DOUBLE PLANIMETRY AS A MEANS OF MEASUREMENT OF CEREBRAL STRUCTURES

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Until recently for the determination of the volume of cerebral structures by measurements on serial sections, the formula proposed in our Institute:

$$V = \beta \cdot h(S_1 + S_2 + \ldots + S_n),$$

has been used, where V = volume; β = a constant; h is the section step i.e., the distance between successive sections studied; S = area of cross section of the structure on each section. With this method of measurement the error is 4-8%, and is greater the smaller the number of sections (n), as will be shown below.

The aim of the present work has been firstly to introduce a mathematical basis for determination of the volumes, and secondly to improve the method so as to reduce the error to a minimum, and to make it independent of the number of sections (n).

The method we have proposed is as follows:

Projections or micrographs are made at a considerable enlargement. A planimeter is used to measure the cross sectional area of the object under study (at high magnification the error due to the planimeter is reduced). A graph is then constructed; along the abscissa is marked off the section step (h), and along the ordinate the values of the area (S) of the object in each section. A smooth line is then drawn through the points by means of a flexible curve. The area of the figure enclosed between this curve and the abscissa is numerically equal to the volume of the object being measured.

The problem is now to determine the area of this figure to a higher degree of accuracy. For this purpose there are several formulae.

The formula for a rectangle is:

$$\int_a^b f(x)\,dx = \frac{b-a}{n}\left(y_{1/2} + y_{3/2} + \ldots + y_{n-1/2}\right). \tag{1}$$

The formula for trapeziums is:

$$\int_a^b f(x)\,dx = \frac{b-a}{n}\left(\frac{y_0 + y_n}{2} + y_1 + y_2 + \ldots + y_{n-1}\right). \tag{2}$$

This formula has been used in the Brain Institute.

Simpson's formula [1] allows the given curve to be integrated by a parabolic interpolation, i.e., sections of the curve are fitted to parabolic curves, and are integrated. In this formula the number of sections (n) does not appreciably affect the percentage error:

$$\int_a^b f(x)\,dx = \frac{b-a}{6\cdot n}[(y_0+y_n) + 2(y_1+y_2+\ldots+y_{n-1}) + 4(y_{1/2} + y_{3/2} + \ldots y_{n-1/2})]. \tag{3}$$

There is a further graphical method (4) in which the integration of the curve is made by means of a planimeter (secondary planimetry). This method is the most easily applied, and the least laborious, and it gives the most accurate result. The theoretical basis of the method is Simpson's formula. After the result has been obtained it must be applied to the actual size of the object, neglecting for the time being the crenation of the brain tissue which occurs as it is passed through the various fixatives and stains. This will be the subject of future articles.

Thus,

$$V = \frac{\beta}{m}\left(\int_a^b f(x)\,dx\right),$$

Where β is a constant related to the scale; m is the magnification of the micro-projection or micrograph.

Example: from preparations of a continuous series of sections stained with cresol violet by the method used at the Brain Institute a micrograph was made of every 20th section at a magnification of 1:30.

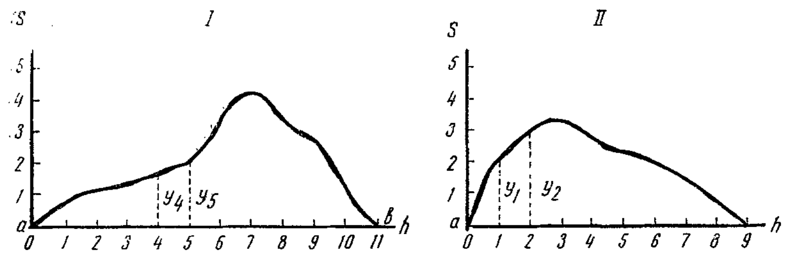
The sections were 20μ thick. The object was to determine the volume of the right paraventricular and supra-optic nuclei of the human hypothalamic region.

The number of sections (1 out of every 20) of the paraventricular nucleus was 12, and of the supra-optic nucleus 10. The distance between every 29th section was 400μ .

The area of cross section of the paraventricular nucleus (in cm^2) was: $S_0 = 0$, $S_1 = 4$, $S_2 = 6$, $S_3 = 7$, $S_4 = 9$, $S_5 = 10$, $S_6 = 17$, $S_7 = 22$, $S_8 = 17$, $S_9 = 9$, $S_{10} = 6$, $S_{11} = 0$.

The area of cross section of the supra-optic nucleus (in cm^2) was: $S_0 = 0$, $S_1 = 10$, $S_2 = 15$, $S_3 = 17$, $S_4 = 14$, $S_5 = 12$, $S_6 = 10$, $S_7 = 8$, $S_8 = 4$, $S_9 = 0$.

A graph is now constructed, one division along the abscissa represented 400 μ , and one division along the ordinate was 5 cm^2. Consequently, in the first case $y_0 = 0$, $y_1 = 0.8$, $y_2 = 1.2$, $y_3 = 1.4$, $y_4 = 1.8$, $y_5 = 2.0$, $y_6 = 3.4$, $y_7 = 4.4$, $y_8 = 3.4$, $y_9 = 1.8$, $y_{10} = 1.2$, $y_{11} = 0$; in the second case $y_0 = 0$, $y_1 = 2.0$, $y_2 = 3.0$, $y_3 = 3.4$, $y_4 = 2.8$, $y_5 = 2.4$, $y_6 = 2.0$, $y_7 = 1.6$, $y_8 = 0.8$, $y_9 = 0$ (see figure).



Graphical representation of the volume of (I) the paraventricular and (II) the supra-optic nucleus of the human hypothalamus.

Let us now calculate the volume of the paraventricular nucleus (in cm^2) by the four methods:

$$\int_a^b f(x)\,dx = \frac{b-a}{n}(y_{1/2} + y_{3/2} + \ldots + y_{n-1/2}) = \frac{12-0}{12}(0{,}4 + 1{,}0 + 1{,}3 + 1{,}6 + 1{,}9 + 2{,}7 + 3{,}9 + 3{,}9 + 2{,}6 + 1{,}5 + 0{,}6) = 21{,}4. \tag{1}$$

$$\int_a^b f(x)\,dx = \frac{b-a}{n}\left(\frac{y_0+y_n}{2}+y_1+y_2+\ldots+y_{n-1}=\frac{12-0}{12}\ 0{,}8+1{,}2+1{,}4\right.$$

$$\left.+1{,}5+2{,}0+3{,}4+4{,}4+3{,}4+1{,}8+1{,}2\right)=19{,}8. \quad (2)$$

$$\int_a^b f(x)\,dx = \frac{b-a}{6\cdot n}\,[(y_0+y_n)+2(y_1+y_2+\ldots+y_{n-1}+4(y_{1/2}+y_{3/2}+\ldots y_{n-1/2})]$$

$$=\frac{12-0^a}{6.12}\ [\,(0{,}8+1{,}2+1{,}4+1{,}5+2{,}0+3{,}4+4{,}4+3{,}4+1{,}8+1{,}2)+4\,]$$

$$(0{,}4+1{,}0+1{,}3+1{,}6+1{,}9+2{,}7+3{,}9+3{,}9+2{,}6+1{,}5+0{,}6)=20{,}4. \quad (3)$$

Planimetry gave a value of 20.6.

The mean value of the area of the given figure (20.56) was calculated as the arithmetic mean of results obtained by the four methods. Formula (1) gave 3.9% error, formula (2) gave 3.9%, formula (3) gave 0.9%, and formula (4) gave 0.19%. Similar operations were carried out for the supra-optic nucleus. Formula 1 gives an area of 20.0 (error 5.3%), formula 2 gave an area of 18.0 and 5.3% error, formula 3 gave 19.2 and 1.05% error, and formula 4, 19.0 and 0.2%. The mean area was 19.4.

From these examples it can be seen that the smallest percentage error (0.19 and 0.2) was obtained by use of the method of secondary planimetry; the number of sections (n) does not exert an appreciable influence on these indices, whereas when formulae (1) or (2) are used the percentage error increases from 3.9 to 5.3% when the number n is reduced only by 2 (when there is already a considerable error).

The results obtained are now brought to the true volume of the paraventricular and supra-optic nucleus. Thus:

$$V=\frac{\beta}{m}\left[\int_a^b f(x)\,dx\right],\quad m=900$$

(magnification of micrograph 1:30, and the area is taken from the projection), $\beta = 5\cdot 0.04$ (1 division on the ordinate = 5 cm^2, 1 division on the abscissa = 400μ, or 0.04 cm), then $V_1 = \frac{5\cdot 0.04}{900}\cdot 20.6 = 0.0046\ cm^3 = 4.6\ mm^3$, = volume of paraventricular nucleus; $V_2 = \frac{5\cdot 0.04}{900}\cdot 19.0 = 0.0042\ cm^3 = 4.2\ mm^3$ = volume of supra-optic nucleus.

In this case we have used only one of the possible ways of determining the volume of the figure, by using the area of cross section. By now a new scientific discipline stereology has developed; it is concerned with problems of determination of the volume of bodies, i.e., of the three-dimensional measurement of a body given only data on cross sectional areas (two-dimensional data). Very few articles on this subject have appeared [2-4].

The method of secondary planimetry applied to a graph of the expression of the volume of the object under study is the most precise. It enables the volume of any cerebral structure to be determined when investigating morphology on serial sections.

SUMMARY

A new method is described for determination of volumes of various cerebral structures from serial sections stained by a method developed at the Brain Institute. The method consists of diphasic planimetry, applied after the volume of the object has been plotted graphically; Simpson's formula is as follows:

$$\int_a^b f(x)\,dx = \frac{b-a}{6\cdot n}[(y_0+y_n)+2(y_1+y_2+\ldots+y_{n-1})]+4\cdot$$

$$\cdot\,(y_{1/2}+y_{3/2}+\ldots+y_{n-1/2}),$$

i.e., the method of parabolic interpolation, was applied as a theoretical basis.

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