

Histochemical Changes at Base Level of Umbilical Cord

I. Behaviour of Alkaline and Acid Phosphatases

G. LO MENZO

Institute of Forensic Medicine and Insurance University of Catania
(Head: Prof. F. NICOLETTI)

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This subject has already drawn many authors' interest, particularly with regard to macroscopical and histological changes of umbilical cord.

The problem is particularly important when we are in the impossibility of surveying the lungs (for example if they have been lost), and it is extremely important to ascertain whether extrauterine life has been lived.

In 1898 KOCKEL reported that a leucocyte infiltrate in the subcutaneous tissue of periumbilical region should be considered as a characteristic of newborn that lived an extrauterine life.

But such an interpretation was, however, restricted by later studies of GLINSKY and HOROSZKIEWICZ (1903) who gave no importance to a superficial infiltrate, except when it, in a notable quantity, marked the boundaries of the funiculus base: that generally is found to be present after about 24 hours of extrauterine life.

GRÄFF (1922) thought that not even this evidence should be taken into account.

Afterwards, GIORDANO (1932) and MANUZA (1935), confirmed the studies of GLINSKY and HOROSZKIEWICZ, pointing out that both a leucocyte slight infiltrate and its absence could not mean that newborn had or had not lived an extrauterine life.

Only a remarkable infiltrate at the funiculus base should be considered as a sign of extrauterine life, prolonged as long as the second or third day.

Subsequently, CAMBA confirmed the data reported by MANUNZA, by VEIGA DE CARVALHO and FITTIPALDI about the finding of plasmacells in the perifunicular infiltrate of hereditary luetic newborn.

Reading once again previous literature about this subject, we considered that a more detailed histochemical study of the skin surrounding the umbilical cord base could be interesting, since sure changes occur after birth and go on as long as funiculus falls.

Material and Methods

The investigation was carried out on 16 either male or female newborn observed in our section.

Among these, 5 lived no extrauterine life (one of these 5 was a fetus in the 6th month); among the remaining 11, two lived 72 hours, one 48 hours, one 24 hours,

one 15 hours, two 12 hours (one of them was premature), one 8 hours, two 2 hours, one a few minutes.

Only subjects in a good state of preservation were used, they had a well known anamnesis as regarding cause, date of death and exact period of time lived.

The removal of periumbilical zone was carried out from 24 to 36 hours after death.

The skin and the initial part of umbilical cord were then fixed for 10 hours in a 10% neutral buffered formaline at $+4^{\circ}\text{C}$.

Then by "Kryomat Leitz" were prepared 10—15 μ thick sections, which were examined according to the following procedures:

Alkaline Phosphatase Activity

1. Incubation for 10 minutes at $+20^{\circ}\text{C}$ in "Tris" buffer 0.2 M at $\text{pH} = 10$ containing 0.1% of sodium α -naphthyl phosphate and 0.1% of Fast Violet B (a diazonium salt of 6-benzoyl-4-methoxy-m-toluidines). This solution prepared extemporaneously in order to avoid auto-oxidation phenomena, was previously shaken and filtered to eliminate precipitates.

2. Washing in distilled water for 3 minutes, and subsequent setting in glycerine jelly. Phosphatase hydrolyzes sodium α -naphthyl phosphate, breaking down it into sodium phosphate and α -naphthol.

The latter reacting with a diazonium salt of α -naphthylamine at strongly alkaline pH, breaks down azoic bonds, producing a brown precipitate where enzymes act.

Acid Phosphatase Activity

Incubation for 10 minutes at 20°C in Veronal Acetate buffer 0.1 M at $\text{pH} = 5$, containing 0.1% of sodium α -naphthyl phosphate and Fast Garnet GBC 0.1 M. The solution, prepared extemporaneously, was shaken and filtered. A dark-reddish precipitate is formed where acid phosphatase acts, through the same process above.

Results

Alkaline Phosphatase Activity

No enzymatic activity was observed either in the skin or in the umbilical cord of newborn that lived no extrauterine life; dermal vessels were well evident.

As regards as these, the newborn that lived a few minutes, or two and eight hours, showed no noteworthy change, but a slight increase of epidermic enzymatic activity.

Newborn who lived 12 or 15 hours presented an enzymatic amount, with variable characteristics, in the connective tissue of the borderline zone between cutaneous stratum and umbilical cord.

Some times it was one's impression that a real leucocyte infiltrate was stratified in the connective tissue under epidermis; or on the other hand, this infiltrate made a transversal layer that seemed to demarcate clearly the skin from the umbilical cord.

A slight enzymatic reaction was always present in the skin. The reaction in the borderline zone become more marked in sections from

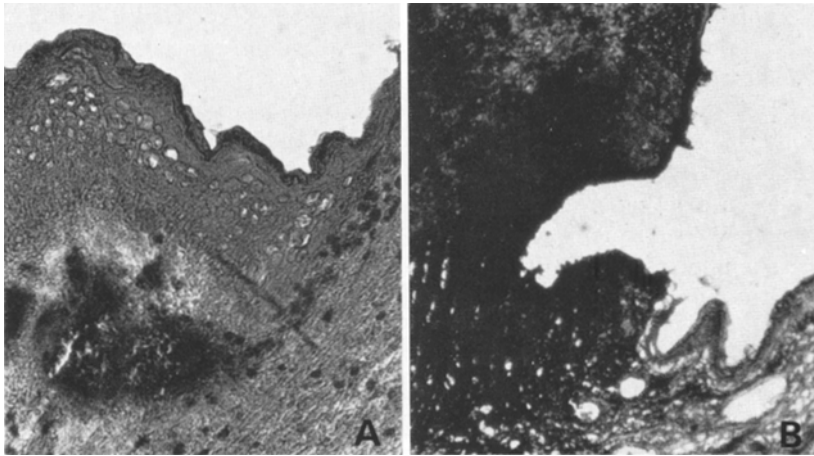


Fig. 1 A and B. Borderline zone between the cutaneous stratum and the funicular cord. A, Acid phosphatase activity. Newborn that had lived for 15 h of extrauterine life: Accumulation in the dermis of the enzyme with well evident leukocyte formations. Slight strengthening of the enzymatic activity at epidermic level (stratum basale, spinosum, granulosum, lucidum and corneum). B, Alkaline phosphatase activity. Newborn that had lived for 72 h of extrauterine life: The enzymatic accumulation greatly affected the epidermis (stratum basale, spinosum, granulosum, lucidum, corneum) and the dermis

newborn that lived 24 and 48 hours while leucocyte formations appeared less clear. Constantly we noted a considerable increase in phosphatase activity in the intermediate zone between skin and funiculus of newborn that lived three days; here an enzymatic pool affected epidermis and dermis.

Acid Phosphatase Activity

Enzymatic behaviour did not undergo any substantial change in relation to what we described for the alkaline enzyme.

A dermal amount, with a well evident infiltrate, was clearly observable in newborn that lived 12 or 15 hours, while no reaction was observable in newborn that lived a less long period of time.

The amount was more and more intense in newborn that died after 24—48 hours, and it became massive in those that lived 72 hours.

Considerations

From the data above we can deduce that, about 12 hours after birth, an alkaline and acid phosphatase activity increase is constantly observable in the borderline of umbilical cord.

The quantity of enzymes becomes greater and greater until it reaches considerable values in those newborn that lived a prolonged extrauterine life (about 72 hours).

We think it is significant to point out that histochemical properties observed follow a characteristic process: in newborn that died about 12 hours after birth, the enzyme amount, essentially at dermal level, is disposed at a tangent to epidermis, or it is perpendicular to this, as though it constitutes a ring separating skin from cord. Another characteristic information is the observation that the enzymatic activity spot is constituted at round formations both free and heaped, which resemble leucocytes.

This observation should be confirmed both in the already known studies of several authors who, with the usual histological techniques, reported the presence of leucocyte infiltrate in the skin surrounding funiculus (WACHSTEIN, 1946, 1955; VALENTINE and coll., 1954), and in the demonstrated alkaline phosphatase activity of neutrophil leucocytes, and in the acid phosphatase activity of polymorphonuclear leucocytes and phagocytes (GROGG and PEARSE, 1952; FAWCETT, 1953).

In newborn that lived a longer extrauterine life, as long as nearly the fall of funiculus, the activity of the two enzymes considered, underwent a considerable increase in the whole perifunicular zone, so much that the various skin strata were notably affected.

Such a behaviour is explained by the well known functional mechanism of the two enzymes. Phosphatases are, in fact, hydrolases and their function is to catalyze the breaking down of phosphoric acid from mono- and disphosphoric esters.

They have been also attributed with a synthetic function (RICHTERICH, 1952; MORTON, 1953, 1955 etc.) through transphosphorilation processes.

Therefore, it would seem that the increase we noted would, firstly attributable to leucocyte and phagocyte accumulation which develops in the border zone between skin and umbilical cord.

With the progression of hydrolase processes and with the establishing of those repairing ones, a further impulse in enzymatic accumulation should crop up that might be explained by transphosphorilation processes responsible for the repairing phenomena themselves.

Therefore, the two actions would be integrated and added to one another, thus providing the reason for the enzymes accumulation which represent the solution to a necessity of elimination while drying up.

From this point of view, this study is interesting for, unlike the results reported by various authors — (who investigated values and limits of umbilical cord docimasy and who also found perifunicular infiltrate in subjects that lived no extrauterine life) — we observed no

alkaline reaction in that ring separating skin from cord in the newborn that lived less than 12 hours.

This is probably in relation to the fact that, in such a case, the infiltrate was constituted of elements in particular functional conditions.

The enzymatic increase in leucocyte system was noted, in fact, in certain conditions as, trauma, surgical operations, etc. If, however, the test is an improvement on preceding results, it does not prove so useful for the various needs of a forensic medicine investigation.

The accumulation of enzymes is certainly evidence of extrauterine life, but only of that life which lasted at least 12 hours.

Therefore, the test has only an orientative value in those cases which, for particular conditions, it is not possible to find more reliable data, allowing, thus, to exclude an infanticide (according to the provisions of Art. 578, Italian C.P.) and to configure instead, a homicide.

It is important to point out how necessary it is to use material in a good state of preservation and still relatively recent (not longer than the third day after death).

Without such a certainty, one must only consider the positivity of the test; its negativity does not allow us to exclude extrauterine life for the loss of enzymes might be the consequence of a long period of time elapsed from the moment of death.

The application of the method in newborn that lived 12 or 15 hours certainly produces the best results for identification purposes, as, after such a period of time, macroscopical alterations of the funiculus occur, that make useless the application of the test described.

In conclusion, considering the inevitable limitations, we can say that an accumulation of alkaline and acid phosphatases in perifunicular zone is to be considered as evidence of a prolonged extrauterine life.

Summary

The author studied the behaviour of alkaline and acid phosphatases at level of the umbilical cord base of 16 newborn that lived or had not extrauterine life.

He noted that, starting from the 12th hour of life, a constant enzymatic reaction was observable with clear leucocyte accumulation characteristics, in the dermis of the borderline coronets. This became more and more intense with the prolongation of extrauterine life until, after 72 hours of life itself, it affected all the cutaneous strata. No reaction at such a level was present in products born dead.

Firstly, the enzymatic accumulation is presented in relation to hydrolytic phenomena and afterwards, to the adding together of the synthesis phenomena (by means of transphosphorilation processes) of which the umbilical cord system would be the site.

The test is indicated, even considering the inevitable limitations (fresh material in a good state of preservation), as being one of those which give evidence of extrauterine life.

Zusammenfassung

Der Verfasser untersuchte das Verhalten der alkalischen und der sauren Phosphatase an der Basis der Chorda umbilicalis bei 16 Neugeborenen, die z. T. extrauterin gelebt hatten.

Er fand, daß nach einer Überlebenszeit von 12 Std eine bestimmte enzymatische Reaktion mit charakteristischer leukocytärer Ansammlung sichtbar wurde, und zwar ringförmig in den äußeren Grenzbezirken der Epidermis. Bei zunehmender Überlebenszeit fand sich eine deutlichere Ausprägung dieser Befunde, nach 72 Std waren sämtliche Schichten der Haut einbezogen. Bei Totgeborenen wurden diese Veränderungen nicht in entsprechendem Maße vorgefunden.

Die Enzymvermehrung wird einmal auf hydrolytische Vorgänge und zum anderen auf synthetisierende Vorgänge (d. h. Transphosphorylierung) zurückgeführt. Diese Prozesse scheinen sich im System der Chorda umbilicalis abzuspielen.

Dieser Test kann zum Nachweis extrauterinen Lebens herangezogen werden, vorausgesetzt, daß es sich um frisches und gut erhaltenes Untersuchungsmaterial handelt.

Riassunto

L'A. ha studiato il comportamento della fosfatasi alcalina e acida a livello della base d'impianto del funicolo ombelicale di 16 prodotti del concepimento che avevano o meno goduto di vita extrauterina.

Ha notato che costantemente, a partire dalla 12^a, ora di vita goduta, era visibile una reazione enzimatica con chiare caratteristiche di accumulo leucocitario, nel derma del cercone di demarcazione. Col protrarsi della vita extrauterina la reazione si faceva sempre più intensa tanto da interessare, dopo 72 ore di vita stessa, tutti gli strati cutanei. Nessuna reazione a tale livello era presente nei nati morti.

L'accumulo enzimatico viene messo in rapporto, in un primo tempo ai fenomeni idrolitici e successivamente al sommarsi di quelli di sintesi (mediante processi di transfosforilazione) di cui sarebbe sede la base d'impianto del cordone ombelicale.

La prova viene segnalata, pur con le inevitabili limitazioni (materiale fresco e in buono stato di conservazione), fra quelle di goduta vita extrauterina protratta.

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Prof. Dott. GIUSEPPE LO MENZO
Libero Docente e Assistente Ordinario
Istituto Medicina Legale e Assicurazioni
Università, 95124-Catania, Via Biblioteca 4