SECTION III. PHYSIOPATHOLOGICAL STUDY

BY

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To define more clearly the nature of articular chondrocalcinosis (AC), biochemical, morphological, and crystallographic studies have been undertaken.

Biochemical Study

We found no abnormalities of serum calcium and phosphate levels (serum calcium 9 to 11.5 mg. per cent.; inorganic phosphate 2.53 to 4.57 mg. per cent.). Plasma alkaline phosphatase levels were also normal (2.4 to 9.3 King-Delory units). The test of Sulkowitch was positive from + to +++; calciuria—in long-term tests—varied within the range of from 100 to 200 mg. per 24 hrs, similar on the whole to that in the control group. Calcium balance, tested in two patients, was positive in both, the utilization exceeding 50 per cent. at intake levels between 916 and 2,478 mg. Ca/d. A certain tendency to retention must therefore be present (Table IV). These results permit us to conclude that the calcification of articular cartilage in patients affected with AC does not develop as a result of

altered calcium metabolism, and therefore that metastatic calcification is not involved.

The serum levels of chlorides, sodium, and potassium were also normal.

Using the method of unidimensional paper chromatography, twelve patients were examined for free amino acids in the serum and urine and nothing abnormal was observed.

Synovial Fluid

In the available literature we have found no detailed analysis of the synovial exudate in AC. Ravault, Ruitton, Maitrepierre, Lejeune, Thomme, and Perrin (1960) reported the aminotripeptidase activity level (3 per cent.) found in a synovial exudate.

We have investigated 21 exudates obtained from fourteen knee joints of eleven patients (Table V, opposite).

The mean values show that these samples meet the criteria for the so-called inflammatory group (Ropes

TABLE IV
RESULTS OF CALCIUM BALANCE IN TWO PATIENTS WITH ARTICULAR CHONDROCALCINOSIS
(Cases 8 and 3)

Case	Day	Total Amount (g.)			Calcium Intake (mg.) in Food	Calcium Output (mg.)			Calcium Balance (mg.)	Intake (Ca/kg.)
		Food	Urine	Stool		Urine	Stool	Together		
Case 8 (56.05 kg.)	1	2,865	780	0	1,265.27	93.55	0	93.55	+ 1,171.72	25.82
	2	2,765	790	45.0	987.78	117.90	188.25	306.15	+ 681.63	20.15
	3	2,965	610	38.0	1,355.10	122.31	266.41	389.22	+ 965.88	27.65
	4	2,655	670	41.0	1,943.98	109.42	237.25	346.67	+ 1,597.31	39.67
	5	2,880	360	23.0	1,051.38	65.89	164.53	230.42	+ 820.96	21.45
	6	2,755	690	22.0	916.03	89.37	131.56	220.93	+ 635.10	18.69
	7	2,675	190	43.0	988.40	44.67	501.13	545.80	+ 442.60	20.17
	8	2,675	170	56.2	1,006.47	40.45	448.60	489.05	+ 517.42	20.54
	9	2,935	780	41.0	1,929.96	166.93	291.53	458.46	+ 1,471.50	39.36
	10	2,900	560	60.0	2,478.01	94.55	490.77	585.32	+ 1,892.69	50.57
	Total ..	28,070	5,600	369.2	13,922.38	945.54	2,720.03	3,665.57	10,256.81	284.07
Case 3 (49 kg.)	1	2,865	1,190	119.0	1,265.27	227.87	243.83	471.70	+ 793.57	22.57
	2	2,765	457	115.0	987.78	74.64	395.39	470.03	+ 517.75	17.62
	3	2,965	1,180	68.0	1,355.10	224.29	349.94	574.23	+ 780.87	24.17
	4	2,655	820	40.0	1,943.98	145.47	170.55	316.02	+ 1,627.96	34.68
	5	2,880	1,000	25.0	1,051.38	283.00	170.44	453.44	+ 597.94	18.75
	6	2,755	1,250	80.0	916.03	239.36	572.71	812.07	+ 103.30	16.34
	7	2,675	1,190	95.0	988.40	196.03	164.86	360.86	+ 627.51	17.63
	8	2,675	1,150	47.7	1,006.47	199.16	334.49	533.65	+ 472.82	17.95
	9	2,935	1,520	69.0	1,929.96	199.03	453.43	652.46	+ 1,277.50	34.43
	10	2,900	1,620	67.0	2,478.01	259.06	380.57	639.63	+ 1,838.38	44.21
	Total ..	28,070	11,377	725.1	13,922.38	2,047.91	3,236.21	5,284.12	8,637.60	248.35

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TABLE V
PRINCIPAL CHARACTERISTICS OF THE SYNOVIAL FLUID IN ARTICULAR CHONDROCALCINOSIS

Index										No. of Tests	Average	Variability	Method
Volume (ml.)										21	28.0	0.2-95.0	Ropes and Bauer, 1953
Turbidity (0-4)										21	1.28	0-4	
Spontaneous Coagulation (0-4)										21	1.95	0-4	
Cellular Elements	Nuclear cells (μ l.)			21	6,900	109-23,200							
	Polynuclear cells ..	(μ l.)	18	5,341	0-18,212								
		(per cent.)	18	55.9	0-90.0								
Difference between Blood and Synovial Sugar (mg. per cent.)										8	-14.5	0--34.5	Luštinec, 1953
Hyaluronic Acid	Intrinsic viscosity			11	23.1	17.6-30.0	Sundblad, 1953						
	Anomalous viscosity			13	55.2	24.7-85.3	Sundblad, 1954						
	Concentration (mg. per cent.)			11	305.0	104-824	Decker* Marković and Hüttl, 1961						
	Acetic acid precipitate			14	2.7	1-4	Ropes†						
Proteins	Total (g. per cent.)			12	3.65	2.5-4.8	Gornall, Bardawill, and David, 1949						
	A/G Synovial fluid			14	1.45	0.82-2.08	Hüttl and Marković, 1962						
	A/G SF : A/G Serum			14	1.28	0.90-1.69							
	SF (g. per cent.) S (g. per cent.)	Total proteins			12	0.50	0.34-0.66	Nettelbladt and Sundblad, 1959					
		Albumin			12	0.57	0.40-0.81						
		Globulin α_1			12	0.47	0.26-0.97						
		Globulin α_2			12	0.32	0.18-0.59						
		Globulin β			12	0.41	0.27-0.58						
		Globulin γ			12	0.49	0.33-0.61						
Synovial fluid			4	45.7	24.5-63.0	Ayala, Moore, and Hess, 1951							
Serum			3	79.3	54.0-109								
SF : S			4	0.51	0.44-0.58								
Aminotripeptidase Activity										5	10.2	3.4-20.3	Ziff‡

* Decker, McGuckin, McKenzie, and Slocumb, 1959.

† Ropes, Robertson, Rossmeisl, Peabody, and Bauer, 1947.

‡ Ziff, Simson, Scull, Smith, Shatton, and Mainland, 1955.

and Bauer, 1953). This is suggested by the development of turbidity, tendency to spontaneous coagulation (increased fibrinogen level), considerably increased number of nuclear cells with a high percentage of polynuclears, reduced polymerization of hyaluronic acid (judging by the values both of intrinsic and anomalous viscosity), and increased protein level with a displacement towards the globulins.

However, if no account is taken of the mean values, the individual findings show a wide range of variation. Apart from the intensely clouded exudates (which show a prompt spontaneous coagulation, with nuclear cell levels reaching 90 per cent., with a very low degree of hyaluronic acid

polymerization, evident displacement towards the globulins, and increased values of sialic acid and aminotripeptidase activity), there also occur clear exudates with a high viscosity level which are not liable to spontaneous coagulation, with nuclear cell levels in 1 μ l. lower than 500, with insignificant polynuclear cell levels, and a relatively retained degree of hyaluronic acid polymerization (hyaluronic acid being, moreover, present in high concentrations), and with relatively satisfactory protein ratios and low levels of sialic acid and aminotripeptidase activity.

Whereas the first group of exudates meets the criteria postulated for "inflammatory" disease (Ropes and Bauer, 1953), the second group is

compatible with the picture of irritant "degenerative" alterations. In Table VI these two groups are reviewed separately. Section I summarizes the characteristics of exudates obtained from joints with marked local inflammation during a fully-developed arthritic episode at the peak of the affection; Section II summarizes those of exudates obtained in periods of remission from joints which showed radiological evidence of advanced osteo-arthritic changes.

The joints which show a fully-developed picture of disease with extensive mineral incrustations in the articular cartilage react by a stormy inflammation ("inflammatory" period); concurrently with the progressive loss of the incrustated cartilage—which represents the anatomic substrate responsible for the

acute inflammation—these inflammatory manifestations become gradually less intense and in the final stage of the disease, after the cartilage has disappeared (being replaced by hyperplastic bone tissue), the joint reacts both qualitatively and quantitatively as in the so-called non-inflammatory affections ("degenerative" period). The demarcation line between these two periods is not sharp, the inflammation subsides gradually so that the synovial fluid passes through the intermediate stages described by Ropes and Bauer (1953).

Morphology

Histological and histochemical examinations were made of the head of the first metatarsus

TABLE VI
CHARACTERISTICS OF SYNOVIAL FLUID IN ARTICULAR CHONDROCALCINOSIS ACCORDING
TO THE STAGE OF THE DISEASE

Index		No. of Tests	Period				
			I. Inflammatory		II. Degenerative		
			Average	Range	No. of Tests	Average	Range
Volume (ml.)	.. .. .	14	33.1	0.2-75	5	16.9	1.5-35
Turbidity (0-4)	.. .. .	14	1.8	1-4	5	0	0-±
Spontaneous Coagulation (0-4)	.. .. .	14	2.8	0-4	5	0.2	0-1
Cellular Elements	Nuclear cells (μl.)	14	9,967	1,200-23,200	5	473	109-1,500
	Polynuclear cells (μl.)	12	7,950	840-18,212	5	96.6	0-450
	(per cent.)	12	77.6	61-90.5	5	10.5	0-30
Difference between Blood and Synovial Sugar (mg. per cent.)		8	14.5	0-34.5	—	—	—
Hyaluronic Acid	Intrinsic viscosity	5	21.23	17.6-25.3	5	25.7	20.6-30.0
	Anomalous viscosity	6	41.8	24.7-52.5	5	70.1	50.5-85.3
	Concentration (mg. per cent.)	5	164.3	104-237	4	462.4	202-824
	Acetic acid precipitate	9	2.2	1-3	5	3.3	3-4
Proteins	Total (g. per cent.)	5	3.97	3.32-4.82	4	3.25	2.49-3.96
	A/G Synovial fluid	7	1.14	0.82-1.66	5	1.89	1.43-2.08
	A/G SF : A/G S	7	1.28	0.90-1.69	4	1.26	1.02-1.52
	Total proteins	5	0.59	0.49-0.67	4	0.44	0.34-0.52
	Albumin	5	0.65	0.50-0.82	4	0.48	0.35-0.59
	Globulin α ₁	5	0.48	0.31-0.64	4	0.40	0.29-0.52
	Globulin α ₂	5	0.40	0.29-0.59	4	0.26	0.20-0.34
	Globulin β	5	0.45	0.35-0.58	4	0.35	0.28-0.42
	Globulin γ	5	0.51	0.41-0.61	4	0.46	0.33-0.61
	Synovial Fluid	2	62.7	62.5-63.0	2	28.7	24.5-33.0
Sialic Acid (mg. per cent.)	Serum	1	109.0	—	2	64.5	54.0-75.0
	SF : S	2	0.575	0.57-0.58	2	0.445	0.44-0.45
Aminotripeptidase Activity		2	17.9	15.5-20.3	3	5.02	3.42-7.09

obtained by resection from two patients operated on for hallux valgus (Fig. 20).

We succeeded in obtaining microscopic sections showing various stages of development from primary incipient changes to the secondary changes progressing from the centre to the periphery of the surface of the joint.

Primary Structural Alterations.—These may be divided into two stages:

(1) A thick condensed film appears on the surface of the cartilage and by amitotic division of separate cells reaches the zone close to the surface (Fig. 21, overleaf).

A tendency to progress is seen in areas where the cell division occurs rapidly. These cells assume the character of connective tissue, while intercellular fibrous structures appear in their vicinity and surround the focus dividing it from adjacent area (Fig. 22, overleaf).

Initially, the metaplastic focal alterations of hyaline cartilage are localized close to the cartilage surface and have a tendency to progress towards the surface. After these alterations have reached the

surface they produce an uneven surface (in all probability because of defective connective tissue substrate) (Fig. 23, overleaf).

(2) Mineral salts are precipitated into the foci which appear after decalcification as cavities. Initially, these incrustations are small and isolated, but they later coalesce below the surface of the film and form a continuous layer (Fig. 24, overleaf).

After the incrustation of the focus the cells disappear from the cavity, but the organic fibrous substrate remains (Fig. 25, overleaf). The cavities extend towards the metaplastic alteration of the cartilage and mineral salts are deposited in the altered intercellular mass, whereas the outer skins of the dividing cells and their septa remain preserved for a long period.

Secondary Structural Alterations.—These are characterized by a reparative process of the metaplastically altered and incrustated cartilage. They start at the periphery, at the site of transition of the cartilage into the capsule. Vascular connective tissue intrudes into the cavities and replaces them,

Fig. 20.—Macroscopic view of calcified articular cartilage of distal head of first metatarsus.

Figs 21 to 26.—Histological and histochemical pattern of calcified cartilage. Distal head of first metatarsus.
Figs 21, 22, 23, Masson; Figs 24, 25, 26, Gömöri.

in many cases up to the full height of the cartilage from the surface to the osteoid line of the subchondral bone (Fig. 26, opposite).

The thickness of the cartilage remains unaffected by the primary structural alterations, being reduced only at sites situated above impressions, whereas in the course of the secondary alterations—after resorption of the pathologically-altered cartilage—the joint surface is covered only with a thin layer of the pannus, the total thickness of the cartilage being reduced. A response to the loss of the functional and mechanical capacity of the joint is the compensatory extension of the joint surface resulting in the development of marginal osteophytes.

The histological findings in the joint capsule are

of great interest. As in the cartilage, focal metaplastic alterations occur in the subsynovial layer, but in the opposite direction. Isogenic cartilaginous zones develop in the connective tissue below the acellular membrane and tend to coalesce and form larger masses (Fig. 27).

This picture of metabolic disturbance may also be partly explained by histochemical examination, using the staining methods of McManus, Mowdry, and Steelman and the combined Ritter-Oleson method.

The thicker surface film of the cartilage and the synovial lining consist mainly of dense mucoproteins. These probably represent the metabolic product of active and dividing cells. The surface

Fig. 27.—Histological finding in joint capsule of first metatarsophalangeal joint.

film of the joint cavity and the contiguous area of isogenous nodes where the mucoproteins are localized, are stained homogeneously, being resistant to the precipitation of mineral salts. This is why the film, the cuticles, and the septa of the cartilaginous cells and the fibrous intercellular structures remain intact for a long time and are even visible after decalcification. The primary metabolic disturbance which precedes the structural impairment probably depends on the metabolism of the intercellular ground substance of the articular cartilage. The principal product of this metabolism is chondroitinsulphuric acid, the synthesis of which is presumably impaired, causing the disintegration of the focal area and denudation of the collagen fibres, with the consequent development of conditions favourable to the precipitation of mineral salts into the metaplastic tissue.

Crystallography

Mineral incrustations localized in the surface layers of the calcified cartilage were separated in the form of white microcrystalline samples and examined by radiological diffraction. We tried to establish the crystalline phases constituting the incrustation, and to find whether the ratio of the components constituting the incrustation varies in different samples taken from the same individual, and in samples from different subjects. The results obtained with a sample taken from the largest focus in the first patient are shown in Table VII.

The diffraction crystallograms were made using $\text{Cu}_K\alpha$ radiation ($\text{Cu}_K\alpha = 1.5418 \text{ \AA}$) in the Guinier's chamber ($1^\circ = 4 \text{ mm.}$); the position and intensity of the lines were measured with the Zeiss non-registering microphotometer. The relatively great number of reflexions permitted some conclusions to be drawn regarding the character of the crystalline phases present, particularly the minerals of the apatite type. Comparing the results with the data on some well-known minerals of the apatite group, we found the carbonate apatite to be the principal component of the incrustation; this was also suggested by the development of CO_2 in the reaction

TABLE VII
RESULTS OF ROENTGEN DIFFRACTION EXAMINATION
OF INCRUSTATIONS

Order	Intensity	$d_{hkl}(\text{\AA})$	Order	Intensity	$d_{hkl}(\text{\AA})$
1	0.0626	8.094	44	0.0301	2.308 U
2	0.0434	7.375 U	45	0.0237	2.296
3	0.0859	7.044 U	46	0.0704	2.263 A
4	0.0167	5.528 U	47	0.0114	2.250
5	0.0071	5.372	48	0.0230	2.217 U
6	0.0072	5.246	49	0.0217	2.191
7	0.0308	4.615	50	very weak	2.169
8	0.0183	4.506 U	51	very weak	2.153
9	0.0095	4.178	52	very weak	2.129 A
10	0.0120	4.120 A	52	very weak	2.129
11	0.0329	4.028 U	53	0.1049	2.111
12	0.0100	3.861 A	54	0.0257	2.082
13	very weak	3.823	55	0.0434	2.068 U A
14	0.0195	3.770	56	0.0252	2.046
15	0.0108	3.728	57	0.0315	2.042
16	very weak	3.688	58	0.0580	2.030
17	very weak	3.590 U	59	0.0950	2.012
18	very weak	3.520	60	weak	2.006 A
19	0.0546	3.457 A	61	0.0245	1.986
20	very weak	3.401	62	0.0281	1.970
21	0.1666	3.247 U	63	0.0200	1.946 U
22	0.0229	3.194 A	64	0.0344	1.937
23	0.1557	3.121	65	0.1114	1.908 A
24	0.0894	3.075 A	66	0.0507	1.877 A
25	0.0742	3.045 U	67	0.0253	1.863
26	0.0891	2.982	68	0.0480	1.853
27	0.0096	2.888 U	69	0.0854	1.839 U A
28	0.0140	2.861	70	0.0296	1.821
29	0.0132	2.843	71	0.0446	1.811 A
30	0.0921	2.786 A	72	very weak	1.793
31	0.0184	2.760	73	0.0388	1.769 A
32	very weak	2.777	74	0.0900	1.753 A
33	0.0301	2.712	75	0.0315	1.738
34	0.0805	2.675 U	76	0.0587	1.720
35	0.0516	2.652	77	0.0774	1.703
36	0.0310	2.614 A	78	weak	1.683
37	0.0511	2.588	79	weak	1.662
38	0.0158	2.564	80	very weak	1.646
39	0.0450	2.524 A	81	moderate	1.630 A
40	0.0109	2.434	82	moderate	1.617 U
40	very weak	2.427	83	moderate	1.604
41	0.0248	2.400 U	84	weak	1.588
42	0.0489	2.350	85	moderate	1.571
43	0.0301	2.332			

U = Calcium urea
A = Carbonate apatite
 $d_{hkl}(\text{\AA})$ = Interplanar spacing

of acids with the sample. A series of reflexions occurring in the diffraction crystallogram have not yet been identified, but the incrustations did not exhibit any presence of uric acid or hydrated calcium oxalates (not even in the modified form of calcium carbonate), whereas the presence of calcium urate might be admitted. An important observation was the variation in quantitative results obtained from individual foci in the same subject.