The Use of the Double Crystal Spectrometer in the Analysis of Bragg Reflections at Very Small Angles*

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A two-crystal spectrometer has been used to investigate x-rays scattered from the large periodicities in wet collagen. Positions and relative intensities are assigned to the first through the seventh orders and to the ninth order. Some advantages of this method over the usual diffraction methods are pointed out.

I. METHOD

THE customary x-ray diffraction methods, using slit or pinhole collimation and photographic registration, become increasingly difficult as the angle between the scattered radiation and the primary beam is decreased. At best, the conflicting demands of resolution and intensity can be compromised at exposure times of hours or days and resolutions of two or three minutes of arc. The very small apertures required are difficult to align, slit edge scattering is a problem, and little power can be dissipated in the small area of the target seen by the slits. Photographic registration, while it has the advantage of simultaneously collecting data at many scattering angles, is not well suited to accurate intensity measurements nor to the measurement of very low intensities. Some investigators have lessened these difficulties by the use of an evacuated camera and very long wave-length radiation,[1] or by the use of a curved crystal monochromator.[2]

The double crystal spectrometer as used in the present work[3] (see Fig. 1) is equivalent to a slit geometry of high resolution, but without the usual intensity difficulties since one can use a wide beam and, therefore, a large focal spot. The first crystal collimates the beam while the second crystal analyzes the angular distribution of the scattered x-rays near the parallel position. The resolution is limited only by the natural width of the parallel position rocking curve. The accuracy with which the angular position of the peaks may be determined is governed by the sharpness of the peak and the precision of the measurement of the angle of rotation of the second crystal.

A Ross-type mounting was used in the $(1,-1)$ position. The spectrometer crystals were of calcite, ground and etched, having a full width at half-maximum of 16 seconds for Cu K_α radiation. The angular position of the second crystal was measurable to within one-half second of arc. A metal, continuously pumped x-ray tube with interchangeable iron and copper targets was used for the present experiments. It was operated at 20 milliamperes and 31 kilovolts with electronic stabilization of current and voltage.[4] The beam irradiating the sample was approximately 4 mm wide, and 3 mm' high. The former figure is the diameter of the focal spot. A thin glass window, argon-alcohol Geiger counter served to record the x-ray intensity reflected by the second crystal.

II. DISCUSSION

The fibrous proteins are among the best examples of very large periodicities to be found in nature. Among these, collagen has been investigated by means of the electron microscope,[5] and by means of x-rays,[6,7] and has been found to

FIG. 1. The geometry of the two crystal spectrometer for small angle scattering.

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[1] R. Hosemann, Zeits. f. Physik **114**, 133 (1939).
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[5] C. E. Hall, M. A. Jackus, and F. O. Schmitt, J. Am. Chem. Soc. **64**, 1234 (1942).
[6] R. S. Bear, J. Am. Chem. Soc. **66**, 1297 (1944) and also **64**, 727 (1942).
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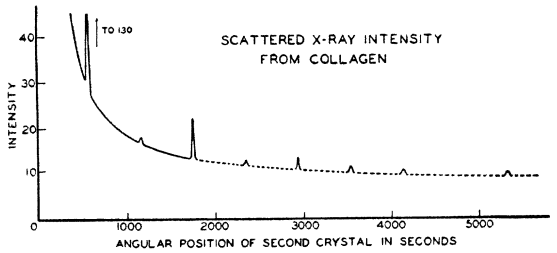

FIG. 2. Scattered intensity of x-rays from wet collagen. The full lines indicate the regions mapped carefully. Points in these regions were taken every five seconds of arc. Points in the dashed regions were taken every 100 seconds of arc.

have a particularly regular structure. The electron microscope studies indicate a fundamental periodicity varying from 522 to 902 angstroms in dried collagen. However, the preparation of the specimen for use in the electron microscope has a considerable effect on its structure. Several investigators have used x-ray diffraction cameras in the study of collagen. Bear[6] and Kratky and Sekora[7] have observed a first-order peak in the region corresponding to 640A for dried collagen, assigning its exact position by measurement of higher orders. Kratky and Sekora used dried kangaroo tail tendon, finding a spacing of 642A. Bear used, among other things, kangaroo tail tendon and beef tendon. He listed periodicities varying from 640 to 645A for air-dried samples, and from 675 to 680A for wet samples.

The collagenous tissues used in the present study were samples of beef tendon approximately

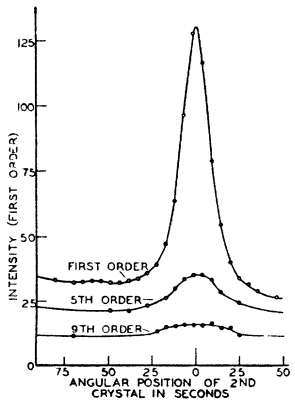

FIG. 3. Detailed curves with experimental points of the first, fifth, and ninth orders. The fifth and ninth orders are plotted to five times the vertical scale of the first order. The zero ordinate for the fifth and ninth orders is not shown, but is apparent from Fig. 2. The slight hump at the left base of the first order peak is from $K\beta$ radiation.

1 mm thick, soaked in water for several hours and then enclosed in cellulose tape. The specimens were placed midway between the spectrometer crystals (Fig. 1). The fiber axes were perpendicular to both the x-ray beam and the axis of rotation of the second crystal, thus permitting the observation of periodicities along the fiber axis.

Diffracted maxima corresponding to several orders were observed from the collagen. These are shown in Fig. 2. The relative intensities of the orders and the lattice spacing as determined from them are given in Table I; the mean value of the latter is 675A in satisfactory agreement with the results of Bear. It should be noted that the relative intensity of the first order (shown in more detail in Fig. 3) is much higher than that found by previous investigators. Another sample was examined in the first and third orders, yielding a spacing of 670A. The data for the first specimen were taken with an iron target; data for the second sample, as well as the data described below, were taken with copper radiation.

The resolution of the instrument and the regularity of the collagen lattice are apparent from the sharpness of the lower order peaks, which were about 20 seconds wide. This is very little more than the $(1,-1)$ width. The higher orders are somewhat wider, due in large part to the $K_{\alpha 1}$, $K_{\alpha 2}$ spacing, which is 10 seconds for iron radiation in the ninth order. In the first-order plot in Fig. 3, the $K\beta$ peak is clearly evident, though weak. Because of the high resolution one can examine the regions between the peaks carefully for further scattering. A check was made for evidence of diffuse small angle scattering of the type obtained with small, randomly spaced particles.[8] The region of the curve between the central maximum and the third-order peak showed no further evidence of scattering, the shape of the curve agreeing very closely with the rocking curve with no sample in place (corrected for absorption). Also the region 0–500 seconds with the fibers perpendicular to the beam but parallel to the axis of rotation of the second crystal was checked and was found to agree closely with the blank rocking curve.

Some observations were made of the position

[8] See, for example, R. Hosemann, Zeits. f. Physik **113**, 751 (1939).

and intensity of the first-order peak as a function of the orientation of the fibers. When the sample was rotated about a vertical axis (parallel to the axis of rotation of the second crystal), the peak moved to larger angles; when rotated about a horizontal axis (parallel to the x-ray beam), the peak moved to smaller angles and widened considerably. In both cases, the height of the peak had decreased by half within 15 degrees of the normal specimen position. This made it difficult to determine the shifts quantitatively, since they are functions of the cosine of a small angle. They were consistent, however, with secant ϕ and cosine ϕ, respectively.

The total intensity available and the ratio of peak to background determine the counting time necessary for a given statistical accuracy. Figures 2 and 3 are from a run in which 6400 counts per point were taken. Counting times averaged between one and five minutes per point for the first three orders. Thus the neighborhood of the first-order peak could be mapped in less than an hour. A peak having an intensity equal to that of the first-order peak could be located easily if it were only 100 seconds from the primary beam, However, the eighth-order peak in this sample was so weak that it was not located, as the counting times for significant statistics would have been excessive.

TABLE I. Intensity in arbitrary units and lattice spacing obtained from various orders. The spacings were calculated from the angular separation of the given order from the central maximum (zero order).

Order	Intensity	Spacing
0	~100,000.0	—
1	100.0	674.7A
2	0.9	674.7
3	8.5	675.1
4	0.5	676.1
5	3.0	674.3
6	0.9	676.1
7	1.1	675.1
8	<0.2	—
9	1.1	675.1
		Av. 675.1A

It is evident that neither the full intensity nor angular precision of the instrument have been realized. The output of the tube could be increased by a factor of two to four without target damage. We believe our lattice spacings are dependable to within one quarter of one percent, but the lines are sufficiently sharp to permit measurements to one-tenth of one percent with a little extra care. The intensity might be considerably increased, with some loss of resolution, by grinding the crystals.

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