SHORT COMMUNICATION

Paraproteinemia: Predictive Value of Kunkel's Zinc Sulfate Turbidity Test

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IMMUNOELECTROPHORETIC analysis (IEPA) is an effective method for determining paraproteinemia. However, because of the scarcity of technologists with the necessary skills, and also because of the cost of equipment and antisera, the technique is routinely available in only a small proportion of hospitals, mostly in the larger centres. Clinicians outside these hospitals must either depend on the results of simple electrophoresis, or send the suspect serum elsewhere for analysis.

A simple and accurate screening test for paraproteinemia would be of value to small hospitals and individual practitioners. One test which has been widely used is the dilution test of Sia and Wu.[12] However, the predictive value of this test is low; about 50% of sera containing an IgM paraprotein give negative reactions.[7] The test may also be positive in hyperimmunoglobulinemic sera without detectable paraproteins.[6]

Preliminary examination of some other nonspecific chemical tests indicated that the zinc sulfate turbidity test of Kunkel[5] might be a useful screening test for paraproteinemia. This test depends on the precipitation of the immunoglobulins by a dilute buffered solution of zinc sulfate. In the absence of paraproteinemia, the turbidity developed varies directly with the immunoglobulin content of the serum. However, some paraproteins may inhibit the reaction,[2] while others give exceptionally high values. This paper reports a study designed to test the value of the zinc sulfate turbidity reaction as a screening test for paraproteinemia.

Materials and Methods

Reagent.—Kunkel's zinc sulfate reagent[5] was prepared as a single solution containing zinc sulfate (heptahydrate), 24 mg. per litre; 5,5′-diethylbarbituric acid, 280 mg. per litre; and sodium 5,5′-diethylbarbiturate, 210 mg. per litre. All chemicals were of analytical grade; the final pH of the reagent was 7.5 ± 0.1. The solution was stored in polyethylene bottles at room temperature; no deterioration was observed after four weeks.

Technique.—A 0.05-ml. portion of serum was pipetted into 3 ml. of zinc sulfate reagent in a clean 12 × 100 mm. test tube. The tube was shaken and allowed to stand at room temperature. After 30 minutes, the optical density was determined at 650 mμ., using a Bausch and Lomb Spectronic 20 colorimeter. The optical density was converted to zinc turbidity units (ZTU), using the barium sulfate standard described by Kunkel.[5] An optical density of 0.48 was accepted as equivalent to 20 ZTU.

Sera.—Four hundred sera, comprising 200 sera from apparently healthy blood donors and 200 pathological sera submitted to this laboratory for IEPA, were tested. All sera were examined by IEPA, using our standard method.[9] Sixty-eight of the pathological sera, but none of the normal (donor) sera, contained paraproteins.

Evaluation.—To assess the predictive value of the test, pathological sera were divided into classes on the basis of their protein levels, as supplied by the referring clinician. Sera with protein levels of 8 g. per 100 ml. or more were classed as hyperproteinemic, those with levels of 9 g. per 100 ml. or more as markedly hyperproteinemic. Where exact protein levels had not been quoted, the evaluation of the referring clinician (moderately or markedly elevated) was accepted as the basis of classification.

The protein level and zinc sulfate turbidity were entered against each patient's control number in a table. No other data were entered at this stage.

As a result of preliminary testing, the following criteria were selected as strongly suggestive of paraproteinemia:

1. Turbidity greater than 20 ZTU.
2. Moderately elevated total protein, with a turbidity less than 9 ZTU.
3. Markedly elevated total protein, with a turbidity between 9 and 15 ZTU.

Predictions made on the basis of these criteria were entered in a fourth column, and the results of IEPA in the last column for comparison.

Results

Table I compares the predicted and actual occurrence of paraproteins in the sera studied.

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The presence or absence of paraproteinemia was predicted correctly in 84.5% of the pathological sera. Of the 68 paraproteinemic sera, 61 (89.7%) were correctly predicted. "False positives" (sera in which a paraprotein was predicted but not found) constituted 12% of the pathological sera. "False negatives" constituted 3.5% of the pathological sera, or 10.3% of the paraproteinemic sera. In none of the normal sera was a paraprotein either predicted or found.

TABLE I.—RESULTS OF ATTEMPTS TO PREDICT THE PRESENCE OF PARAPROTEINS IN THE SERA STUDIED

	Predicted positive		*Predicted negative*	
Sera	*Positive*	*Negative*	*Positive*	*Negative*
Pathological				
—hyperproteinemic.....	60	23	4	38
—normoproteinemic.....	1	0	3	53
—hypoproteinemic.....	0	1	0	17
Normal.....	0	0	0	200

Table II shows the distribution of zinc sulfate turbidities among the sera studied. Ninety-nine per cent of the normal sera gave values between 0 and 10 ZTU; 98.2% of the normoproteinemic and all but one of the hypoproteinemic pathological sera also fell within this range. One hypoproteinemic serum gave a turbidity reading of 27 ZTU; gross hemolysis was evident on visual examination. Subsequent analysis of normal sera to which hemoglobin had been added confirmed that the presence of free hemoglobin in the serum can cause marked elevation of the zinc sulfate turbidity.

TABLE II.—DISTRIBUTION OF ZINC SULFATE TURBIDITY VALUES IN PATHOLOGICAL AND NORMAL SERA

	Percentage of sera with turbidity in the range				
Sera	*0 - 5*	*6 - 10*	*11 - 15*	*16 - 20*	*20*
Pathological					
—hyperproteinemic, paraprotein.....	34.4	12.5	9.4	10.9	32.8
—hyperproteinemic, no paraprotein.....	8.3	26.2	27.8	26.2	11.5
—normoproteinemic..	64.9	33.3	1.8	0.0	0.0
—hypoproteinemic...	94.4	0.0	0.0	0.0	5.6
Normal.....	93.0	6.0	1.0	0.0	0.0

Seventy-five per cent of the hyperproteinemic sera with turbidities greater than 20 ZTU, and all sera with turbidities greater than 40 ZTU, contained paraproteins. Of the 51 hyperproteinemic sera giving turbidities between 0 and 10 ZTU, 30 (58.8%) contained paraproteins; of the 56 normoproteinemic sera in the same range, only 4 (7.3%) contained paraproteins. Of the 46 hyperproteinemic sera with turbidities between 11 and 20 ZTU, 13 (29.8%) contained paraproteins, but in 10 of these cases the discrepancies between the protein levels and the zinc sulfate turbidities were sufficient to suggest that the reaction was being inhibited by a paraprotein.

DISCUSSION

Recent studies[3, 4] indicate that the occurrence of paraproteinemia in disease states may have both diagnostic and prognostic significance.

A screening test for paraproteinemia should be simple, economical and rapid. It should distinguish, with a high degree of accuracy, those sera requiring examination by IEPA from those unlikely to contain a paraprotein. Of the screening techniques in current use, simple electrophoresis is probably the most accurate.[10] However, it has been shown that about one-fifth of cases of myeloma may present with a relative hypogammaglobulinemia, and these cases may well be missed on simple electrophoresis.[13]

Kunkel's zinc sulfate turbidity test, if combined with a routine total protein determination, appears to satisfy the criteria for a screening test well. The relatively high incidence of "false positives" is not a major disadvantage because the subsequent IEPA will distinguish these sera from the actual paraproteinemias. The incidence of "false negatives" is higher than desirable, but compares favourably with the results obtainable by other screening methods.[1, 6-8] In addition, it must be noted that of the seven patients whose sera gave "false negative" reactions, four had been tentatively diagnosed as paraproteinemias by the referring clinicians, on the basis of the clinical picture. These sera would undoubtedly have been referred for IEPA regardless of the zinc sulfate turbidities. Thus the diagnosis would have been missed in only three cases (4.4% of the patients with paraproteinemia).

In using the zinc sulfate turbidity test, two sources of error must be kept in mind. Schultze and Heremans[11] have noted that abnormally low values occur using sera from patients who have received intravenous injections of heparin. The present study shows that free hemoglobin in the serum may give rise to abnormally high zinc turbidities.

Summary Comparison of the zinc sulfate turbidity with the protein level in 200 pathological sera was used to predict the presence or absence of paraproteinemia. Prediction was correct in 84.5% of the cases. Seven cases of paraproteinemia out of 68 were not detected by the technique.

It is suggested that the zinc sulfate turbidity test, combined with protein determination, provides a valuable screening test for paraproteinemia.

Résumé On a comparé l'épreuve de floculation au sulfate de zinc avec les valeurs de la protéinémie dans 200 spécimens de sérum pathologique, pour pouvoir prédire la présence ou l'absence de paraprotéinémie. La corrélation était exacte dans 84.5% des cas. Par cette méthode, sept cas de paraprotéinémie sur 68 n'ont pas été décelés. On estime que la réaction de floculation au sulfate de zinc, associée au dosage des protéines sériques, constitue un moyen précieux de dépistage de la paraprotéinémie.

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CASE REPORTS

"Anonymous" Mycobacterial Infection Causing Disseminated Osteomyelitis and Skin Lesions

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"ANONYMOUS" mycobacteria have been reported as causing mainly pulmonary disease in adults[1-4] and cervical adenitis in children.[5-8] In contrast, there have been relatively few references to concomitant bone and skin involvement[9-12] or to bone lesions[13] with visceral manifestations.[14-16]

The purpose of this paper is to report the occurrence in a child of disseminated granulomatous skin and bone lesions from which a strain of Battey-type mycobacterium was isolated repeatedly and which appeared to be the etiological agent. Although *Staphylococcus pyogenes* was the only organism that could initially be isolated from bone fragments, it was only after antituberculous therapy had been instituted that definite improvement and healing of both types of lesions was observed.

P.B., a 5½-year-old white girl, was admitted to St. Justine Hospital in March 1965 with a presumptive diagnosis of generalized osteomyelitis.

Ten months before admission, she had developed erythema and edema of the right forearm, left ankle and right heel. Her family physician referred her to the local hospital with a tentative diagnosis of rheumatic fever. According to the information obtained, the patient's temperature varied between 98° and 101° F. during the first week of hospitalization; her white blood cell count (WBC) was 13,000 with a predominance of lymphocytes, and her erythrocyte sedimentation rate (ESR) was 42 mm. Upon treatment with penicillin, acetylsalicylic acid and steroids, the inflammatory reaction quickly disappeared. Three weeks later, however, radiological evidence of osteomyelitis was noted and there was a coincidental appearance of cutaneous lesions. Steroid therapy was discontinued. The patient remained in hospital for a further three months and was then discharged with a diagnosis of acute osteomyelitis of the right forearm, left ankle and right foot. She was readmitted to the same hospital in December 1964, at which time improvement in the

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