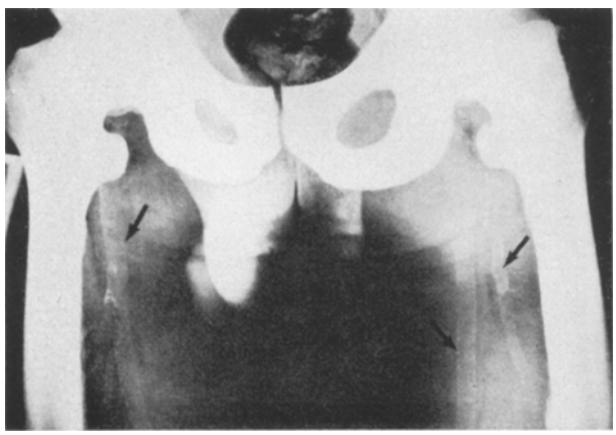

Fig. 1. Skeletal fluorosis in a 44-year-old man with calcifications of arteries from an endemic area in Arabia. (Courtesy of Drs. S. P. KUMAR and R. A. KEMP HARPER, St. Bartholomew's Hospital, London, England.)

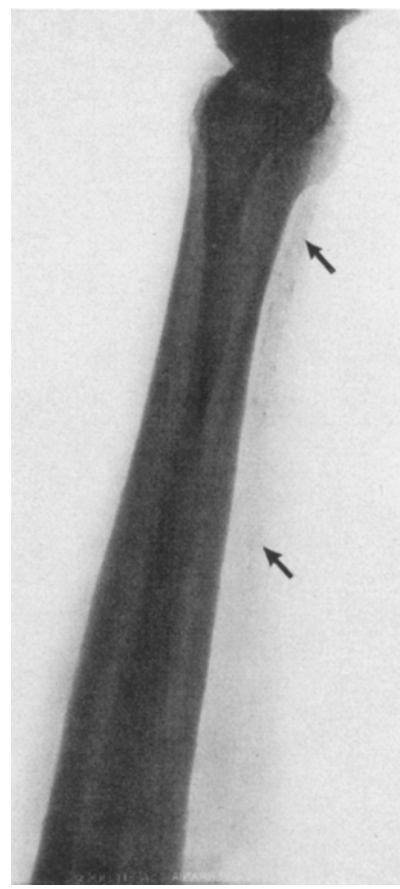

Fig. 2. Calcification of the posterior tibial artery. Skeletal fluorosis in a 49-year-old man residing in Sicily where water contains F^- naturally at 5 ppm. (Courtesy of Drs. G. NALBONE and F. PARLATO, University of Palermo, Italy.)

[14] The statistical work was performed on the basis of the 1958 N.I.H. Research Report prior to the appearance of the published paper in Public Health Report *80*, 529 (1965), by J. M. LUCAS and Prof. L. J. SAVAGE of the Department of Statistics at Yale University.

Aorta F^- and disease: CALL et al.[2] noted a significantly higher storage of bone F^- in cases with pyelonephritis, but no relationship of aorta F^- levels with the causes of death. In the present study, no correlation could be established between aorta F^- levels and the disease.

Discussion. As indicated in Table I, F^- levels in the aorta depend little, if at all, on the amount of Ca^{++} present. That F^- does not seem to be bound in appreciable amounts as calcium-fluoride (CaF_2), has been recognized by others, with respect to bones and teeth[15]. The erratic fluctuations of F^- levels in the aorta from person to person are noteworthy in this as well as in CALL's study. Some samples of aorta tissue contained virtually no F^-, and others up to 258 ppm. In a single organ such as the placenta[16] or in the skin of patients with various dermatological lesions[17] F^- levels vary widely in closely adjoining tissue areas.

Since the F^- content of the aorta does not parallel F^- levels in the skeleton, bone F^- cannot be considered a criterion of F^-'s presence elsewhere in the system nor can possible ill effects in the system be precluded on the basis of low F^- levels in bones.

Zusammenfassung. Fluor- und Kalziumwerte in verkalktem Aortengewebe wurden mit denen von makroskopisch normal erscheinendem Aortengewebe verglichen. Der Fluorgehalt der Aorta verschiedener Personen zeigte grosse individuelle Schwankungen, die unabhängig vom Kalziumgehalt waren. Eine direkte Korrelation des Aortenfluors mit dem Lebensalter wurde festgestellt. Die statistische Auswertung der Untersuchungen von CALL et al.[2] ergab, dass der Fluorgehalt der Aorta unabhängig von demjenigen des Skelettes ist.

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Site of Action of Dopamine and Apomorphine on Compulsive Gnawing Behaviour in Rats

According to early publications, injection of apomorphine into rodents results in gnawing behaviour, which effect is dependent on the presence of the corpus striatum[1]. Recently it has been shown that apomorphine shares this effect with DOPA (as a precursor of dopamine) and that the presence of a phenylethylamine configuration with OH-groups at the *para-* and *meta*-positions of the phenol ring is obligatory for provoking a compulsion to gnaw[2].

Injection of DOPA results in an accumulation of dopamine in the brain, especially in extrapyramidal structures[3]. It could be anticipated, therefore, that the site of action of dopamine and apomorphine would be situated within the extrapyramidal system. This report provides data supporting this assumption.

Experimental. Crystalline DOPA or apomorphine was tamped into a stainless steel cannula, which was introduced stereotaxically into the brain of male albino rats (140–160 g) under light ether anaesthesia. When the tip of the cannula had reached the desired position, the compound was delivered by pushing a stylet down the cannula. The amount of implanted material was about 100 µg. After implantation, the animals were placed in metal cages with a wire-mesh floor, on which the rats were able to gnaw, and their behaviour was observed for several hours.

Results. Implantation sites are shown in Figures 1–3. Effective implantations of DOPA resulted, after a delay of 1–2 h, in intense compulsive gnawing behaviour, lasting for 3 h and sometimes longer. After apomorphine implantation gnawing started within 30–40 min, lasting for about 2 h. Positive effects were observed with both compounds after implantation in the dorsal part of the cau-

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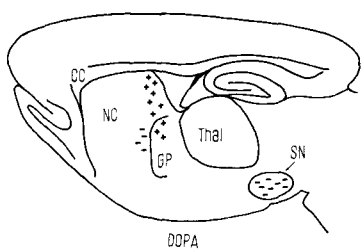

Fig. 1. Implantation sites of l-DOPA, shown in sagittal section of rat brain. + = evoking gnawing behaviour; − = ineffective.

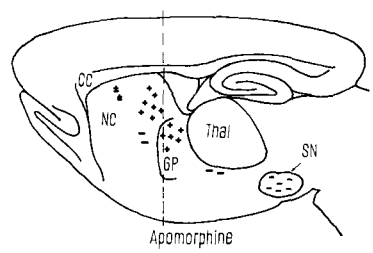

Fig. 2. Implantation sites of apomorphine, shown in sagittal section, at plane indicated in Figure 3 by broken line.