

# A MODEL FOR THE SHAPE OF THE ERYTHROCYTES IN BIOLOGY

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## MODEL OF THE EQUILIBRIUM OF THE MEMBRANE IN THE SHAPE OF THE ERYTHROCYTES IN MAN AND ANIMALS

### 0.1. *Introduction*

In number 110 of this Bulletin was published a study (20) of the human erythrocyte where a model of a toral surface of circular section was proposed, which was deduced using a formula of equilibrium of the membrane. Assuming that in the mammals, birds and reptiles, the shape of the erythrocyte varies —some have nucleus— it seemed interesting to try to study the possibility of a general model which could be applied to the different cases, having a base in the equilibrium of the membrane. The results of this study are given in the present work, together with the necessary formulae as model which seems to adapt itself to the different cases.

In order to facilitate the reading, the study has been divided in five parts:

- 1) General idea, examples and uses of Tables.
- 2) The necessary formulae and the results.
- 3) The test made to check the equilibrium of the adapted membrane, and some Tables.
- 4) Historical summary.
- 5) Observations.

## PART I

### GENERAL EXPOSITION

#### 1.1. *General shape adopted* (Fig. 1)

It was supposed, as in the first study published, the equilibrium of a membrane of rotation OCB, submitted to a presión  $p$  from inwards to outwards, so that the tensions in it in the sense of its perimeter in the vertical section as in the rotation circles be equal; from a point C up to 0 is supposed a charge working parallel to the axis  $yy$ , which equilibrates the reaction from  $p$  transmitted from the membrane; the point C varies in different cases. From estimate calculation, making use of the measurements of (11) it was evident that a charge of hyperbolic distribution between 0 and C resulted convenient; it is possible that it is, in fact, a combination of function  $y = Ce^{AX}$ , as these appear in nearly all natural phenomenonons.

#### 1.2. *Formulae for the membrane*

According to the theory which was adopted in the work published previously, the formula which governs for the curve of the membrane, taking as coordinates the distance from the normal of the point to the axis YY is

$$\text{Sen } \varnothing = \frac{Ar^2 + B}{2}$$

knowing the values of  $r$  y from the angle to two points of the curve, the coefficient A and B can be calculated.

For the part of the curve between 0 and C, it resulted more convenient

$$\text{sen } \varnothing = Cr + D$$

the coefficients of which are calculated beginning from the final point C and from the point 0 taking the angle there zero or very near zero.

The formulas were transformed to cartesian coordinates. With the aid of an electronic machine the curves for the erythrocytes were evaluated from 5 to 3 unities (microms) which are given in Table 1.

*Example:* One draws the erythrocyte of radio 4 for diverse values of  $r_1$  (Fig. 1-2) with a curve of hyperbolical transition down to 0. It can be seen that for  $r_1 = 2,50$ ,  $r_2 = 2,00$  it is similar to the human erythrocyte. If one diminishes  $r_1$  the shape approximates that of birds and reptiles; it can also be observed that for  $r_1$  nearing zero the shape resembles an

ellipsoid of revolution 3 which sometimes appears when the erythrocytes are subjected to a negative osmotic pression.

*Tests:* In order to make proofs of the equilibrium of the membrane with a central uniform charge or of other type, tests were made with the device which can be seen in the photos 1 and 2. The results are considered to be in accord with the adopted theory.

### 1.3. *Erythrocytes in other animals*

In mammals the erythrocytes as in man have no nucleus and the prevailing shape is similar; the elliptical shape predominates in some of them. In the other species of animals the erythrocytes are nucleate.

In the following Table is given approximately the principal dimensions of the erythrocytes and also its number in  $\text{mm}^3$ .

TABLE 1

| <i>TYPE</i> | <i>SHAPE</i>                   | <i>NUCLEUS</i> | <i>DIAMETER<br/>MICRONS</i> | <i>NUMBER<br/><math>\text{mm}^3</math></i> |
|-------------|--------------------------------|----------------|-----------------------------|--------------------------------------------|
| MAMMALS     | CIRCULAR<br>SOME<br>ELLIPTICAL | HAVE NONE      | 4-8                         | $5 \cdot 10^6$ - $8 \cdot 10^6$            |
| FISHES      | ELLIPTICAL                     | HAVE           | MIN. 7-10<br>MAX. 10-20     | $5 \cdot 10^5$ - $2 \cdot 10^6$            |
| ANPHIBIANS  | ELLIPTICAL                     | HAVE           | MIN. 18-36<br>MAX. 19-65    | $2 \cdot 10^5$ - $8 \cdot 10^5$            |
| REPTILES    | ELLIPTICAL                     | HAVE           | MIN. 5-7,5<br>MAX. 13-15    | $5 \cdot 10^5$ - $1 \cdot 10^6$            |
| BIRDS       | ELLIPTICAL                     | HAVE           | MIN. 6-10<br>MAX. 10-15     | $3 \cdot 10^6$ - $4 \cdot 10^6$            |

### 1.4. *Possible experiments in biophysics*

From the study which was made, I consider that could be investigated:

- 1) The characteristic mechanics of the remains of erythrocytes which are to be found in the blood and in the reticuloendoteical tissue with the object of comparing them with those of the animal erythrocyte. It is to be hoped that the membrane subjected to repeated variable efforts of tension will weaken with time; this would explain to a great extent the rupture in the average life of the erythrocyte (approximately 120 days).

- 2) To determine the central adherence of the membrane: this adherence will be proportional to the number of erythrocytes which pass to the spherical shape in variable solutions of osmolarity. A way should be followed similar to the way to obtain the index of fragility of the erythrocyte, but the solutions will have to be taken wider apart for those used for this one. The index of central adherence should be fixed for the case of 50% of changes of the erythrocytes to the spherical shape. There after could the index be obtained in diverse anormal states of the blood in order to conclude whether or not it can have any application in medical analysis.

## PART II THEORY

### 2.1. *Chief membrane of revolution*

The theory exposed in (20) is repeated here and more extension is given to it.

One takes the equilibrium of a surface of revolution submitted to an uniform pression  $p$  from inwards to outwards, so that the tensions in the elements in the generating curve, in the tangent  $y$  and to the circle perpendicular to it with radio  $r$  are equals.

That is  $YY$  the rotation axis

$C$  a point of the curve in the plan

$CD$  the normal in the curvature in  $C$

$r_1$  radio of the curvature

$r_2 = CD$

$r$  the radio of rotation

$\emptyset$  the angle of the normal and the axis

According to the theory of the membranes of rotation (2) we have

$$\frac{6\varphi}{r_2} + \frac{6r}{r_1} = -p \quad (\alpha)$$

As we selected  $6\varphi = 6r = C$  constant

gives

$$\frac{1}{r_2} + \frac{1}{r_1} = \frac{p}{C} = K \quad (1)$$

we pass the formula in function of 2.  
we have

$$r_2 = \frac{r}{\text{sen } \varphi}$$

The radio of the curvature comes to be

$$r_1 = \frac{dr}{d\varphi \cdot \cos \varphi}$$

replaced it results

$$\frac{\text{sen } \varphi}{r} + \frac{d\varphi \cos \varphi}{dr} = K \quad (2)$$

$$\text{sen}\varphi \cdot dr + r \cos\varphi \cdot d\varphi = K \text{ } r dr$$

The first member is d (r senφ)

then we can write

$$d (r \text{ sen}\varphi) = K r dr \quad (2-1)$$

Integrating it results

$$r \text{ sen}\varphi = \frac{K r^2}{2} + B \text{ con } \frac{K}{2} = A$$

$$\text{sen}\varphi = \frac{A r^2 + 2B}{r} \quad (3)$$

which is the formula which shall be used.

## 2.2. Part between A and C

It will be studied with different types of charge between A and O, in order to see which is the most acceptable.

## 2.3. General formulae

1) p

That is  $q = F(r)$  (fig. 2-2)

The area of the q between 0 and C will be

$$Q = \int_0^{r_2} f(r) \text{ } dr \quad (4)$$

The center of gravity of the area comes to be

$$d = \frac{\int_0^{r_2} f(r) r dr}{\int_0^{r_2} f(r) dr}$$

This total charge  $p$  in the rotational surface of 0 to  $C$  is from the theorem of Pappus.

$$P = 2\pi Q d = 2\pi \frac{\int_0^{r_2} f(r) \int_0^{r_2} r dr (r) dr}{\int_0^{r_2} f(r) dr}$$

$$= 2\pi \int_0^{r_2} f(r) r dr \quad (5)$$

#### 2.4. Tensions in $C$

Comes to be from

$$6\varphi = 6r = \frac{P}{2rr \sin \varphi}$$

and of (5)

$$6\varphi = \frac{\int_0^{r_2} f(r) r dr}{r \sin \varphi} \quad (6)$$

#### 2.5. Normal Component of $q$

In an area of area 1 of membrane  $OC$  see (fig. 2-3) it produces, as vertical component  $y \cos \varphi$  as a normal component  $y \cos^2 \varphi = f(r) \cos^2 \varphi$  with  $q = f(r)$

In accordance with the formula (2), transformed to (2-A), it must have

$$\frac{d(r \operatorname{sen} \varphi)}{r dr} = \frac{\zeta(f(r) \operatorname{sen}^2 \varphi)}{M} \quad (7)$$

and of (6)

$$\frac{d(r \operatorname{sen} \varphi)}{r dr} = \frac{M f(r) \cos^2 \varphi}{\int_0^{r^2} f(r) r dr} \times \frac{1}{C} \quad (7A)$$

$$\frac{d(r \operatorname{sen} \varphi)}{r \operatorname{sen} \varphi} = \frac{K f(r) \cdot r dr}{\int_0^{r^2} f(r) dr} \quad (7-A)$$

and of (7)

$$M = \text{Constant}$$

$$\frac{M \cos^2 \varphi}{C} = K$$

$$\frac{d(r \operatorname{sen} \varphi)}{r \operatorname{sen} \varphi} = \frac{K f(r) \cdot r dr}{\int_0^{r^2} f(r) dr} \quad (7-A)$$

The numerator of the 2nd member is the derivative of the denominator integrated

$$L r \operatorname{sen} \varphi = L K \int_0^{r^2} f(r) r dr$$

$$r \operatorname{sen} \varphi = K \int_0^{r^2} f(r) r dr + A \quad (8)$$

which will be the formula used

EXAMPLE:

a) *Uniform charge* (fig. 2-4)

$$f(r) = q$$

$$r \operatorname{sen} \varphi = K \int_0^r q r dr + A$$

$$\text{sen}\varphi = \frac{M r^2 + A}{r}$$

$$\frac{Kq}{2} = M$$

Taking  $\text{sen}\varphi = 0$  at  $0$   $\text{sen}\varphi = Mr$

b) *Trapezoidal charge* (fig. 2-5)

Applying

$$f(r) = \frac{q_0 - q_1}{r_1} \cdot r + q_1$$

$$f(r) = Kr + q_1$$

$$r \text{sen}\varphi = Kf(Kr + q_1) \quad (10)$$

$$\text{sen}\varphi = \frac{Cr^2 + Dr^3 + A}{r}$$

$$\text{sen}\varphi = Cr + Dr^2 \quad (10-A)$$

c) *Exponential charge*

$$f(r) = Ae^{Br}$$

$$r \text{sen}\varphi = A \int e^{Br} r dr = \frac{A}{B} e^{Br} r - \frac{A}{B} \int e^{Br} dr$$

$$r \text{sen}\varphi = \frac{A}{B} r e^{Br} - \frac{A}{B^2} e^{Br} + D$$

$$\frac{A}{B} = M$$

$$\frac{A}{B^2} = N$$

$$\text{sen}\varphi = e^{\text{Br}} \frac{(rM + N) + D}{r} \quad (11)$$

$$\text{sen}\varphi = e^{\text{Br}} \left( M + \frac{N}{r} \right)$$

d) *Parabolic charge*

$$f(r) = Ar^2 + B$$

$$r \text{sen}\varphi = f(Ar^2 + B) r dr$$

$$r \text{sen}\varphi = \frac{Ar^4}{4} + \frac{Br^2}{2} + E \quad (12)$$

$$\text{sen}\varphi = \frac{Mr^4 + Nr^2 + E}{r} \quad (12-A)$$

e) *Hyperbolic charge*

$$fr = \frac{A}{r} + B$$

$$r \text{sen}\varphi = \int \left( \frac{A}{r} + B \right) r dr + \text{sen}\varphi = Ar + \frac{Br^2}{2} + D$$

$$\text{sen}\varphi = \frac{Ar + E\sqrt{2} + D}{r} \quad (13)$$

Taking  $\text{sen}\varphi = 0$  in 0

$$\text{sen}\varphi = A + \frac{E}{r} \quad (13-A)$$

## 2.6 Formulae in cartesian coordinates

For the drawing of the curvey it was found more convenient to use the cartesian coordinates taking the value of  $\frac{dy}{dx}$ , deducting from the preceding formulae. (Fig. 2-6).

In systems of coordinates x, and in a point D we have (fig. 2-6)

$$\frac{dy}{dx} = \operatorname{tg} \varnothing \quad \varnothing = 180^\circ - \phi$$

$$\operatorname{tg} \varnothing = \frac{\operatorname{sen} \varnothing}{\sqrt{1 - \cos^2 \varnothing}} = -\operatorname{tg} \phi = \frac{dy}{dx}$$

In the preceding formulae we have found  $\operatorname{sen} \varnothing = f(r)$ , with  $x=r$ .

$$\frac{dy}{dx} = \frac{f(r) \, dr}{\sqrt{1 - (fr)^2}} \quad (14)$$

For example, in the case of the curve (3)

They give

$$\begin{aligned} \frac{dy}{dx} &= \frac{Ar^2 + B}{r} \\ \frac{dy}{dr} &= \frac{\frac{Ar^2 + B}{r}}{\sqrt{1 - \frac{(Ar^2 + B)^2}{r^2}}} = \\ &= \frac{Ar^2 + B}{\sqrt{1 - \frac{(Ar^2 + B)^2}{r^2}}} \quad x = r \\ y &= \int \frac{(Ar^2 + B) \, dr}{\sqrt{r^2 - (Ar^2 + B)^2}} \quad (15) \end{aligned}$$

this formula did not result integrable by the means of the usual functions, (78-79) and for its valuation, the integration by areas of the tangents was used, taking trapezoidal areas for the following examples; for the tables 1 calculation with electronic machine was used (9).

*Note:* The tangential component is  $f(r) \cos\phi \sin\phi$ ; with a Poisson's modulus approximately equal 1 (is a characteristic of the membrane's material), the effect of this component will be very small and was not considered.

### 2.7. Example 1

In order to study how the supposed curves were adapted to the real shapes of the erythrocyte with a base to the drawing N° 4 of (11) which we consider ought to approach the real mediations given there (fig. 2-7).

We took

$$\begin{aligned} r_0 &= 8.20 & r_1 &= 5.50 \\ r_2 &= 4.25 \end{aligned}$$

of the formula (15)  
for the connection

we have

$$\operatorname{tg}\phi = \frac{Ar^2 + B}{\sqrt{r^2 - (Ar^2 + B)^2}}$$

For

$$r_0 = 0 \quad 0 = 5.50^2 A + B$$

$$8.20^2 = A \cdot 8.20^2 + B$$

por

$$r = 8.20 \quad \phi = 90^\circ$$

result

$$A = \frac{8.20^2}{8.20^2 - 5.50^2} = 0.22168$$

$$B = 5.50^2 \times 0.22165 = 6.7058$$

which gives the data of the table 2 between 8,20 and 8,00, divisions were made of 0,0025 and 0,05 in order to be able to appreciate the variations.

TABLE 2

| $r$   | $tg \emptyset$ | $\Delta y$ | $y$   |
|-------|----------------|------------|-------|
| 8,19  | 10,581         | 0,150      |       |
| 8,115 | 7,82579        | 0,124      |       |
| 8,150 | 5,50345        | 0,167      |       |
| 8,10  | 3,83927        | 0,233      |       |
| 8,05  | 3,09299        | 0,173      |       |
| 8,00  | 2,64925        | 0,164      | 1,018 |
| 7,75  | 1,63258        | 0,534      | 1,549 |
| 7,50  | 1,20100        | 0,354      | 1,896 |
| 7,25  | 0,93310        | 0,268      | 2,164 |
| 7,00  | 0,73797        | 0,209      | 2,373 |
| 6,75  | 0,58180        | 0,165      | 2,538 |
| 6,50  | 0,44850        | 0,129      | 2,667 |
| 6,25  | 0,36539        | 0,101      | 2,768 |
| 6,00  | 0,21740        | 0,072      | 2,840 |
| 5,75  | 0,10910        | 0,04       | 2,801 |
| 5,50  | —0—            | 0,01       | 2,891 |
| 5,25  | —0,11420       | —0,0       | 2,891 |
| 5,00  | —0,23933       | —0,02      | 2,871 |
| 4,75  | —0,35613       | —0,070     | 2,801 |
| 4,50  | —0,56606       | —0,115     | 2,696 |
| 4,25  | —0,82350       | —0,174     | 2,529 |
| 4,00  | —1,28734       | —0,264     | 2,244 |
| 3,75  | —3,29545       | —0,573     | 1,671 |

### 2.8. *Hyperbolic curve of connection*

For  $r_2 = 4,50$

$$\text{sen } \emptyset = 0 \quad r = 0$$

$$\text{The formula } \text{sen } \emptyset = \frac{Ar^2 + Br + C}{r}$$

$$\text{For } C = 0 \quad \text{sen } \emptyset = Ar + B$$

We have

$$\begin{array}{ll} 0.01 = 0.4 DA + B & r = 0.40 \quad \emptyset = 0.60^\circ \\ 0.42961 = 4.50 A + B & r = 4.50 \quad \emptyset = 29.5^\circ \end{array}$$

TABLE 3

For

| $r$  | $\text{sen } \varnothing$ | $\text{tg } \varnothing$ | $\Delta y$ | $y$   |
|------|---------------------------|--------------------------|------------|-------|
| 4,50 | 0,49261                   | 0,56606                  | 0          | 2,696 |
| 4,25 | 0,46314                   | 0,52257                  | 0,132      | 2,564 |
| 4,00 | 0,43372                   | 0,48135                  | 0,125      | 2,439 |
| 3,75 | 0,40429                   | 0,44203                  | 0,115      | 2,324 |
| 3,50 | 0,37487                   | 0,40436                  | 0,106      | 2,218 |
| 3,25 | 0,34544                   | 0,36811                  | 0,096      | 2,122 |
| 3,00 | 0,31602                   | 0,33309                  | 0,087      | 2,035 |
| 2,75 | 0,28659                   | 0,29914                  | 0,079      | 1,956 |
| 2,50 | 0,25717                   | 0,26612                  | 0,070      | 1,886 |
| 2,25 | 0,22774                   | 0,23389                  | 0,060      | 1,826 |
| 2,00 | 0,19832                   | 0,20233                  | 0,054      | 1,772 |
| 1,75 | 0,16889                   | 0,17136                  | 0,047      | 1,725 |
| 1,50 | —0,13947                  | 0,14085                  | 0,039      | 1,686 |
| 1,25 | —0,11004                  | 0,11072                  | 0,031      | 1,655 |
| 1,00 | —0,08062                  | 0,08088                  | 0,015      | 1,640 |
| 0,50 | —0,05885                  | 0,05895                  | 0,011      | 1,630 |
| 0    | —0,03708                  | 0,03710                  | 0,01       | 1,620 |

TABLE 3-A

| PUNTO | SEN             | TANG           | A          | ORD.  |
|-------|-----------------|----------------|------------|-------|
| $r$   | $sen \emptyset$ | $tg \emptyset$ | $\Delta y$ | $y$   |
| 4,50  | 0,49261         | 0,56606        | 0          | 2,736 |
| 4,25  | 0,46275         | 0,52200        | 0,136      | 2,600 |
| 4,00  | 0,43590         | 0,48433        | 0,126      | 2,474 |
| 3,75  | 0,40752         | 0,44652        | 0,116      | 2,358 |
| 3,50  | 0,37195         | 0,40069        | 0,106      | 2,252 |
| 3,25  | 0,35075         | 0,37457        | 0,097      | 2,155 |
| 3,00  | 0,32224         | 0,34639        | 0,089      | 2,066 |
| 2,75  | 0,29402         | 0,30766        | 0,081      | 1,985 |
| 2,50  | 0,26565         | 0,27555        | 0,073      | 1,912 |
| 2,25  | 0,23273         | 0,23930        | 0,064      | 1,848 |
| 2,00  | 0,20890         | 0,21361        | 0,056      | 1,792 |
| 1,75  | 0,18052         | 0,18354        | 0,050      | 1,842 |
| 1,50  | 0,15215         | 0,15394        | 0,042      | 1,700 |
| 1,25  | 0,12377         | 0,12479        | 0,035      | 1,665 |
| 1,00  | 0,04540         | 0,09583        | 0,027      | 1,648 |
| 0,75  | 0,06702         | 0,06772        | 0,020      | 1,628 |
| 0,50  | 0,03860         | 0,03860        | 0,013      | 1,615 |
| 0,40  | 0               | 0              | 0          | "     |
| 0,25  | —0,01027        | —0,01027       | 0          | "     |
| 0     | —0,01810        | —0,01810       | —0,005     | 1,62  |

In order to obtain a better approximation to the curve taken as a guide, an electronic calculation was made varying  $r_1$  from 0,10 in 0,10, and it was found that for  $r_1 = 5,40$  the curve nearly coincides, as indicates Table 4.

A curve of hyperbolic connection was taken, beginning with  $r = 4,50$ , and good results were obtained.

### 2.9. Other examples

A study was made of curves with  $r_0 = 7,50$   $r_1 = 2$  and  $r_0 = 7,50$   $r_1 = 0,50$  which have approximately the same longitude as that of example 1; the curves obtained are seen in figure 2-8. We observe that the model of the erythrocyte considered in this way passes to similar forms as have those of the experiences in which the erythrocytes are put to osmotic pressures in hypotonic liquids (5) and also to the shape of the erythrocytes of birds and reptiles; therefore it has been considered that the model presented can be used as a base of explanation to the different forms of erythrocytes in biology.

TABLE 4

| $r$  | MEASURES | CALC. |
|------|----------|-------|
| 8,20 | 0        | 0     |
| 8,00 | 1,50     | 1,50  |
| 7,50 | 2,10     | 2,12  |
| 7,00 | 2,40     | 2,44  |
| 6,50 | 2,80     | 2,74  |
| 6,00 | 2,90     | 2,93  |
| 5,50 | 2,95     | 2,99  |
| 5,00 | 2,90     | 2,97  |
| 4,50 | 2,80     | 2,80  |
| 4,00 | 2,65     | 2,46  |

### 2.10. Tables for the ordinary erythrocytes

The Tables N° 1 were made, as indicated in the first part. The tables can be used, for example that of  $r_0 = 5,00$  to determine any other element of radio, it suffices to take the relation  $r_1/r_0$  and to use for instance the  $r_0 = 5,00$  with the same relation, multiplying all the ordinates by that relation.

### 2.11. Approximate formula for the principal curve

The formula given in (5) for the oval of Cassini is

$$y = B [(C^4 + A^2r^2)^{1/2} - \Lambda^2r^2]^{1/2} \quad (16)$$

it was taken the example already studied with  $r_0 = 8,20$ , and the oval 5,50 with Tg null was made to pass in that point: It was obtained

$$C^2 = 938.74$$

$$A^2 = 36.68$$

$$4A^2 = 146.72$$

and were calculated the ordinates which are given in Table N° 4 in comparison with the electronic calculation; the maximum ordinate of the electronic calculation gives 2,88; that of the oval gives 2,547 taking  $B = \frac{2,88}{2,547}$

$= 1,13$ , and multiplying the ordinates of the oval, we obtain the good results which are given as far as  $r = 4,50$ ; the electronic calculated curve is annulled for  $r = 3,20$ ; the oval for  $r = 2,50$ .

TABLE 5

| <i>r</i> | <i>elect.</i><br><i>y</i> | <i>oval</i><br><i>y<sub>1</sub></i> | <i>y<sub>2</sub> x 1.13</i> |
|----------|---------------------------|-------------------------------------|-----------------------------|
| 8,20     | 0                         | 0                                   | 0                           |
| 8,00     | 1,04                      | 0,933                               | 1,054                       |
| 7,50     | 1,898                     | 1,714                               | 1,936                       |
| 7,00     | 2,37                      | 2,114                               | 2,388                       |
| 6,50     | 2,693                     | 2,362                               | 2,669                       |
| 6,00     | 2,836                     | 2,488                               | 2,811                       |
| 5,50     | 2,88                      | 2,547                               | 2,878                       |
| 5,00     | 2,832                     | 2,488                               | 2,811                       |
| 4,50     | 2,626                     | 2,364                               | 2,671                       |
| 4,00     | 2,209                     | 2,159                               | 2,434                       |
| 3,50     |                           | 1,830                               |                             |
| 3,00     |                           | 1,36                                |                             |
| 2,50     |                           | 0                                   |                             |
| 2,00     |                           |                                     |                             |
| 1,90     |                           |                                     |                             |

### 2.12. Calculation of tension in the membrane

In accordance with the initial theory we have

$$\delta r = \delta \varphi$$

$$\frac{-p}{\delta r} = K$$

But we made  $\frac{K}{2} = A$  see (2-1)

Then  $K = 2A$

$$\frac{-p}{\delta r} = 2A \quad \delta r = \delta \varphi = \frac{p}{2A} \quad (17)$$

For the example

$$A = 0,22168$$

$$\delta \varphi = \frac{p}{2 \times 0,22168} = 2,25 p$$

Immediately we deduce, given p  
 In the case of a reticulocyte with a spherical shape

$$\delta\varphi = \delta r = \frac{pr_0}{e} = \frac{p \times 8.20}{4} = 4,10 p$$

### 2.13. Curve of transition

With the uniform distribution of charge adopted we have:

$$R = 9 f(r) = q$$

The reaction in the circle of connection of the membrane of the erythrocyte will be:

$$R = q (r_0^2 - r_1^2)$$

for lineal unity in the circle of connection of the two curves, it will be:

$$P = \frac{q (r_0^2 - r_1^2) \Pi}{2\Pi r_2} = \frac{r_0^2 - r_1^2}{2r_2} \quad (18-1)$$

We know the angle  $\varnothing$  in that circle, then there

$$\delta x = \delta y = \frac{q (r_0^2 - r_1^2)}{2 r \text{ sen } \varnothing}$$

The adapted distribution for the vertical charge between  $\varnothing$  and  $r_2$  is better considered hyperbolic

$$q = \left( \frac{C}{r} + B \right) K$$

According to (5)

$$P = 2 \Pi K \int_0^r \left( \frac{C}{r} + B \right) r dr = 2\Pi K \left( Cr + \frac{Br^2}{2} \right) \quad (18-2)$$

This allows us to determine K, comparing (18-1) and (18-2)

$$K = \frac{q r_0^2 - r_1^2}{2r \left( Cr + \frac{Br^2}{2} \right)} \quad (18-3)$$

C and B being known through the tangential conditions, as has been seen previously.

(18)

In the point 0 there is a point theoretically to the infinity; it will be convenient to calculate up to 0,40  $r_0$  and then take up uniform in that radio.

#### 2.14. Longitude of the principal curve

We have

$$\begin{aligned}
 dL &= dr \sqrt{1 + \frac{(dy)^2}{(dx)^2}} \\
 dL &= dr \frac{1 + (Ar^2 + B)^2}{\sqrt{1 + \frac{(Ar^2 + B)^2}{r^2 - (Ar^2 + B)^2}}} = \\
 &= \frac{r dr}{\sqrt{-Ar^4 + r^2(1-2AB) - B^2}}
 \end{aligned} \tag{19}$$

Making

$$\begin{aligned}
 t &= r^2 & dt &= 2r dr \\
 dr &= \frac{dt}{2r}
 \end{aligned}$$

$$dL \frac{1}{2} = \frac{dt}{\sqrt{-At^2 + t(1-2AB) - B^2}} \tag{19-A}$$

The tables of integrals (9) with X give  
 $(X = ar^2 + br + C)$

$$L = \int_{a_1}^{a_2} \frac{dt}{\sqrt{t}} \left| \frac{1}{\sqrt{ta}} \operatorname{arc} \operatorname{sen} \frac{2at + b}{\sqrt{\Delta}} \right.$$

$$\begin{aligned}
 \Delta &= 1 - 4ab \\
 \Delta &= 1 - 4AB
 \end{aligned}$$

or that is

$$L = \frac{a_2}{a_1} \left| \begin{array}{l} -1 \\ 2A \end{array} \right. = \frac{-1}{2A} \text{ arc sen } \frac{-2(At)^2 + (1-2AB)}{\sqrt{1-4AB}} \quad (20)$$

or replacing t for r

$$L = \frac{a_2}{a_1} \left| \begin{array}{l} 1 \\ 2A \end{array} \right. = \frac{1}{2A} \text{ arc sen } \frac{-2(Ar^2) + (1-2AB)}{\sqrt{1-4AB}}$$

*Example:* Taking the example

$$\begin{aligned} r_0 &= 8.20 \\ A &= 0.22168 \\ B &= 6.7058 \\ r_1 &= 5.50 \end{aligned} \quad (21)$$

$$\begin{aligned} L &= \left| \begin{array}{l} 51.5 \\ 1 \\ 8.2 \quad 0.44336 \end{array} \right. \text{ arc} \\ &\text{sen } \frac{-2(0.22168 \times 8.2)^2 + (1+297308)}{\sqrt{1+5.94616}} \\ &= + \frac{1}{L \cdot 0.44336} \\ &\text{arc sen } \frac{-2(0.22168 \cdot 5.50)^2 + (1+2.97308)^2}{\sqrt{1+5.94616}} = 6,21 \end{aligned}$$

Give with less because it begins with  $r_0 = 8,20$   
Until 4,50

$$\begin{aligned} L &= \frac{-1}{0.44238} \text{ arc} \\ &\text{sen } \frac{(0.22168 \times 4.50)^2 + 3.97308}{2.65555} \\ L &= 5.476 \end{aligned}$$

### 2.15. Auxiliary curve

Of usage

$$\frac{dy}{dx} = \frac{Ar+B}{\sqrt{1-(Ar+B)^2}}$$

Then

$$dL = \sqrt{1 + \frac{(Ar+B)^2}{1-(Ar+B)^2}}$$

$$dr = \frac{dr}{\sqrt{1-(Ar+B)^2}}$$

Making

$$Ar+B = t \quad A dr = dt \quad dr = \frac{dt}{A}$$

$$L = \frac{1}{A} \text{arc sen } t.$$

We have:

$$dL = \frac{1}{A} \frac{dt}{\sqrt{1-t^2}}$$

Example for the curve of example 1

$$L = \frac{1}{A} \left| \begin{array}{l} t_2 \\ \text{arc sen } t \\ t_1 \end{array} \right| = \frac{1}{A} \left| \begin{array}{l} r_2 \\ \text{arc . sen } (Ar+B) \\ r_1 \end{array} \right|$$

between 4,50 and 0 the longitude will be

$$L = \frac{1}{0.11770} \text{arc } (0.11770 \times 4.50 - 0.0370)$$

$$L = 4.69$$

### 2.16. Membranes of elipsoidal surface

In fig. 2-9, we have in the horizontal plane in the nucleus, the ellipse of axes a and b with the axes of coordinates xx and tt, (yy is conserved

for the vertical axe). The surface can be considered as formed by ellipses, in horizontal plans, comparing it with the surface of rotation. Fig. 1-2: conserving the constants  $x$  and  $y$ , varying only  $t$ , the ellipsoidal surface could be considered as proceeding from the rotational and be designated as related to it. In this case, and following that which has been studied about the surface of revolution, it will be proceeded as follows:

- 1) The curve of the section OX is determined as was studied before; it suffices to know the points of tangency  $r_0$  and 1.
- 2) For a section of any angle one divides OD in a number of parts equal to those taken in OA, 1, 2, 3 . . .  $n-1$ ,  $n$ ; and the ordinates of OA are brought to OD in the corresponding divisions; we will have the  $y_1, y_2 \dots y_n -1$ , and  $n$  of OD.
- 3) For OB one draws, as OA and proceeds as in OD.

### 2.17. Tensions in the chief membrane

Now the tensions are not constant as they were in the surface of revolution.

For in accordance with (72) related shells,  $b$ , changing the circles of revolution to ellipse, we have with (1) the new values.

$$(22) \quad ds'y = ds\varphi \sqrt{n^2 \cos^2 \varnothing \cos^2 \varphi + \text{sen}^2 \varnothing \cos^2 \varphi + \text{sen}^2 \varnothing}$$

$$ds'e = ds\epsilon \sqrt{\text{sen}^2 \varnothing + n^2 \cos^2 \varnothing}$$

$n$  is the relation of the original  $y$  in the surface of revolution to the new  $y$  in the ellipsoidal surface.

The pressions in the surface of revolution, which we suppose in the unity of surface come given for

$$(23) \quad N^1\varphi = N\varphi \sqrt{\frac{n^2 \text{sen}^2 \varnothing \cos^2 \varphi + \text{sen}^2 \varnothing \cos^2 \varphi + \text{sen} \varphi^2}{n^2 \varnothing + \cos^2 \varnothing}}$$

$$N^1\varnothing = N\varnothing \sqrt{\frac{\text{sen}^2 \varnothing + n^2 \cos^2 \varnothing}{n^2 \cos^2 \varphi \text{sen}^2 \varnothing + \cos^2 \varphi \text{sen}^2 + \text{sen}^2 \varnothing}}$$

Remembering that in the surface of revolution  $N_y = N_0$  (in the surface unity) and we shall call it  $T$ , we can obtain the charges for lineal unity by dividing the (2) by the longitudes.

We have:

$$T'\varphi = \frac{T\varphi}{\sqrt{n^2\text{sen}^2\varnothing + \text{cos}^2\varnothing}}$$

$$T'\varnothing = \frac{T\varnothing}{\sqrt{\text{cos}^2\varnothing (\text{cos}^2\varnothing + n^2\text{sen}^2\varnothing) + \text{sen}^2\varnothing}}$$

In the ellipse in plant Fig. 2-11

We have

$$\frac{x^2}{a^2} + \frac{y^2}{b^2} = 1$$

$$x^2 + \frac{a^2}{b^2} y^2 = a^2$$

$$\frac{a^2}{b^2} = n^2$$

$$x^2 + n^2 y^2 = a^2$$

$$x = \rho \text{ sen}\varphi$$

$$y = \rho \text{ cos}\varphi$$

(24)

Then replacing in (24)

$$\text{cos}^2\varphi + n^2\text{sen}^2\varphi = \frac{a^2}{\rho^2}$$

The tension  $T\varphi$  can be written

$$T'\varphi = \frac{T\varphi}{\sqrt{\text{cos}^2\varphi + n^2\text{sen}^2\varphi}} = \frac{T\varphi}{a} \rho$$

Then  $T'\varphi$  varies directly proportional to  $\rho$ .

## 2.18. Tension in the membrane of the nucleus

Adopting an hyperbolical curve, as was done in the case of the rotational membranes, the tracing of the ellipses is done according to the given indication. The vertical pressions will be shown by

$$P'y = \frac{P_y}{\sqrt{K^2 \cos^2\varphi + \sin^2\varphi}}$$

with the same proceeding K.

## PART III

### TESTS

#### 3.1. *Measuring apparatus*

With the object of verifying the theory of equilibrium of the membrane adopted as a model for the erythrocyte, tests were made as further described.

An apparatus was built, shaped by a wooden frame which has 4 vertical boards (figs. 3-1 and 3 a-A and photos 1, 2, 3), in them are some holes are at distances in the circles which vary from 1,5 to 2,5 cm.

Through the holes can be slid rods of bronze of 45 cm in length and 3 mm in diameter united near the extremes by circular crowns of wood a, b, c, d, e, in the various circles. Horizontal forces can be applied to the crowns by means of weights which transfer their action through threads with a horizontal part, passing through rings which cause little friction. The spherical globe for testing is put between the two sets of rods, and it is easy to determine the distances of its surface to the two interior boards, measuring the longitude of the rods which stand out of the two exterior boards; the measurements were made with an exactitude of approximately one millimeter.

For the study globes of plastic like those used in commerce were used, one for the shore and another like a foot-ball, the latter results to be more spherically perfect. Were used also globes of rubber which are better as they have no joinings, but it was necessary to use small diameters because the big ones generally recede from the spherical shape.

For the measurements we took a horizontal plan HH and another vertical VV, determining the coordinates of the globe surface every 1,5 cm.

#### 3.2. *Tests*

The first tests gave intolerable differences between the measurements in the axes HH and VV, and they served to correct the defects in the construction of the apparatus. In total, 22 measurements were made; in all the shapes

the surface resulted towards, but the adjustment in order to obtain that the measurements in the horizontal and vertical axes nearly coincide is difficult. Here are 4 examples which are considered acceptable.

#### Case 1

*Diameter of the globe:* 42 cm plastic material, shore type, charged circle 5.

Charge type: hyperbolic (for this, it sufficed to take 600 gr performing in each wheel).

The joined picture and drawing indicate a concordance which is considered acceptable. The greatest difference is always in the measuring near the utmost point of the diameter; this is due to the difficulty in measuring because of the sudden change in the curve. The exact calculation was done by electronic machine.

#### Case 2

*Diameter of the globe:* 27,5 cm. Plastic material, type similar to that of a foot-ball.

Charged circles: 5.

Type of hyperbolic charge: 600 gr. in each wheel. Direct calculation with areas of tangents.

Observations: The same as in case N° 1.

#### Case 3

*Diameter of the globe:* 28 cm. Rubber material; usual type used in commerce. The results are acceptable.

#### Case 4

*Diameter of the globe:* 33,4 cm. Same as the former but with internal air different.

Observations: The same as in case N° 1.

### 3.3. Curve of transition

In case 3 the curve of transition was accentuated and it could be studied taking

$$\text{sen } \emptyset = Ar + B$$

which is the hyperbolic charge gave

$$\text{sen } \emptyset = 0,04 r + 0,08$$

In the other two cases the curve is lightly accentuated and it is practically confused with a straight line.

It should be noted that in the region near the axe there is an inclination contrary to the curve of transition studied; this is to be interpreted as an influence of the reinforcement of the globes at the extremities, at which they have a circle of plastic material.

### *Conclusion of the tests*

It is considered that the tests, within the possible precision used, indicate that the applied formulae for the study of equilibrium of membrane correspond to the reality, and are therefore applicable to the model used for the erythrocyte.

In order that the tests be more precise, it would have been necessary to possess globes of rubber especially constructed to make them the most spherical possible, and a machine with a dispositive capable of much greater charges transmissible to the globe.

*NOTE:* I must give thanks to the master carpenter José N. Ruiz for his valuable help in the construction of the apparatus and in the measuring work.

## PART IV

### HISTORICAL SUMMARY

In the following an approximate historical development is given of the biophysical study of the erythrocyte's membrane.

- 1) Ponder gives a method of microscopical photography to determine the dimensions of the erythrocyte. He describes the procedure in detail and specifies the various dimensions of the human erythrocyte as well as those of the rabbit and of the goat; he also specifies the dimensions of the cylinders of erythrocytes rouleaux. This is an important work as it is the first time that the dimensions of the erythrocyte are taken with precision, as Ponder himself points out, and as other masters indicate later. For the determination of the surface he fixes 16 points and he considers that there may be an utmost error of measurement of between 0,10 and 0,20 microns.

- 1A) Ponder also gives a detailed study of the phenomenon of hemolysis of the erythrocyte. He indicated that there have been various attempts to explain the shape of the erythrocyte and quotes one which says that the equilibrium of the membrane must be between a cylinder and a sphere.
- 2) Mitchinson and Swan use an instrument which consists of a micro-pipette communicating with a deposit of water; a cell is adhered to the pipette and a suction is made which produces a projection in the wall of the cell; the measuring of the deformations allow to determine the tensions in the membrane of the cell.
- 3) Rand and Burton use the method indicated in (1) with modifications to apply it to the membrane of the erythrocyte. They find, interesting detail, that the internal pression is stronger than the external one in about 2,6 mm of water. They measure the tensions and the deformation up to the bursting point of the membrane, in tension and flexion in normal and crenated erythrocytes.
- 4) It is the same foregoing theme continued by Rand; he arrives at the conclusion that, as far as its breaking point, the membrane has a visco-elastic comportment. From various experiences, he deduces for Young's module between  $7,3 \times 10^6$  to  $3 \times 10^8$  dynes/cm<sup>2</sup>; he quotes a value of the same module from Katchalsky and col. of  $2,7 \times 10^7$  dynes/cm<sup>2</sup>.
- 4A) Murphy makes a determination of the cholesterol in the membrane of the erythrocyte; he uses a system of radioactive markers, and with the aid of ingenious procedures determines the position of the cholesterol; the result is that the distribution is not uniform but is such that he considers can influence, though not as the only cause, the shape of the erythrocyte, as this distribution modifies the powers which act between the membranes next to the erythrocyte.
- 5) In (5) Y. C. Fung makes an excellent exposition of the mechanical problem of the equilibrium of the membrane of the erythrocyte: He supposes that the interior is liquid and that the membrane alone acts; he adopts a surface of revolution as can be seen in figure A, of which the generatrix is formed by a circle in the utmost exterior part, and by two other circles of superior radio in the central part. For the equilibrium he indicates that there must be tensions on one direction in the external circle, and others in the contrary sense

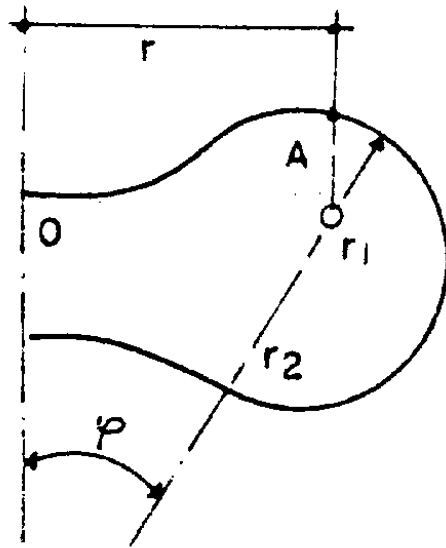


Fig. A

in the internal circle. He studies it first as a membrane almost smooth and comes to the conclusion that, in order not to result in a theoretical incompatibility of infinite tension in 10, the pression must be  $P_i = P_v = 0$ , and he uses for that purpose the theory of the shells without resistance to flexion; then he considers the case of shells resistant to flexion, and he comes to the conclusion that, even when the shell resists the flexion, considering its canty thickness, the greater part of the tensions will be taken by the action of the membrane, that is to say the stress and cutting effort. In the case of flexion, in which there would be tensions of compression, he makes a study of stability, and he arrives at the approximation that a tension of compression equal to 1,2 mm of water would produce a bulging in the shell. He makes experiments with rubber balls of thin texture, filled with water and submerged in a tank of water subjected to pression: The spherical shape changes to an ellipsoidal one, and wrinkles are formed in the walls through the buldging of the membrane.

- 6) Burton and Shravastav indicate that the shape of the membrane must be governed by the mechanics of the shells, and they take the case of non-resistance to the flexión, that is to say the equilibrium of the soap-bubbles; they come to the conclusion that, in order to equilibrate the pressions made upon the membrane of the erythrocyte, there must exist attractive forces between the membrane of the erythrocyte. They make experiments with condensing plates loaded with electricity, placing fibers of nylon between them; they measure the

attraction at various points of the plate with a maximum result in the centre; minimum at a certain distance, and zero on the edge which corresponds approximately to the shape, of the erythrocyte.

- 7) Canham and Burton study the distribution of the erythrocyte in the blood. Their study contains the interesting fact that the erythrocyte can go through canals of an approximate minimum of 3,7 mic. of width or diameter which corresponds to the canalicules of the spleen. The erythrocytes of larger diameter are more exposed to rupture; in the tests that were made, there appears erythrocytes between 7 to 9,5 mic. of diameter. They quote clinical cases of eliminated spleen in which the proportion of erythrocytes of greater size was superior to the normal, which confirms the foregoing, and which indicates the effect of the control of the vessel.
- 8) Y. C. Fung and P. Tong study the erythrocyte on the theory of the shells without assistance to the flexion, in order to find the condition required to pass from its normal shape to the spherical one, they use polar coordinates and they deduce formulas for the tensions and deformations in the membrane. For the shape of the erythrocyte, they use a polynomial of grade 8 with three coefficients; in the example which they present, they use the coefficients given by Ponder (1). They make a detailed exposition of the formulas employed, and they present the examination of other theories of the erythrocyte, among which that of Murphy who found that the distribution of the cholesterol in the membrane of the erythrocyte is not uniform, and therefore the superficial tension is not uniform and the would give it the characteristic shape. In an appendix he asserts that the effect of flexion, which he does not consider, is negligible.
- 9) L. López and collaborators assume that the equilibrium of the membrane is due to hydrostatic pressures and to those of an electrical charge which they take as a pole electrically charged supposing a surface of equipotential revolution around an axis. They obtain an expression of density of charge which is in volts, an integral elliptic. They make numerical examples and they obtain shapes of surface very similar to the erythrocyte.
- 10) Shrivastav and Burton place erythrocytes in an isotonical solution, and with a polarised light and the use of a microscope, they can photograph the erythrocyte through different angles of the light; this

allows them to determine the surface of the erythrocyte. Thereafter they take measurements in erythrocytes placed in hypotential solutions; passing from the biconcave shape to the ellipsoidal and to the spherical one. They indicate that from the central point to a certain radio, there is a birefringent material which they consider can be assimilated to fibers which maintain the opposite points of the membrane and they refer to (6) with respect to the equilibrium of membrane which must be used, and they maintain the theory proposed there.

- 10A) Shrivastav gives a more detailed explanation of what is exposed in (6) and they accurately describe the equipment used of an electric condenser with two short distanced plates, and the measurements, using a layer of air or nylon fibers.

They describe the microscope of polarized light mentioned in 10, and the results of the tests and conclusions given before. They make a series of experiments using erythrocytes in an isotonical solution (PA7,4 Bsm 306), adding a chemical product (tween) which alters the exterior tension of the membrane of the erythrocyte; the tween decreases the usual tension of 2,3 mm. of height of the water which act from outside to inside, and the erythrocytes get transformed into a toral with the central cavity slightly accentuated. They take measurements of both normal and deformed erythrocytes and they find a disminution in the minimum thickness and an augmentation in the separation of the distance between the points which confront themselves facing the erythrocyte. They interpret this as a confirmation of the existence of forces between the membranes of the erythrocyte. They consider that the existence of the difference of tension of 2,3 mm of water existing in the membrane is important in order to maintain the shape of the erythrocyte; mentioning that Rand had already indicated in (4)-1964 that this tensión does not change, even when the erythrocyte has varied its shape in hypotonical solutions.

- 11) Canham considers the energy necessary for a shell resisting flexion with a minimum energy of that energy. He takes the measurement of 23 erythrocytes, making 50 measurements in each one so as to determine its shape. For the shape of the erythrocyte he uses the formula of the oval of Cassini. He makes tests in isotonical and hypotonical solutions, and he makes calculations of minimum energy, verifying at the same time if the measured shapes and the anticipated ones coincide well.

- 11A) E. A. Lebland specifies the dimensions of the erythrocytes by means of very ingenious processes of holography, and he determines the different shapes which they take passing from the normal to the spherical one; he uses solutions of variable tonicity, and he gives a precision of 1% in his measurements.
- 12) Hochmut and his collaborators present a method by which to measure Young's modulus, arriving at fixing it  $1 \times 10^4$  dynes/cm<sup>2</sup>, that is to say of the order  $10^3$  to  $10^4$  less than that given by Rand-Katchalsky (4).
- 13) Evans takes for the membrane of the erythrocyte an undeformable material in a lamina subjected to compression in two octagonal directions in one plan. For the equilibrium of the shell without flexion he uses the usual formulas of equilibrium of the theory of the shells and he gives formulas of approximation for the radii of curvature and tensions at each point.  
He made tests with three erythrocytes passing them from their normal shape to the ellipsoidal, and the measurements allowed him to specify the coefficients of the formulas; the surfaces of the membranes remained constant. He obtains for the modulus of elasticity in the three cases tested between  $10^3$  and  $10^4$  dynes/cm<sup>2</sup>, quotes the value of Rand of  $10^5$  dynes/cm for the rupture.
- 14) Using the technique of the micropipette indicated in (2) Evans, basing himself on his conception of material of membrane (13) develops the necessary formulas; he takes the data of Rand and Burton (3) and arrives at the conclusion that the modulus of elasticity is between 1 to  $5 \times 10^5$  dynes/cm<sup>2</sup> and on the basis of Hensinkveld's and Lebland's works, he comes to a modulus for the constant effort of  $1 \times 10^4$  dynes/cm<sup>2</sup>. He quotes a very high modulus of elasticity obtained by Katchalsky and collaborators (1960 in Flow properties of Blood and other biological systems Perg. O.N.Y.).
- 15) K. H. Adams considers the equilibrium of membrane basing himself on the total electrostatic charge contained in a biological membrane and the shape of equilibrium for a minimum of the total of energy would correspond to the shape of the erythrocyte. He gives the formula of the total energy; the latter is simplified and expressed for the condition of a minimum. He considers the volume of the erythrocyte and the area of the membrane to be constant, which allows him to give two more equations; he uses the formula of the oval of Cassini used by Canham (11) and he concludes that the biconcave shape of the erythrocyte minimises the electrostatical energy in the

same way that it minimises the energy of deformation already demonstrated by Canham (11); he considers more simple the explanation through electrostatical energy for the shape of the membrane of the erythrocyte.

- 16) Jay uses a method of photography to obtain profile views of erythrocytes with an augmentation of 15.000, and making marks in them, a digital computer gives him an information of area volume, thickness maximum and minimum of membrane, etc. He places some erythrocytes in a solution of albumin of variable concentration. It is to be observed that the area decreases with the concentration of albumins in the solution, but he believes that this cannot be attributed to an osmotical pression, but he attributes it rather to a change in the substance of the membrane; the volume decreases as well as the area and he considers that this is due to a change of permeability of ions  $+K$  and  $-N$ .

The number of erythrocytes in cup shape augments in solution of albumin in comparison with those placed in the solution of Ringe.

- 17) Deuling and Helfrich start from the energy of a curvature of a unit of area of a membrane formed by two layers of lipid with structure of support, which contains the two principal radii of curvature in that element as well as elastic moduls in the two principal directions, they include a possible initial curvature. They take a surface generated by a curve which rotates round an axis; the surface generated and the volume contained in it is taken as a constant. They apply the calculus of variations with the conditions that the total energy be minimum, this allows them to come to a solution for the generating curve. They calculate specimens and they obtain values very similar to those obtained by Evans and Lebland in the measurement of 500 erythrocytes; they study the case when the volume does not fill the whole of the surface and the case of the cup shape arriving at a good approximation to the measurements made with similar shapes by the authors already mentioned.

- 18) Evans and collaborators, using the method of the improved pipette decide the elastic characteristics of the membrane and arrive at the values of 95 dynes/cm and 193 dynes/cm for the modulus of elasticity (by lineal cm) of the layers of lipid and structure.

- 19) Evans makes some considerations concerning the formulas used in (13), modifying the exposition.

- 20) Is a study of the equilibrium of membrane of a surface of revolution submitted to a uniform pression with the condition that the tension in the meridional sense and in the sense of rotation be equal; a surface expressed, by an elliptic integral is obtained. An approximation to a total surface engendered by a circle is made. Elemental tests are made and it is considered that the shape of the membrane obtained corresponds to that of the erythrocyte and allows to explain its abnormal shapes as well.

## PART V

### OBSERVATIONS

#### 5.1. *General Considerations*

In accordance with what is said in Chapter 4, it can be seen that the methods indicated by Ponder (1) in 1930, used to determine the dimensions of the erythrocyte improve progressively. The idea of the equilibrium of the membrane already cited in (1) and (3) is developed in (6) with the complete theory of the resistant or not resistant shells to the flexion, and it is this theory which is used in the following studies.

In 9 it is determined that the shape of the erythrocyte must be the corresponding one to the equilibrium of electrostatic forces in the membrane, and of osmotic charge.

In 10 it is confirmed that the shape of the erythrocyte approaches well enough the state of equilibrium for a minimum of energy of the flexion.

In 13 is assumed a material of membrane indeformable to the traction or compression, and the theory is used of the husks not resistant to the flexion and in 15, for the surface is used that which gives the minimum of electric energy.

In 17, with an erythrocyte of constant volume and area, one takes the energy of deformation or flection of each element of surface, and, as the surface of the erythrocyte, that which gives a minimum for the energy, and one deducts formulas, arriving at a good approximation.

In 20, one supposed husks without flection, submitted to an osmotic pression, with equal tangencial and meridian tensions, arriving at a formula of hyperbolic integral which is simplified for the exposition.

### 5.2. Resistance of the material of membrane

The average module of elasticity varies with the state of load, the apparent value given by the various experimenters is as follows:

In (3)  $E=7,3 \times 10^6$  to  $E=3 \times 10^8$  dynes/cm<sup>2</sup> (quotes a Kachalsky) that gives  $E=2,4 \times 10^7$  dynes/cm<sup>2</sup>.

In (12)  $E=1,7 \times 10^7$  for the elastic part  $E=2,5 \times 10^4$  dynes/cm<sup>2</sup>.

In (14) with his conception of special material he interprets the tests of (2) and (4) and gives  $E=1 \times 10^4$  dynes/cm<sup>2</sup>.

In (18) is supposed a membrane with two substances, one stiffer than the other; one arrives at 100 dynes cm/lineal, which would give  $E=6,6 \times 10^7$ , supposing the membrane to be of 150 Å° in thickness.

I consider that from the foregoing data there can be taken an average of  $E=2 \times 10^7$  dynes/cm<sup>2</sup>, that is 20,20 kg/cm<sup>2</sup> for the modulus of apparent elasticity of the material and the proper modulus of elasticity would be  $2 \times 10^4$  di/cm<sup>2</sup>, or that is hardly 0,02 kg/cm<sup>2</sup>.

In accordance with (18) the maximum tension in the membrane must not exceed 10 dynes/cm/1, that is to say that with a membrane of 100 Å° this is equivalent to 10 kg/cm<sup>2</sup>, but there is already danger of lisis with less than 6 dynes/cm/1, that is, let us say, for 5 dynes/cm/1 or is 5 kg/cm<sup>2</sup> the membrane would resist.

### 5.3. Comparison of the theories

From the theories of equilibrium, applying the theories of the husks very clearly exposed in (5); the experiences with electric camp of (6) with nylon fibres and the curves drawn, those of (9) with electric charge, and the (20), they all come to a curve of hyperbolical type. It is to be noted that in (20) the curve is closed, it does not arrive to the origin, and in (9) the equation given

$$V.i = \sum_{j=i}^N \frac{4 r_j d\theta}{1_j} \int_0^{2\pi} \frac{d\theta}{(1-K^2 \text{sen}^2\theta)^{1/2}} \quad j \neq i$$

must have a singular point for

$$1-K^2 \text{sen}^2\theta = 0$$

The curves of (6) are also practically detained at a certain distance from the origin; so that from different points of view, one arrives at the same curve.

In the verification of the minimum energy of flexion done in (11) the shape of the oval of Cassini used coincides practically with an hyperbolic curve which would correspond to the equilibrium of membrane, and as this called attention, it was considered convenient to make an estimation of the initial energy before the deformation of the reticulocyte, for which an equivalent spherical membrane was chosen. Adopting a coefficient of Poisson of 0,99 and the same module of elasticity of the erythrocyte  $E=1 \times 10^7$ , one arrives at the conclusion that the energy of deformation because of tensions in the membrane is approximately 100 times greater than the necessary flexion in order to overcome the energy of curvature existing in the membrane; this leads to believe that the equilibrium of membrane which dominates is that of the interior tensions, that is to say that of the membrane, and it would be somewhat influenced by the flexion; this would correspond to what has already been cited by Fung (5), indicating the small effect of the flexion. The study of Deuling and Helfrich (17) is also based on the energy of minimum flexion, and it comes to very interesting results, specially as refers to the biconcave and similar shapes. Because of what has just been seen, it has occurred to me to make the suggestion of studying the equilibrium of membrane of the erythrocyte, taking in consideration the flexion and the simultaneous action of the traction or of the compression which, to simplify, although not exact these could be the ones given for the theory of the husks without flexion. I tried to study the problem taking only the energy of the tensions without flexion, applying the method of the variations for the minimum of energy, but without being able to obtain satisfactory results.

In the work presented, it is presumed that there are ties depending on an axis, and in a certain zone of the membrane which equilibrate the action of tensions from inside to outside. This tension according to (3) exists and is from 2,6 mm of water in isotonic solution. Initially, the pressures produced by osmotic pressure or by electric camp can contribute or initiate the curvature of the spherical reticulocyte; but there should exist a tie depending on a certain axle, as there exists in the nucleated erythrocytes of fishes, amphibians, reptiles and birds, which would be as a rest of nucleus through the biological process of evolution. The central zone stabilises the mechanical function and diminishes the tensions if compared with the espherocyte.

BIBLIOGRAFIA  
BIBLIOGRAPHY

A) BIOFÍSICA  
BIOPHYSIC

- 1930 / 1. PONDER E. The measurement of the diameter of erythrocytes V. The relation of the diameter pg. 29-39. Quarterly Journal of Experimental Physiology V.20-1930.
- 1A. PONDER E. Hemolysis and related phenomena, New York Greene & Stranton. N.Y. Cap. 3.
- 1954 / 2. J. M. MITCHISON AND M. M. SWANN. The mechanical properties of the cell surface. I The cell elastimeter I. Exp/Biol 31-443.
- 1964 / 3. R. P. RAND AND A. C. BURTON. Mechanical properties of the red cell membrane I: Membrane stiffness and intracellular pressure Bioph J. 4-115.
4. R. P. RAND II. Viscoelasticity breakdown of the membrane. Bioph. J. 4-303.
- 1965 / 5. JOHN B. MURPHY. Erythrocyte metabolism VI. Cell shape and the location of cholesterol in the erythrocyte membrane. J. Lab. Clin. Med. 1965 N° 5.
- 1967 / 6. ALAN C. BURTON AND B. SHRIVASTAV. Active forces between red cells and within a cell. Hemorheology Proceedings of the first International Conference. The University of Reykjavick 10-16th. July 1966. Pergamon Press. Oxford. New York.
- 1968 / 7. P. B. CANHAM AND ALAN C. BURTON, distribution of size in populations of normal red cells. Circulation Research. March 1968.
8. Y. C. FUNG AND P. TONG. Theory of the sphering of the red cells. Bioph. J. 8-175.
9. L. LÓPEZ Y. M. DUCE AND W. A. HUNT. On the shape of the erythrocyte. Bioph. J. 8-1229.
10. SHRIVASTAV AND BURTON. Evidence of the refringence of struture across the dimple region of red cells. J. Cell Phisiol. 674 101-114.
- 10A. DE IL SHRIVASTAV, B. B. Studies on the physical forces required to explain the equilibrium of the membrane of the red cell. D. Thesis. August. 1968 University of Western Ontario. Canada.
- 1970 / 11. P. B. CANHAM. The minumun energy of bending as a possible explanation of the biconcave shape of the human red blood cell. J. Theoret Biol. 970-61-8.
- 1972 / 11A. E. A. LEBLAND. Geometric properties of individual red blood cell discocyte-spherocyte transformations. Biorheology 1973. Vol 10 pp 405-410.
- 1973 / 12. R. M. HOCHMUTH, N. MOHANDAS AND P. L. BLACKSHEAR JR. Measurement of the elastic modulus for red cell membrane. Bioph J. 13-147.
13. E. A. EVANS. A new material concept for the red cell membrane. Bioph. J. 13-926.

14. E. M. EVANS. New membrane concept applied to the analysis of fluid shear and micropipette-deformed red blood cells. *Bioph J.* 13-841.
15. E. A. ADAMS. Theory of the shape of the red blood cell. *Bioph H.* 13-1049.
- 1978 / 16. U. ALFRED, N. L. JAY. Geometry of the human erythrocyte. Effect of albumin on cell geometry. *Bioph J.* 15-205.
- 1976 / 17. H. F. DEULING AND W. HELFRICH. Red blood cell shapes as explained on the basis of curvature elasticity. *Bioph J.* 16-861.
18. E. A. EVANS, R. WAUGH AND L. MELNIK. Elastic area & compressibility modulus of red cell membrane. *Bioph J.* 16-585.
19. Evans & Evans Constitutive Relation for red cell membrane. Correction. *Bioph J.* 16-597.
- 1977 / 20. ALBERTO E. OLIVARES. Relation between the shape and the equilibrium of the membrane of the erythrocyte. *Boletín de la Academia de Ciencias Físicas Matemáticas y Naturales de Caracas.* 112-23.

#### REFERENCIAS EN LOS TRABAJOS MENCIONADOS

#### REFERENCES IN THE PAPERS OF THE QUOTED AUTHORS

21. De 2  
KATCHALSKY Y COL. (1960). Theological consideration of the haemologysing red blood cell in flow properties and other biological systems (A. L. Copley and G. Stainsby editors) New York Pergamon Press Inc.
22. De 3  
PONDER E. (1930). Measurements of diameters of erythrocytes. V. The relation of diameter to thickness. *Quart J. Exp. Physich* 20; 29.
23. PONDER E. (1945). The physical structure of the red cell membrane with special reference to its shape. *Tr. Fradays So.* 33-947.
24. RAND R. P. BOURTON. 1963. Area and volume charge in haemolysis of single erythrocytes. *Biol J. J. Cell and Comp. Physiol*, 61; 245.
25. De 7  
HOUGHIN D. N., MUNN J. I., AND PARNELL, I. L. Method for the measurement of the red cell dimensions and calculation of mean corpuscular volume and surface area. *Blood.* 13; 1185.
26. De 8  
KOCHE J. A. (1966). Visco-elastic properties of the red cell membrane. *Proc. Ist. Int. conf. Haematology.* Ireland.
27. De 10  
LEWIS J. S. OSBORN AND P. STUART. (1968). Demonstration of an internal structure within te red cell by Jon etching and sceanning electron microscopy. *Nature London* 220; 614-618.

- 28 De 10 A  
 BELL, D. J. GRANT, J. K. (EDS). (1963). The structure and function of the membranes and surfaces of cells Biochem. Soc. Sympos. 22 - Cambridge, England. University Press.
29. COOK, G. M. W., HEARD, D. H. AND SEAMAN, G. V. E. (1961). Sialic acids and the electrokinetic charge of the human erythrocyte. *Nature*. 191; 44-47.
30. DANON, D. (1967). Structural changes associated with red cell aging. XVIII John G. Gibson II Lecture; College of Physicians and Surgeons, Columbia University.
31. DAVIES, J. T. AND RIDEAL, E. K. (1961). The physics of the surfaces. In *Interfacial Phenomena* Academic Press N. Y. pp. 1-55.
32. ELUL, R. (1967). Fixed charge in the cell membrane. *J. Physiol.* 189; 351-365.
33. FRIEDENBERG, R., BLATT, A., GALLUCCI, V., DANIELLS, J. F. AND SHAMES I. (1966). Electrostatics of membrane systems - I. A non-statistical approach to cellular membrane system. *J. Theoret. Biol.* 11; 465-477.
34. FURCHGOTT, R. F. (1940). Disc sphere transformation in mammalian red cells. *J. Exp. Biol.* 17; 30-44.
35. GLAESER, R. M. (1963). The electric charge and surface properties of intact cells. Ph.D. Thesis. University of California.
36. HARRIS, J. W. (1965). *The red Cell*. 3rd. ed. Harvard University Press. Mass.
37. HARTRIDGE, H. (1919). Shape of red blood corpuscles *J. Physiol.* 53; P1xxi.
38. HEARD, D. H. AND SEAMAN, G. V. F. (1960). The influence of PH and ionic strength of the electrokinetic stability of the human erythrocyte membrane. *J. Gen. Physiol.* 43; 635-654.
39. HILLER, J. AND HOFFMAN, J. F. (1953). On the ultrastructure of the plasma membrane as revealed by the electromicroscope. *J. Cell. Com. Physiol.* 42; 203-247.
40. De 10A  
 HOFFMAN, J. F. (1958). Physiological characteristics of the human red cell ghosts. *J. Gen. Physiol* 42; 9-28.
41. HUNTER, R. J. (1960). Constancy of the surface density of the human erythrocytes at different ionic strengths. *Arch. Biochem. Biophys.* 88; 308-312.
42. JAY, A. W. L. (1968). Direct measurement of potential difference across the red cell membrane. Presented at the 12th annual meeting of the Biophysical Society. (*Biophys. J. Sco. Abstr.* Vol. 8.)
43. KAVANAU, J. L. (1965). *Structure and function in biological membranes*. Vol. I and II. San Francisco. Holden-Day.
44. KAVANAU, J. L. (1966). Membrane structure and function. *Fed. Proc.* 25; 1096-1107.
45. KORN, E. D. (1929) *The anatomy and Physiology of capillaries*. Revised and enlarged edition. New Haven Yale University Press.
46. LATA, J. A. (1952). The surface of the mammalian erythrocyte. *Blood* 7; 508-521.
47. NAKAO, M., NAKAO, T. AND YAMAZOE, S. (1960). Adenosine triphosphate and maintenance of shape of the human red cells. *Nature* 187; 945.

48. NAKAO, M. T. AND YAMAZOE, S. (1961). Adenosine triphosphate and shape of te erythrocytes. *J. Biochem.* 49; 487-498.
49. NEVO, A. (1960). On the estimation of the thickness of the red cell membrane from ultracentrifuge studies. *Exp. Cell. Res.* 21; 286-291.
50. De 10A  
PONDER, E. (1940). The red cell as an osmometer. *Sympos. Quant. Biol.* 8; 133-143.
51. PONDER, E. (1944). Red cell dimensions: measurement, in Glaeser, O. ed. *Medical Physics. Chicago, III. The yearbook Publishers Inc.* 1; 1203-1214.
52. PONDER, E. (1955). Red cell structure and its breakdown. *Protoplasmatologia.* 10 N° 2 Wien. Springer-Verlag.
53. PRANKERD, T. A. J. (1961). *The red cell.* 1st ed. Blackwell. Scientific Publications. Oxford.
54. RAND, R. P. (1967). Some biophysical considerations of the red cell membrane. *Fed. Proc.* 26; 1780-1784.
55. RAND, R. P., BURTON, A. C. AND CANHAM, P. B. (1965). Reversible changes in shape of red cells in electrical fields. *Nature.* 205; 977-978.
56. ROBERTSON, J. D. (1959). The ultra structure of cell membranes and their derivations. *Biochem. Soc. Sympos.* 16; 3-43.
57. SEAMAN, G. V. F. AND UHLENBRUCK, G. (1963). The surface structure of erythrocytes from some sources. *Arch. Biochem. Biophys.* 100; 493-502.
58. De 10A  
SEEMAN, P. (1966). II. Erythrocyte membrane stabilization by local anesthetic and tranquilizers. *Biochemical Pharmacology,* 15; 1767-1774.
59. WAUGH, D. F. AND SCHOMITT, E. O. (1940). Investigations of the thickness and ultrastructure of cellular membranes by the analytical leptoscope. *Sympos. Quant. Biol.* 8; 233-241.
60. WEISS, L. (1965). Studies on cell deformability. I. Effect of surface charge. *J. Cell. Biol.* 26; 735-739.
61. WILLIAMS, T. F., FORDHAM, C. C. AND HOLLANDER, W. (1959). A study of the osmotic behavior of human erythrocytes. *J. Clin. Invest.* 38; 1587-1597.
62. De 14  
MARCHESI, V. T., E. STEERS, T. V. TILLACK AND J. L. MARCHESI (1969). Red cell membrane, C. X. Jamieson and I. J. Greenwalt, editors, J. B. Lakineent Co. Philadelphia. 117. .
63. WEED R. I. AND P. L. LACELLE (1959). Red Cell Membrane, as (29)-318.
64. De 17  
HELLFRICH WAND H. J. DEULING (1978). Some theoretical shapes of red blood cells *J. Phys.* 3G; C1. 327.
65. De 17  
SHALAK, E. A., TOZEREN, R. P. ZARDA AND J. CHEON (1973). Strain energy function of red blood cell membranes. *Bioph J.* 13; 245.

66. De 17  
 BASIS M. (1973). ZARDA P. R. AND SKALAK (1975). Sphering and formation of red cell. Biomechanics Symposium. R. Skalak and R. M. Nerem editors A.M.P. 10; 49.
67. De 18  
 EVANS AND Y. C. FUNG (1972). Improved measurements of erythrocyte geometry. Microsasc. Res. 4; 335.
68. De 18  
 EVANS E. A. AND P. F. LEBLAND (1973). Geometric properties of individual red blood cell discocyte spherocyte transformations 10; 893.
69. Living blood cells and their ultrastructure. Springer Verlag N. York. 1973.
70. EVANS E. A. (1975) b. Composite material structure of the red cell membranes. In Erythrocyte structure and function. G. J. Brewer editor. Alan Liss. N. Y. 491.
71. EVANS E. A. AND R. M. HOCHMUTH (1976). b. Membrane viscoplastic flow. Bioph J. 16; 13.

B) HEMATOLOGÍA  
 HAEMATOLOGY

72. Several authors. The red blood cell. (1964). Bishop Surgenon. Acad. Press. N. York.
73. DANGHERTY WILLIAM C. (1976). Introduction to Haematology. Second Edition the C. Mosby Company.
74. WARREN ANDREW. Comparative Haematology (1965). Grunestratun. N. York.

TEORÍA DE LAS CÁSCARAS  
 THEORY OF SHELLS

75. TIMOSHENKO S. (1940). Theory of Plates and Shells. New York. McGraw Hill Book Company.
76. GIRKMAN KARL. (1954). Flächen Fragwerke.
77. FLUGGE WILHELM. (1957). Static and Dinamik der Schalen. Springer Verlag. Berlín.

MATEMÁTICAS  
 MATHEMATICS

78. G. PETIT BOIS. (1961). Infinite Integrals. Dover Publications Inc. N. York.
79. Handbook of Mathematical Functions. (1965). P. M. Morse and col. Edited Milton Abramowith and Irene A. Stegun. Dover Publications Inc. N. York.
80. Taschenbuch der Mathematic Bronstein - Semdjajev. (1961). B. G. Teubner Verlag Eeipzig.
81. CYPECA. Cálculos Electrónicos. Caracas.

TABLA I

$R_0 = 5$   
 $A = 0,3125$   
 $B = 2,8125$

| Curva Principal<br>Principal Curve  |         |      | 5-2, 00 |
|-------------------------------------|---------|------|---------|
| 5.0                                 | 3.0     | 4.75 | 0.57072 |
|                                     |         | 4.50 | 0.96012 |
|                                     |         | 4.25 | 1.22458 |
|                                     |         | 4.00 | 1.41641 |
|                                     |         | 3.75 | 1.55548 |
|                                     |         | 3.50 | 1.65119 |
|                                     |         | 3.25 | 1.70798 |
|                                     |         | 3.00 | 1.72711 |
|                                     |         | 2.75 | 1.70687 |
|                                     |         | 2.50 | 1.64148 |
|                                     |         | 2.25 | 1.51690 |
| 2.00                                | 1.29161 |      |         |
| Curva de Enlace<br>Transition Curve |         |      | 2,25-0  |
|                                     |         | 2.25 | 1.56900 |
|                                     |         | 2.00 | 1.41912 |
|                                     |         | 1.75 | 1.29392 |
|                                     |         | 1.50 | 1.19075 |
|                                     |         | 1.25 | 1.10774 |
|                                     |         | 1.00 | 1.04353 |
|                                     |         | 0.75 | 0.99714 |
|                                     |         | 0.50 | 0.96791 |
|                                     |         | 0.25 | 0.95544 |

$R_0 = 5$   
 $A = 0,266666$   
 $B = 1,66666$

| Curva Principal<br>Principal Curve  |         |      | 5-1,00  |
|-------------------------------------|---------|------|---------|
| 5.0                                 | 2.50    | 4.75 | 0.65166 |
|                                     |         | 4.50 | 1.10660 |
|                                     |         | 4.25 | 1.42619 |
|                                     |         | 4.00 | 1.66936 |
|                                     |         | 3.75 | 1.85889 |
|                                     |         | 3.50 | 2.00615 |
|                                     |         | 3.25 | 2.11742 |
|                                     |         | 3.00 | 2.19610 |
|                                     |         | 2.75 | 2.24367 |
|                                     |         | 2.50 | 2.25988 |
|                                     |         | 2.25 | 2.24253 |
| 2.00                                | 2.18634 |      |         |
| 1.75                                | 2.07974 |      |         |
| 1.50                                | 1.89206 |      |         |
| Curva de Enlace<br>Transition Curve |         |      | 1,75-0  |
|                                     |         | 1.75 | 2.07174 |
|                                     |         | 1.50 | 1.94623 |
|                                     |         | 1.25 | 1.84570 |
|                                     |         | 1.00 | 1.76781 |
|                                     |         | 0.75 | 1.71091 |
|                                     |         | 0.50 | 1.67394 |
|                                     |         | 0.25 | 1.65620 |

TABLA I

$R_0 = 5$   
 $A = 0,238092$   
 $B = 0,9523808$

$R_0 = 5$   
 $A = 0,21978$   
 $B = 0,494505$

| Curva Principal<br>Principal Curve  |         |         | 5 - 1,50 |
|-------------------------------------|---------|---------|----------|
| 5.0                                 | 2.0     | 4.75    | 0.72106  |
|                                     |         | 4.50    | 1.23165  |
|                                     |         | 4.25    | 1.59744  |
|                                     |         | 4.00    | 1.88297  |
|                                     |         | 3.75    | 2.11334  |
|                                     |         | 3.50    | 2.30132  |
|                                     |         | 3.25    | 2.45425  |
|                                     |         | 3.00    | 2.57661  |
|                                     |         | 2.50    | 2.67106  |
|                                     |         | 2.25    | 2.73900  |
|                                     |         | 2.00    | 2.78064  |
|                                     |         | 1.75    | 2.79500  |
|                                     |         | 1.50    | 2.77938  |
|                                     |         | 1.25    | 2.72810  |
| 1.00                                | 2.62883 |         |          |
| Curva de Enlace<br>Transition Curve |         |         | 2- 0     |
|                                     | 1.25    | 2.62883 |          |
|                                     | 1.00    | 2.51448 |          |
|                                     | 0.75    | 2.43168 |          |
|                                     | 0.50    | 2.37720 |          |
|                                     | 0.25    | 2.34916 |          |

| Curva Principal<br>Principal Curve  |         |         | 5- 1,00 |
|-------------------------------------|---------|---------|---------|
| 5.00                                | 1.50    | 4.75    | 0.77794 |
|                                     |         | 4.50    | 1.33393 |
|                                     |         | 4.25    | 1.73718 |
|                                     |         | 4.00    | 2.05682 |
|                                     |         | 3.75    | 2.31978 |
|                                     |         | 3.50    | 2.53991 |
|                                     |         | 3.25    | 2.72531 |
|                                     |         | 3.00    | 2.88111 |
|                                     |         | 2.75    | 3.01067 |
|                                     |         | 2.50    | 3.11614 |
|                                     |         | 2.25    | 3.19877 |
|                                     |         | 2.00    | 3.25901 |
|                                     |         | 1.75    | 3.29645 |
|                                     |         | 1.50    | 3.30955 |
| 1.25                                | 3.29480 |         |         |
| 1.00                                | 3.24525 |         |         |
| 0.75                                | 3.14273 |         |         |
| 0.50                                | 2.90365 |         |         |
| Curva de Enlace<br>Transition Curve |         |         | 0,75-0  |
|                                     | 0.75    | 3.14275 |         |
|                                     | 0.50    | 3.03142 |         |
|                                     | 0.25    | 2.97333 |         |

|                 |         |        |      |
|-----------------|---------|--------|------|
| Curva Principal |         | 5 - 00 |      |
| Principal Curve |         |        |      |
| 4.75            | 0.84707 | 5.00   | 4.75 |
| 4.50            | 1.45810 |        | 4.50 |
| 4.25            | 1.90667 |        | 4.25 |
| 4.00            | 2.26741 |        | 4.00 |
| 3.75            | 2.56951 |        | 3.75 |
| 3.50            | 2.82805 |        | 3.50 |
| 3.25            | 3.05201 |        | 3.25 |
| 3.00            | 3.24723 |        | 3.00 |
| 2.75            | 3.41777 |        | 2.75 |
| 2.50            | 3.56650 |        | 2.50 |
| 2.25            | 3.69556 |        | 2.25 |
| 2.00            | 3.80651 |        | 2.00 |
| 1.75            | 3.90052 |        | 1.75 |
| 1.50            | 3.97835 |        | 1.50 |
| 1.25            | 4.04045 |        | 1.25 |
| 1.00            | 4.08679 |        | 1.00 |
| 0.75            | 4.11568 |        | 0.75 |
| 0.50            | 4.12779 |        | 0.50 |
| 0.25            | 4.11164 |        | 0.25 |

$R_0 = 5$   
 $A = 0,20202$   
 $B = 0,050505$

|                 |         |        |      |
|-----------------|---------|--------|------|
| Curva Principal |         | 5 - 00 |      |
| Principal Curve |         |        |      |
| 4.75            | 0.82056 | 5.00   | 4.75 |
| 4.50            | 1.41049 |        | 4.50 |
| 4.25            | 1.84170 |        | 4.25 |
| 4.00            | 2.18670 |        | 4.00 |
| 3.75            | 2.47383 |        | 3.75 |
| 3.50            | 2.71768 |        | 3.50 |
| 3.25            | 2.92691 |        | 3.25 |
| 3.00            | 3.10710 |        | 3.00 |
| 2.75            | 3.26202 |        | 2.75 |
| 2.50            | 3.39431 |        | 2.50 |
| 2.25            | 3.50578 |        | 2.25 |
| 2.00            | 3.59760 |        | 2.00 |
| 1.75            | 3.67038 |        | 1.75 |
| 1.50            | 3.72413 |        | 1.50 |
| 1.25            | 3.75810 |        | 1.25 |
| 1.00            | 3.77023 |        | 1.00 |
| 0.75            | 3.75582 |        | 0.75 |
| 0.50            | 3.70248 |        | 0.50 |
| 0.25            | 3.54999 |        | 0.25 |

$R_0 = 5$   
 $A = 0,26833$   
 $B = 0,26833$

TABLA

TABLA 1

$R_0 = 4,50$   
 $A = 0,3472218$   
 $B = 2,5312467$

$R_0 = 4,50$   
 $A = 0,299999$   
 $B = 1,8749981$

| Curva Principal<br>Principal Curve  |         |      | 4,50-2,25 |
|-------------------------------------|---------|------|-----------|
| 4.50                                | 2.7     | 4.25 | 0.53830   |
|                                     |         | 4.00 | 0.90104   |
|                                     |         | 3.75 | 1.14258   |
|                                     |         | 3.50 | 1.31244   |
|                                     |         | 3.25 | 1.42903   |
|                                     |         | 3.00 | 1.50074   |
|                                     |         | 2.75 | 1.53036   |
|                                     |         | 2.50 | 1.51690   |
|                                     |         | 2.25 | 1.45416   |
|                                     |         | 2.00 | 1.32518   |
| 1.75                                | 1.07001 |      |           |
| Curva de Enlace<br>Transition Curve |         |      | 2,00- 0   |
|                                     |         | 2.00 | 1.32318   |
|                                     |         | 1.75 | 1.16578   |
|                                     |         | 1.50 | 1.03815   |
|                                     |         | 1.25 | 0.93650   |
|                                     |         | 1.00 | 0.85827   |
|                                     |         | 0.75 | 0.80172   |
|                                     |         | 0.50 | 0.76570   |
|                                     |         | 0.25 | 0.74952   |

| Curva Principal<br>Principal Curve  |         |      | 4,50-2,00 |
|-------------------------------------|---------|------|-----------|
| 4.50                                | 2.50    | 4.25 | 0.57396   |
|                                     |         | 4.00 | 0.96598   |
|                                     |         | 3.75 | 1.23257   |
|                                     |         | 3.50 | 1.42628   |
|                                     |         | 3.25 | 1.56702   |
|                                     |         | 3.00 | 1.66416   |
|                                     |         | 2.75 | 1.72203   |
|                                     |         | 2.50 | 1.74161   |
|                                     |         | 2.25 | 1.72065   |
|                                     |         | 2.00 | 1.65201   |
| 1.75                                | 1.51735 |      |           |
| 1.50                                | 1.24931 |      |           |
| Curva de Enlace<br>Transition Curve |         |      | 1,75-0,00 |
|                                     |         | 1.75 | 1.51735   |
|                                     |         | 1.50 | 1.37128   |
|                                     |         | 1.25 | 1.25586   |
|                                     |         | 1.00 | 1.16730   |
|                                     |         | 0.75 | 1.10312   |
|                                     |         | 0.50 | 1.06171   |

TABLA I

$R_0 = 4,50$   
 $A = 0,2769228$   
 $B = 1,1076912$

$R_0 = 4,50$   
 $A = 0,249999$   
 $B = 0,5624993$

| Curva Principal<br>Principal Curve  |      |      | 4,50-1,50 |
|-------------------------------------|------|------|-----------|
| 4.50                                | 2.00 | 4.25 | 0.65362   |
|                                     |      | 4.00 | 1.11032   |
|                                     |      | 3.75 | 1.43151   |
|                                     |      | 3.50 | 1.67625   |
|                                     |      | 3.25 | 1.86736   |
|                                     |      | 3.00 | 2.01620   |
|                                     |      | 2.75 | 2.12900   |
|                                     |      | 2.50 | 2.25780   |
|                                     |      | 2.25 | 2.27452   |
|                                     |      | 2.00 | 2.25632   |
|                                     |      | 1.75 | 2.19616   |
|                                     |      | 1.50 | 2.07703   |
|                                     |      | 1.25 | 1.83762   |
| 1.00                                |      |      |           |
| Curva de Enlace<br>Transition Curve |      |      | 1,25-0    |
|                                     |      | 1.25 | 3.07703   |
|                                     |      | 1.00 | 1.93727   |
|                                     |      | 0.75 | 1.83921   |
|                                     |      | 0.50 | 1.77397   |
|                                     |      |      | 1.74134   |

| Curva Principal<br>Principal Curve  |         |      | 4,50-1,00 |
|-------------------------------------|---------|------|-----------|
| 4.50                                | 1.50    | 4.25 | 0.71916   |
|                                     |         | 4.00 | 1.22864   |
|                                     |         | 3.75 | 1.59389   |
|                                     |         | 3.50 | 1.87925   |
|                                     |         | 3.25 | 2.10977   |
|                                     |         | 3.00 | 2.29818   |
|                                     |         | 2.75 | 2.45160   |
|                                     |         | 2.50 | 2.57509   |
|                                     |         | 2.25 | 2.67066   |
|                                     |         | 2.00 | 2.73980   |
|                                     |         | 1.75 | 2.78254   |
|                                     |         | 1.50 | 2.79744   |
|                                     |         | 1.25 | 2.78075   |
| 1.00                                | 2.72390 |      |           |
| 0.75                                | 2.60390 |      |           |
| Curva de Enlace<br>Transition Curve |         |      | 0,75-0    |
|                                     |         | 0.75 | 2.60390   |
|                                     |         | 0.50 | 2.47320   |
|                                     |         | 0.25 | 2.40672   |

TABLA I

$R_0 = 4,50$   
 $A = 0,233766$   
 $B = 0,233766$

$R_0 = 4,50$   
 $A = 0,225$   
 $B = 0,05625$

| Curva Principal<br>Principal Curve  |         |      | 4,50-0,75 |
|-------------------------------------|---------|------|-----------|
| 4.50                                | 1.00    | 4.25 | 0.76864   |
|                                     |         | 4.00 | 1.31784   |
|                                     |         | 3.75 | 1.71609   |
|                                     |         | 3.50 | 2.03172   |
|                                     |         | 3.25 | 1.29142   |
|                                     |         | 3.00 | 2.50886   |
|                                     |         | 2.75 | 2.69212   |
|                                     |         | 2.50 | 2.84630   |
|                                     |         | 2.25 | 2.97475   |
|                                     |         | 2.00 | 3.07965   |
|                                     |         | 1.75 | 3.16224   |
|                                     |         | 1.50 | 3.22294   |
|                                     |         | 1.25 | 3.26115   |
|                                     |         | 1.00 | 3.27478   |
|                                     |         | 0.75 | 3.25859   |
| 0.50                                | 3.19829 |      |           |
| 0.25                                | 3.01212 |      |           |
| Curva de Enlace<br>Transition Curve |         |      | 0,50-0    |
|                                     |         | 0.50 | 3.19829   |
|                                     |         | 0.25 | 3.13173   |

| Curva Principal<br>Principal Curve |         |      | 4,50- 00 |
|------------------------------------|---------|------|----------|
| 4.50                               | 0.50    | 4.25 | 0.79964  |
|                                    |         | 4.00 | 1.37370  |
|                                    |         | 3.75 | 1.79258  |
|                                    |         | 3.50 | 2.12709  |
|                                    |         | 3.25 | 2.40495  |
|                                    |         | 3.00 | 2.64043  |
|                                    |         | 2.75 | 2.84203  |
|                                    |         | 2.50 | 3.01526  |
|                                    |         | 2.25 | 3.16386  |
|                                    |         | 2.00 | 3.29050  |
|                                    |         | 1.75 | 3.39704  |
|                                    |         | 1.50 | 3.48477  |
|                                    |         | 1.25 | 3.55445  |
|                                    |         | 1.00 | 3.60629  |
|                                    |         | 0.75 | 3.63964  |
| 0.50                               | 3.65202 |      |          |
| 0.25                               | 3.63401 |      |          |

TABLA I

$R_0 = 4,00$   
 $A^0 = 0,4102564$   
 $B = 2,5641025$

$R_0 = 4,00$   
 $A = 0,33333$   
 $B = 1,33333$

| Curva Principal<br>Principal Curve |      |                                     | 4-2, 25 |
|------------------------------------|------|-------------------------------------|---------|
| 4.00                               | 2.50 | 3.75                                | 0.48383 |
|                                    |      | 3.50                                | 0.80108 |
|                                    |      | 3.25                                | 1.00272 |
|                                    |      | 3.00                                | 1.13338 |
|                                    |      | 2.75                                | 1.20866 |
|                                    |      | 2.50                                | 1.23375 |
|                                    |      | 2.25                                | 1.20687 |
|                                    |      | 2.00                                | 1.11675 |
|                                    |      | 1.75                                | 0.92408 |
|                                    |      | Curva de Enlace<br>Transition Curve |         |
|                                    |      | 2.00                                | 1.11675 |
|                                    |      | 1.75                                | 0.99752 |
|                                    |      | 1.50                                | 0.89868 |
|                                    |      | 1.25                                | 0.81864 |
|                                    |      | 1.00                                | 0.75624 |
|                                    |      | 0.75                                | 0.71065 |
|                                    |      | 0.50                                | 0.68128 |
|                                    |      | 0.25                                | 0.66778 |

| Curva Principal<br>Principal Curve |      |                                     | 4-1, 75 |
|------------------------------------|------|-------------------------------------|---------|
| 4.00                               | 2.00 | 3.75                                | 0.57704 |
|                                    |      | 3.50                                | 0.97164 |
|                                    |      | 3.25                                | 1.24045 |
|                                    |      | 3.00                                | 1.43619 |
|                                    |      | 2.75                                | 1.57882 |
|                                    |      | 2.50                                | 1.67764 |
|                                    |      | 2.25                                | 1.73682 |
|                                    |      | 2.00                                | 1.75699 |
|                                    |      | 1.75                                | 1.73502 |
|                                    |      | 1.50                                | 1.66149 |
|                                    |      | 1.25                                | 1.50921 |
|                                    |      | Curva de Enlace<br>Transition Curve |         |
|                                    |      | 1.50                                | 1.66149 |
|                                    |      | 1.25                                | 1.56682 |
|                                    |      | 1.00                                | 1.49280 |
|                                    |      | 0.75                                | 1.43813 |
|                                    |      | 0.50                                | 1.40189 |
|                                    |      | 0.25                                | 1.38354 |

TABLA 1

$R_0 = 4,00$   
 $A = 0,2909088$   
 $B = 0,6545448$

$R_0 = 4,00$   
 $A = 0,260666$   
 $B = 0,26666$

| Curva Principal<br>Principal Curve |      |                                     | 4-1,00  |
|------------------------------------|------|-------------------------------------|---------|
| 4.00                               | 1.50 | 3.75                                | 0,65370 |
|                                    |      | 3.50                                | 1,11084 |
|                                    |      | 3.25                                | 1,43278 |
|                                    |      | 3.00                                | 1,67840 |
|                                    |      | 2.75                                | 1,87065 |
|                                    |      | 2.50                                | 2,02051 |
|                                    |      | 2.25                                | 2,13508 |
|                                    |      | 2.00                                | 2,21669 |
|                                    |      | 1.75                                | 2,26671 |
|                                    |      | 1.50                                | 2,28408 |
|                                    |      | 1.25                                | 2,26462 |
|                                    |      | 1.00                                | 2,19775 |
|                                    |      | 0.75                                | 2,05108 |
|                                    |      | Curva de Enlace<br>Transition Curve |         |
|                                    | 0.75 | 2,05108                             |         |
|                                    | 0.50 | 1,89040                             |         |
|                                    | 0.25 | 1,81212                             |         |

| Curva Principal<br>Principal Curve  |      |         | 4-0,75  |
|-------------------------------------|------|---------|---------|
| 4.00                                | 1.00 | 3.75    | 0,71203 |
|                                     |      | 3.50    | 1,21649 |
|                                     |      | 3.25    | 1,57819 |
|                                     |      | 3.00    | 1,86090 |
|                                     |      | 2.75    | 2,08944 |
|                                     |      | 2.50    | 2,27646 |
|                                     |      | 2.25    | 2,42926 |
|                                     |      | 2.00    | 2,55227 |
|                                     |      | 1.75    | 2,64810 |
|                                     |      | 1.50    | 2,71798 |
|                                     |      | 1.25    | 2,76174 |
|                                     |      | 1.00    | 2,77730 |
|                                     |      | 0.75    | 2,75881 |
|                                     |      | 0.50    | 2,68926 |
| Curva de Enlace<br>Transition Curve |      |         | 0,50-00 |
|                                     | 0.50 | 2,68926 |         |
|                                     | 0.25 | 2,61249 |         |

TABLA 1

$R_0 = 3,50$   
 $A = 0,4464285$   
 $B = 1,9687495$

| Curva Principal<br>Principal Curve  |      |      | 3,50-2,00 |
|-------------------------------------|------|------|-----------|
| 3.50                                | 2.10 | 3.25 | 0.46680   |
|                                     |      | 3.00 | 0.76947   |
|                                     |      | 2.75 | 0.95781   |
|                                     |      | 2.50 | 1.07468   |
|                                     |      | 2.25 | 1.13424   |
|                                     |      | 2.00 | 1.13955   |
|                                     |      | 1.75 | 1.08416   |
|                                     |      | 1.50 | 0.94137   |
| Curva de Enlace<br>Transition Curve |      |      | 1,80-0    |
|                                     |      | 1.80 | 0.94137   |
|                                     |      | 1.60 | 0.78957   |
|                                     |      | 1.40 | 0.66580   |
|                                     |      | 1.20 | 0.56558   |
|                                     |      | 1.00 | 0.48599   |
|                                     |      | 0.80 | 0.42503   |
|                                     |      | 0.60 | 0.38136   |
|                                     |      | 0.40 | 0.35409   |
|                                     |      | 0.20 | 0.34268   |

$R_0 = 3,50$   
 $A = 0,4242423$   
 $B = 1,6969692$

| Curva Principal<br>Principal Curve  |      |      | 3,50-1,75 |
|-------------------------------------|------|------|-----------|
| 3.50                                | 2.00 | 3.25 | 0.48756   |
|                                     |      | 3.00 | 0.80781   |
|                                     |      | 2.75 | 1.01188   |
|                                     |      | 2.50 | 1.14456   |
|                                     |      | 2.25 | 1.22138   |
|                                     |      | 2.00 | 1.24715   |
|                                     |      | 1.75 | 1.21905   |
|                                     |      | 1.50 | 1.12236   |
|                                     |      | 1.25 | 0.89570   |
| Curva de Enlace<br>Transition Curve |      |      | 1,50-0    |
|                                     |      | 1.50 | 1.12233   |
|                                     |      | 1.25 | 0.94610   |
|                                     |      | 1.00 | 0.94940   |
|                                     |      | 0.75 | 0.87910   |
|                                     |      | 0.50 | 0.83310   |
|                                     |      | 0.25 | 0.81050   |

TABLA 1

$R_0 = 3,50$   
 $A = 0,35$   
 $B = 0,7875$

| Curva Principal<br>Principal Curve  |      |      | 3,50-1,25 |
|-------------------------------------|------|------|-----------|
| 3.50                                | 1.50 | 3.25 | 0.57910   |
|                                     |      | 3.00 | 0.97566   |
|                                     |      | 2.75 | 1.24636   |
|                                     |      | 2.50 | 1.44403   |
|                                     |      | 2.25 | 1.58860   |
|                                     |      | 2.00 | 1.68931   |
|                                     |      | 1.75 | 1.75010   |
|                                     |      | 1.50 | 1.77104   |
|                                     |      | 1.25 | 1.74756   |
|                                     |      | 1.00 | 1.66553   |
|                                     |      | 0.75 | 1.47017   |
| Curva de Enlace<br>Transition Curve |      |      | 1,00-0    |
|                                     |      | 1.00 | 1.66530   |
|                                     |      | 0.75 | 1.56317   |
|                                     |      | 0.50 | 1.49589   |
|                                     |      | 0.25 | 1.46031   |

$R_0 = 3,50$   
 $A = 0,311111$   
 $B = 0,31111$

| Curva Principal<br>Principal Curve  |         |      | 3,50--0,75 |
|-------------------------------------|---------|------|------------|
| 3.50                                | 1.00    | 3.25 | 0.64925    |
|                                     |         | 3.00 | 1.10349    |
|                                     |         | 2.75 | 1.42362    |
|                                     |         | 2.50 | 1.66829    |
|                                     |         | 2.25 | 1.86011    |
|                                     |         | 2.00 | 2.01044    |
|                                     |         | 1.75 | 2.12541    |
|                                     |         | 1.50 | 2.20815    |
|                                     |         | 1.25 | 2.25952    |
|                                     |         | 1.00 | 2.27769    |
|                                     |         | 0.75 | 2.25607    |
| 0.50                                | 2.17347 |      |            |
| Curva de Enlace<br>Transition Curve |         |      | 0,50-0     |
|                                     |         | 0.50 | 2.17344    |
|                                     |         | 0.25 | 2.08219    |

TABLA 1

$R_0 = 3,00$   
 $A = 0,520833$   
 $B = 1,6874998$

| Curva Principal<br>Principal Curve |      |                                     | 3-1, 00 |
|------------------------------------|------|-------------------------------------|---------|
| 3.00                               | 1.80 | 2.75                                | 0.42659 |
|                                    |      | 2.50                                | 0.69444 |
|                                    |      | 2.25                                | 0.85056 |
|                                    |      | 2.00                                | 0.93322 |
|                                    |      | 1.75                                | 0.98409 |
|                                    |      | 1.50                                | 0.90216 |
|                                    |      | 1.25                                | 0.74743 |
|                                    |      | Curva de Enlace<br>Transition Curve |         |
|                                    |      | 1.25                                | 0.74743 |
|                                    |      | 1.00                                | 0.54767 |
|                                    |      | 0.75                                | 0.41448 |
|                                    |      | 0.50                                | 0.33103 |
|                                    |      | 0.25                                | 0.28965 |

$R_0 = 3,00$   
 $A = 0,44444$   
 $B = 0,999999$

| Curva Principal<br>Principal Curve  |      |      | 3-1, 25 |
|-------------------------------------|------|------|---------|
| 3.00                                | 1.50 | 2.75 | 0.49124 |
|                                     |      | 2.50 | 0.81466 |
|                                     |      | 2.25 | 1.02144 |
|                                     |      | 2.00 | 1.15655 |
|                                     |      | 1.75 | 1.23534 |
|                                     |      | 1.50 | 1.26204 |
|                                     |      | 1.25 | 1.23205 |
|                                     |      | 1.00 | 1.12346 |
| Curva de Enlace<br>Transition Curve |      |      | 1,00-0  |
|                                     |      | 1.00 | 1.12346 |
|                                     |      | 0.75 | 0.98608 |
|                                     |      | 0.50 | 0.89889 |
|                                     |      | 0.25 | 0.85387 |

TABLA I

$R_0 = 3,00$   
 $A = 0,375$   
 $B = 0,375$

| Curva Principal<br>Principal Curve  |      |      | 3- 0,75 |
|-------------------------------------|------|------|---------|
| 3.00                                | 1.00 | 2.75 | 0.57778 |
|                                     |      | 2.50 | 0.97395 |
|                                     |      | 2.25 | 1.24495 |
|                                     |      | 2.00 | 1.44347 |
|                                     |      | 1.75 | 1.58936 |
|                                     |      | 1.50 | 1.69179 |
|                                     |      | 1.25 | 1.75437 |
|                                     |      | 1.00 | 1.77632 |
|                                     |      | 0.75 | 1.75017 |
|                                     |      | 0.50 | 1.64728 |
| Curva de Enlace<br>Transition Curve |      |      | 0,50-0  |
|                                     |      | 0.50 | 1.64728 |
|                                     |      | 0.25 | 1.53325 |

$R_0 = 3,00$   
 $A = 0,3428571$   
 $B = 0,0857142$

| Curva Principal<br>Principal Curve |      |      | 3-0, 25 |
|------------------------------------|------|------|---------|
| 3.00                               | 0.50 | 2.75 | 0.63397 |
|                                    |      | 2.50 | 1.07695 |
|                                    |      | 2.25 | 1.38873 |
|                                    |      | 2.00 | 1.62674 |
|                                    |      | 1.75 | 1.81330 |
|                                    |      | 1.50 | 1.95958 |
|                                    |      | 1.25 | 2.07180 |
|                                    |      | 1.00 | 2.15322 |
|                                    |      | 0.75 | 2.20470 |
|                                    |      | 0.50 | 2.22362 |
|                                    |      | 0.25 | 2.19594 |