Colorimetric Method for Measuring Pseudocholinesterase, Acetylcholinesterase and Whole Blood Cholinesterase. Its Use in the Presumptive Diagnosis of Cancer

We first started to determine cholinesterase activity in plasma, whole blood and cells in 1958. We calculated the latter, starting with the units obtained in plasma and whole blood, using the colorimetric method of BIGGS et al.[1]. We found increased cholinesterase activity in asthmatic patients as compared with the normal subjects, there being also increased activity following the administration of ACTH; these differences were statistically significant ($P < 0.001$)[2].

Following the same technique we later administered ACTH and corticosterone to Wistar rats and here also the increase was statistically significant ($P < 0.001$)[3,4]. Furthermore, following adrenalectomy in these animals there was a definite decrease ($P < 0.001$) of cholinesterase activity. These levels became almost normal after the injection of corticosterone[5].

In 1964[6], we started our studies on cholinesterase activity in patients suffering from cancer in different organs and found a decided decrease of these enzymes as compared with normal subjects ($P < 0.001$). We used the aforementioned colorimetric technique during these investigations[7,8]. We then proceeded to perfect this method of dosage of the colinesterase enzymes in order to increase precision and sensitivity by introducing the direct assay of acetylcholinesterase into the blood cells.

Material and methods. The determinations were made on 100 normal adults and on 30 male patients with pulmonary cancer. The method is based on the changes in colour produced in a Buffer of Bromothymol blue due to acetic acid released from the acetylcholine of the substrate. This is released by the cholinesterase present in the material to be analyzed.

Our technique is now the following: Reagents. (1) Buffer Stock: Sodium Barbital 16.4944 g KH_2PO_4 1.361 g and NaCl 175.35 g dissolved in 1 l of distilled water. (2) Stock buffer-indicator solution (Stock BI): Bromothymol blue 100 mg dissolved in 2 ml of $2N$ NaOH. Add 150 ml of the stock buffer solution, completing with distilled water up to 950 ml and then HCl $0.5N$ to obtain pH 8 which must be exact. As a general rule less than 16 ml of $0.5N$ HCl are used. (3) Working plasma: Stock BI 476.2 to 1000 ml with distilled water. Working whole blood and cells: Stock BI 400 to 1000 ml with distilled water. (4) 0.01% saponin solution. (5) Substrate: $1.5 \times 10^{-2} M$ of final concentration of acetylcholine bromide. Store in refrigerator and change every 15 days.

Calibration curve. Starting with a solution of $0.020N$ acetic acid, a scale is prepared as follows: 20 tubes are used, in which increasing quantities of acetic acid $0.020N$ (from 1–20 ml) and decreasing quantities of distilled water (from 19 ml to 0) are put in such a way that the first tube contains 1 µmole of acetic acid corresponding to 10 µ/ml. The final result is expressed in µmoles per millilitre of free acetic acid.

Plasma calibration curve. 21 numerated tubes are used from 0–20. To each one 0.1 ml of plasma, 1.4 ml of distilled water, 2 ml of Stock BI and 1 ml of the dilution of the corresponding acid is added. Tube 0 is treated the same, with the exception of putting 2.4 ml of distilled water and not adding the dilution of acetic acid $0.02N$. Mix well and read the absorbance at 620 nm, using distilled water as a blank. Divide the absorbance obtained in each tube (starting with tube No. 1) by the absorbance obtained in tube No. 0. This will give values which, carried to the ordinate axis and putting the corresponding units in the abscissa, allows us to obtain the calibration curve (Figure 1).

Whole blood calibration curve. 21 numerated tubes from 0–20 are used. Tube 0 contains 1 ml of saponin, 0.1 ml of the sample, 3.0 ml of Stock BI and 4.9 ml of distilled water. Tubes 1–20: 1 ml of saponin, 0.1 ml of pooled normal whole blood; 3.0 ml of Stock BI, 2.9 ml of distilled water and 2 ml of the corresponding dilution of acetic acid. Blank: 1 ml of saponin, 0.1 ml of the sample and 7.9 ml of distilled water. Mix well and read the absorbance at 620 nm and continue as for the plasma curve, using the contents of the blank tube as the reference blank (Figure 2).

Cellular calibration curve. The procedure is exactly the same as for whole blood but using 0.1 ml of cells obtained after centrifuging the blood and washing twice with physiological solution. Each centrifugation is done at 4500 rpm for 10 min each. The curve is prepared as for whole blood (Figure 2).

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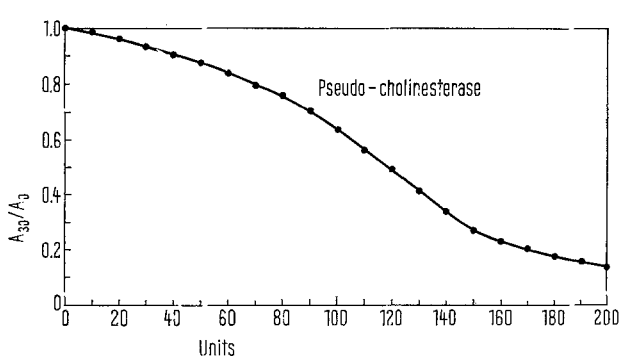

Fig. 1. Plasma calibration curve.

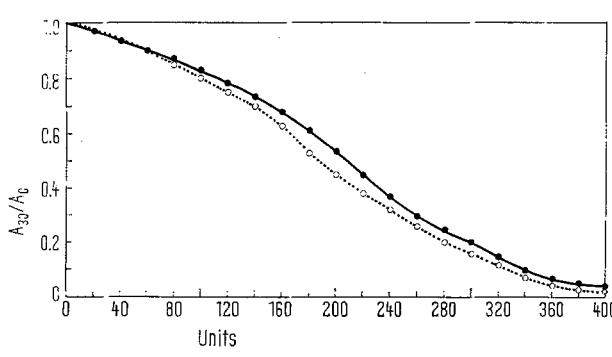

Fig. 2. Whole blood and cellular calibration curve. (·····) whole blood cholinesterase; (——) acetylcholinesterase.

Units of cholinesterase activity found in 100 normal adult males and 30 males suffering from cancer of the lung

ChE plasma		AChE ml of blood		AChE ml of cells		ChE whole blood	
Normal	Cancer	Normal	Cancer	Normal	Cancer	Normal	Cancer
98 ± 19	44 ± 20[a]	136 ± 14	94 ± 20[b]	285 ± 25	254 ± 29[c]	233 ± 25	167 ± 42[d]

Average and standard deviation. [a] $P<0.001$. [b] $P<0.001$. [c] $P<0.001$. [d] $P<0.001$.

Plasma. Contains: Working plasma 4.2 ml, 0.1 ml of the sample; 0.20 ml of substrate. Whole blood. Contains 1 ml of saponin, 0.1 ml of the sample, 7.5 ml of working blood, 0.40 ml of substrate. The corresponding blank contains 1 ml of saponin, 0.1 ml of the sample, 7.9 ml of distilled water. Cells. The procedure is exactly the same as for whole blood but using the corresponding sample.

In all three, immediately after adding the substrate, the spectrophotometer is read at 620 nm. Incubation for 30 min at 25 °C and the reading is repeated. The ratio between the final and initial readings is obtained and the units of pseudocholinesterase or acetylcholinesterase obtained found on the curve. To express the concentration of acetylcholinesterase per millilitre of cells, the following formula is applied:

$$\frac{\text{Value obtained in cellular curve} \times \text{hematocryt}}{100}$$

Care should be taken of the following: (1) The pH of the buffer indicator (Stock BI) must be exact. (2) The salt of acetylcholine used should be kept in a dryer to avoid hydration; once the solution has been prepared it should be kept in a refrigerator. It should be renewed every 15 days. (3) The reagents should be kept at 4–6 °C.

Results and discussion. The results obtained with this technique in normal subjects and in patients with pulmonary cancer are represented in the corresponding Table. The differences were statistically significative ($P<0.001$).

It is indispensable to fix the correct normal limits by making the statistical analysis of a sufficient number of normal subjects adding and subtracting from the mean value obtained twice the amount of standard deviations. It is also necessary to make these calculations in both men and women as the values in the latter are slightly lower than those of the males.

To use these determinations as a test for the presumptive diagnosis of neoplastic diseases, there are some circumstances that modify the values of the cholinesterase enzymes that should be kept in mind; liver insufficiency, anticholinesterase drugs, administration of ACTH or corticosteroids. The results of a positive reaction for the presumptive diagnosis of cancer should be expressed: 1 cross (+), 2 crosses (++), 3 crosses (+++) or 4 crosses (++++), according to whether the abnormal decrease is present in one or all of the different determinations.

Résumé. On décrit une méthode colorimétrique perfectionnée pour la détermination simultanée de l'activité pseudocholinestérasique, de l'acétylcholinestérase par millilitre de globules et de sang et de la cholinestérase sanguine totale, méthode actuellement employée par les auteurs pour le diagnostic présomptif du cancer. Les différences entre 100 sujets normaux et 30 malades de cancer pulmonaire ont été statistiquement significatives ($P<0.001$) dans les quatre déterminations.

J. R. VACCAREZZA, D. C. RUIZ and A. C. DOMÍNGUEZ

Laboratorio de Investigaciones Fisiopatológicas, Cátedra de Tisiología, Facultad de Medicina de Buenos Aires, Buenos Aires (Argentina), 25 February 1969.

On the Dependence of Nuclear Oxidative Phosphorylation on Glycolysis in Isolated Rat Thymus Nuclei

As has been established by several authors[1–8], cell nuclei isolated from the thymus glands of calf and rat contain cytochromes and show an oxygen dependent ATP synthesis. Therefore oxidative phosphorylation is no longer a process exclusively associated to mitochondria. The cell nucleus of the thymus appears to be a complex organelle, also containing enzymes and metabolites of glycolysis and of the citric acid cycle[9–10]. MCEWEN[11] demonstrated the dependence of respiration and ATP synthesis on glycolysis. In his experiments he found a decreased respiration and ATP content when he used the glycolytic inhibitor iodoacetate (IAA), which blocks glyceraldehydephosphate dehydrogenase (1.2.1.12). From his figures it was striking that ATP content was lowered by 89%, whereas oxygen uptake was decreased by only 31%. In his experiments 1 mM IAA was used; the nuclei were isolated from calf thymus. BETEL[12] used 1.5 mM IAA

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