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The Location of the Mercury Atoms in Crystals of Ribonuclease-Parachloromercuribenzoate

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The positions of the mercury atoms in ribonuclease–parachloromercuribenzoate (Ri–*pcmb*) have been determined from a study of Patterson difference projections on (010) and sharpened Patterson sections. This has permitted the calculation of the structure factors of the mercury atoms, leading to the synthesis of Fourier series for the metal-free crystal.

1. Introduction

Bernal, Carlisle and Rosemeyer (1959) showed that when crystals of ribonuclease–parachloromercuribenzoate containing chemically 1 atom of Hg per protein molecule (Dix's crystals) were soaked in 60% aqueous alcohol solutions containing 0·5% Na–*pcmb*, the symmetry of the crystals was unaffected. This diffusion process gave approximately 2 Hg atoms per protein molecule, as chemically determined by Rosemeyer. There was some evidence, from a comparison of the Patterson sections at $v=\frac{1}{2}$ for the soaked Dix's crystal and the metal-free one, that the molecules of one had moved slightly with respect to the other, showing that the crystals were not strictly isomorphous. We therefore decided to work on the soaked Dix's crystals because of the increased intensity changes in the X-ray reflections with respect to those from the metal-free one. In what follows we shall show that in the soaked Dix crystals the Hg atoms occupy two sites per protein molecule and that in the Dix crystal itself these sites are statistically filled. Since the work on the former was carried out before that on the latter, it is convenient to retain this order in the present account.

2. Location of the Hg atoms in the soaked Dix crystal

Fig. 1(*a*) shows a Patterson difference map on (010) (Bernal, Carlisle and Rosemeyer, 1959) using $(\Delta|F(h0l)|)^2$ between the soaked-Dix and normal crystal, incorporating reflections to spacings of 2Å. This map was not interpretable. We next followed the procedure of Green, Ingram, and Perutz (1954) using a smoothing function of the type $\exp[-B\sin^3\theta]$, to limit the reflections to spacings of 6Å; the resulting map is shown in Fig. 1(*b*). This map can be interpreted in terms of the vector relation $(A+B)^2=A^2+2AB+B^2$ relating 2 pairs of atoms through a centre, which is shown graphically by the parallelogram inscribed on it. The peaks $2AB$, showing vectors between atoms not related by the centre, must fall half way between the peaks A^2 and B^2. The additional peak seen on this map cannot be included in this scheme and is not therefore associated with a Hg–Hg vector but is more likely to be due to a relative movement of the molecules between the two crystals.

Fig. 1(*b*) can only give very approximate x and z coordinates for the Hg atoms occupying sites A and B. Furthermore it is necessary to know the relative distance Δy of site A with respect to B along the b axis of the crystal (space group $P2_1$) and for this purpose we were strongly advised by Professor D. M. Hodgkin, F.R.S., to look at the individual fully-sharpened three-dimensional Pattersons of the two crystals. These calculations were very kindly carried out for us by Professor K. N. Trueblood on the Electronic Computer S.W.A.C. of the University of California.

The coefficients of the two Patterson series, each containing about 7,000 terms and scaled approximately with respect to each other, were sharpened by dividing each term by an average f^2 (averaged according to atomic percentages), multiplying by $\exp[3B\sin^2\theta/\lambda^2]$, followed by modification with a function

$$\tfrac{1}{2}\sin^4\theta\,.\exp[-50\sin^2\theta/\lambda^2]$$

which reduces the effects of the low order terms and enhances those in the region of 3*A*. The functions were calculated at 1/60 intervals of the cell edges, for the $\frac{1}{4}$ unit cell, on sections perpendicular to b, which correspond to intervals of approximately 0·5, 0·65, and 0·9 Å along a, b and c respectively. The origin peak was also removed. For economy of space and for purposes of the argument only sections $v=6/60$, 24/60, and 30/60 for the two crystals have been reproduced (Figs. 2 and 3).

In the space groups $P2_1$, the equivalent points are (x, y, z) and $(\bar{x}, y+\frac{1}{2}, \bar{z})$. For 2 Hg atoms per asymmetric unit, the equivalent points are:

Sites A, x_1, y_1, z_1 and $\bar{x}_1, y_1+\frac{1}{2}, \bar{z}_1$
Sites B, x_2, y_2, z_2 and $\bar{x}_2, y_2+\frac{1}{2}, \bar{z}_2$

Excluding the vectors at the origin, the 12 remaining vectors between these sites are of two types:

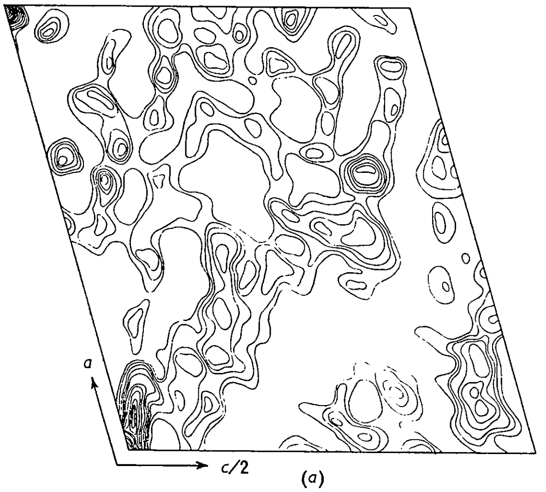

Fig. 1.

(1) Those lying on $v=\frac{1}{2}$ which may be called the A^2 and B^2 type, have components

$$2x_1, \tfrac{1}{2}, 2z_1 \quad \text{and} \quad \overline{2x_1}, \tfrac{1}{2}, \overline{2z_1}$$
$$2x_2, \tfrac{1}{2}, 2z_2 \quad \text{and} \quad \overline{2x_2}, \tfrac{1}{2}, \overline{2z_2}\,.$$

(2) Those of the AB type situated on the planes $v=\pm(y_1-y_2)$ and $\pm(y_1-y_2)-\frac{1}{2}$ having components:

$$(x_1-x_2),\ \pm(y_1-y_2),\ (z_1-z_2);\ \overline{(x_1-x_2)},\ \pm(y_1-y_2),\ \overline{(z_1-z_2)}$$
$$\text{and}\ (x_1+x_2),\ \pm\{(y_1-y_2)-\tfrac{1}{2}\},\ (z_1+z_2)$$
$$\text{and}\ \overline{(x_1+x_2)},\ \pm\{(y_1-y_2)-\tfrac{1}{2}\},\ \overline{(z_1+z_2)}\,.$$

The AB type of vectors, owing to Patterson symmetry, have mirror image relationships to each other about $v=0$ and $v=\frac{1}{2}$; hence the appearance of vectors of the type $2AB$ when seen in projection on (010), see Fig. 1(*b*).

This means that the comparisons of the Patterson sections of the crystals, with and without the metal atoms, *must be done by examining pairs of planes*, e.g. $v=1/60$ and 29/60, 2/60 and 28/60, and so on to find sufficiently heavy peaks of the AB type, *of the same height*, whose vector relationships are consistent with the appearence of the A^2 and B^2 peaks on $v=\frac{1}{2}$. It is advisable to begin the interpretation by looking for the former vectors rather than the latter because of the large number of peaks between related atoms

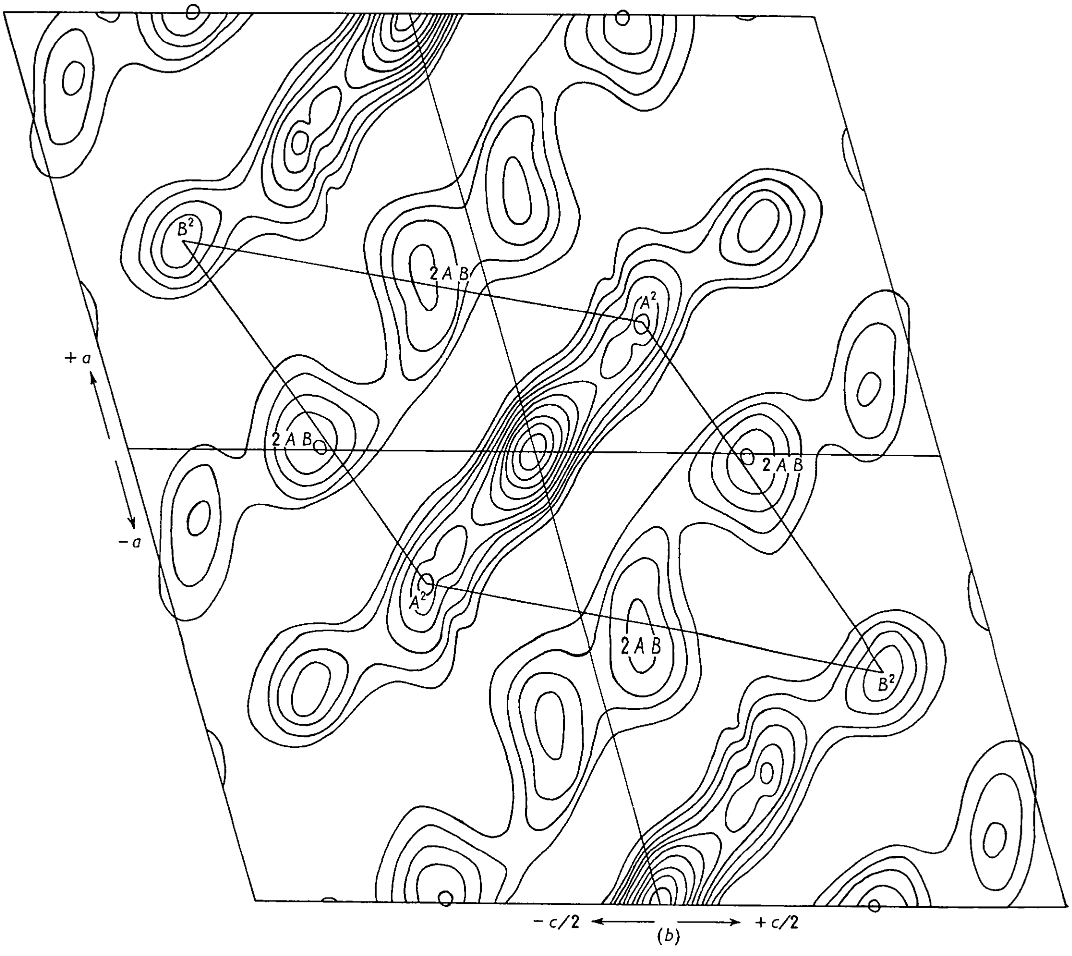

Fig. 1.

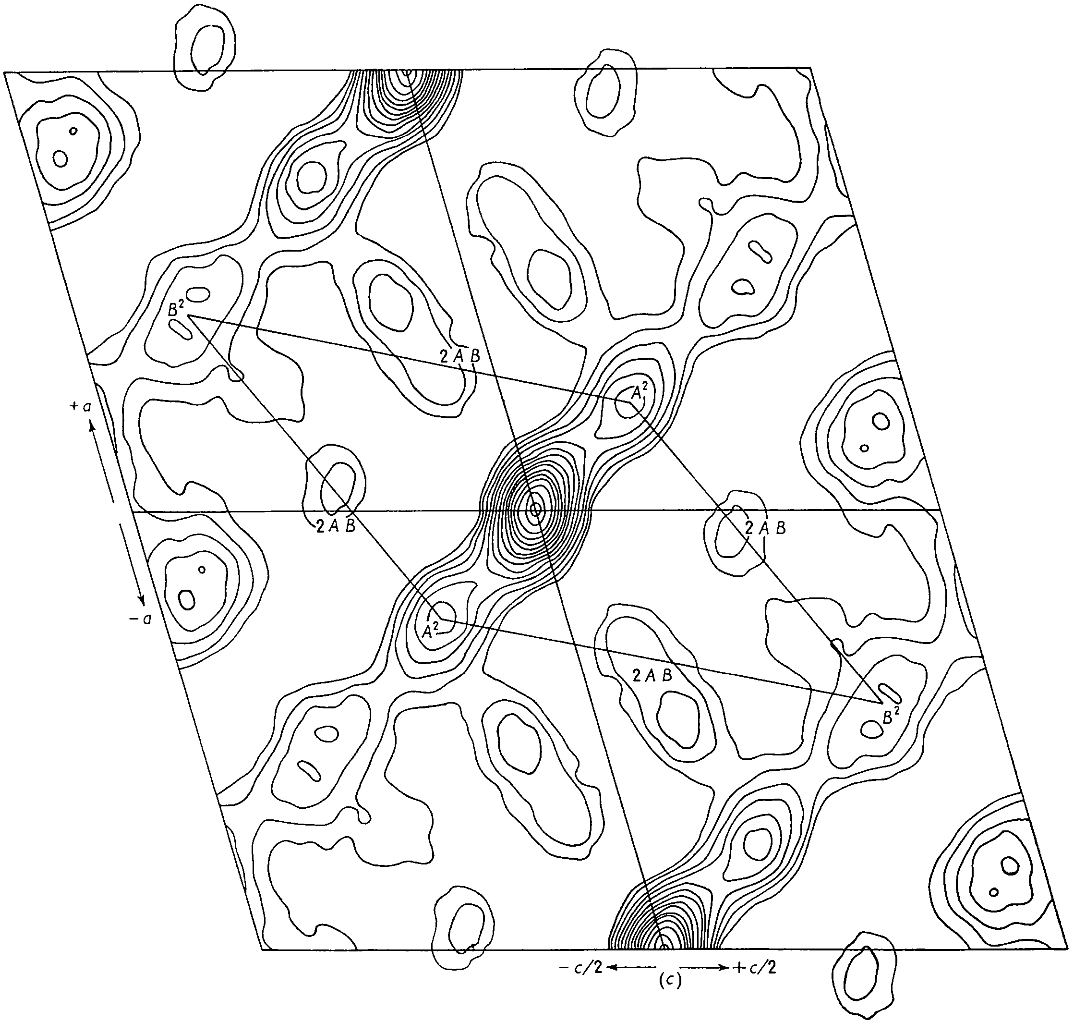

Fig. 1. Patterson difference maps projected on (010) for: (*a*) Soaked Dix crystal–normal crystal, using reflections with $d > 2$ Å. (*b*) Soaked Dix crystal–normal crystal, using smoothing function to terminate series at $d = 6$ Å. (*c*) Dix crystal–normal crystal, using smoothing function to terminate series at $d = 6$ Å.

(there being about 1000 in each molecule) which would fall on $v = \frac{1}{2}$ and so make interpretation difficult. Added to this there must also be a large number of non-Harker peaks falling on this plane, which would tend to reduce the effects of the A^2, B^2 peaks of the Hg–Hg vectors.

If one accepts the interpretation of the Patterson difference map, Fig. 1(*b*), as reasonably correct, then it is only necessary to look in the $2AB$ regions of the two 3-dimensional Pattersons along b on pairs of planes equidistant from $v = 0$ and $\frac{1}{2}$ to find the AB vectors. *This was not done.* Instead, we took about half the magnitude of the average peak height for the Ri-*pcmb* Patterson function as a lower limit and any peak below this was rejected. Then we drew up a list of all peaks above this height which appeared on the Ri–*pcmb* Patterson sections but not on the corresponding Ri-sections. Fig. 4 shows a plot of these peak positions on the Patterson difference map; the number beside each position corresponds to the section v on which it was located. For any given pair of planes, e.g., $v = 1/60$, $29/60$, we took all combinations of these AB vectors, determined the x and z co-ordinates of each A and B position and attempted to locate the A^2, B^2 vectors on $v = \frac{1}{2}$, by comparison of the corresponding Patterson maps. (Figs. 3 (*a*) and (*b*)). Out of the many pairs of AB vectors that arose, there was only one that yielded vectors A^2 and B^2 that fell on positive areas on $v = \frac{1}{2}$ for the Ri–*pcmb* Patterson. In every other case either A^2 or B^2 (and sometimes both vectors) fell in negative areas on this section, thus providing sufficiently stringent conditions for their rejection.

The arrows on Figs. 2(*a*) and (*b*) indicate the AB peaks on the Ri–*pcmb* Patterson sections $v = 6/60$ and $24/60$ that were finally selected, and Figs. 2(*c*) and (*d*) show the corresponding sections for the ribonuclease crystal. The two indicated peaks on Figs. 2(*a*) and (*b*)

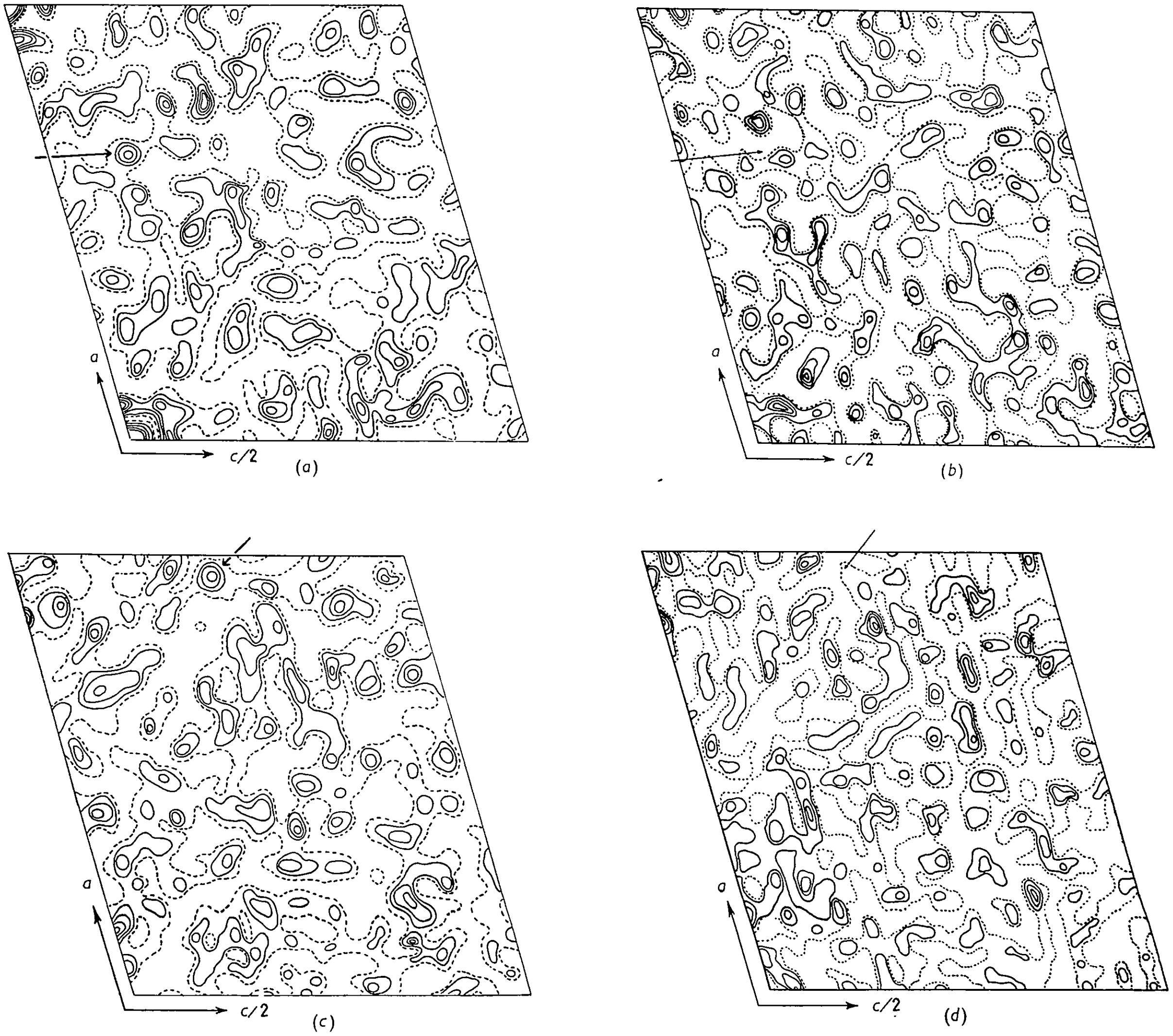

Fig. 2. Fully-sharpened Patterson sections: (*a*) Soaked Dix crystal, $v=6/60$. (*b*) Normal crystal, $v=6/60$. (*c*) Soaked Dix crystal, $v=24/60$. (*d*) Normal crystal, $v=24/60$. The arrows indicated the positions of the *AB* peaks of the mercury atoms in the soaked-Dix crystal.

are of the same height (and their positions have also been indicated by crosses on Fig. 4). Though they are by no means the highest in the functions, they lie well above the lower limit set in our investigation.

Figs. 3(*a*) and (*b*) show the section $v=\frac{1}{2}$ for the soaked Ri-*pcmb* and ribonuclease crystals respectively. The comparison of these two sections by themselves shows how difficult it would have been to identify the A^2, B^2 vectors which are shown by the arrows on Fig. 3(*a*). The interesting and important finding is that the sharpening of the two Patterson functions has revealed the effect of the Hg–Hg vectors on this section, and for comparison we reproduce the corresponding unsharpened sections of the two crystals, Figs. 3(*c*) and (*d*). It is easily seen what sharpening has accomplished.

The *AB* vector on $v=6/30$ and the other on 24/30 for the Ri–*pcmb* crystal give more reliable *x* and *z* co-ordinates for the Hg atoms than those that would have been obtained from Fig. 1(*b*). Using these vector relationships, the two Hg atoms in the asymmetric unit have the following co-ordinates:

	x	y	z
A	0·153	0·049	0·075
B	0·806	0·951	0·175

The *y* coordinates of the Hg atoms are only relative to one another and $\Delta y=0{\cdot}098\simeq 6/60=1/10$. It is of interest in this connection to compare the $0k0$ reflections of the Ri– and Ri-*pcmb* crystals. This is shown in Fig. 5. The biggest changes are at 040, 0,10,0, and 0,12,0. The reversal of the distribution of intensity in

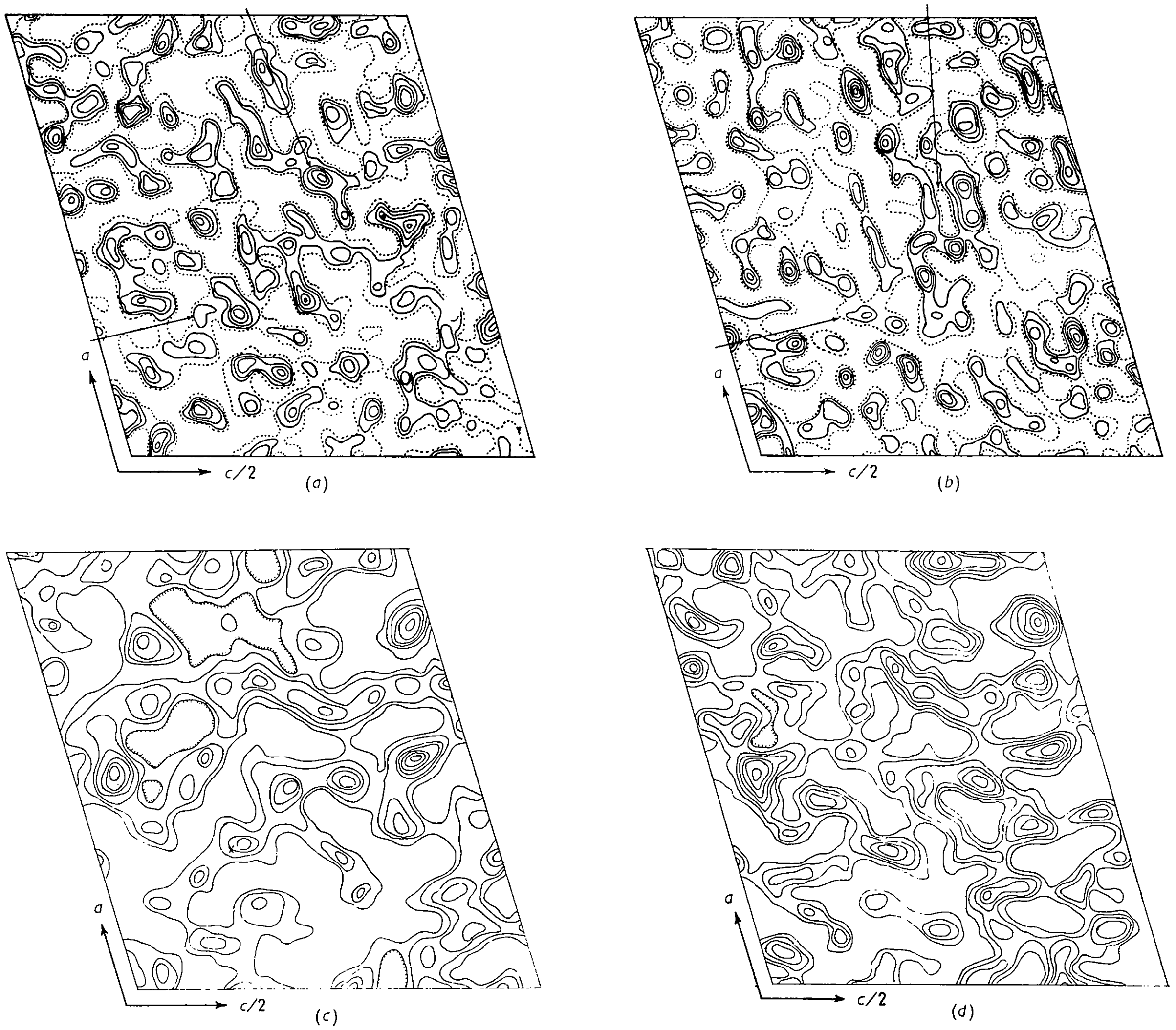

Fig. 3. Fully-sharpened Patterson sections: (*a*) Soaked Dix crystal, $v = 30/60$. (*b*) Normal crystal, $v = 30/60$ compared with the corresponding unsharpened Patterson sections: (*c*) Soaked Dix crystal, $v = 30/60$. (*d*) Normal crystal, $v = 30/60$. The arrows indicate the positions of the A^2 and B^2 peaks of the mercury atoms in the soaked Dix crystal.

going from ribonuclease to the soaked Ri–*pcmb* crystal for 0,10,0 and 0,12,0 is consistent with the Δy value found from the 3-dimensional Pattersons, and the change in 040 is due to the fact that the two Hg atoms have such a small Δy separation, and must be lying on planes approximately between the two molecules.

Table 1, column 5, shows the calculated $F(h0l)$ structure factors for the two Hg atoms to spacings of 2·5 Å. Column 4 shows the $|\Delta F(h0l)|$ of the crystals with and without the heavy atoms. As expected, the overall agreement is not very good, indicating that the relative movement of the molecules in the two crystals is possibly responsible for the discrepancies between the amplitudes of the corresponding reflections in these two columns. Some indication that this is likely to be so is shown by Fig. 6.

3. The location of the Hg atoms in the unsoaked Dix's crystal from Patterson-difference maps on (010)

Dix crystals, as stated earlier, contain 1 Hg atom per protein molecule. Since the soaked Dix crystals contain two heavy-atom sites per molecule, it is not unreasonable to suggest that the Hg atom in Dix crystal is statistically divided between the two positions. The intensities of the X-ray reflections from ribonuclease and soaked Ri–*pcmb* crystals were visually estimated. As the intensity differences of the corresponding reflections between the unsoaked Dix crystal and ribonuclease itself are smaller than the intensity differences between the soaked Dix crystal and ribonuclease for the same reflections, it is necessary

Table 1. *The calculated structure factors for the* Hg *atoms in the soaked* Ri-*pcmb crystals compared with the observed differences in structure amplitudes between the crystals with and without the metal atoms.*

l	$\lvert F_{\mathrm{Ri}}\rvert$	$\lvert F_{\mathrm{RiHg}}\rvert$	ΔF	$4F_{\mathrm{Hg}}$
		$00l$		
1	221	491	270	214
2	194	147	-47	0
3	455	516	61	-129
4	244	311	67	-94
5	147	294	147	0
6	85	199	114	0
7	224	134	-90	-119
8	180	180	0	-225
9	216	159	-57	-181
10	0	0	0	0
11	448	285	-163	166
12	85	85	0	191
13	95	112	17	93
14	134	104	-30	0
15	127	43	-84	1
16	104	0	-104	60
17	224	134	-90	75
18	189	120	-79	0
19	147	95	-52	-106
20	0	180	-180	-147
		$\bar{1}0l$		
20	112	95	-17	-69
19	74	0	-74	-91
18	175	95	-80	-20
17	180	164	-16	111
16	0	95	95	192
15	60	104	-44	146
14	281	463	182	19
13	208	104	-104	-63
12	493	794	301	-36
11	159	337	178	32
10	60	159	99	15
9	60	368	308	-118
8	112	104	-8	-249
7	0	397	397	-226
6	285	258	-27	-37
5	0	112	112	164
4	297	120	-177	214
3	0	262	262	110
2	441	265	-176	6
1	329	236	-93	32
		$10l$		
0	393	515	122	145
1	339	308	-31	178
2	194	85	-109	35
3	294	465	171	-186
4	0	303	303	-295
5	74	208	134	-207
6	190	95	-95	-26
7	288	465	177	76
8	95	104	9	39
9	255	268	13	-35
10	248	424	176	-15
11	493	794	301	106
12	127	0	-127	205
13	199	0	-199	171
14	291	447	156	26
15	0	134	134	-106
16	0	95	95	-128
17	153	194	41	-60
18	127	95	-32	-3
19	185	104	-81	-16
		$\bar{2}0l$		
21	60	120	60	58
20	0	74	74	86
19	127	43	-84	42
18	74	134	60	-1
17	199	325	126	31
16	180	0	-180	115
15	120	321	201	147
14	60	0	-60	53
13	0	127	127	-117
12	336	303	-33	-221
11	357	228	-129	-174
10	0	318	318	-38
9	233	549	316	40
8	398	224	174	-5
7	258	443	185	-81
6	95	0	-95	-44
5	420	258	-162	124
4	112	134	22	281
3	332	969	637	265
2	85	60	-25	73
1	358	248	-110	-125
		$20l$		
0	400	320	-80	-171
1	244	262	18	-77
2	104	0	-104	2
3	60	0	-60	-50
4	147	248	101	-172
5	323	405	82	-204
6	258	0	-258	-67
7	43	104	61	138
8	127	0	-127	240
9	185	74	-111	173
10	417	347	-70	35
11	127	0	-127	-34
12	325	351	26	5
13	221	212	-9	60
14	127	199	72	30
15	185	95	-90	-78
16	60	74	14	-164
17	43	85	42	-143
18	95	0	-95	-37
19	0	0	0	-58
		$\bar{3}0l$		
21	134	95	-39	52
20	0	43	-43	143
19	170	43	-127	150
18	194	216	22	58
17	134	185	51	-51
16	459	597	138	-79
15	159	0	-159	-25
14	104	331	227	15
13	445	566	121	-43
12	294	43	-251	-162
11	443	585	142	-207
10	251	0	-251	-95
9	112	112	0	103
8	112	0	-112	225
7	74	141	67	178
6	175	0	-175	46
5	258	74	-184	-15
4	719	1200	481	51
3	380	424	44	132
2	221	236	15	81
1	407	337	-70	-107
		$30l$		
0	0	104	104	-275
1	104	85	-19	-268
2	318	194	-124	-96
3	281	345	64	78
4	85	0	-85	112
5	120	203	83	32
6	208	281	73	-19
7	85	180	95	49
8	85	312	227	170
9	0	120	120	201
10	265	0	-265	85
11	275	329	54	-85
12	441	393	-48	-171
13	489	499	10	-125
14	294	262	-32	-30
15	74	60	-14	9
16	170	120	-50	-30
17	95	85	-10	-70
18	224	95	-129	-40
		$\bar{4}0l$		
21	0	60	60	1
20	60	208	148	46
19	141	244	103	98
18	120	0	-120	71
17	159	281	122	-48
16	164	0	-164	-171
15	342	400	58	186
14	395	705	310	-83
13	164	0	-164	35
12	337	380	43	56
11	180	244	64	-9
10	153	0	-153	-42
9	358	551	193	44
8	281	265	-16	194
7	164	104	-60	254
6	120	285	165	136
5	308	477	169	-73
4	170	190	20	-196
3	244	323	79	-154
2	224	228	4	-38
1	268	208	-60	-3
		$40l$		
0	236	112	-124	-87
1	407	194	-213	-169
2	120	0	-120	-113
3	203	281	78	73
4	104	104	0	235
5	85	112	27	236
6	104	43	-61	97
7	85	43	-42	-37
8	0	95	95	-55
9	0	43	43	9
10	268	134	-134	37
11	203	0	-203	-35
12	180	0	-180	-144
13	281	423	142	-173
14	236	74	-163	-86
15	365	199	-166	43
16	0	43	43	107
17	0	0	0	77
		$\bar{5}0l$		
20	175	0	-175	-83
19	127	74	-53	-66
18	164	85	-79	-11

Table 1 (*cont.*)

l	$\|F_{\mathrm{Ri}}\|$	$\|F_{\mathrm{RiHg}}\|$	ΔF	$4F_{\mathrm{Hg}}$
		$\bar{5}0l$		
17	147	0	−147	−5
16	385	358	−27	−76
15	0	0	0	−147
14	153	300	147	−114
13	391	127	−264	33
12	244	0	−244	180
11	141	473	332	203
10	337	175	162	97
9	212	120	−92	−16
8	315	281	−34	−23
7	147	159	12	53
6	203	307	104	79
5	0	104	104	−30
4	288	347	59	−200
3	194	297	103	−269
2	216	104	−112	−159
1	153	208	55	36
		$50l$		
0	120	0	−120	146
1	0	141	141	108
2	304	159	−145	17
3	212	141	−71	8
4	120	43	−77	101
5	449	626	177	180
6	0	0	0	129
7	185	288	103	−34
8	324	352	28	−174
9	175	0	−175	−182
10	141	141	0	−80
11	43	0	−43	12
12	134	212	78	15
13	194	153	−41	−36
14	170	85	−85	−48
15	85	104	19	17
16	43	74	31	107
		$\bar{6}0l$		
20	120	0	−120	−104
19	224	228	4	−157
18	0	74	74	−107
17	185	170	−15	3
16	147	0	−147	73
15	275	329	54	50
14	437	495	8	−8
13	216	0	−216	2
12	185	332	147	100
11	208	0	−208	190
10	216	95	121	155
9	451	599	148	−6
8	262	297	35	−165
7	255	294	39	−192
6	141	74	−67	−94
5	303	258	−45	2
4	0	104	104	−11
3	127	180	53	−94
2	397	488	97	−113
1	0	60	60	5
		$60l$		
0	435	268	−167	177
1	405	461	56	249
2	588	591	3	157
3	549	465	−84	−4
4	240	127	−113	−91
5	236	329	93	−57
6	532	300	−232	9
7	194	306	112	−2
		$60l$		
8	74	60	−14	−94
9	95	251	156	−165
10	0	95	95	−125
11	233	221	−12	4
12	0	112	112	114
13	306	281	−25	126
14	0	43	43	56
15	95	104	9	−1
		$\bar{7}0l$		
20	371	486	115	−20
19	0	0	0	−77
18	306	288	−18	−93
17	153	297	144	−16
16	175	268	93	111
15	43	74	31	180
14	127	255	128	129
13	285	203	−82	14
12	194	244	50	−51
11	0	0	0	−19
10	134	0	−134	38
9	104	185	81	12
8	398	291	−107	−109
7	281	312	31	−213
6	561	809	248	−182
5	199	85	−114	−24
4	43	0	−43	129
3	153	180	27	156
2	221	281	60	72
1	0	74	74	2
		$70l$		
0	185	112	−73	33
1	153	85	−68	118
2	0	127	127	133
3	0	60	60	21
4	433	473	40	−135
5	475	342	−133	−202
6	141	439	298	−134
7	422	0	−422	−13
8	0	153	153	46
9	112	153	41	15
10	164	0	−164	−30
11	141	134	−7	−9
12	281	85	−196	74
13	203	236	33	133
		$\bar{8}0l$		
19	43	43	0	74
18	164	208	44	31
17	482	352	−130	−3
16	141	85	−56	34
15	127	0	−127	110
14	60	0	−60	131
13	0	104	104	42
12	194	147	−47	−102
11	0	203	203	−180
10	447	376	−71	−133
9	413	589	186	−25
8	216	0	−216	26
7	300	509	209	−16
6	112	0	−112	−71
5	185	60	−125	−33
4	318	463	145	99
3	566	502	−64	208
2	391	329	−62	185
1	255	190	−65	46
		$80l$		
0	85	185	100	−86
1	120	141	21	−107
2	445	399	−46	−41
3	268	141	−127	4
4	275	251	−24	−40
5	175	43	−132	−121
6	134	185	51	−133
7	120	60	−60	−39
8	199	159	−40	89
9	127	228	101	145
10	199	120	−79	99
11	95	95	0	17
		$\bar{9}0l$		
18	74	95	21	126
17	74	74	0	45
16	112	74	−38	−42
15	104	60	−44	−57
14	60	60	0	−11
13	60	0	−60	15
12	134	43	−91	−40
11	0	85	85	−133
10	120	74	−46	−160
9	278	297	19	−67
8	0	0	0	79
7	74	0	−74	158
6	320	306	−14	117
5	85	0	−85	26
4	224	255	31	−6
3	95	95	0	46
2	300	236	−64	96
1	104	141	37	53
		$90l$		
0	120	95	−25	−75
1	0	147	−147	−178
2	104	0	−104	−164
3	255	208	47	−54
4	0	104	104	47
5	74	74	0	59
6	0	0	0	10
7	112	0	−112	−14
8	104	147	43	34
9	34	159	25	105
		$\overline{10},0l$		
16	272	324	52	−42
15	332	199	−133	−133
14	85	0	−85	−137
13	0	0	0	−56
12	112	134	22	25
11	127	104	−23	32
10	194	180	−14	−16
9	147	85	−62	−33
8	112	255	143	35
7	258	342	84	138
6	120	175	55	169
5	255	351	96	84
4	244	228	−16	−49
3	134	74	−60	−119
2	194	251	57	−86
1	74	43	−31	−18
		$10,0l$		
0	275	255	−20	−5
1	127	127	0	−61
2	0	104	104	−106
3	0	60	60	−65

Table 1 (*cont.*)

l	$\lvert F_{Ri}\rvert$	$\lvert F_{RiHg}\rvert$	ΔF	$4F_{Hg}$
		10,0l		
4	216	104	−112	46
5	199	164	−35	135
6	0	60	60	129
		$\overline{11}$,0l		
13	0	43	43	−79
12	112	180	68	26
11	60	95	35	122
10	240	95	−145	129
9	159	43	−116	57
8	258	422	164	10
7	159	134	−25	6
6	0	0	0	42
5	203	190	−13	51
4	190	112	−78	−22
3	112	60	−52	−124
2	60	0	−60	−157
1	127	134	7	−87
		11,0l		
0	85	60	−25	23
1	95	0	−95	78
2	0	95	95	52
3	306	228	−78	4
		$\overline{12}$,0l		
8	0	43	43	−7
7	0	127	127	−97
6	95	0	−95	−107
5	0	0	0	−48
4	306	228	−78	1

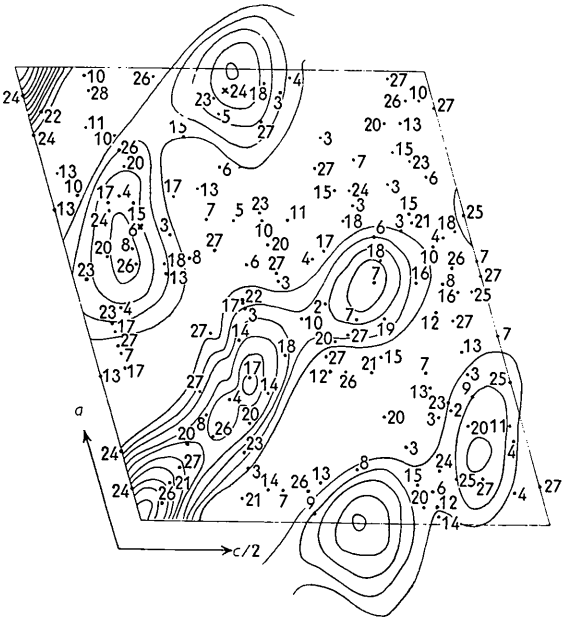

Fig. 4. Plot on Fig. 1(*b*) of all peaks occurring on fully-sharpened Patterson sections of soaked Dix crystal having peak height greater than half the average peak height. The crosses indicate the positions of the *AB* peaks of the mercury atoms in the soaked-Dix crystal as determined from the fully-sharpened Patterson sections.

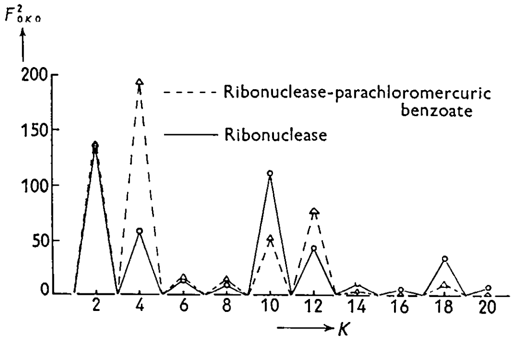

Fig. 5. Comparison of the intensities of the 0*k*0 reflections between the soaked-Dix and normal crystals.

to photometer the reflections from Dix crystals and ribonuclease if a reliable conclusion is to be drawn about Hg positions that are now making $\frac{1}{4}$ of their full contribution to the intensities of the reflections. The following work briefly describes the procedure adopted.

The investigation was confined to the *h*0*l* reflections of the two crystals, whose diffraction patterns were recorded with a Buerger Precession camera which facilitated the measurement of intensities by means of a JCL—Walker Automatic Recording Microdensitometer.

Crystals of about the same size, of both types, were carefully selected for the intensity measurements, and the intensities were corrected for Lorentz-polarisation effects by Waser's function given in Volume II of the *International Tables for Crystallography*. The $F^2(h0l)$ values from the two crystals were independently scaled by the Wilson method (1942), with due allowance made for the presence of the Hg atoms in Dix's crystal. The Patterson difference map using $(\Delta|F|)^2$ as coefficients was calculated for spacings to 2·5 Å; it showed similarities to Fig. 1(*a*) and was also uninterpretable. A smoothing function was then used which terminated the series at 6 Å, and Fig. 1(*c*) shows the resulting Patterson difference map projected on (010).

The similarity between Figs. 1(*b*) and (*c*) is at once apparent, though the peaks on 1(*c*) show different relative heights compared with those on 1(*b*). The pattern however is again interpretable in terms of 2 pairs of atoms related through a centre. The *x* and *z* coordinates of the *A* and *B* sites, which can only be approximate, are given in the table below together witht the corresponding *x* and *z* psotitions determined from the analysis of the 3-dimensional Patterson sections of the soaked crystals.

	A (site)		*B* (site)	
Crystals	*x*	*z*	*x*	*z*
Unsoaked Dix crystal	0·12	0·08	0·78	0·18
Soaked Dix crystal	0·153	0·075	0·806	0·150

The agreement is sufficiently close to show that the mercury atoms occupy the same sites in both the unsoaked and soaked Dix crystal. The Δy values for the *A* and *B* sites in Dix's crystal have not been determined, but it is unlikely that they will be any different from those already found for the soaked ones.

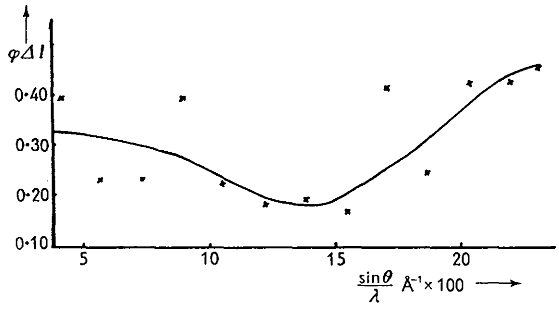

Fig. 6. Plot of the fractional change in intensity, $\varphi\Delta I = 2\{(\Delta|F|)^2/\bar{I}_{\mathrm{Ri}}\}^{\frac{1}{2}}$, for Dix crystal—normal crystal, versus $\sin\theta/\lambda$. The initial fall in $\varphi\Delta I$ is due to the mercury atoms in the Dix crystal and the increase for $\sin\theta/\lambda > 0{\cdot}14$ is attributed to a shift in the molecules of one crystal relative to those of the other.

Fig. 6 shows a plot of $\varphi\Delta I = 2\{(\Delta|F|)^2/\bar{I}_{\mathrm{Ri}}\}^{\frac{1}{2}}$ against $\sin\theta/\lambda$ (Crick and Magdoff, 1956). The initial decrease in the function is due to the effect of the heavy atoms, but the small increase towards higher $\sin\theta/\lambda$ values indicates that there must be a slight shift of the molecules in the Dix crystals relative to those in the metal-free ones. This is in agreement with the previous findings of Bernal, Carlisle and Rosemeyer (1959) for the soaked crystals.

4. Conclusions

This work has shown how the positions of heavy atoms in protein crystals, particularly when there is more than one per asymmetric unit, may be found by using fully sharpened three-dimensional Patterson functions, and this is all the more important where there is a likelihood that isomorphism is not strictly obeyed owing to the relative movement of the molecules in the two crystals.

The structure factors of the mercury atoms in the soaked crystal have now been calculated to spacings of $2A$, leading to the synthesis of two three-dimensional Fourier distributions for the metal-free crystal incorporating some 60% of the reflections in each case to (*a*) the 6Å region, and (*b*) the 2·5Å region. From this it has been possible to gain some knowledge of the configuration of the single polypeptide chain in this molecule and also the positions of the four cystine sulphurs in it.

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