EXCLUSION OF PROTEIN FROM HIGH POLYMER MEDIA

I. DERIVATION OF PROBABILITY DISTRIBUTION FOR THE NUMBER OF FIBER CENTERS WITHIN ANY SPHERE OF RADIUS *r*

PAUL W. CHUN, J. I. THORNBY, *and* J. G. SAW

From the Department of Biochemistry, University of Florida College of Medicine, Gainesville, Florida 32601. Dr. Thornby's present address is the Division of Behavioral Sciences, University of Florida College of Medicine. Dr. Saw's present address is the Department of Statistics, College of Arts and Sciences, University of Florida.

ABSTRACT Ogston's (1958) fiber model based on Poisson's distribution function gives the average number of fibers making contact and no contact inside a sphere of radius *r*. The probability of penetration of spherical particles within a fibrous network was derived from the moment generating function

$$E(Z)^A = \exp 4\pi\nu(Z - 1) \int_0^\infty 1\, \alpha(\mu)\mu^2\, d\mu.$$

A is the number of particles that intrude into a sphere of radious r. $\alpha(\mu)$ is the probability that a particle, whose center is μ units away from the origin, intrudes into a sphere of radius r. A has a Poisson distribution with a mean value $E(A) = 4\pi\nu\alpha(\mu)\mu^2\, d\mu$. The theoretical derivation of the distribution function of A gives Ogston's fiber model.

INTRODUCTION

The exclusion of protein from high polymer media, one aspect of protein precipitation which holds many possibilities, remains relatively unexplored in its theoretical aspects. The technique of exclusion has rarely been applied in research on biologically important macromolecules.

Preliminary research using the exclusion technique includes the efficient separation of single- and double-stranded DNA (Alberts, 1967), in a dextran-polyethylene glycol two-phase system (Albertsson, 1962, 1965), crystallization by fractional precipitation of alcohol oxidase with polyethylene glycol (Janssen and Ruelius, 1968), and fractional precipitation of plasma protein with polyethylene glycol (Polson et al., 1964; Iverius and Laurent, 1967; Chun et al., 1967).

Ogston (1958), Ogston and Phelps (1960), and Laurent (1963, 1967 *a*) in independent studies on the exclusion of macromolecules from high polymer media, have each reached the conclusion that exclusion depends on the steric factors operating in a three-component system.

Such a three-component system reflects the interaction of solvent-solute in vivo, in which one kind of solute excludes another in a continuing interaction which regulates the various physiological processes.

Thus, for example, high molecular weight polysaccharides in the extracellular spaces of connective tissue are linked within a structural network of protein fibers to the protein themselves. Laurent (1967) identifies this as a polysaccharides phase separate from other tissue compartments. Globular proteins moving through such a system will interact with the polysaccharides, by the mechanism of steric exclusion, thus affecting their properties and distribution. The three components operating here, then, are the solvent, high molecular weight polysaccharides, and the globular proteins.

Ogston (1958) has considered the nature of the proein fiber network in such a three-component system and the possibility of an object penetrating such a suspension. The theoretical derivation of probability distribution for the number of fiber centers within any sphere of radius r based on a Poisson distribution function is a modification of Ogston's fiber model.

THEORETICAL DERIVATION

I. Probability Distribution for the Number of Fiber Centers (n) Within any Sphere of Radius r

Assumptions. The assumptions on which this derivation is based are similar to those outlined by Ogston in 1958: (1) the probability of n-fiber center inside a sphere of radius r is determined by a Poisson distribution function $P(n; r)$ and (2) the probability that a fiber center occurs inside any increment dv of volume is given by $\nu\, dv$ + (small order of dv), where ν is the average number of fibers per cm^3. Each fiber is considered to be a straight segment of negligible thickness. The moment generating function was applied to Ogston's fiber model to determine the distribution of spaces in the fiber network.

By assumption 2 the probability of one fiber center inside the spherical shell between the sphere within radius r and the sphere within radius $(r + dr)$ equals $4\pi\nu r^2\, dr$. The probability that n fibers have their center within radius r of the origin is given by

$$P(n;r) = \frac{[\lambda(r)]^n e^{-\lambda(r)}}{n!} \tag{1}$$

where $\lambda(r) = \frac{4}{3}\pi\nu r^3$ is the average number of fiber centers within a sphere of radius r.

Let the distances between the origin and fiber centers be denoted as

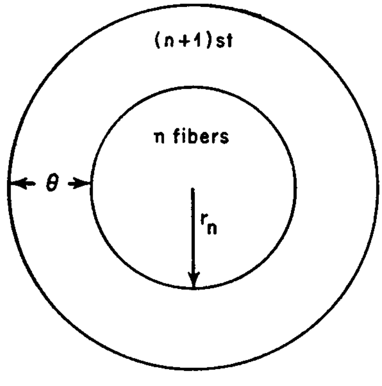

FIGURE 1 Model based on the probability that $(n + 1)$st fiber occurs between r_n and $(r_n + \theta)$ as given by Equation 2.

$$0 < r_1 < r_2 < r_3 \cdots < r_n < \cdots r_{n+1} \cdots$$

From Fig. 1, the probability that the distance between nth and $(n + 1)$st fiber center is less than θ when first n distances are given may be formulated by the following expression:

$$P\{r_{n+1} - r_n \leqq \theta \mid (r_1 \cdots r_n)\} = P\{N(r_n + \theta) - N(r_n) \geqq 1 \mid (r_1 \cdots r_n)\}$$
$$= 1 - P\{N(r_n + \theta) - N(r_n) = 0 \mid (r_1 \cdots r_n)\} \quad (2)$$

where $N(r_n + \theta) - N(r_n)$ represents the random number of fiber centers in the spherical shell. From Equation 2 one determines the probability that $(n + 1)$st fibers occur between r_n and $(r_n + \theta)$, given $r_1 \cdots r_n$. It can be shown that

$$P\{N(r_n + \theta) - N(r_n) = k \mid r_1 \cdots r_n)\} = \frac{[\lambda(r_n + \theta) - \lambda(r_n)]^k e^{-[\lambda(r_n+\theta)-\lambda(r_n)]}}{k!}.$$

From Equation 2

$$P\{(r_{n+1} - r_{n \leq \theta}) \mid (r_1 \cdots r_n)\} = 1 - e^{\lambda(r_n)-\lambda(r_n+\theta)}. \quad (3)$$

Letting $r = (r_n + \theta)$, then define the conditional cumulative distribution function

$$F\{r \mid (r_1 \cdots r_n)\} = P\{r_{n+1} \leqq r \mid (r_1 \cdots r_n)\} = 1 - e^{\lambda(r_n)-\lambda(r)}$$

then the probability density function, $f\{(r_{n+1}) \mid (r_1 \cdots r_n) \mid \}$ is given by derivation with respect to r:

$$\frac{dF\{r \mid (r_1 \cdots r_n)\}}{dr} = f\{r_{n+1} \mid (r_1 \cdots r_n)\} = (4\pi\nu)r_{n+1}^2 e^{\lambda(r_n)-\lambda(r_{n+1})}$$

$$\text{since } \frac{d}{dr}\lambda(r) = \frac{d}{dr}\left(\frac{4}{3}\pi\nu r^3\right) = 4\pi\nu r^2. \quad (4)$$

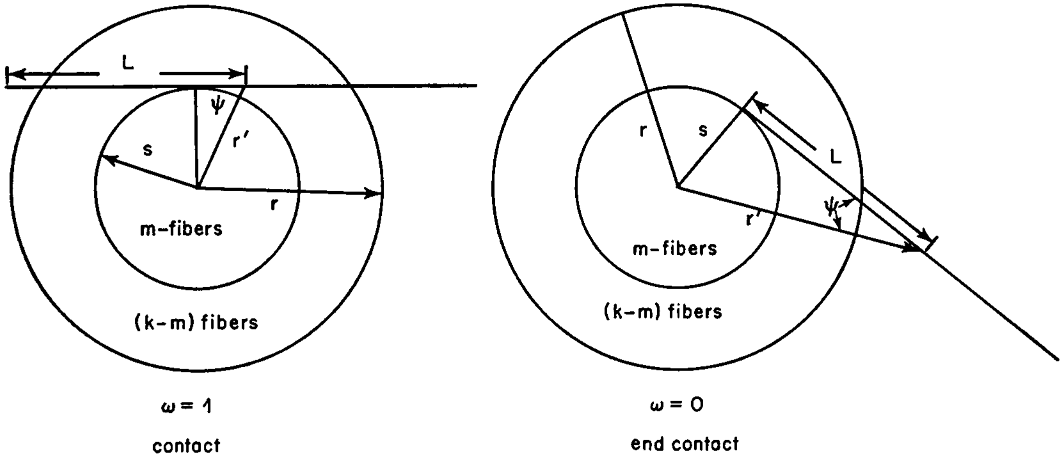

FIGURE 2 Average number of fibers making contact inside a sphere.

The joint probability density function can be evaluated from Equation 4 by applying $n = 1, 2, \cdots i$, then:

$$f\{r_1\} = 4\pi\nu r_1^2 e^{-\lambda(r_1)}$$

$$f\{r_2 \mid (r_1)\} = 4\pi\nu r_2^2 e^{\lambda(r_1)-\lambda(r_2)}$$

$$f\{r_3 \mid (r_1, r_2)\} = 4\pi\nu r_3^2 e^{\lambda(r_2)-\lambda(r_3)}$$

$$\vdots$$

$$\{r_{n+1} \mid (r_1 \cdots r_n)\} = 4\pi\nu r_{n+1}^2 e^{\lambda(r_n)-\lambda(r_{n+1})}$$

$$f(r_1 \cdots r_{n+1}) = f\{r_1\} f\{r_2) \mid (r_1)\} \cdots f\{r_{n+1}) \mid (r_1 \cdots r_n)\}$$

$$= (4\pi\nu)^{n+1}(r_1^2 r_2^2 r_3^2 \cdots r_{n+1}^2) e^{-\lambda(r_{n+1})}. \tag{5}$$

Equation 5 expresses a joint probability density function. Letting the number of fiber centers within radius s as shown in Fig. 2 be given by m and within radius r be given by n:

$$P\{N(s) = m; N(r) = k\} = P\{N(s) = m\} P\{N(r) = n) \mid N(s) = m)\}$$

$$= \frac{[\lambda(s)]^m e^{-\lambda(s)}}{m!} \cdot \frac{[\lambda(r) - \lambda(s)]^{n-m} e^{-[\lambda(r)-\lambda(s)]}}{(n-m)!}$$

then

$$f\{r_1 \cdots r_n \mid N(s) = m; N(r) = n)\} = \frac{f(r_1 \cdots r_n)}{P\{N(s) = m); N(r) = n)\}} \tag{6}$$

$$= \left[\frac{(4\pi\nu)^n (r_1^2 \cdots r_n^2) e^{-\lambda[r(n)]}}{\left(\frac{4}{3}\pi\nu s^3\right)^m \left[\frac{4}{3}\pi\nu(r^3 - s^3)^{n-m}(e^{-\lambda[r(n)]})\right]} \right] [m!(n-m)!]$$

$$= m!(n-m)! \prod_{i=1}^{m} \left(\frac{3r_i^2}{s^3}\right) \prod_{j=m+1}^{n} \left(\frac{3r_j^2}{r^3 - s^3}\right). \qquad (7)$$

Thus, from Equation 7

$$f\{r_1 \cdots r_n) \mid N(s) = 0; N(r) = n\} = n! \prod_{j=1}^{n} \left(\frac{3r_j^2}{r^3}\right). \qquad (8)$$

II. The Average Number of Fibers Making Contact Inside a Sphere (See Fig. 2)

Let spheres of radius s and r be given, let a fiber be located at fixed distance r' from the center and the variable be defined:

$$\omega = \begin{cases} 1, & \text{if the fiber makes contact with the sphere of radius } s \\ 0, & \text{otherwise.} \end{cases}$$

Four possibilities exist:

(a) $s \leqq r' \leqq \sqrt{s^2 + L^2}$, L = half length of fiber

$0 \leqq \psi \leqq \sin^{-1}\frac{s}{r}$,

then $E[\omega] = P\,[\text{contact}] = \int_0^{\sin^{-1} s/r} \sin\psi\, d\psi = 1 - \sqrt{1 - s^2/r'^2}$

(b) $\sqrt{s^2 + L^2} \leqq r' \leqq s + L$

$$0 \leqq \psi \leqq \cos^{-1}\left(\frac{r'^2 + L^2 - s^2}{2r'L}\right) \qquad (9)$$

then $E(\omega) = P\,[\text{contact}] = \int_0^{\cos^{-1}(r'^2+L^2-s^2)/2r'L} \sin\psi\, d\psi = \dfrac{s^2 - (r' - L)^2}{2r'L}$

(c) $r' \leqq s$, then $E(\omega) = P\,[\text{contact}] = 1$

(d) $r' > s + L$, then $E(\omega) = P\,[\text{contact}] = 0$.

Suppose a sphere with radius s is given and n centers lie inside a sphere with given radii of $r_1 \cdots r_n$. Then letting $A(r)$ be the number of these n particles that intrude into a sphere of radius s,

$$A(r) = \omega_{(r_1)} + \omega_{(r_2)} + \omega_{(r_3)} + \cdots + \omega_{(r_n)}$$

and for an arbitrary parameter Z, the moment generating function of $A(r)$ is given by the following expression:

$$E\{Z^{A(r)} \mid (r_1 \cdots r_n); N(r) = n\}$$

$$= \prod_{j=1}^{n} E\{Z^{\omega(r_j)} \mid (r_1 \cdots r_n); N(r) = n\} = \prod_{j=1}^{n} [(1 - \alpha(r_j)Z^0 + \alpha(r_j)Z^1)]$$

$$= \prod_{j=1}^{n} [(1 - \alpha(r_j) + Z\alpha(r_j))] = \prod_{j=1}^{n} [1 + \alpha(r_j)(Z - 1)] \qquad (10)$$

where $[1 - \alpha(r_j)]$ is a probability of no contact and $\alpha(r_j)$ is the probability of contact.

From Equations 8 and 10 one obtains, after simplification:

$$E\{Z^{A(r)}\} = \sum_{n=0}^{\infty} \frac{[\lambda(r)]^n e^{-\lambda(r)}}{n!} \prod_{j=1}^{n} \int_0^r \frac{3r_j^2}{r^3} [1 + \alpha(r_j)(Z - 1)]\, dr_j$$

$$= \sum_{n=0}^{\infty} \frac{[\lambda(r)]^n e^{-\lambda(r)}}{n!} \left\{1 + \frac{3(Z-1)}{r^3} \int_0^r \alpha(\mu)\mu^2\, d\mu\right\}^n$$

$$= \exp\left[\frac{4}{3}\pi\nu r^3 \left\{1 + \frac{3(Z-1)}{r^3} \int_0^r \alpha(\mu)\mu^2\, d\mu - 1\right\}\right]$$

$$= \exp\left[4\pi\nu(Z-1) \int_0^r \alpha(\mu)\mu^2\, d\mu\right].$$

Therefore

$$E(Z^{A(r)}) = \exp\left[4\pi\nu(Z-1) \int_0^r \alpha(\mu)\mu^2\, d\mu\right] \tag{11}$$

and letting r become infinite

$$E(Z^{A}) = \exp\left[4\pi\nu(Z-1) \int_0^\infty \alpha(\mu)\mu^2\, d\mu\right] \tag{11 a}$$

$E(Z^A)$ is the moment generating function for the Poisson distribution with mean of $4\pi\nu \int_0^\infty \alpha(\mu)\mu^2\, d\mu$. Hence

$$E(A) = 4\pi\nu \int_0^\infty \alpha(\mu)\mu^2\, d\mu. \tag{12}$$

Therefore, solution of Equation 12 gives the required distribution of the probability of contact with a given sphere of radius r.

Using the conditions of Equation 9,

$$\int_0^\infty \alpha(\mu)\mu^2\, d\mu = \int_0^s \mu^2\, d\mu + \int_s^{\sqrt{s^2+L^2}} \left[1 - \sqrt{1 - \frac{s^2}{\mu^2}}\right] \mu^2\, d\mu$$

$$+ \int_{\sqrt{s^2+L^2}}^{s+L} \left[\frac{s^2 - (\mu - L)^2}{2\mu L}\right] \mu^2\, d\mu + 0$$

$$= \frac{s^3}{3} + \frac{1}{3}[(s^2 + L^2)^{3/2} - s^3] - \frac{L^3}{3} + \frac{(s^2 - L^2)}{4L}[(s + L)^2 - (s^2 + L^2)]$$

$$- \frac{1}{8L}[(s + L)^4 - (s^2 + L^2)^2] + \frac{1}{3}[(s + L)^3 - (s^2 + L^2)^{3/2}]$$

$$= \frac{1}{2}Ls^2 + \frac{1}{3}s^3 = \frac{1}{6}(2s^3 + 3Ls^2).$$

Thus,

$$P[A = 0] = e^{-4\pi\nu(1/6)(2s^3+3Ls^2)}$$

$$-\frac{dP[A = 0]}{ds} = 4\pi\nu(s^2 + Ls)e^{-4\pi\nu(1/6)(2s^3+3Ls^2)}$$

$$= [4\pi\nu s^2 + 4\pi\nu Ls]e^{-[(2\pi\nu Ls^2+(4/3)\pi\nu s^3)]}. \quad (13)$$

Equation 13 gives the required distribution of the probability that there shall be at least one tangential or end-contact between s and $(s + ds)$ and no contact within s. In fact, $P[A = 0] =$ the probability of no fibers being in contact with a sphere of radius s. This Equation 13 is consistent with the Ogston's fiber model.

III. The Average Number of Spheres Making Contact Inside a Sphere

The probability that n spheres have their centers within radius r of the origin is shown in Fig. 3. The occurrence of a sphere whose center lies within $r + L$ from the origin is shown in Fig. 3. The probability distribution of the number of centers within radius $r + L$ is a Poisson distribution as described in the text. If the distance between origin and nearest sphere is R, then the probability of none within $(r + L)$ can be expressed in the following manner

$$P\{N(r + L) = 0\} = P\{R \geqq (r + L)\} = e^{-(4/3)\pi\nu(r+L)^3}$$

$$-\frac{dP[R \geqq (r + L)]}{dr} = 4\pi\nu(r + L)^2 e^{-(4/3)\pi(r+L)^3}. \quad (14)$$

Equation 14 represents the probability of distance R between origin and nearest sphere. If an arbitrary sphere were located at the origin as indicated in Fig. 3 (as dotted line), the probability distribution for the distance r to its nearest neighbor is similarly given by

$$4\pi\nu(r + 2L)^2 e^{-(4/3)\pi\nu(r+2L)^3}. \quad (15)$$

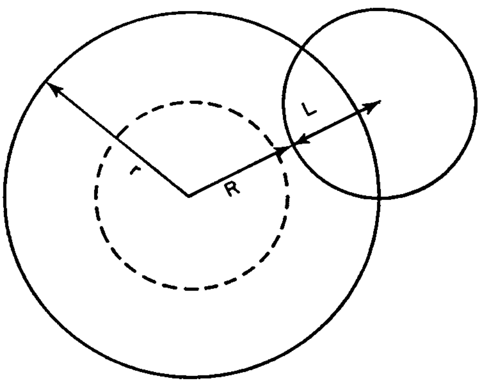

FIGURE 3 Average number of spheres making contact inside a sphere.

IV. The Probability of Penetration of Spherical Particles within Fibers and Spherical Network

From Equation 13, probability that the distance R from point at origin to closest fiber is greater than s is

$$P[R > s] = e^{-[(2\pi\nu L s^2 + (4/3)\pi\nu s^3)]}. \quad (16)$$

Letting $L \gg s$

$$P[R > s] = e^{-(2\pi\nu L s^2)}. \quad (17)$$

DISCUSSION

The probability of penetration of spherical particles within a fibrous network was derived from the moment generating function, the Poisson distribution having a mean value of

$$E(Z)^A = e^{4\pi\nu(Z-1)\int_0^\infty \alpha(\mu)\mu \, d\mu}.$$

This method of derivation yields results which are consistent with those proposed by Ogston from his fiber model (1958).

Ogston and Phelps (1960) used Equation 16, but making the assumption that the spherical particles were penetrating a "string of beads" network, providing that the radii of the beads are greater than the radius of a penetrating sphere. Equation 16 becomes

$$P_{s<R} = e^{-(4/3)\pi(s)^3}, \quad \text{where} \quad r = (r_s + r_b), \frac{4}{3}\pi\nu = \frac{\bar{v}}{r_b^3} W$$

and $W = \dfrac{(4/3)\pi r_b^3 \nu}{\bar{v}} = \dfrac{\text{volume occupied by beads/ml of solute}}{\text{volume}_b/\text{gram}_b}$
$= \text{weight of beads/ml of solute.}$

Thus, the expected excluded volume/weight of beads will be

$$\frac{P_{s<R}}{\text{wt}_b/\text{ml of solute}} = \frac{\left(1 - \frac{1}{K_D}\right)}{\omega}, \quad \text{where}$$

$$\log \; K_D = \left[\frac{\bar{v}}{2.303} \frac{(r_b + r_s)^3}{r_b^3}\right] \omega = J\omega \quad (18)$$

(Ogston and Phelps, 1960).

The results enable one to calculate the length of the beads and diameter of the penetrating sphere.

Laurent's manipulation (1963, 1967) has been effectively used in studies on the exclusion of protein from high polymer media and studies of agarose gel by gel chromatography to determine the radius of the penetrating sphere (Laurent, 1967 *b*). The assumption is made that the radius of the penetrating sphere is greater than the radius of the fiber, these fibers being very thin. The probability based on the moment generating function yields Equation 17. In this case, the length of fiber, $L' = (2\ Lv)$ and the partition coefficient, $K_D = P_{s<R}$. Thus the weight

$$W = \frac{(\pi r_b^2 2L)v}{\bar{v}} = \frac{\pi r_b^2 L'}{\bar{v}}$$

$$\log\ K_D = \left[\frac{\bar{v}}{2.303}\left(\frac{r_b + r_s}{r_b}\right)^2\right] W = fW \qquad (19)$$

where f is obtainable from a plot of log K_D vs. W.

Since the partition coefficient K_D is inversely proportional to the probability of penetration when the radius of the penetrating sphere is less than the radius of the fiber, the coefficient can be used to calculate fiber length as well as the diameter of the penetrating sphere (Equation 18). If a reverse case occurs, then the partition coefficient is directly proportional to K_D (Equation 19).

The utility of our present derivation is in the applicability of the moment generating function to Ogston's fiber model. The moment generating function derivation will yield the required distribution of probability of tangential contact and no contact with a given sphere of radius "*r*".

The validity of Equation 13 is presently being investigated using the known partition function K_D in order to evaluate the volumes of protein excluded from high polymer media.

The conclusion to which one is led by the research results (Laurent, 1967 *a*) is that macromolecules such as proteins and large polysaccharides are unevenly distributed between blood and cellular compartments. Protein molecules may penetrate the intracellular compartments by a gel sieve or exclusion phenomena operating in specific tissue such as the intima of the aorta, connective tissue, and cartilage. Such an abnormal distribution will usually result in an overestimate of the amount of extracellular space present. This conclusion is supported by the fact that polysaccharide–protein complexes are found to resist the passage of solutes such as protein from blood to lymph through tissue spaces, by a process of molecular exclusion (Laurent, 1967 *a*).

This work was supported by National Institutes of Health Research Grant FR-05362-06, 07.

Reprint requests should be addressed to the Department of Biochemistry, University of Florida College of Medicine, Gainesville, Fla. 32601

Received for publication 5 July 1968 and in revised form 24 October 1968.

REFERENCES

ALBERTS, B. M. 1967. *Biochemistry.* **6**:2527.

ALBERTSSON, P. A. 1962. *Arch. Biochem. Biophys.* **98**(Suppl. 1):264.

ALBERTSSON, P. A. 1965. *Biochim. Biophys. Acta.* **103**:1.

CHUN, P. W., M. FRIED, and E. F. ELLIS. 1967. *Anal. Biochem.* **19**:481.

IVERIUS, P. H., and T. C. LAURENT. 1967. *Biochim. Biophys. Acta.* **133**:371.

JANSSEN, F. W., and H. W. RUELIUS. 1968. *Biochim. Biophys. Acta.* **151**:330.

LAURENT, T. C. 1963. *Biochem. J.* **89**:253.

LAURENT, T. C. 1967 *a*. The Chemical Physiology of Mucopolysaccharides, Little, Brown and Co., Boston, Mass. In press.

LAURENT, T. C. 1967 *b*. *Biochim. Biophys. Acta.* **136**:199.

OGSTON, A. G. 1958. *Trans. Faraday Soc.* **54**:1754.

OGSTON, A. G., and C. F. PHELPS. 1960. *Biochem. J.* **78**:827.

POLSON, A., G. M. POTGIETER, J. F. LARGIER, G. E. F. MEARS, and E. J. JOURHERT. 1964. *Biochim. Biophys. Acta.* **82**:463.