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AN X-RAY-DIFFRACTION STUDY OF CRYSTALLINE CHOLESTEROL IN SOME PATHOLOGICAL DEPOSITS IN MAN

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SUMMARY

Cholesterol crystallized in some different organs in man has been studied. Only one crystal form has been found, which is that of the monohydrate of cholesterol. All examined crystals of this form were twins, and it is very probable that the specimens of the different forms of cholesterol monohydrate described in the literature are not single crystals, as has been assumed, but twins of the same crystal form as the one described here. The monohydrate loses its water readily, giving a crystal form of anhydrous cholesterol. X-ray powder data are given for these two crystal forms for identification purposes.

INTRODUCTION

A great deal of work on cholesterol deposits in man is reported in the literature, but very little attention has been paid to studies of the actual crystal form that separates. The conditions for this type of cholesterol crystallization can only be completely investigated *in vitro* if it is possible to identify the obtained crystal form with that in the deposits.

X-ray-diffraction methods have earlier been used for identification of pathological cholesterol deposits by comparison with some sample, which, however, has not been adequately specified[1,2].

EXPERIMENTAL

A pure sample of cholesterol was prepared[3]. X-ray powder patterns were recorded in a Guinier camera using Cu$K\alpha$ radiation. Unit-cell data were derived from rotation and Weissenberg photographs using Cu$K\alpha$ radiation.

RESULTS AND DISCUSSION

Cholesterol monohydrate in pathological deposits

Cholesterol may crystallize in biliary calculi, in atheromatous plaques and cholesteatomas. X-ray powder patterns of these different cholesterol deposits were recorded almost immediately after they were removed at operation or autopsy. Diffractograms were recorded from 100 biliary calculi, consecutively collected from

patients operated upon in the Surgical Clinic I, Sahlgren's Hospital, Gothenburg. Two atheromatous plaques from different patients and two cholesteatomas were investigated in the same way. Cholesterol occurred in the same crystal form in all these deposits. The interplanar spacings and corresponding relative intensities of this form are listed in Table I. Beautiful crystals may be obtained from some biliary calculi consisting of almost pure cholesterol. Many crystals were examined, all showing

TABLE I

INTERPLANAR SPACINGS AND RELATIVE INTENSITIES FOR CHOLESTEROL MONOHYDRATE FROM DEPOSITS IN MAN

s, strong; m, medium; w, weak.

d (Å)	*Relative intensity*	*d* (Å)	*Relative intensity*	*d* (Å)	*Relative intensity*
5.99	s	3.81	s	2.44	w
5.92	s	3.69	w	2.37	w
5.68	m	3.53	m	2.30	w
5.40	m	3.39	w	2.19	w
5.23	w	3.29	w	2.13	w
5.05	m	3.07	w	2.09	w
4.85	w	3.02	w	2.01	w
4.76	m	2.91	m	1.98	w
4.58	m	2.83	w	1.95	w
4.40	w	2.74	w	1.90	w
4.28	w	2.66	w	1.85	w
3.90	m	2.59	w		

systematic absences in the reciprocal lattice, which did not correspond to any possible space group. This suggested that twins might have been examined instead of single crystals. An analysis of the symmetry and absences in the reciprocal lattice established that the crystals were twins. The following X-ray data were derived:

Crystal system: triclinic. $a = 6.25 \pm 0.03$ Å; $b = 68.0 \pm 0.4$ Å; $c = 16.04 \pm 0.08$ Å; $\alpha = 90° \pm 0.2°$; $\beta = 129.5° \pm 0.6°$; $\gamma = 90° \pm 0.2°$.

The choice of unit cell corresponds to a centered cell with space group $C1$. The calculated density of 1.03 ± 0.01 g·cm^{-3} corresponding to 8 molecules of cholesterol and 8 molecules of water in the unit cell is in agreement with the observed density of 1.04 g/cm^3 (determined by flotation in KBr solution). The water content was also determined in another way. A relatively large number of crystals collected from a biliary calculus were heated above the melting point. The reduction in weight corresponded to a loss of 1 molecule of water per molecule of cholesterol.

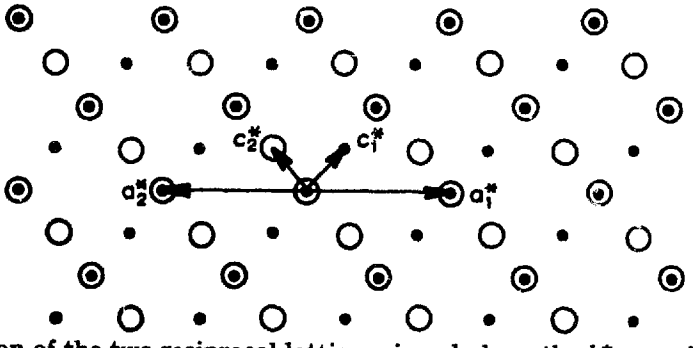

Fig. 1. Superposition of the two reciprocal lattices viewed along the b^*-axes. The a^*- and c^*-axes are shown for the two individuals having their b^*-axes in opposite directions. Rows with constant (h,l) are denoted by open circles in one lattice and filled circles in the other.

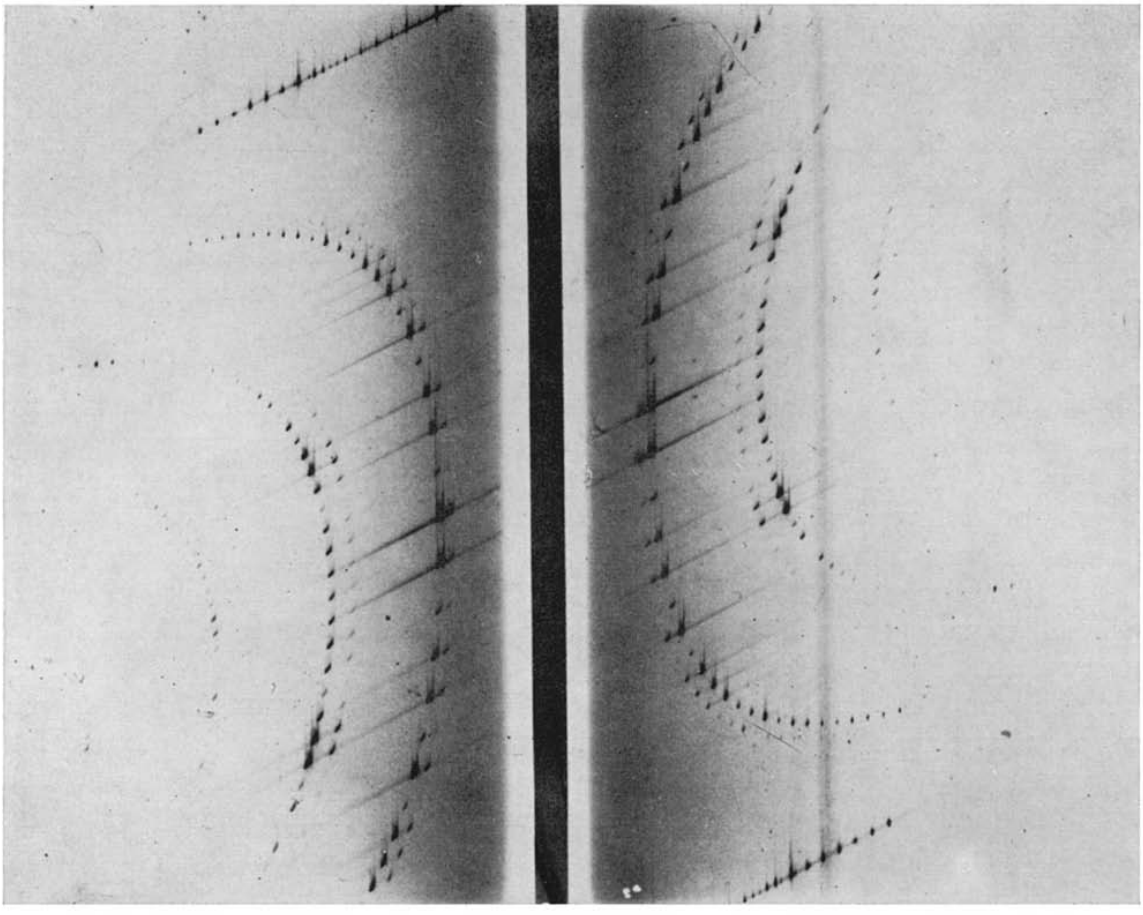

Fig. 2. Weissenberg photograph of the (*hk*O)-zone showing the Laue symmetry corresponding to plane group *pmm* due to the twinning.

The crystals twin on the (010)-plane and the orientation of the individuals in relation to each other is illustrated in Fig. 1, showing the reciprocal lattices. A Weissenberg photograph of the (*hk*O)-zone is shown in Fig. 2. The twinning seems to occur very frequently, as the symmetry of all examined crystals showed that individuals of both orientations were present to about the same extent. According to a rule introduced by MALARD (thoroughly discussed by BUERGER[4]), the occurrence of twins is very common when the dimensional symmetry is higher than the actual point-group symmetry. This condition is fulfilled here, as a unit cell with monoclinic dimensions can be chosen. There is in fact still higher dimensional symmetry as a non-primitive unit cell with orthorhombic dimensions can be chosen, which is obtained from the unit cell given above by the transformation matrix:

$$\begin{bmatrix} 4 & 0 & 1 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

This is the condition for the coincidence of the lattices shown in Fig. 1. This twinning gives some information on the crystal structure. The interaction over the contact surface between the individuals of different orientation does probably not differ much from the interaction over the corresponding plane within the individual.

TAZAKI *et al.*[5] give the following X-ray data for cholesterol monohydrate from biliary calculi: $a = 12.3$ Å; $b = 33.3$ Å; $c = 12.3$ Å; $\beta = 100°32'$; $Z = 8$ molecules per

cell. These data would agree with ours, if we neglected the twin formation. Their conclusion that the crystals are monoclinic may be based on the apparent (false) symmetry due to the twinning.

BERNAL AND CROWFOOT[6] have given unit-cell data for cholesterol monohydrate crystallized from an alcohol–water solution. A comparison with their oscillation photographs kindly provided by Professor CROWFOOT HODGKIN shows that their crystal form must be the same or very nearly the same as that investigated by us. The only difference, except for our twinning interpretation of the lattice, is that their *c*-axis is twice as long as ours.

KLÖTZER[7] gives data for three crystal forms of cholesterol monohydrate, two of which have the same dimensions, but different intensity distribution. The intensity data of these two indicate that they both are twins of the same type we have described here. The difference between the two seems to be that individuals of both orientations are present to the same extent in his form II, while individuals of one orientation seem to dominate in form I. The crystal form III is incompletely described. Another comment on KLÖTZER's work should be made. He suggests centrosymmetric space groups for the crystal forms, which is impossible, as the substance is an optical antipod.

Cholesterol is almost insoluble in water. EKVALL AND MANDELL[8] have reported an approximate value of 0.06–0.7 mg of cholesterol in 1000 g of water. It is therefore remarkable that crystals of anhydrous cholesterol in the presence of water undergo a transition into the monohydrate described here. This observation made by KLÖTZER has been confirmed by us. The crystallizations were carried out using the earlier described pure sample of cholesterol.

On an anhydrous crystal form of cholesterol

When cholesterol monohydrate crystallized in man is kept in air for some time, it loses its water. Beside the formation of an anhydrous crystal form, the X-ray powder pattern indicated that more or less of the cholesterol was transformed into an amorphous state when the water left the lattice. One biliary calculus kept in air for a long time, which consisted of about equal amounts of cholesterol and calcium bilirubinate (shown by infrared absorption after separation) was completely amorphous. The time taken until all the water has left the monohydrate, when kept in air, varies with the crystal size. Crystals of the hydrate are stable for up to 5 years under certain conditions, while samples consisting of very small crystallites have been found, where a large part is transformed after a few days[9]. Only one crystal form of anhydrous cholesterol has been found in the different deposits after the transformation from the hydrate. The same crystal form was present in the examined sample of pure cholesterol described earlier, and a comparison with X-ray single crystal photographs provided by Professor CROWFOOT HODGKIN shows that it is identical with that of anhydrous cholesterol described by BERNAL AND CROWFOOT[6]. The interplanar spacings together with the corresponding relative intensities of this anhydrous crystal form are given in Table II. Identification of cholesterol crystals in man using X-ray-diffraction methods should be made by comparison with the data of the two crystal forms described (Tables I and II) and not with the diffraction pattern of some sample of unknown crystalline character. The observation that cholesterol can exist in an amorphous state should also be kept in mind.

The X-ray data for cholesterol in biliary calculi given by EPPRECHT *et al.*[1] seem to correspond to this anhydrous crystal form as also do the data for cholesterol in brain fluid given by PARSONS AND BARTONE[2].

TABLE II

INTERPLANAR SPACINGS AND RELATIVE INTENSITIES FOR ANHYDROUS CHOLESTEROL FORMED FROM DEPOSITS IN MAN

s, strong; m, medium; w, weak.

d (Å)	*Relative intensity*	*d* (Å)	*Relative intensity*	*d* (Å)	*Relative intensity*
13.94	s	4.65	m	3.00	w
13.64	w	4.50	m	2.90	w
12.53	w	4.39	w	2.84	w
11.37	m	4.27	w	2.77	w
10.24	w	4.12	m	2.71	m
9.73	w	4.07	m	2.65	w
9.16	m	3.98	w	2.59	m
8.12	m	3.94	w	2.51	w
7.82	m	3.89	w	2.47	w
7.48	m	3.75	m	2.41	w
7.01	m	3.72	m	2.36	w
6.72	s	3.55	m	2.31	w
6.21	s	3.49	m	2.24	w
5.72	s	3.44	m	2.10	w
5.57	w	3.41	w	2.07	w
5.46	m	3.32	w	2.05	w
5.17	s	3.28	w	2.01	w
5.05	s	3.10	w	1.98	w
4.83	s	3.07	w		

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