Reliability of enzymatic lysozyme determinations

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Concentrations of human lysozyme (HL) in serum and urine are usually determined by measuring bacteriolytic activity. Several compounds interfere with the bacteriolytic action of lysozyme. *In vitro* studies have shown the inhibiting effects of substances like heparin (Kaiser, 1953), hyaluronic acid, DNA, and RNA (Skarnes and Watson, 1955), and aminoglycosides (neomycin, gentamycin, streptomycin) (Eudy and Burrous, 1972). Compounds like protamin and histones activate the lytic action of lysozyme (Kaiser, 1953; Skarnes and Watson, 1955).

The presence of activators and inhibitors of the bacteriolytic action has been demonstrated in dog serum and urine by Harrison and Barnes (1970) and Harrison and Swingler (1971). These authors added egg-white lysozyme to varying dilutions of dog serum and urine after which the recovery was assayed. It was observed that the influence of activators and inhibitors was dependent on the dilution factor.

Our study reports on the recovery of HL from human serum and urine. The reliability of enzymatic lysozyme determinations in serum and urine is discussed.

Methods and Material

The lysozyme determinations in this study were carried out turbidimetrically according to the automated method of Terry *et al* (1971).

From a solution of a weighed amount of standard HL in water, dilutions of 1, 5, 10, and 15 μg/ml in water were prepared.

Lysozyme concentrations in serum or urine were measured as bacteriolytic activities per μg standard HL. Standard Technicon Auto Analyzer equipment was used and all reagents were of 'Zur Analyse' quality (Merck, Darmstadt, GFR).

HL was isolated from the urine of a patient suffering from nephrocalcinosis and renal tubular acidosis. The isolation was carried out according to the procedure of Johansson and Malmquist (1971). The purity of the lysozyme preparation was checked electrophoretically in agarose gel. One single band was obtained.

Lyophilized *Micrococcus lysodeikticus* cells were purchased from Sigma Chemical Corporation (USA).

The variability of the method was tested by assaying two different sera 20 times on the same day.

For both sera a coefficient of variation of 6·7% was calculated, which is in good agreement with the results reported by Terry *et al* (1971).

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Results

RECOVERY OF HL FROM HUMAN SERUM

The samples from all individuals were diluted four times with water, and the recovery of 5 μg added HL per ml diluted serum was estimated. For 35 patients with normal or increased endogenous lysozyme concentrations the mean recovery was 101 ± 19% (SD) (figure). To investigate individual day-to-day variations, recoveries were estimated in serum samples obtained from three patients during 7 to 10 consecutive days. Recoveries of 105 ± 9·2% (SD), 83 ± 8·1% (SD), and 105 ± 9·7% (SD) were found respectively (figure).

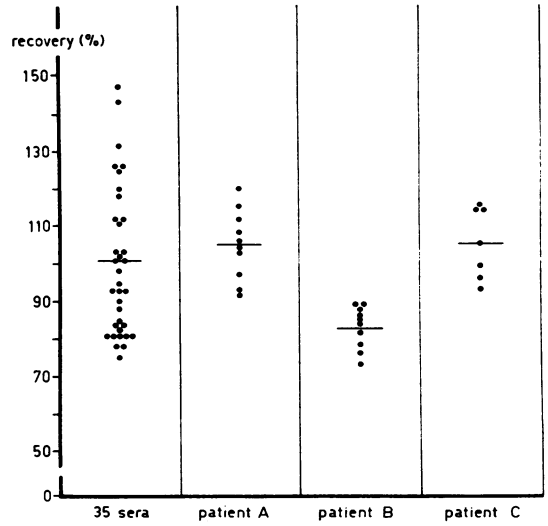

Figure *Recovery of human lysozyme added to serum samples obtained from 35 individuals. From patients A, B, and C recoveries of human lysozyme were assayed on 10 or 7 (patient C) consecutive days.*

RECOVERY OF HL FROM HUMAN URINE

To each of 14 urine samples of patients with normal lysozyme concentrations HL was added in quantities of 5, 10, and 15 μg per ml of urine. Recoveries varied between 88% and 150%, as is demonstrated in the table. In some urines (1-5), the recovery of HL decreased if the added quantities increased, whereas in others (6-9) the opposite was found. In samples

Sample	Amount of Lysozyme added per ml Urine (μg)		
	5	10	15
1	150	135	117
2	144	138	131
3	139	121	89
4	121	111	99
5	120	100	99
6	112	117	132
7	104	110	121
8	102	110	119
9	88	100	100
10	119	117	114
11	109	111	105
12	109	107	104
13	107	105	101
14	105	102	98

Table *Recovery of human lysozyme from urine* (%)

10-14 the recovery was not dependent on the added quantity.

Discussion

Our results demonstrate considerable variations in recovery of HL from human serum and urine. These variations appear to be greater between individual patients than within the same patient during 7 to 10 consecutive days. Apart from the variability of the method (6·7%), the variations could be considered as the result of the presence of activators and inhibitors. If this assumption is correct, the preponderant presence of inhibitors is responsible for the low recovery of HL from the serum of patient B, whereas in patients A and C the actions of inhibitors and activators would equal each other (figure).

Individual day-to-day variations in the ratio of activities of inhibitors and activators are smaller than the variations in a group of individuals.

In the urine samples the situation is apparently more complex: the recovery of HL depends on both the added amount of HL and the patients.

A conclusive explanation for the observations cannot be given unless activators and inhibitors are isolated from serum and urine.

Harrison and Swingler (1971) carried out a few orientating experiments in an attempt to identify activating and inhibiting factors. They isolated inhibiting and activating fractions by Sephadex G-200 chromatography of human serum.

No information concerning the purity of the fractions was given however. As our results show, bacteriolytic lysozyme determinations are afflicted with considerable errors, and great care must therefore be taken in their interpretation. Our results made us decide to reject the bacteriolytic lysozyme determination in lysozyme clearance studies. According to the clearance formula, the errors in the measured lysozyme concentrations can enhance each other. This may result in a total error in the clearance value up to 35%.

Immunochemical lysozyme determinations, as described by Johansson and Malmquist (1971), can be expected to give more accurate information about the true lysozyme concentration in serum and urine.

However, the bacteriolytic method is satisfactory enough for longitudinal studies in the serum of individual patients because of the relatively small (10-15%) differences in recovery from serum.

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