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Total blood paper chromatography for amino-acid balance

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A modification of the amino-acid paper chromatographic method using fresh preserved blood on filter paper is presented. The modification allows a wider utilization of the method, with a greater number of samples in each determination. This makes it more reliable for field work.

The study for amino-acid balance of 200 non-selected children showed a mean value of 0.92 ± 0.3 and a normal range of between 0.62 and 1.22.

The comparison of the results obtained with the original and the modified technique in different laboratories showed that their results were identical.

Among 80 children with chronic encephalopathy, amino-acid disturbances were found in 29%.

States of malnutrition are easily detected on the basis of clinical signs and symptoms and of anthropometric measurements but the use of reliable biochemical methods becomes necessary when we wish to assess a community [3, 8]. The numerous parameters studied include haemoglobin, blood cholesterol, cholesterol esters, total blood proteins, serum albumin, amino-acid balance and the hydroxyproline/creatinine ratio with weight and height indices [3, 14], serum transferrin [6], and zinc [5]. In addition, enzymatic studies [7] are invaluable, especially for detecting marginal or subclinical undernutrition.

Amino-acid balance is one of the most widely used methods [11, 12, 13] due to its easy technique and to the fact that other methods are less suitable for mass screening. The method relates the non-essential and essential amino-acids in such a way that their ratio is high in undernourished children. Thus, the method offers an index for the early detection of protein-calorie malnutrition, even when no clinical signs are evident [1].

The original procedure being too complex for field work, we have modified it in order to facilitate screening. Normal values were determined in a non-selected sample; in addition, in a group of chronic encephalopathics; and, finally, we have correlated the results with those of the original method in a group of hospitalized malnourished children. A quality control of the modified method has also been performed during the entire course of the study.

Material and Methods

Blood samples were taken by finger punction, collected on Whatman's 31 paper, dried at room temperature and then subjected to autoclaving at 15 pounds pressure for 3 minutes. The same paper served for identification of the samples.

For chromatography, Whatman's 3 MM paper, 14 cm wide and 33 cm long, cut in 2.5 cm strips according to Fig. 1 was used.

Blood was applied in disks 12 mm in diameter inserted into 12 mm holes made in the paper strips.

The chromatograms were placed on a metal support Datta type for multiple development of adequate proportions. Simultaneous development of 24 chromatograms was performed in a Shandon chamber of $17 \times 33.5 \times 35.5$ cm internal size, in isopropanol/water (3 : 1) solvent [9]. Running was allowed until the front of the solvent had reached the widest part of the strip (approximately one hour in ascending form). Then the chromatograms and the blood disks were dried, and a second run was made in N-butanol/acetic acid/water (12 : 3 : 5) solvent during the night (about fifteen hours in ascending form). This solvent can only be used twice consecutively.

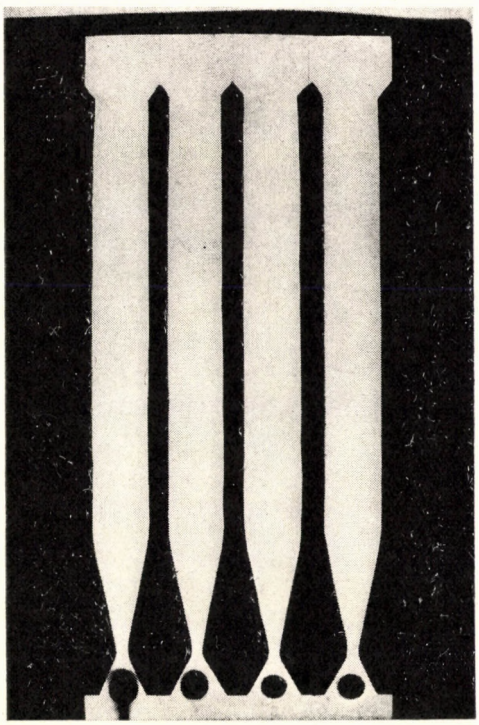

Fig. 1. Paper strips cut as explained in text. On the lower end of the first one at the left, the paper disk with dry blood is seen in the corresponding hole

Developing was made in 2% ninhydrine in acetone, then the strips were dried and oven heated at 110 °C during 5 minutes. Of the two upper spots, the highest one corresponded to leucine and isoleucine, and the other to valine and methionine; they were pencil marked. The same operation was performed with glycine, serine, glutamine and taurine with the corresponding spot adjacent to a third spot from the point of application. Since this spot was larger than the others, it was easy to differentiate it.

The chromatogram was then passed through a 10% copper nitrate solution; then the spots were allowed to dry and processed in the following way.

Two ml 80% ethanol was added to each tube and they were placed in boiling water for 15 minutes under continuous shaking. Then the operation was repeated with another 2 ml of 80% ethanol and the two eluates were pooled. Of the mixture, a 4 ml sample was subjected to spectrophotometry (SP 600 Unicam) at 510 mU wavelength against an ethanol blank.

With the purpose of establishing normal values in a non-selected sample, blood was taken from children different in age, sex and race, all of them attending the William Soler Pediatric Hospital Laboratory. In order to compare the original method performed in that laboratory with our modified method, another group was taken from children hospitalized in the Section of Nutrition of the same Hospital. Finally, samples were collected from chronic ence-

phalopathy patients interned at homes for mentally and physically disabled children. In these patients we expected to find pathological values and, at the same time, to utilize the procedure for amino-acidopathy screening.

Results

Using the procedure as described, there was no interference resulting from total blood utilization; all spots remained clearly separated, with the exception of the higher one, which did not always separate from phenylalanine.

Comparison of the results yielded by the original method in the Hospital Laboratory with those yielded by our procedure, showed no statistically significant difference, both procedures offering identical results. Pooled blood obtained from the controls was subjected to 20 determinations during 20 days; the values obtained were 55% within the limits of one standard deviation, with a 15.5% variation coefficient (Fig. 2).

As a mean value for 200 children, 0.92 ± 0.3 was obtained and the normal range was found between 0.62 and 1.22. Values above 1.82 were considered pathological. These data were obtained by rectification of the mean values taken from 200 original subjects, using the Thielman modification of Newman's method.

Among 80 encephalopathic children, 29% had an amino-acid balance value greater than 1.34; while 51% fell in the range of normal values.

Discussion

The main advantage of the presented method is the fact that dried blood samples preserved on filter paper can directly be used in amino-acid balance chromatography, avoiding in this way the initial steps of sample preparation and its preservation. This allows a wider utilization of the method.

As far as the original and the modified procedures are concerned,

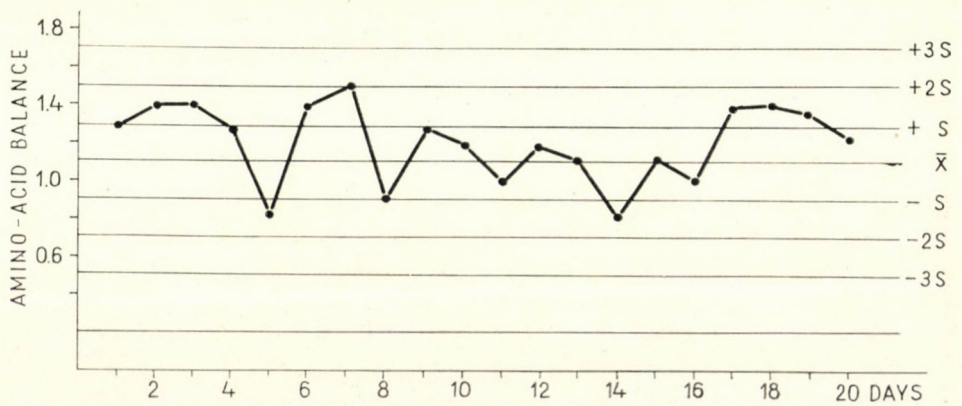

Fig. 2. Results of quality control carried out during twenty consecutive days in pooled blood obtained from controls

their results are not always well correlated with the clinical judgement of malnutrition. To conduct a screening, the use of other diagnostic acids is therefore necessary, and in this sense we have used the hydroxyproline/creatinine coefficient with weight and height indices [3, 14].

Application on paper forming a wedge offers the advantage of saving time and material, which allows a study of a greater number of cases than with bar application of such a number of samples.

Data obtained for the mentally retarded group showed a relatively high percentage of children with an amino-acid balance higher than 1.34, more than twice of those found in mixed population.

The normal values obtained for Habana children were well below those reported by other authors for other countries. These values could presumably be accounted for by nutritional habits in Cuba.

To sum up,

1) fresh blood preserved on filter paper can be used for amino-acid balance analysis;

2) normal values obtained for Habana children were inferior to those reported for other countries;

3) a comparison of the original and the modified methods showed that the latter was more advantageous for application in a greater number of cases;

4) encephalopathic children showed a disturbed amino-acid balance in a high percentage.

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