by the incident and reflected beams for any reflection *hkl* is indicated by the relative intensity of 200 when the X-ray beams are parallel to the reflecting planes *hkl*. This is a fair approximation for reflections at low angles. This method could not be used, however, for the measurements made from crystals mounted to rotate about the [001] axes, since a full rotation about this axis was not possible. These measurements were not corrected for absorption errors.

Finally, the diffractometer was reset manually at regular intervals during data collection to measure the intensities of a number of reference reflections. These measurements were used to monitor the stability of the system and the extent of irradiation damage to the crystal, and they were also recorded on the paper-tape output for analysis by the data-processing program. An attempt was made to minimize irradiation damage by using a shutter to expose the crystal only during the measuring cycle.

26.1.2.6.2. *Data processing*

The measurements N, n_1 and n_2, together with the indices of the reflections, *hkl*, were all printed out in plain language on a teleprinter and punched in paper tape for direct transfer to a computer (Fig. 26.1.2.7). The plain-language record was important during measurement of the low-angle reflections, when the diffractometer had to be adjusted by hand. Not all imperfections in the measurements were easily spotted at this stage, however, and ACTN's data-processing program (North, 1964) therefore incorporated systematic checks on the quality of the measurements.

The program checked for the following contingencies:

(1) malfunction of the diffractometer-output mechanism leading to the paper tape being an inaccurate record of the measurements, generally because the tape punch had failed to perforate the tape or had 'stuttered';

(2) errors by the pulse counters, detected by the 'ring-of-three' circuit;

(3) peak counting rate so high that counting-loss errors were appreciable;

(4) count on reflection not significantly above background;

(5) failure of diffractometer to set crystal or counter correctly; and

(6) gradual drift in the experimental parameters, including movement of the crystal within its mounting and irradiation damage to the crystal.

These checks were made while the diffractometer tape was being read into the computer, and a monitor output was produced simultaneously, as shown in Fig. 26.1.2.8. The checks depended in large part on the fact that the significance of an intensity measurement may be assessed in terms of counting statistics. The standard deviation of a background-corrected count, N_o $(= N - n_1 - n_2)$, is given by $\sigma^2(N_o) = N + n_1 + n_2$, and the ratio $\sigma(N_o)/N_o$ may be taken as an indication of the significance of the measurement. Measurements were rejected when this ratio exceeded unity. N_o might then have been taken as zero but, following Hamilton (1955), we considered it preferable to replace N_o by a fraction (0.33 for centric and 0.5 for acentric reflections) of the minimum background-corrected count that we should have considered acceptable. Reflections were treated in the same way whether the net count N_o was positive or negative, but measurements were rejected if N_o was negative and $|N_o| < \sigma(N_o)$.

Mis-setting of the crystal was frequently revealed by marked inequality of the background counts. Measurements were therefore rejected if the difference between the two backgrounds exceeded three or four standard deviations, that is if $(n_1 - n_2)^2 > b^2(n_1 + n_2)$, where b is the appropriate constant.

After monitoring the quality of the data in this way, the program proceeded: (i) to extract background-corrected counts; (ii) to apply a correction for irradiation damage derived from any systematic variation in the intensities of the reference reflections; (iii) to sort the reflections into a specified sequence of indices; (iv) to apply Lorentz–polarization factors; and (v) to apply absorption corrections (the data for which were read separately from a specially prepared punched tape, Fig. 26.1.2.6). The outputs from this program comprised data sets from a number of individual crystals of the native protein and the three derivatives. The scale factors needed to bring the measurements from the individual crystals of

```
*02+13+00+
:000234 002500 000284

*01+13+00+
:000260 001660 000279

*00-13+00+
:000181 000522 000212

*00-12+00+
:000225 000651 000236

*01+12+00+
:000327 005860 000362

*02+12+00+
:000281 006717 000260

*03+12+00+
:000236 000507 000240

*04+12+00+
:000225 000488 000241

*05+12+00+
:000244 003763 000263
```

(*a*)

```
*    7    2
   0   4912   98
   1   4836   97
   2    276   33
   3  12710  127
   4   1216   49
   5    802   40
   6    398   32
   7   1551   47
   9    935   37
  10   1257   38

*    8    2
   0   9855   99
   1   1450   44
   2   6117   61
   3   4733   95
   4     61   26
   5    395   32
   6   1304   39
   7    129   28
   8    183   27
   9   1725   52
```

(*b*)

Fig. 26.1.2.7. Typical output from the linear diffractometer. (*a*) Indices *h*, *k*, *l* followed by background (n_1), peak (N), background (n_2) counts. (*b*) Listing ready for the next stage in data processing with indices * *h k* followed by *l*, background corrected peak and standard deviation. Reproduced with permission from North (1964). Copyright (1964) Institute of Physics.

```
PBDN 3/2
TLY  PDBR 4  D 2 * 1/5    K = 1 2 : 1 4 : 1 6    24/2/64
 01   01   14 :=  SD  966
 01   00   14 PE 2
 01  -01   14 :-  SD  999  :=  SD  156
 01  -04   14 :-  BG  :+  BG
 01  -13   14 :=  N-  SD  578  BG
 01  -13   14 :-  N-  :=  N-  BG
 01  -13   14 :-  SD  391  :=  N-  SD  234
 01  -14   14 :-  SD  132  :+  SD  216
 01  -15   14 :=  SD  813
 00  -15   14 :-  BG  :=  BG  :+  BG
 00  -12   14 :=  BG
 00  -10   14 :+  BG
 00  -10   14 :=  BG
 00  -04   14 :=  BG
 00  -04   14 :+  BG
 00   04   14 :-  BG  :=  BG  :+  BG/

TOTAL  PE1  PE2  PE*  SD  N-  BG  N>H
  42    0    1    0   9   4  15    0
```

Fig. 26.1.2.8. Format of monitor output in which the computer lists reflections that fail the tests for format or significance. PE1 signifies punching error, indices; PE2, punching error, measurements; PE3, failure of electronic check on counting circuits; SD, standard deviation greater than set limit; N-, net count negative; BG, backgrounds significantly different; N > H, gross counts exceed counting-loss limit. This output was from the version of the program designed to be used with the diffractometer fitted with three counters. The symbols: −, =, + refer, respectively, to reflections measured by the lower, central and upper counters. Reproduced with permission from Arndt *et al.* (1964). Copyright (1964) Institute of Physics.

references