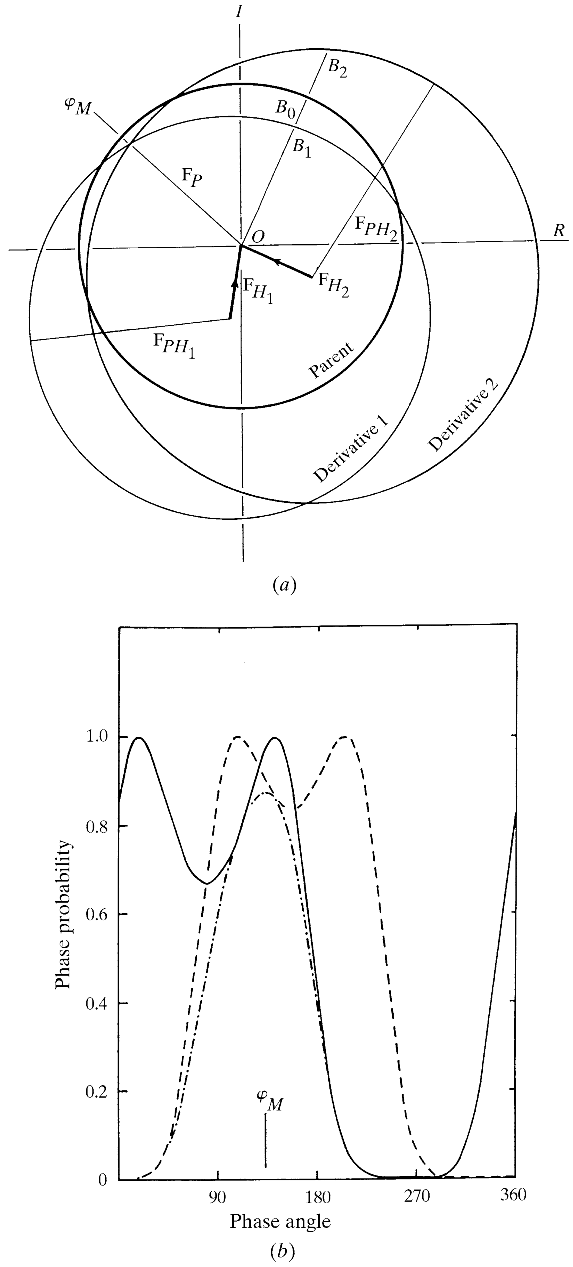

Figure 14.1.3.1
(*a*) Harker construction for a double isomorphous replacement. φ_M is the 'most probable' phase for $\mathbf{F}_P$. (*b*) Phase probability distribution corresponding to the double isomorphous replacement shown in part (*a*). The curve for derivative 1 is solid, that for derivative 2 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 φ, 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. 14.1.2.1*a*), 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 φ 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 φ 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 φ only.

For an arbitrary phase angle φ, 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

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

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 φ being the true phase is

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

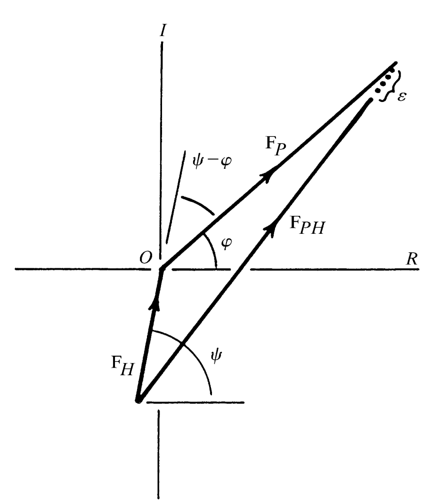

Figure 14.1.4.1
Vector diagram illustrating the lack of closure, ε, of an isomorphous-replacement phase triangle.