scanning slide was offset for upper levels so that, for example, the centre of the scanning slide was the $H = -4$ for the level $K = 8$.

Crystals could be set fairly well by eye. Viewed through the diffractometer microscope, they looked roughly square in the [001] direction, and the arcs of the goniometer head were set so that the edges were horizontal and vertical. When the crystal was turned through 90° from this position, the reflection of a light held level with the microscope could often be seen in the true (110) face. Setting the crystals on the goniometer head with [001] parallel to one of the arcs facilitated subsequent adjustments.

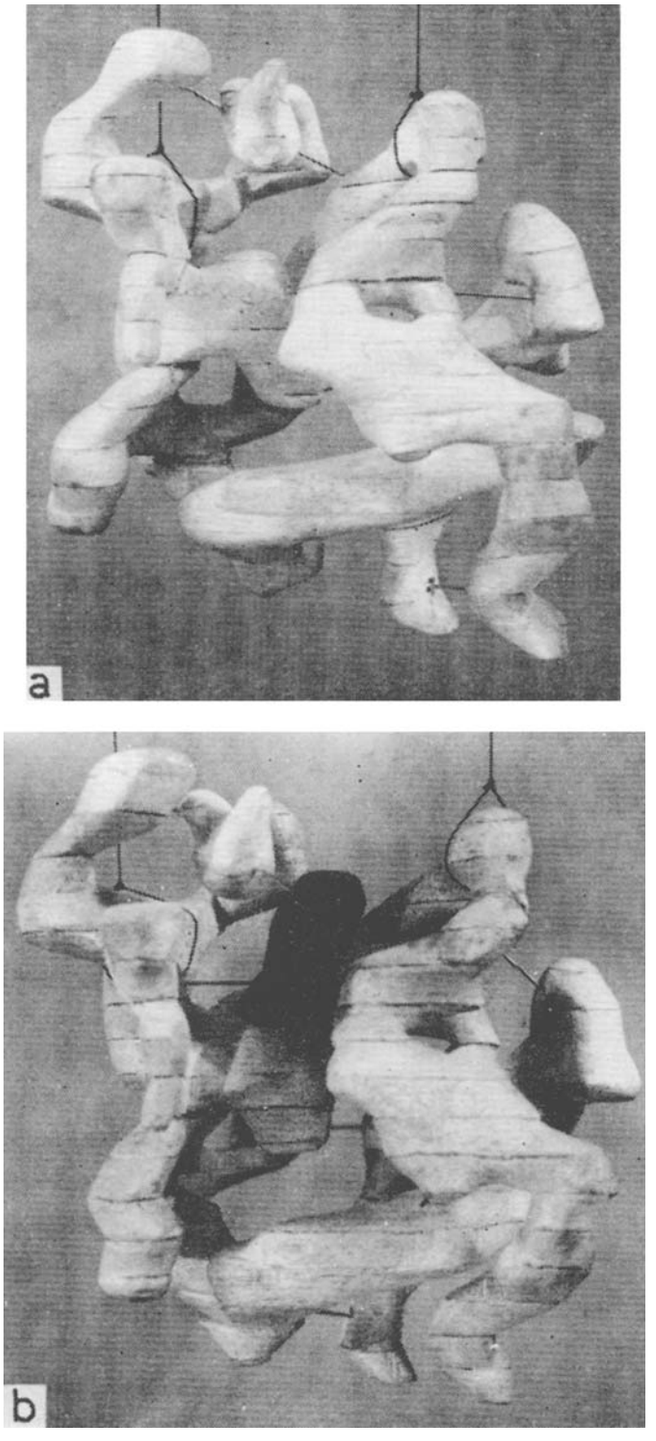

Fig. 26.1.3.3. Solid model of the electron density greater than about 0.5 e Å^{-3} in the second study of lysozyme at 6 Å resolution. This view of the model is equivalent to a view of the original model seen horizontally from the right of Fig. 26.1.2.13(*c*). (*a*) The new model has a marked cleft running roughly vertically down the other side of the model, corresponding to the one that can be seen in Fig. 26.1.2.13(*c*). (*b*) The cleft was shown to bind inhibitor molecules. The black density is that observed for the lysozyme–GlcNAc complex at 6 Å resolution.

Fine adjustment of crystal orientation was achieved by setting on the $\bar{4}40$ reflection. For this purpose, C – the vertical slide – was set to 0.1104 and the inclination angle to $\mu = 3°\ 5'$. Then, if one arc was set fairly well by eye, the other could be oriented parallel to the incident X-ray beam and adjusted to locate the reflection. The two arcs were then adjusted in turn to give the optimum setting, in which the crystal was rotated about the normal to the $(\bar{4}40)$ plane.

The orientation in the AC reciprocal-lattice plane was determined by returning the vertical slide to zero and setting the upper (scanning) slide to 0.1104 with the lower (stepping) slide at zero. The crystal was then rotated until the monoclinic 400 reflection appeared. At this stage, checks were made with the top/bottom and left/right slits to make sure that the crystal was well centred in the X-ray beam and that the counter apertures were well positioned. Similar checks were made with the crystal rotated about 180° to the monoclinic $\bar{4}00$ reflection.

The final check on crystal orientation was to locate the 008 reflection near 0.3248 on the lower (stepping) slide, with the other slides set at zero. There was some variation in the value of c^* for different crystals, and c^* was often closer to 0.0404 than to 0.0406 r.l.u.

At this stage, the first measurements were made of the intensities of the reflections (monoclinic) 400, 16,0,0 and 008. These reference reflections were remeasured at intervals during the measurement of each triplet of reciprocal-lattice levels as a check on the stability of the whole system and irradiation damage to the crystal. The measurements were manually entered on the diffractometer output tape and monitored by the data-processing program.

In order to set the diffractometer for a particular triplet of levels, we found it convenient to leave the lower slide set for the 008 reflection, to adjust the vertical slide and the tilt angle (μ) for the levels in question, and then to run out along the stepping slide until a suitable high-angle reflection was found. The crystal rotation angle was then optimized for this reflection.

Finally, careful checks were made to ensure that the reflections to be measured fell in the reciprocal-lattice hemisphere with L positive, and that they were indexed in a right-handed axial system. The automatic run was then begun at the 2 Å limit on the stepping (C^*) slide, and all reflections were scanned from H_{max} to $-H_{max}$ on the scanning slide. Virtually all reflections within (and in some directions a little beyond) the 2 Å limit were measured in this way in fourteen triplet levels, seven even and seven odd. About 2000 reflections were measured in a typical overnight run, and data collection for each species of crystal took slightly more than two weeks.

The derivative crystals required for these measurements were prepared by the diffusion method described above, and about 20 of them were mounted at the same time at the beginning of the data-collection process to ensure that they were all the same. At the end of a run, careful measurements were made of the peak intensity of the $\bar{4}40$ reflection as the crystal was rotated through steps of 15° about the crystal-mounting axis (φ). These measurements were used during data processing in an improved method of absorption correction devised by North *et al.* (1968).

26.1.3.4.2. *Diffractometer output*

The output from the triple-counter diffractometer again consisted of a plain-language output for immediate checking of the results and output at this stage on eight-hole-punched paper tape for immediate

references