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A note on Burbank's paper on 'Intrinsic and systematic multiple diffraction'.

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Burbank (1965) has recently discussed under the title *Intrinsic and Systematic Multiple Diffraction* the conditions giving rise to multiple diffraction for the single-crystal orienter and for the precession camera. He concludes that there are important differences between the two techniques. For the single-crystal orienter, multiple diffraction will always occur if the crystal is mounted with a symmetry axis parallel to, or a symmetry plane normal to, the rotation axis (φ axis). For the precession camera, the conditions for multiple diffraction can be created or avoided at will by the choice of $\bar{\mu}$, regardless of crystal orientation. Burbank suggests that the zero-level precession technique is the only method in common use which offers the possibility of direct experimental observation of the magnitude of multiple diffraction effects.

The purpose of this note is to point out that there are no such differences between the two techniques if the orienter is used as a four-circle instrument with independent motion of the ω and 2θ axes. Although the original single-crystal orienter described by Furnas (1957) was a three-circle diffractometer, the majority of instruments commercially available today have four independent axes: the direct experimental observation of multiple diffraction with these four-circle instruments has been fully described by Willis (1962) and by Santoro & Zocchi (1964). The situation discussed by Burbank corresponds to the use of the orienter as a three-circle instrument, with the angle ε between the χ plane and the normal to the *hkl* plane under observation as zero. This $\varepsilon=0$ (or 'symmetrical *A*') setting is formally equivalent to the equi-inclination Weissenberg setting (Phillips, 1964) and gives rise, therefore, as Burbank shows in another way, to multiple diffraction under the same conditions as for the equi-inclination setting (Yakel & Fankuchen, 1962).

The limitation of Burbank's analysis to the $\varepsilon=0$ setting is apparent in his answer to the question: Is there a reciprocal lattice plane coincident with the vertical circle of reflexion? Burbank states that, if the crystal is oriented with a reciprocal lattice vector along the rotation axis (the φ axis of the single-crystal orienter), there will be a reciprocal lattice plane coincident with the vertical circle of reflexion. This is true only if the φ axis is in the plane of the vertical circle of reflexion. The φ axis is mechanically constrained to lie in the vertical χ plane, and the vertical circle of reflexion and the vertical χ plane are in coincidence for $\varepsilon=0$ but not in the general case, $\varepsilon\neq0$.

The essential *similarity* of the precession and single-crystal orienter techniques as regards observing multiple diffraction effects is strikingly illustrated by reference to the last section of Burbank's paper. The procedure described there for creating or avoiding at will multiple diffraction with the precession technique is exactly paralleled for the orienter technique. If a symmetry axis of the crystal is parallel to the φ axis of the orienter, the *hkl* intensity is first recorded with a zero off-set angle ε, corresponding to the condition for intrinsic multiple diffraction. ε is then given a small increment, positive or negative, sufficient to destroy the multiple diffraction condition, and the intensity remeasured. Any difference in the two intensities is due to multiple diffraction.

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Disorder in erythrocyte catalase crystals.

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Introduction

Catalase is an enzyme which decomposes hydrogen peroxide into oxygen and water. It consists of protein, combined with four haem groups, and has a molecular weight of 238,000, as calculated from the sedimentation constant and iron content (Lamberg & Legge, 1949).

The catalase crystals

Horse erythrocyte catalase was crystallized according to Bonnichsen (1947). Crystals grew in two to three weeks in

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2·3 *M* ammonium sulphate solution. At first sight the crystals appeared to be rectangular prisms, but close examination showed the {110} faces to be curved. Crystals were elongated along [100] and some of them seemed to be terminated by {100} faces, but most crystals had oblique terminal faces. In polarized light the crystals are weakly dichroic with the direction of strong absorption parallel to [100]. They show marked birefringence and, as far as can be judged, straight extinction.

X-ray diffraction pictures were taken of crystals mounted in thin-walled glass capillaries, in equilibrium with their mother liquor. A Buerger precession camera was used with filtered Cu $K\alpha$ radiation from a Broad rotating anode tube. On 9° precession photographs, corresponding to a sphere of reflexion of 4·9 Å, spots were observed to the limit of the photograph. The X-ray pictures have orthorhombic symmetry of space group $P22_12_1$ ($0k0$ and $00l$ absent for k or $l=2n+1$) with $a=180{\cdot}0$, $b=116{\cdot}7$, $c=114{\cdot}1$ Å, giving a unit-cell volume of 240×10^4 Å^3. If the space taken up by one catalase molecule is the same, in proportion, as that taken up by one haemoglobin molecule ($17{\cdot}5\times10^4$ Å^3) in the lattice of horse haemoglobin (Perutz, 1942) then it would occupy $62{\cdot}0\times10^4$ Å^3. Hence, there must be four molecules per unit cell.

The diffraction pattern contains some unusual features. A still photograph with the X-ray beam along [100] fails to show the usual sets of concentric rings, but looks much like a precession photograph. On precession photographs taken along other zone axes, some of the reciprocal lattice lines contain sharp spots while others contain diffuse streaks drawn out along **a***. Moreover, the sharp spots exhibit absences of a peculiar kind. These phenomena are summarized in Table 1.

Table 1. *Description of the systematic absences and diffuse streaks*

S and *D* signify sharp and diffuse respectively

Reciprocal lattice plane	Absences	Spots	
$h0l$ (00l absent for $l=2n+1$ on all layer lines both sharp and diffuse)	$h=2n+1$	$l=0$ to 4	*S*
		$l=5$	*D*
	$h=2n$	$l=6$ to 14	*S*
		$l=15$	*D*
	$h=2n+1$	$l=16$ to 24	*S*
$hk0$ (0k0 absent for $k=2n+1$ on all layer lines both sharp and diffuse)	$h=2n+1$	$k=0$ to 6	*S*
		$k=7$ and 8	*D*
	$h=2n$	$k=9$ to 18	*S*
		$k=19$ to 21	*D*
	$h=2n+1$	$k=23$?	*S*
$h1l$	$h=2n+1$	$l=0$ to 6	*S*
		$l=7$	*D*
	$h=2n$	$l=8$ to ?	*S*
$hk1$	$h=2n+1$	$k=0$ to 4	*S*
		$k=5$ to 7	*D*
	$h=2n$	$k=7$ to 13	*S*
		$k=14$	*D*
	$h=2n+1$	$k=15$ to ?	*S*
hkk	$h=2n+1$	$k=0$ to 3	*S*
		$k=4$	*D*
	$h=2n$	$k=5$	*S*
		$k=6$ and 7	*D*
	$h=2n+1$	$k=8$ to 13	*S*
		$k=14$	*D*
	$h=2n$	$k=15$ and 16	*S*
		$k=17$	*D*
$h\,2k\,k$	$h=2n+1$	$k=0$ to 5	*S*
		$k=6$ to ?	*D*
$h\,k\,2k$	$h=2n+1$	$k=0$ to ?	*S*

The nature of the disorder

The strange absences and the streaks suggest that the crystals are disordered, and may be thought of as consisting of ordered layers perpendicular to [100] stacked in a random fashion. The $h00$ reflexions are sharp to the limit of the photograph, indicating that the perpendicular distances between successive layers are constant throughout the crystal. The streaks and absences might be explained by successive layers failing to be in register as far as their y and z coordinates of the molecule are concerned. A situation of this kind was encountered in imidazole methaemoglobin (Howells & Perutz, 1954; Bragg, 1954; Cochran & Howells, 1954) and in pig lactic dehydrogenase (Pickles, Jeffery & Rossmann, 1964). In the former a knowledge of a related ordered diffraction pattern led to the understanding of the packing arrangement in the disordered lattice. In the absence of such a clue for catalase we shall propose here only a system of packing which is consistent with the main features of the diffraction patterns.

We shall assume (i) that the space group of each layer is $P22_12_1$ with two molecules per unit cell, each containing a twofold axis and (ii) that each successive layer is displaced by $\pm\varepsilon_b$ and $\pm\varepsilon_c$ parallel to **b** and **c** with respect to the previous layer. Thus every alternate layer can have its origin on the twofold axis of the original layer, but the origin of successive layers will have a statistical distribution about the central twofold axis. This twofold axis is maintained statistically throughout the crystal and requires two layers for one statistical repeat. The statistical space group is, therefore, $P22_12_1$ with four molecules in the unit cell, as was observed.

The contribution to a structure factor due to a unit point atom at (x,y,z) can now be written as

$$S(h,k,l)=\exp\{2\pi i[hx+ky+lz]+\exp\{2\pi i[h(x+\tfrac{1}{2})+k(y\pm\varepsilon_b)+l(z\pm\varepsilon_c)]\}\,.$$

The coordinates in the second term may have any one of four values according to the combination of $\pm\varepsilon_b$ with $\pm\varepsilon_c$. Any one of these combinations is equally probable, hence we should rewrite the second term as a sum of four terms each with only a quarter weight. In the layer at $x+1$ we must consider all possible combinations with each of the four positions at $x+\frac{1}{2}$, and so on for each successive layer. It is easy to show, by extending the one-dimensional arguments of Cochran & Howells (1954) into two dimensions, that the probability of finding the equivalent atom in the nth layer at $(n-2q)\varepsilon_b$ and $(n-2r)\varepsilon_c$ is given by $\{C^n_{n-q}\,.\,C^n_{n-r}\,.\,(\frac{1}{2})^{2n}\}$, when q and r are integers less than or equal to n. Hence the contribution to the structure factor of every equivalent atom in all $N/2$ layers of cells is given by

$$S(h,k,l)=\sum_{n=0}^{N}\sum_{q=0}^{n}\sum_{r=0}^{n}C^n_{n-q}C^n_{n-r}(\tfrac{1}{2})^{2n}\times \exp\left\{2\pi i\left[h\left(x+\frac{n}{2}\right)+k\{y+(n-2q)\varepsilon_b\}+l\{z+(n-2r)\varepsilon_c\}\right]\right\},$$

that is,

$$S(h,k,l)=G(h,k,l)\exp\{2\pi i(hx+ky+lz)\}$$

where

$$G(h,k,l)=\sum_{n=0}^{N}\sum_{q=0}^{n}\sum_{r=0}^{n}C^n_{n-q}C^n_{n-r}(\tfrac{1}{2})^{2n}\times \exp\left\{2\pi i\left[\frac{hn}{2}+k(n-2q)\varepsilon_b+l(n-2r)\varepsilon_c\right]\right\}. \quad (1)$$

G is a factor common to every atom in the cell. Hence the total structure factor for plane (hkl) would be

$$F(h,k,l)=G(h,k,l)\ \Sigma f_i \exp\{2\pi i(hx_i+ky_i+lz_i)\}\ ,$$

the summation being taken over all atoms in one layer, that is, half the atoms in the unit cell. The ith atom has a scattering factor of f_i.

We shall now consider the nature of the multiplier G. Summing (1) over r and q independently, we have, by the use of the binomial theorem,

$$G(h,k,l)=\sum_{n=0}^{N}(\tfrac{1}{2})^{2n}\times$$

$$\exp\left\{2\pi in\left(\frac{h}{2}+k\varepsilon_b+l\varepsilon_c\right)\{1+\exp(4\pi ik\varepsilon_b)\}^n\{1+\exp(4\pi il\varepsilon_c)\}^n\right\}$$

$$=\sum_{n=0}^{N}(\cos 2\pi k\varepsilon_b \cos 2\pi l\varepsilon_c)^n \exp(\pi inh)\ .$$

This is the sum of a geometric progression, and may be written as

$$G(h,k,l)=\frac{1-K^N\exp(\pi iNh)}{1-K\exp(\pi ih)} \qquad (2)$$

where

$$K=\cos 2\pi k\varepsilon_b \cos 2\pi l\varepsilon_c\ . \qquad (3)$$

Inspection shows that K is confined to the limits

$$-1\le K\le +1.$$

When $K=+1$

$$G(h,k,l)=\frac{\exp(-\pi iNh/2)}{\exp(-\pi ih/2)}\cdot\frac{\sin(\pi Nh/2)}{\sin(\pi h/2)}\ ,\ \text{thus } |G|=N$$

for even values of h.

When $K=0$

$$G(h,k,l)=1\ .$$

When $K=-1$

$$G(h,k,l)=\frac{\exp(-\pi iNh/2)}{\exp(-\pi ih/2)}\cdot\frac{\cos(\pi Nh/2)}{\cos(\pi h/2)}\ ,\ \text{thus } |G|=N$$

for odd values of h.

The function $\sin N\theta/\sin\theta$ is well known in diffraction theory. Since N is a very large number, when $K=1$, G is close to zero unless h is an even integer; while when $K=-1$, G is non-zero only when h is an odd integer. However, clearly when $K=0$, G is unity for all values of h, integral or non-integral. While it is more difficult to examine (2) for intermediate values of K, the above results suffice to interpret the disorder effects we have observed in the catalase crystals.

For the $h0l$ reflexions, $K=\cos 2\pi l\varepsilon_c$. When l is small, $K=+1$; thus reflexions with h odd are systematically absent. Similarly when l is larger, so that $\cos 2\pi l\varepsilon_c\rightarrow -1$, reflexions with h even are systematically absent. When l is intermediate, K is close to zero causing weak diffuse streaks parallel to the a^* axis. It then follows from Table 1 that $2\pi\ .\ 5\ .\ \varepsilon_c=\pi/2$, *i.e.* $\varepsilon_c=1/20$. A similar interpretation applies to the diffuse reflexions and absences for the $(hk0)$ zone; here $2\pi\ .\ 7\ .\ \varepsilon_b=\pi/2$, and therefore $\varepsilon_b=1/28$. We have now determined the two unknown parameters ε_b and ε_c, and it becomes necessary to check whether their values are consistent with the other observations given in Table 1.

Let us first take the hkk reflexions. For these, $K=\cos \pi k/10\ .\ \cos \pi k/14$. We then see that K approaches $+1$, that is, h can only be even for $K<5$, $7<k<10$, $14<k<20$, *etc.*; while K approaches -1, that is h must be odd, for $5<k<7$, $10<k<14$, *etc.* Table 1 shows this to be the general appearance of the hkk reflexions, although the theoretical expected limits are not in complete agreement with the experimental results. The $h1l$ and $hk1$ reflexions should follow the same general pattern as $(h0l)$ and $(hk0)$ zones, although the diffuse bands might be expected to be broader in view of the fact that K cannot attain its maximum value of ± 1. Again observations are in general agreement, although the position of the diffuse streaks are not in exactly the predicted positions. Our two assumptions are thus able to predict the approximate, but not exact, position of diffuse streaks on the photographs and the nature of the unusual systematic absence.

We are grateful to Dr D. M. Blow for first pointing out the similarity of the diffraction pattern described in this paper to that observed by Dr M. F. Perutz in his study of imidazole methaemoglobin. We are also grateful to Dr M. F. Perutz for stimulating discussions as to the possible nature of the disorder. Finally we greatly appreciated not only the help Dr R. E. Marsh has given us in making the manuscript more readable, but particularly some astute comments which have put the interpretation of the disorder effect on a sounder basis.

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The crystal structure of tetraethylammineplatinum(II) dibromotetraethylammineplatinum(IV) tetrabromide.

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The crystal structure determination of Wolffram's red salt (Craven & Hall, 1961) confirmed earlier suggestions (Reihlen & Flohr, 1934) that it is correctly formulated $Pt^{II}(etn)_4\ .\ Pt^{IV}(etn)_4Cl_2\ .\ Cl_4\ .\ 4H_2O$, where etn represents an ethylamine ligand. The octahedral Pt(IV) complex ion and the square planar Pt(II) ion alternate in chains of the form $Cl\text{–}Pt^{IV}\text{–}Cl$ ------ Pt^{II} ------ $Cl\text{–}Pt^{IV}\text{–}Cl$ ------ Pt^{II} ------ characterized by weak charge transfer bonds from the halogen to the Pt(II) ion. The details of the molecular

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