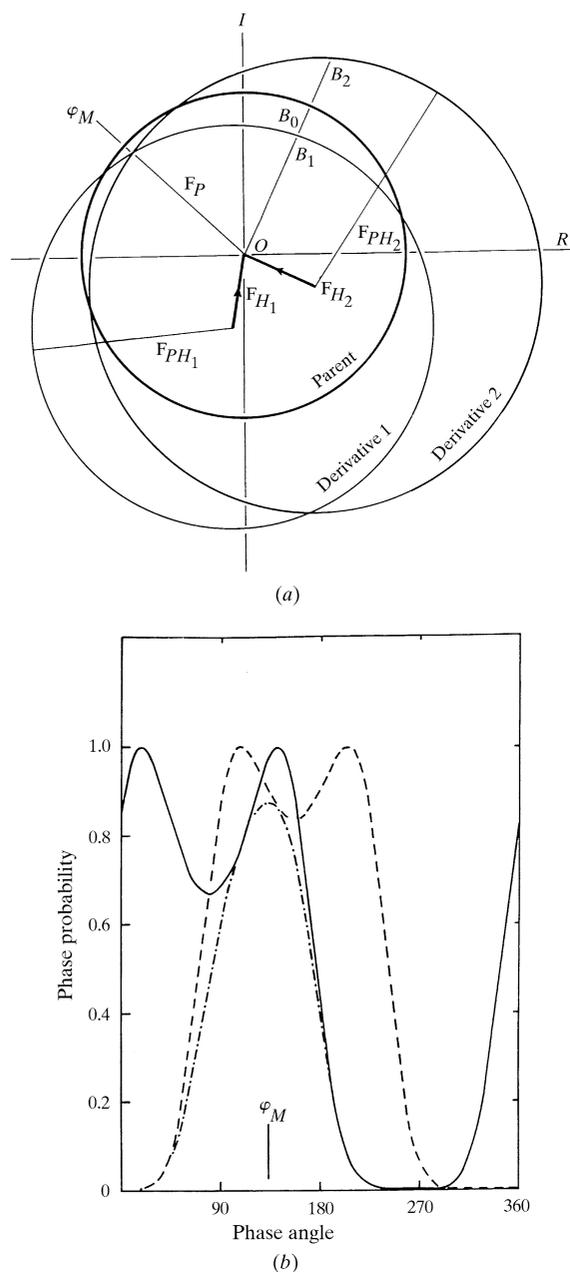


## 14. ANOMALOUS DISPERSION



**Figure 14.1.3.1**  
 (a) Harker construction for a double isomorphous replacement.  $\varphi_M$  is the 'most probable' phase for  $\mathbf{F}_p$ . (b) Phase probability distribution corresponding to the double isomorphous replacement shown in part (a). The curve for derivative 1 is solid, that for derivative 2 is dashed, and that for the combined distribution is drawn as a dotted-and-dashed line.

From the heavy-atom parameters, the corresponding structure factor  $\mathbf{F}_H(\mathbf{h})$  is calculated. To determine  $\varphi$ , the phase of  $\mathbf{F}_p(\mathbf{h})$ , we construct a set of phase circles, as proposed by Harker (1956). From a chosen origin  $O$  (Fig. 14.1.2.1a), the vector  $OA$  is drawn equal to  $-\mathbf{F}_H$ . Circles of radius  $F_p$  and  $F_{PH}$  are then drawn about  $O$  and  $A$ , respectively. The intersections of the phase circles at  $B$  and  $B'$  define two possible phase angles for  $F_p$ . Note that the angles are symmetrical about  $\mathbf{F}_H$ . This ambiguity may in principle be resolved in two ways: (a) by using a second heavy-atom isomorphous derivative or (b) by utilizing the anomalous-scattering effects for the first isomorph.

### 14.1.3. The method of multiple isomorphous replacement

The phase information provided by a second isomorph is illustrated in Fig. 14.1.3.1(a). In theory, the three phase circles will

intersect at a point and the phase ambiguity will be resolved. In practice, there will be errors in the observed amplitudes  $F_p$  and  $F_{PH}$  and in the heavy-atom parameters (and thus in  $\mathbf{F}_H$ ). Also, the isomorphism may be imperfect. As a result, the intersections of the three phase circles may not coincide. Another complication arises from the fact that for reflections where  $\mathbf{F}_H$  is small, the circles will be essentially concentric and will not have well defined points of intersection. In other words, the phase determination will become indeterminate. The method of Blow & Crick (1959) was introduced as a way to take all these factors into account. It has had an extraordinary impact, not only as a practical method for protein phase determination, but also in influencing all subsequent thinking in this area.

### 14.1.4. The method of Blow & Crick

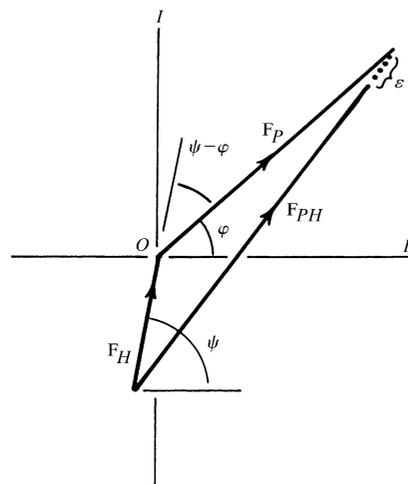
Blow & Crick pointed out that in practice the phase angle  $\varphi$  can never be determined with complete certainty. Rather, there is a finite probability that any arbitrary phase angle may be the correct one. Consider the vector diagram shown in Fig. 14.1.4.1, in which  $\mathbf{F}_H$  is known and we wish to determine the probability  $P(\varphi)$  that the arbitrary phase angle  $\varphi$  is the correct phase of  $\mathbf{F}_p$ . Strictly, one should allow for the possibility of errors in  $\mathbf{F}_H$ ,  $F_p$  and  $F_{PH}$ , and should consider the probability that the vector  $\mathbf{F}_p$  occupies all possible positions in the Argand diagram. However, Blow & Crick suggested that the analysis might be considerably simplified by assuming that  $F_p$  and  $\mathbf{F}_H$  are known accurately and that all the error lies in the observation of  $F_{PH}$ . In other words, it was assumed that the vector  $\mathbf{F}_p$  must lie on the circle of radius  $F_p$ , and the probability distribution of  $F_p$  could be evaluated as a function of  $\varphi$  only.

For an arbitrary phase angle  $\varphi$ , the phase triangle (Fig. 14.1.4.1) will not close exactly. If we define  $F_C$  to be the vector sum of  $\mathbf{F}_H$  and  $F_p \exp(i\varphi)$ , then the lack of closure of the phase triangle is given by

$$\varepsilon = F_C - F_{PH}. \quad (14.1.4.1)$$

Following Blow & Crick, if  $E$  is the r.m.s. error associated with the measurements, and the distribution of error is assumed to be Gaussian, then the probability  $P(\varphi)$  of the phase  $\varphi$  being the true phase is

$$P(\varphi) = N \exp(-\varepsilon^2/2E^2), \quad (14.1.4.2)$$



**Figure 14.1.4.1**  
 Vector diagram illustrating the lack of closure,  $\varepsilon$ , of an isomorphous-replacement phase triangle.