

## THE CONTRIBUTION OF ELECTROSYNERESIS TO IMMUNOLOGIC DIAGNOSIS OF HYDATIDOSIS

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*This paper evaluates how electrosyneresis can contribute to the immunologic diagnosis of human hydatidosis. The work reported here indicates its advantages over other techniques, and provides an estimate of its effectiveness in relation to the location and condition of the cysts.*

### Introduction

Multiple immunologic techniques—particularly the hemagglutination, immunoelectrophoresis, and immunofluorescence tests—have made a definite contribution to the diagnosis of hydatidosis (1, 2). Used together, these three methods assure adequate sensitivity in the immunodiagnosis of this zoonosis, while immunoelectrophoresis assures a high degree of specificity (3).

Immunoelectrophoresis, however, has certain drawbacks. It consumes large quantities of reagents, and it takes 24 hours to yield primary results. Hence, quicker and more economical precipitation reactions are being sought.

Electrosyneresis, described by Bussard (4), would eliminate these disadvantages, since it can detect precipitating antibodies in a few minutes using only small amounts of antigens and antisera. This has been confirmed by various authors (5-8) who, while calling this technique by different names, have used it in studying various antigens, in diagnosing different diseases, and in doing research on the legal aspects of medical problems (9).

The method is based on simultaneous and

opposite displacement of antigens and antisera subjected to the action of an electric field in an alkaline solution. Under these conditions, the antigen fractions with diagnostic value that have so far been identified in extracts of hydatid fluid (10, 11) are negatively charged, while most immunoglobulins remain neutral and are displaced toward the cathode by the electro-endosmotic current.

By moving the hydatid antigen toward the cathode and the antiserum toward the anode, the passage of the current accelerates the meeting of reagents, thus facilitating the rapid formation of complex precipitates. This also "purifies" the hydatid antigen, since its positively charged fractions tend to move in the same direction as the antiserum and thus participate very little in these reactions. Castagnari and Sorice (12), working with sera from hydatidosis patients, have found electrosyneresis to be more sensitive than hemagglutination or immunoelectrophoresis.

In the past, serologic tests for hydatidosis have been found to prompt positive non-specific reactions in sera from patients with other diseases (1). To examine this matter, a type of electrosyneresis was used to test sera from patients with non-hydatid diseases as well as sera from patients with confirmed hydatidosis and from healthy blood donors. Since different helminths are known to possess similar groups of antigens (13), electrosyneresis was

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used to test the same sera against antigens of *Echinococcus granulosus*, *Fasciola hepatica*, and *Taenia saginata*.

## Materials and Methods

### Antigens

The basic materials used were lyophilized hydatid fluid obtained from hepatic cysts of bovine origin, and lyophilized antigens of *F. hepatica* and *T. saginata*. Pursuant to earlier recommendations (3, 10), they were all qualitatively standardized by immunoelectrophoretic testing against hyperimmune rabbit sera. The final concentration of antigens was 50 mg/ml.

### Sera

A total of 66 sera from patients with surgically confirmed hydatidosis were studied; these had been preserved at -20°C for periods ranging from one month to two years. In addition, the study included 23 sera from patients with non-hydatid diseases and 22 sera from healthy blood bank donors. The hydatidosis patients were grouped according to the position of the parasite, and their cysts were classified as follows:

- 1) *Hyaline*—when the parasite's membranes were intact at the time of surgery, and the fluid was clear and transparent.
- 2) *Infected*—when there was evidence of suppuration between the hydatid and the cystic adventitia, and the parasitic fluid was cloudy, but there was no apparent rupture of the larval membranes.
- 3) *Recently ruptured*—when the larval membranes had already ruptured at the time of surgery, but the clinical record made it possible to place the rupture within the six-month period preceding removal.
- 4) *Residual*—when the larval membranes had already ruptured, and the rupture had occurred six months or more before the sample

was taken; or else when the cyst showed extensive calcification.

In all cases the sera were subjected to comparative study in their natural form and after concentration to one-third of their initial volume by lyophilization. The sera were not inactivated.

### Electrosyneresis (ES)

Electrosyneresis was carried out on a 3 ml layer of 0.9 per cent agarose prepared in sodium barbital (veronal) buffer, pH 8.2, which was placed on a 76 x 26 mm glass slide. Two rows of three holes were placed 6 mm apart on each slide (see Figure 1), perpendicular to the axis of electrophoretic migration. Samples of the particular serum being studied were put in the three holes nearest the anode, and extracts of the three antigen groups were put into the three cathode holes.

Electrophoretic separation of the antigens and sera was then carried out in sodium barbital buffer (pH 8.2) for ten minutes at 4°C. A difference in potential of 40 volts was maintained between the two ends of each slide. All the slides were then incubated for 24 hours at 22°C, after which the usual procedure for immunoelectrophoresis was followed (10).

Three systematic readings were taken to observe whether or not precipitation bands had formed. The first reading was taken 24 hours after the electrophoretic separation and the second after 48 hours. The slides were then stained with amidoschwarz and a final reading was obtained. Besides this, an initial reading was taken 60 minutes after completion of electrosyneresis in some cases. All sera that gave rise to one or more precipitation bands insoluble in 5 per cent trisodium citrate were considered positive. A positive result is shown in Plate 1.

### Immunoelectrophoresis (IEP)

The serum samples analyzed by ES were

FIGURE 1—A sketch of the layout used in the tests.

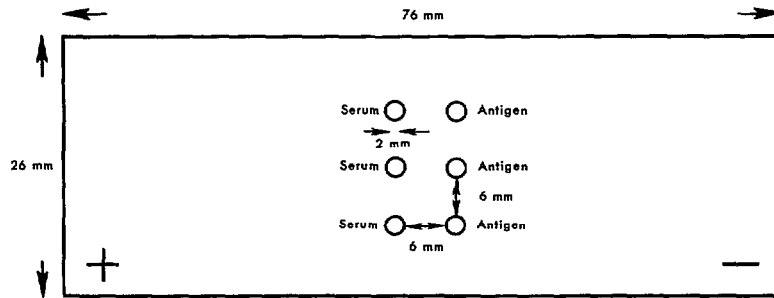
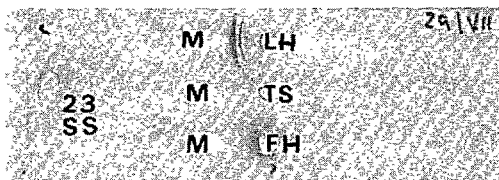


PLATE 1—Electrosyneresis, showing a definitely positive result. On the left is the patient's unconcentrated serum (M), matched against hydatid fluid (LH), *Taenia saginata* extract (TS), and *Fasciola hepatica* extract (FH).



studied by IEP within seven days of the date on which they had been obtained. The micro-technique of Capron *et al.* (2) was used for this purpose.

## Results

### *Reproducibility*

Four reactive and four non-reactive sera were tested by electrosyneresis on three separate occasions during the study. Identical results were obtained each time.

### *Sensitivity*

When concentrated sera were used, ES detected precipitating antibodies in 47 of the 50 sera that had given positive results with IEP. With unconcentrated sera, the percentage of

positive results was considerably lower (see Table 1).

Two of the three ES-negative sera for which IEP obtained positive results showed only one band after IEP (see Table 2). ES did not give rise to any bands of precipitation when carried out with sera which had given negative IEP results.

### *Specificity*

No sera from healthy individuals or patients with non-hydatid diseases generated the characteristic precipitation bands. However, two sera produced diffuse, low-intensity precipitates (Plate 2) that were clearly distinguishable from genuinely positive precipitation bands. One of these sera was from a patient with portal cirrhosis, a diagnosis confirmed by needle biopsy of the liver (Table 3); the other was

TABLE 1—Sensitivity of electrosyneresis (ES) in diagnosing 66 confirmed cases of hydatidosis previously analyzed by immunoelectrophoresis (IEP).

Cases analyzed by ES	Electrosyneresis of concentrated sera <sup>a</sup>		Electrosyneresis of unconcentrated sera	
	Positive	Negative	Positive	Negative
Positive by IEP (50)	47	3	43	7
Negative by IEP (16)	0	16	0	16
Total (66)	47	19	43	23

<sup>a</sup>Sera were concentrated to one-third of original volume by lyophilization.

TABLE 2—Sensitivity of electrosyneresis in relation to the number of precipitating systems shown by immunoelectrophoresis of 50 concentrated<sup>a</sup> sera from hydatidosis patients.

Immunoelectrophoresis		Electrosyneresis	
No. of bands <sup>b</sup>	No. of cases	Positive	Negative
1	4	2	2
More than 1	46	45	1
Total	50	47	3

<sup>a</sup>Sera were concentrated to one-third of original volume by lyophilization.

<sup>b</sup>Including band 5 of Capron *et al.*, 1967 (10).

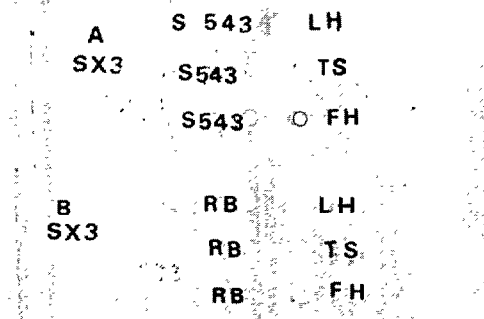


PLATE 2—Electrosyneresis, non-specific reactions. Concentrated sera of two individuals without evidence of hydatidosis, matched against hydatid fluid (LH), *Taenia saginata* (TS), and *Fasciola hepatica* (FH). The upper part of the picture shows the serum of a blood donor (S 543), and the lower part shows that of a portal cirrhosis patient (RB).

TABLE 3—Specificity of electrosyneresis in the analysis of 23 concentrated<sup>a</sup> sera from non-hydatid disease cases.

Disease	No. of cases	Antigens					
		Hydatid fluid		<i>T. saginata</i> extract		<i>F. hepatica</i> extract	
		Posi- tive	Nega- tive	Posi- tive	Nega- tive	Posi- tive	Nega- tive
Pulmonary aspergillosis	6	0	6	1	5	0	6
Pulmonary tuberculosis	6	0	6	0	6	0	6
Cirrhosis	5	1	4	0	5	0	5
Myeloma	2	0	2	0	2	0	2
Chronic leukosis	2	0	2	0	2	0	2
Lung cancer	1	0	1	0	1	0	1
Chronic rheumatoid polyarthrititis	1	0	1	0	1	0	1
Total	23	1	22	1	22	0	23

<sup>a</sup>Sera were concentrated to one-third of original volume by lyophilization.

from a healthy individual in whom no evidence of concomitant hydatidosis was found (Table 4). If these two cases are considered positive, the non-specificity index for the ES test results would still be less than 4.4 per cent.

Some of the sera from hydatidosis patients that showed a positive ES reaction to hydatid fluid also possessed antibodies against various fractions of the *T. saginata* extract and against one component of the *F. hepatica* antigen mosaic (see Table 5). However, the number of crossed reactions with *T. saginata* declined significantly for the group of sera from hydatidosis patients that showed more than three precipitation bands.

#### Sensitivity and Parasite Location

Like IEP, ES showed greater sensitivity for hepatic cysts than for pulmonary cysts (Table 6). Neither test yielded positive results for cysts with cerebral or renal locations.

#### Cyst Condition

Examination of each cyst's condition showed that those which had recently ruptured produced the highest percentage of reactive sera (Table 7). The percentage of positive sera was decidedly lower among patients with hyaline cysts.

TABLE 4—The specificity of electrosyneresis indicated by analysis of 22 concentrated<sup>a</sup> sera from blood bank donors.

No. of cases	Antigens					
	Hydatid fluid		<i>T. saginata</i> extract		<i>F. hepatica</i> extract	
	Posi- tive	Nega- tive	Posi- tive	Nega- tive	Posi- tive	Nega- tive
22	1	21	1	21	0	22

<sup>a</sup>Sera were concentrated to one-third of original volume by lyophilization.

TABLE 5—Reactivity of hydatidosis patients' sera shown positive by electro-syneresis. The sera are grouped according to the number of bands generated when they were tested against various groups of antigens.

No. of bands	Positive to hydatid fluid		Positive to <i>T. saginata</i> extract		Positive to <i>F. hepatica</i> extract	
	Concentrated <sup>a</sup>	Unconcentrated	Concentrated	Unconcentrated	Concentrated	Unconcentrated
1	6	9	21	18	4	1
2	10	12	14	4	0	0
3	11	11	6	4	0	0
4	9	6	1	0	0	0
5	7	2	1	1	0	0
6	3	1	0	0	0	0
7	0	2	1	0	0	0
8 or more	1	0	0	0	0	0
Total	47	43	44	27	4	1

<sup>a</sup>Sera were concentrated to one-third of original volume by lyophilization.

TABLE 6—Results of electro-syneresis and immunoelectrophoresis with hydatid antigen in relation to location of the cysts.

Location	No. of cases	Electro-syneresis		Immunoelectrophoresis	
		Positive	Negative	Positive	Negative
Lungs	37	23	14	26	11
Liver	16	15	1	15	1
Heart	2	2	0	2	0
Spleen	2	1	1	1	1
Multiple	2	2	0	2	0
Bone	2	2	0	2	0
Brain	2	0	2	0	2
Peritoneum	1	1	0	1	0
Kidneys	1	0	1	0	1
Thymus	1	1	0	1	0
Total	66	47	19	50	16

TABLE 7—Results of electro-syneresis and immunoelectrophoresis with hydatid antigen in relation to the condition of the cysts.

State of the cysts	No. of cases	Electro-syneresis		Immunoelectrophoresis	
		Positive	Negative	Positive	Negative
Hyalin	22	10	12	10	12
Infected	3	2	1	3	0
Recently ruptured	15	14	1	14	1
Residual	5	3	2	4	1
Data unavailable	21	18	3	19	2
Total	66	47	19	50	16

## Discussion

The experiments reported here demonstrate that ES can be used as a reproducible, simple, and rapid method for detecting precipitating antibodies against hydatid fluid, without consuming the relatively large quantity of reagents needed for IEP. Given the physical arrangements actually used, one could process 54 serum samples per hour, since three slides could undergo electrophoresis at the same time.

The level of ES specificity was found to be very satisfactory, since only one serum from a healthy donor and one from a patient with non-hydatid disease reacted to the hydatid fluid. The index of non-specificity would thus be 4.4 per cent, similar to that for hemagglutination (1). However, the non-specific precipitation bands produced by these sera (Plate 2) are very different from those of the hydatid serum group (Plate 1), and this appears to offer a basis for reducing the index to an insignificant level without much difficulty. However, the appreciable number of precipitating systems formed when reactive hydatid sera contact *Taenia saginata* extract (Table 5) corroborates the evidence of Capron, et al. (13) that there are antigen fractions common to this organism and *E. granulosus* (see Figure 4), and should alert us to the possibility of crossed reactions. Lack of sera from patients with *Taenia* cestodes

or *F. hepatica* has made it difficult to explore this point more fully.

The overall sensitivity of ES when concentrated sera were used was 71.2 per cent, a proportion slightly inferior to that attained by immunoelectrophoresis (75.7 per cent) for the same group of patients. This result was probably influenced by the fact that the two techniques were not used simultaneously and that some of the sera used for ES had been subjected to repeated freezing and thawing. The rate of positive results with ES when unconcentrated sera were used was 65.1 per cent; and here the number of precipitation bands formed (Plate 3) declined significantly.

The higher rate of positive results observed in cases of hepatic hydatidosis than in cases of pulmonary hydatidosis confirms earlier findings obtained by other serologic tests (1, 2, 14, 15).

Our results also agree with the conclusions of earlier investigations (2, 3, 16) concerning the influence of the cyst's condition on the sensitivity of immunologic tests. In our study, 12 of the 19 sera that did not show a positive ES reaction to hydatid fluid were from patients with hyaline cysts, and two others were from patients with parasitic residues (Table 7).

Our observation of complex precipitates in some slides 60 minutes after electrophoretic migration indicates that adjustment of the concentrations of reagents and the distances

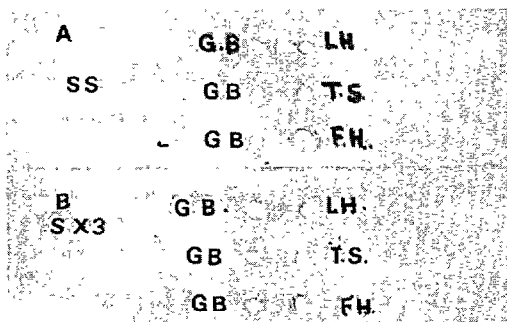


PLATE 3—Electrosyneresis of serum from a hydatidosis patient (GB) matched against hydatid fluid (LH), *Taenia saginata* extract (TS), and *Fasciola hepatica* extract (FH). Unconcentrated serum is at A (SS) and concentrated serum is at B (S x 3).

between them could make this method faster than any precipitation test now used for diagnosing hydatidosis.

Our results and the findings of Castagnari and Sorice (12) indicate that ES should be used in diagnosing this zoonosis, since it is more economical, specific, and rapid than the double gel diffusion used by Guisantes and Yarzabal (17) for detecting the disease.

The fact that our variety of electrosyneresis is less sensitive than that used by Castagnari and Sorice (12) could be due to differences in the

layout on the slide and to a possible denaturing of the sera.

With regard to specificity, these authors do not provide information on the possible effects of other helminthiasis on the results of the test. Therefore, in view of proven cross-reactions, the technique will have to be evaluated with sera from cases of teniasis and distomatosis before it can be regularly used for diagnosing hydatidosis in areas where other helminthiasis are found.

#### ACKNOWLEDGMENTS

The authors wish to thank Drs. Víctor M. Varela-Díaz and Emilio A. Coltorti of the Pan

American Zoonoses Center for their advice and assistance in carrying out this study.

#### SUMMARY

This paper points out the advantages of the new technique of electrosyneresis (ES) in immunologic diagnosis of human hydatidosis. The technique was applied in studying 66 unconcentrated and triply concentrated sera from patients with surgically confirmed hydatidosis, as well as 55 reference sera, all of which had been analyzed by immunoelectrophoresis.

ES, which is simple, rapid, and reproducible, showed an overall sensitivity of 71.2 per cent for sera concentrated to one-third of their initial volume. However, this sensitivity de-

creased significantly when unconcentrated sera were used. A higher rate of positive results was obtained for cases of hepatic hydatidosis than for cases of pulmonary hydatidosis; in addition, the technique's sensitivity was found to be related to the biological state of the cysts. The ES results also showed a low degree of non-specificity (4.4 per cent), comparable to that of the indirect hemagglutination test. Immunoelectrophoresis showed slightly higher sensitivity (75.7 per cent), with respect to the same group of sera.

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