J. Clin. Chem. Clin. Biochem.
Vol. 16, 1978, pp. 343–347

Diagnostic Value of Lysozyme Activity Estimation in the Feces of Infants with Acute Diarrhoea

By *J. Krawczuk, Z. Sawicki* and *J. Krawczyński*

Department of Laboratory Diagnostics (Director: Prof. J. Krawczyński M.D.) Postgraduate Medical School, Warsaw, Poland

(Received April 4/November 28, 1977)

Summary: The reliability of lysozyme (EC. 3.2.1.17) activity estimations with the lysoplate assay method of *Osserman & Lawlor* ((1966), J. Exp. Med. *124*, 921–952) in the feces of infants was investigated. It was found that the precision of the method is relatively high, despite the influence of inhibitors present in material investigated.

The activity of lysozyme in feces was estimated in a control group of 50 healthy infants and in a group of 152 infants with acute diarrhoea. All infants investigated were artificially nourished. In the latter group the activity of lysozyme was estimated twice: a) at the beginning of clinically active phase of the disease and b) in the convalescence period immediately after withdrawal of clinical symptoms.

The range of normal values was 14.9–77.0 (average 44.0) of egg-white lysozyme units/g dry feces. In acute diarrhoea the activity of lysozyme in feces was found to be elevated in 72.4% of cases in the first determination and in an additional 7.6% of cases in the second determination (i.e. a total of 80% of cases in both determinations). The average elevations of lysozyme activity in the feces and the dynamics of their normalization after withdrawal of clinical symptoms were generally related to the severity of the disease.

Diagnostische Bedeutung der Bestimmung von Lysozym in Faeces bei Kindern mit akuter Diarrhoe

Zusammenfassung: Die Zuverlässigkeit der Bestimmung von Lysozym (EC. 3.2.1.17) mit dem „lyso-plate-assay" von *Osserman & Lawlor* ((1966), J. Exp. Med. *124*, 921–952) in Faeces von Kindern wurde untersucht. Ungeachtet des Einflusses von Inhibitoren im Untersuchungsmaterial wurde festgestellt, daß die Präzision der Methode relativ hoch ist.

Lysozym wurde in Faeces von 50 gesunden Kindern (Kontrollgruppe) und 152 Kindern mit akuter Diarrhoe bestimmt. Alle Kinder wurden mit künstlicher Nahrung ernährt. In der Gruppe der kranken Kinder wurde Lysozym a) zu Beginn der klinisch aktiven Phase der Erkrankung und b) in der Gesundungsphase unmittelbar nach Verschwinden der klinischen Symptome bestimmt.

Der Normalbereich lag bei 14,9–77,0 ($\bar{x}$ = 44,0) Eiklar-Lysozym-Einheiten/g Trockenfaeces. Bei akuter Diarrhoe war Lysozym in 72,4% der Fälle bei der ersten und in zusätzlich 7,6% der Fälle bei der zweiten Bestimmung (insgesamt also 80% der Fälle) erhöht. Die erhöhten Werte für Lysozym in Faeces und der Verlauf der Normalisierung und Verschwinden der klinischen Symptome waren generell mit der Schwere der Erkrankung korreliert.

Introduction

Acute diarrhoea in infants is considered today as a general pathological condition in which the most characteristic feature is the elimination of untolerable substances of endogenic and/or exogenic origin (1–4).

Usually the attention of pathologists is directed towards the disturbances affecting the whole organism (2–7) and the local pathological changes of the intestinal wall are often neglected (1, 2, 4, 6, 7), despite their importance in the development and progress of the disease.

The typical, basic local pathological changes in small intestine in acute diarrhoea of infants can be morphologically characterized as enlargement of intestinal capillaries (8–10) and inflammatory infiltrations with typical, cellular components, such as plasmatic cells,

lymphocytes (11–15), neutrophilic (11, 15–19) granulocytes, monocytes and macrophages (20–22) of which the three last are known to be rich in lysozyme (23, 24).

Our previous observations (25) as well as those of other authors (26) suggest that it should be possible to evaluate indirectly the morphotic state of intestinal mucosa by the estimation of lysozyme activity in the feces.

According to *Braun* (27–29) and *Haneberg & Finne* (30) there is no or very little lysozyme activity in the feces of healthy artificially nourished infants. (In nursed infants the lysozyme activity in the feces derives mainly from mother milk). This activity may be increased in acute diarrhoea (27, 28, 29).

On the basis of the observations of *Senn* et al. (24) it was possible to conclude that in inflammatory infiltrated intestinal mucosa the amount of lysozyme may increase in parallel with the immigration of neutrophilic granulocytes, monocytes and macrophages. It seemed likely that lysozyme would be liberated into intestinal fluid from the above mentioned cellular elements as a result of its increased production within these cells (23, 31–33) or as a result of the destruction of the cells.

Taking this in account it was of interest to investigate the potential, diagnostic value of lysozyme estimations in the feces of infants with acute diarrhoea.

Methods and Materials

Our investigations were performed using the lysoplate diffusion method of *Osserman & Lawlor* (34). The activity of lysozyme was estimated in the extracts of feces samples, dried at 37 °C in order to eliminate the influence of varying amounts of water in diarrhoic feces. Lysozyme was extracted from 1 g of dried feces with 20 ml of 0.067 mol/l sodium phosphate buffer, pH 6.3, containing acetone (5 ml/l of buffer).

The results calculated from the diameter of the clearing zone were expressed in egg white lysozyme units (μg/g of dried feces).

In control experiments the precision of the method under different experimental conditions was less than 15%. The influence of interfering substances present in feces was investigated, as well as the influence of analytical and extraction procedures (mean inhibition – 78%). Taking in account the results of these experiments we can assume the satisfactory information value of estimations of lysozyme activity in feces of infants, at least for clinical purposes.

Our clinical investigations were performed on 50 healthy, artificially nourished infants (control group) and on 152 artificially nourished infants with acute diarrhoea of different severity.

The infants with diarrhoea were divided in 4 groups according to the severity of the disease (WHO classification) (2, 7):

48 with "light diarrhoea";
68 with "medium severe diarrhoea";
24 with "severe diarrhoea";
12 with "toxic diarrhoea".

Lysozyme activity in feces extracts was estimated twice: at the begin of the acute phase of the disease (during the first 1–3 days) and after withdrawal of clinical symptoms.

Because the distributions of results obtained in the control group and in groups of patients were neither normal nor log-normal, the statistical significance of their differences were calculated using the χ^2-test.

In 5 cases the morphological changes in the intestine were also investigated on autopsy.

Results and Discussion

The reference values (normals) and reference ranges of lysozyme activity in feces of healthy infants belonging to the control group were as follows: mean value: 44 units/g, range: 14.9–77.0 units/g.

The changes of lysozyme activity in feces during 1 week in artificially nourished infants: healthy and with acute diarrhoea before and during medical treatment are shown in figure 1.

It can be seen that (fig. 1) in normal conditions the changes of lysozyme activity in the feces, if any, are

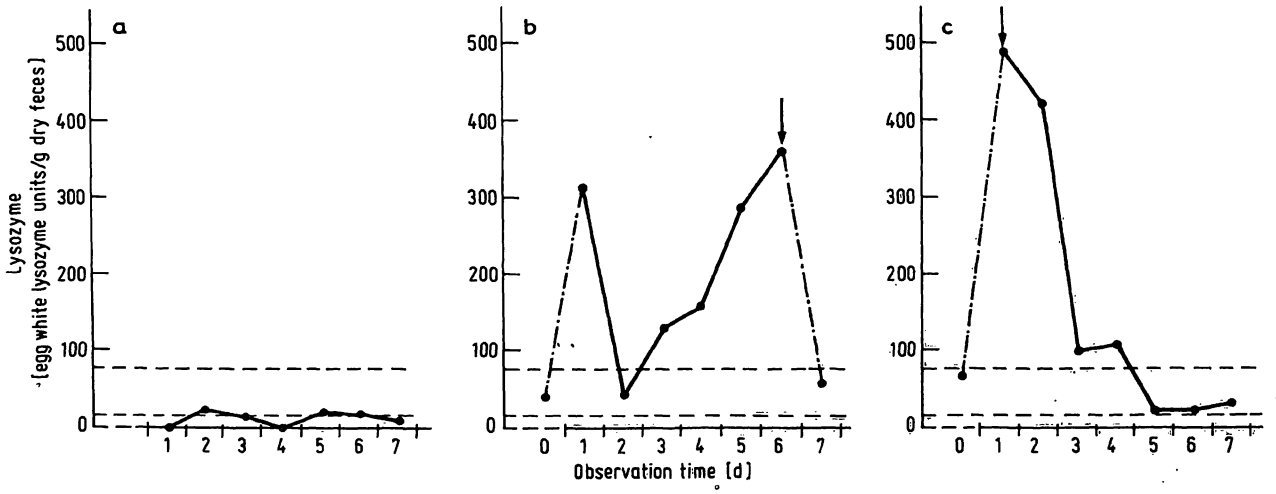

Fig. 1. The examples of lysozyme activity changes in feces of artificially nourished infants; a) healthy infant Z. M. b) infant Z. E. with acute diarrhoea untreated during first 6 days; c) infant J. R. with acute diarrhoea immediatelly treated. Remarks: 1) "0-day" – the last day before onset of the disease (infants Z. E. and J. R.); 2) "7-day" – the first day of the treatment (infant Z. E.).

relatively small, but in some cases of acute untreated diarrhoea, the activity of fecal lysozyme can be very high on the first day of the disease, undergoing considerable changes later. In other cases the activity of lysozyme in the feces increased simultaneously with the intensity of clinical symptoms, and the highest values were observed later. The effective treatment of the disease leads to the permanent decrease of lysozyme activity in the feces.

The frequency of elevated activities of lysozyme in the feces of infants with acute diarrhoea at the beginning of the clinically active phase of the disease was significantly higher than in the control group: $\chi^2_{\nu=1} = 58.89$ ($\chi^2_{0.0005;\nu=1} = 12.116$). This frequency also showed a significant increase with the severity of the illness: $\chi^2_{\nu=4} = 78.92$ ($\chi^2_{0.0005;\nu=4} = 19.997$). The mean values of lysozyme characterizing each group changed in a similar way (fig. 2).

A statistically greater frequency of elevated lysozyme activities in feces was already present in the group of infants with light diarrhoea: $\chi^2_{\nu=1} = 15.17$ ($\chi^2_{0.0005;\nu=1} = 12.116$) and statistically greater than in this last group it was also found in other groups of infants with medium severe: $\chi^2_{\nu=1} = 23.82$ ($\chi^2_{0.0005;\nu=1} = 19{,}997$), severe: $\chi^2_{\nu=1} = 13.18$ ($\chi^2_{0.0005;\nu=1} = 12{,}116$) and toxic diarrhoea: $\chi^2_{\nu=1} = 7.45$ ($\chi^2_{0.01;\nu=1} = 6.635$). The differences of frequences of elevated lysozyme activities in feces between groups of infants with medium severe, severe and toxic diarrhoea were statistically non-significant, being lower than the critical value of the χ^2-test: $\chi^2_{0.05;\nu=1} = 3.841$.

In the convalescence period (according to the WHO-recommendations: no more than 3 fluid stools or 1 stool with mucus- or blood-admixture per 24 hours) (2,7)- the frequency of elevated lysozyme activities in feces were significantly lower than in the clinically active phase of the disease: $\chi^2_{\nu=1} = 18.33$ ($\chi^2_{0.0005;\nu=1} = 12.116$) but always significantly higher than in the control group: $\chi^2_{\nu=1} = 21.25$ ($\chi^2_{0.0005;\nu=1} = 12.116$). The statistical significance of frequences of elevated results in separate groups of patients was generally similar to that found in the clinically active phase of the disease but with considerably lower values in the χ^2-test (tab. 1).

The mean values for lysozyme activity in separated groups of patients behaved similarly to the changes of frequences of the elevated lysozyme activities. In the acute phase of the "light diarrhoea" the mean activity of lysozyme in the feces was 121.1 units/g and in the convalescence period 81.7 units/g. In "medium severe diarrhoea" the respective values were 246.4 units/g and 148.6 units/g, in "severe diarrhoea" 815.6 units/g and 277.7 units/g and in "toxic diarrhoea" 750.1 units/g and 292.1 units/g.

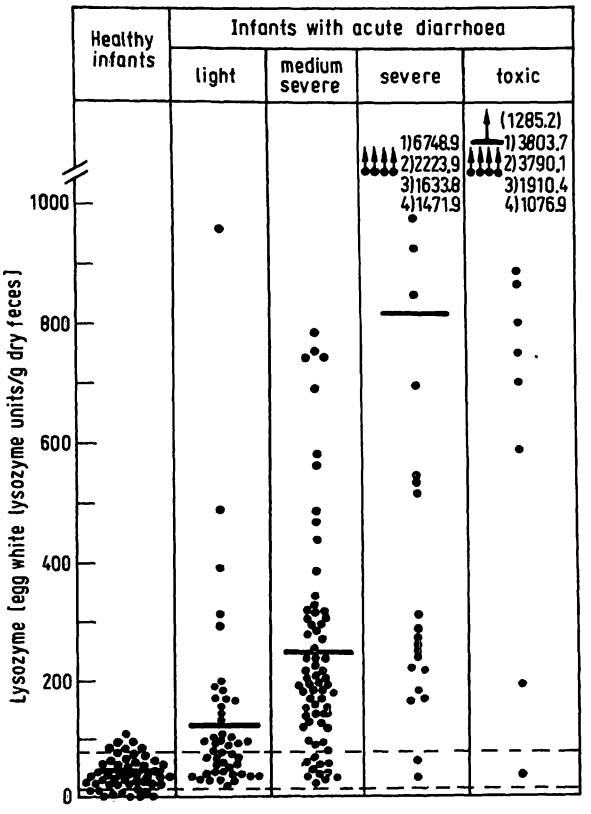

Fig. 2. Lysozyme activity in feces of 202 artificially nourished infants: healthy and with acute diarrhoea of different severity – at the begin of acute phase of the disease. Dotted line ----: upper and lower limits of normals.

Summarizing the results obtained in different groups of patients we can conclude that in the acute phase of the disease the relative frequency of cases with elevated lysozyme activities in the feces increases until the group of "medium severe diarrhoea". In more severe diarrhoeas, in which almost all results are elevated, the mean values of lysozyme activity increase further.

After the disappearance of clinical symptoms of diarrhoea the normalization of lysozyme activity in feces is observed. However, the frequency of this normalization decreases with the severity of the disease and in "severe diarrhoea" only 1/4 of cases show this response. This is also shown in the mean values of lysozyme activity in feces, which are higher, depending on the severity of the diarrhoea.

In the second estimation, we have also found pathological elevations of lysozyme activities in the feces of about 1/5 of the infants with "light diarrhoea" and in about half of the infants with "medium severe diarrhoea", in whom the first investigation showed an activity of lysozyme within the reference limits (tab. 1).

Tab. 1. The classification of infants with acute diarrhoea according to the elevated or normal lysozyme activities in feces at the begin of acute phase of the disease (1st estimation) and after withdrawal of clinical symptoms (2nd estimation).

		Number of cases with lysozyme activity in feces:									
		elevated in the 1st estimation:					within normals in the 1st estimation:				
			in whom the activity in the 2nd estimation:					in whom the activity in the 2nd estimation:			
			further increased:	decreased:				increased:		decreased:	
Acute diarrhoea	Total number of cases in each group	Total		remaining yet elevated	to the range of normal values	below the lower limit of normals	Total	remaining in the range of normals	above the upper limit of normals	remaining in the range of normals	below the lower limit of normals
light	45	20	3	5	9	3	25	8	5	12	0
medium severe	68	56	9	23	21	3	12	3	5	4	0
severe	23	21	6	10	4	1	2	0	0	2	0
toxic	9	8	1	3	3	1	1	0	1	0	0
Total:	145	105	19	41	37	8	40	11	11	18	0
Percentage:		72,4%	13,1%	28,3%	25,5%	5,5%	27,6%	7,6%	7,6%	12,4%	0,0%

These last cases, as well as the infants in whom the lysozyme activity in the second investigation did not return to reference values must be discussed separately. In the majority of these cases (52 out of 71) the exacerbations of clinical symptoms were observed in the course of the disease, despite the continued treatment. In 16 of these 71 infants, already discharged from the hospital as healthy, the recidive of diarrhoea was observed.

In 25 cases of this group we later found disturbances of intestinal absorption, digestion and excretion with following laboratory tests: *D*-xylose, lactose and glucose loading tests and the estimation in feces of serum protein, neutral fat, fatty acids, and soaps.

In two infants with "light diarrhoea" who died from inborn heart defects, no pathological changes were found on autopsy in the small intestine, and the lysozyme activities in the feces were only slightly elevated. In three infants who died in the course of "toxic diarrhoea" the morphological changes in the small intestine wall at autopsy were very pronounced (ulcerations and inflammatory infiltrations) and the lysozyme activities in feces were very high (820.6 units/g, 1079.9 units/g and 3803.7 units/g.).

Our present observations as well as our results concerning the sources of lysozyme in the gastric wall published earlier (25) justify the conclusion that the main source of normal and pathological elevated activities of lysozyme in the feces are the cellular elements of intestinal wall especially the neutrophilic granulocytes, monocytes and macrophages present in "physiological" and "pathological" infiltrations.

Conclusions

Summarizing the results of our investigations we can formulate the following conclusions:

1. The estimation of lysozyme activity in the feces of infants with acute diarrhoea gives in about 80% of cases elevated results which are generally related to the clinical severity of the disease.
2. The elevated lysozyme activities in the feces are probably connected with pathological changes in the intestinal wall such as infiltrations and, in severe cases even ulcerations.
3. The serial estimation of lysozyme activity in the feces of infants with acute diarrhoea seems to have some prognostic value.
4. The estimation of lysozyme activity in feces with the use of the simple and economic lysoplate diffusion method should find wide practical application.

References

1. Bincer, W. (1965), Klinika chorób zakaźnych. PZWL/Med. Staatsverlag), Warszawa.
2. Bożkowa, K. (1972), Biul. Inf. "Cefarm-Polfa" *22*, 241–249.
3. Fordtran, J. S. (1967), Fed. Proc. *5*, 1405–1414.
4. Kubicki, S. (1966), Pol. Arch. Med. Wewn. *37*, 207–218.
5. Ejsmont, L., Grabowska-Lubaszko, U., Gierasimowicz, M. & Iwaniukowicz, A. (1975), Pediatr. Pol. *50*, 753–759.
6. Piszczatowska, T. (1974), Pediatr. Pol. *49*, 493–498.
7. Szotowa, W. & Kowalczyk, Z. (1973), Podstawy oceny klinicznej, leczenie i zapobieganie w ostrych stanach biegunkowych u dzieci w wieku od 0 do 2 lat. PZWL (Med. Staatsverlag), Warszawa.
8. Da Costa, L. R., Croft, D. N. & Creamer, B. (1971), Gut *12*, 179–183.
9. Pruszczyński, A., Sroczyński, K., Kapuścińska, W., Kurnatowski, A., Piotrowski, A., Pszenicka-Gundlachowa, Z., Rapacka, W., Sobis, H., Torzecki, Z., Wieczorek, M. & Wojaczyńska, A. (1957), LTN, Wydz. IV, Lódź.
10. Schussheim, A. (1972), Gastroenterology *58*, 124–132.
11. Toccalino, H., Licastro, R., Garcia Gardo, A. & Williams, M., (1971), J. Pediat. *78*, 907.
12. Soltoft, J. & Soeberg, B. (1972), Gut *13*, 535–538.
13. Doe, W. F. (1972), Gut *13*, 572–578.
14. Taylor, K. B. (1965), Fed. Proc. *24*, 23–28.
15. Trier, J. S. (1967), Fed. Proc. *000*, 1391–1404.
16. Dammin, G. J. (1965), Fed. Proc. *24*, 35–38.
17. Formal, S. B., LaBred, E. H. & Schneider, H. (1965), Fed. Proc. *24*, 29–34.
18. Takeuchi, A., Formal, S. B. & Sprinz, H. (1968), Am. J. Pathol. *52*, 503–529.
19. Bradford, W. D., Noce, P. S. & Gutman, L. T. (1974), Arch. Pathol. *98*, 17–22.
20. Michael, E. (1974), Histochemistry *38*, 361–371.
21. Deane, H. W. (1964), Anat. Rec. *149*, 453–473.
22. Lojda, Z., Frič, P., Jodl, J. & Chmelik, V. (1970), Curr. Top. Pathol. *52*, 1–63.
23. Gordon, B. S., Todd, J. & Cohn, Z. A. (1974), J. Exp. Med. *139*, 1228–1247.
24. Senn, H. J., Chu, B., O'Malley, J. & Holland, J. F. (1970), Acta Haemat. *44*, 65–77.
25. Sadowska, H., Sawicki, Z., Czorniuk, A. & Krawczyński, J. (1976), Diagn. Lab. *12*, 197–208.
26. Hiatt, R. B., Engle, C., Flood, C. & Karush, A. (1952), J. Clin. Invest. *31*, 721–726.
27. Braun, O. H. (1958), Z. Kinderheilk. *81*, 742–751.
28. Braun, O. H. (1969), Dtsch. Med. Wochenschr. *94*, 1458–1462.
29. Braun, O. H. (1971), "Lysozym und Säuglingsernährung" in "Moderne Aspekte der künstlichen Säuglingsernährung." Internationales Symposion. (Berger, H. ed.) Georg Thieme Verlag. Stuttgart.
30. Haneberg, B. & Finne, P. (1974), Acta Paediatr. Scand. *63*, 588–594.
31. Speece, A. J. (1964), J. Histochem. Cytochem. *12*, 384–391.
32. Wiener, J. (1970), Curr. Top. Pathol. *52*, 143–208.
33. Wright, D. G. & Malawista, S. E. (1972), J. Cell. Biol. *53*, 788–796.
34. Osserman, E. F. & Lawlor, D. P. (1966), J. Exp. Med. *124*, 921–952.

Prof. Dr. med. J. Krawczyński
ul. Banacha 1 A
Zaklad Diagnostyki Laboratoryjnej CMKP
PL-02-097 Warszawa