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Immunoreactivity in pulmonary echinococcosis *

2. Evaluation of antibody response

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The part played by certain factors in determining the antibody response in pulmonary echinococcosis has been studied. Five immunodiagnostic procedures were used—complement fixation, latex agglutination, bentonite flocculation, passive haemagglutination, and intradermal tests—and parasitological and pathological examinations were carried out. The number of hydatid cysts had only a small influence on the qualitative nature of the immune response while the quantitative effect was considerable. The immune response did not vary significantly in relation to the size of hydatid cysts but it was affected by changes within the cysts and the surrounding lung tissue. In patients with cysts full of clear hydatid fluid the proportions with negative results and relatively low antibody titres were highest. In these patients the size of hydatid cyst appeared to be significant—the smaller the size, the lower the antibody level. The immune response is weak or completely absent when the hydatid cyst has a thick fibrous capsule. When suppurative changes are present in the hydatid fluid and/or in the fibrous capsule, and when there is inflammatory involvement of the surrounding lung tissue an easily detectable immune response may be seen at an early stage, sometimes even with a high antibody level, but later it usually decreases and may disappear completely. A hypothesis for the explanation of immunological reactivity in pulmonary hydatid disease is discussed.

Evidence exists to suggest that the sensitivity of serological tests is lower in pulmonary hydatid disease than in liver echinococcosis (*2–4, 7–9, 13, 14*), and various hypotheses have been put forward in an attempt to explain this phenomenon (*1, 3, 6, 7*). In some instances antibody levels have been found to be high whereas in others they have been low or negligible. In this paper an attempt is made to explain these differences and to identify factors that might affect immunoreactivity.

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MATERIALS AND METHODS

The study was carried on 140 patients operated on for pulmonary hydatid disease. Clinical examination revealed liver cysts in nine patients and more than one pulmonary cyst in ten others. Nine persons had already undergone surgery for echinococcosis of the lung, liver, or other sites.

The patients were grouped according to the size of the cysts (2–5 cm, 6–10 cm, and over 10 cm in diameter) and also according to the type of hydatid fluid removed (clear, turbid/opaque, or suppurative).

The complement fixation (CF), latex agglutination (LA), bentonite flocculation (BF), passive haemagglutination (PHA), and intradermal (ID) tests were performed as described in the preceding paper (*16*).

Forty-two lung cysts were removed intact and examined for the presence of protoscolices and brood capsules. Their viability was also determined (*15*). Ten of these hydatid cysts, including their fibrous capsules and associated pulmonary tissue

Table 1. Comparison of geometric mean titres (GMT) of antibody response measured by the complement fixation (CF), latex agglutination (LA), bentonite flocculation (BF) and passive haemagglutination (PHA) tests in patients with single or multiple echinococcosis

Type of infection	No. examined	CF		LA		PHA		BF		
		% positive	GMT	% positive	GMT	% positive	GMT	No. examined	%	GMT
Total	140	65.7	1:9	73.6	1:21	70.7	1:576	108	72.2	1:15
Single cyst	121	62.8	1:8	71.9	1:17	68.6	1:258	91	69.2	1:12
Multiple echinococcosis	10	80.0	1:45	70.0	1:92	80.0	1:3855	10	80.0	1:16
Pulmonary & hepatic echinococcosis	9	88.8	1:61	100.0	1:144	88.8	1:20890	7	100.0	1:260

underwent pathomorphological examination. The stains used were: haematoxylin–eosin, Van Gieson's stain, Mallory's aniline blue stain for connective tissue (*12*), Gomori's stain for reticular fibres, toluidine blue at pH 4.5, and the PAS reaction according to Hotchkiss (*11*).

RESULTS

Number of cysts

The immune reaction was weaker in patients with only one pulmonary cyst than in those with multiple pulmonary cysts or both lung and liver cysts (Table 1). The difference in the sensitivity of the tests between patients with single cysts and those with multiple pulmonary cysts was not significant ($t < 1.96$), whereas that between patients with single cysts and those with both lung and liver cysts was significant ($t > 1.96$). The geometric mean titres of those with both lung and liver cysts were significantly higher than those of persons with one lung cyst. The highest percentage of immediate hypersensitivity in the ID test was in those with multiple pulmonary echinococcosis (Table 2).

Table 2. Results of the intradermal test in patients with pulmonary hydatid disease

Type of infection	No. examined	No. positive			
		Immediate		Delayed	
		No.	%	No.	%
Single cysts	77	63	81.8	42	54.5
Multiple echinococcosis	9	9	100.0	7	77.8
Pulmonary and hepatic echinococcosis	9	7	77.8	6	66.7

Size of cysts

The sensitivity of all four serological tests increased with cyst size (Table 3). However, there were no significant differences between the results of the different tests for the same cyst size nor between the results of individual tests for different cyst sizes. Immediate hypersensitivity in the ID test in those with small, medium sized, and large cysts was 81.8%, 80.5%, and 81.8%, respectively, and delayed hypersensitivity was 57.5%, 60.0%, and 54.5%, respectively; the differences were not significant.

Table 3. Comparison of geometric mean titres (GMT) of antibody response measured by the complement fixation (CF), latex agglutination (LA), bentonite flocculation (BF) and passive haemagglutination (PHA) tests in patients with pulmonary hydatid cysts in relation to the size of cysts

Diameter of hydatid cysts (cm)	No. examined	CF		LA		PHA		BF		
		% positive	GMT	% positive	GMT	% positive	GMT	No. examined	% positive	GMT
2–5	33	51.5	1:4	60.6	1:6	60.6	1:100	31	61.3	1:5
6–10	34	64.7	1:10	70.6	1:26	67.6	1:610	30	70.0	1:24
>10	17	64.7	1:4	76.4	1:14	70.6	1:206	16	75.0	1:14

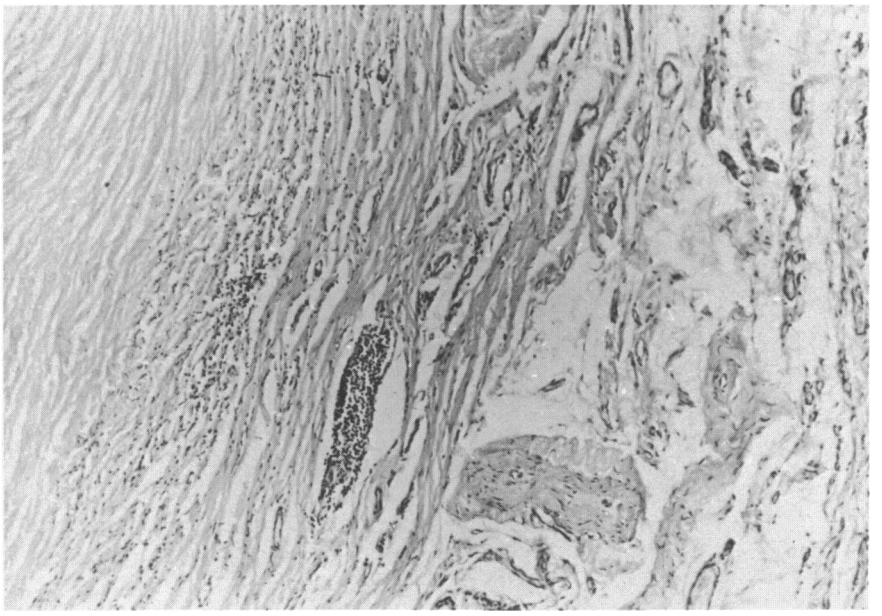

Fig. 1. Little change within the fibrous capsule surrounding the hydatid cyst. Haematoxylin–eosin stain. Magnification, 12.5×10.

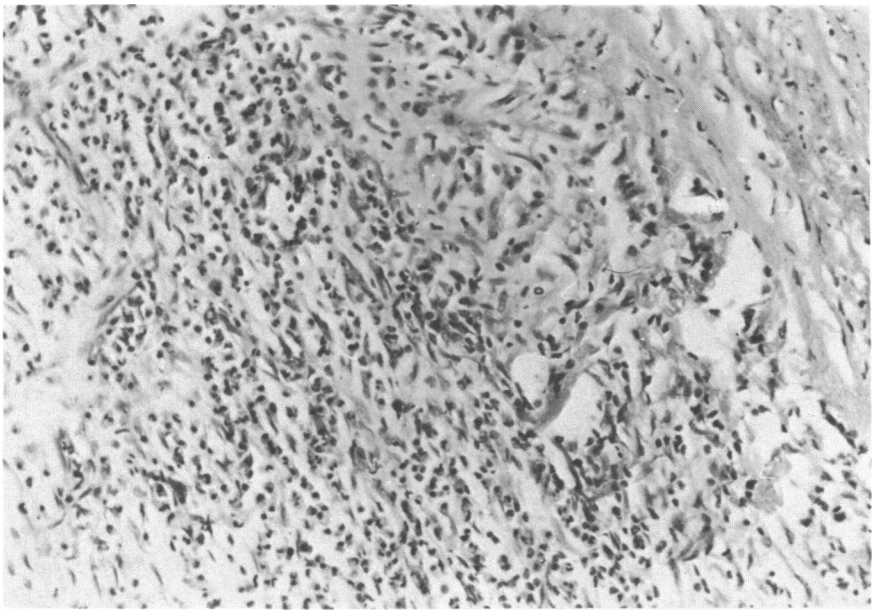

Fig. 2. Inflammatory changes of the fibrous capsule. Proliferation of granulation tissue. Haematoxylin–eosin stain. Magnification, 12.5×25.

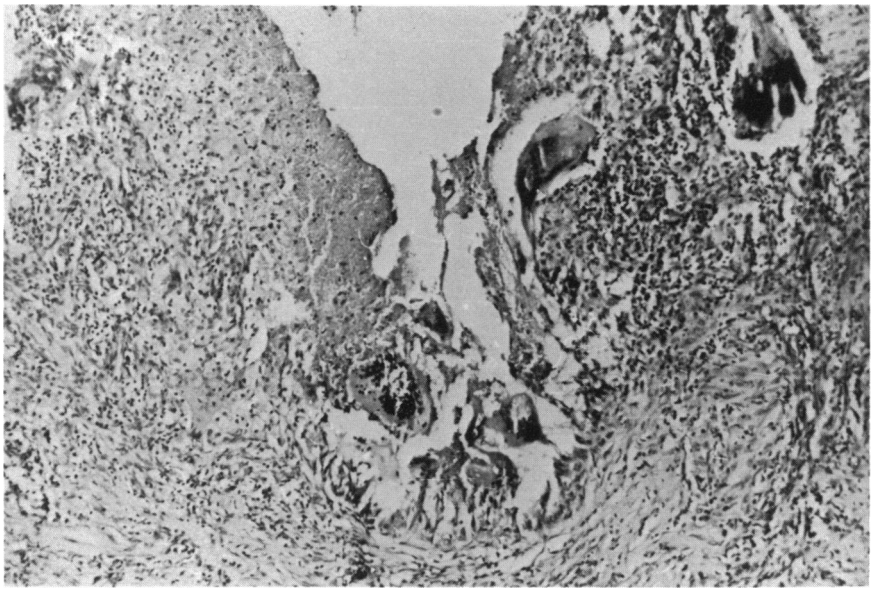

Fig. 3. Suppurative and necrotic changes within the hydatid cyst. Proliferation of granulation tissue with corpus alienum-type giant cells. Haematoxylin–eosin stain. Magnification, 12.5×10.

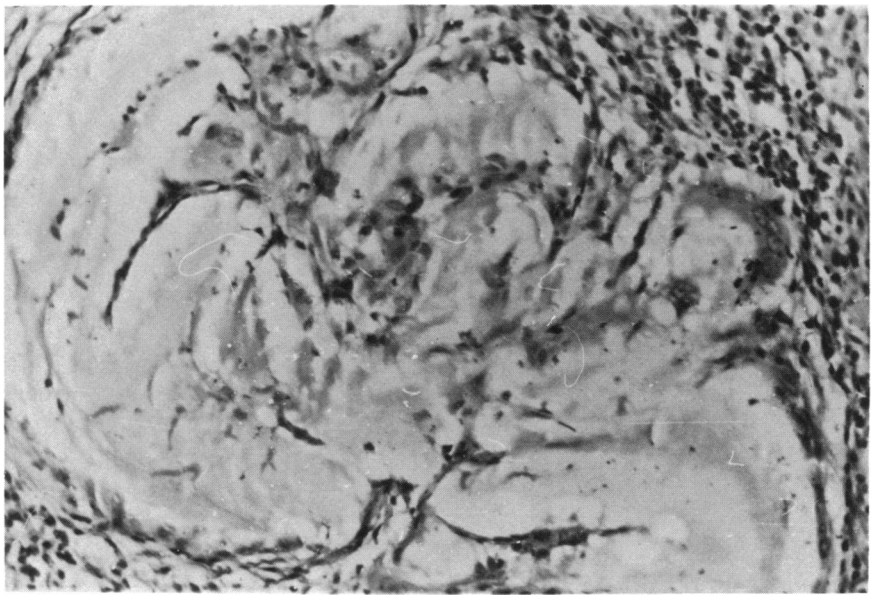

Fig. 4. A small hydatid cyst next to a large one full of hydatid membrane particles and surrounded by corpus alienum-type giant cells. Haematoxylin–eosin stain. Magnification, 12.5×25.

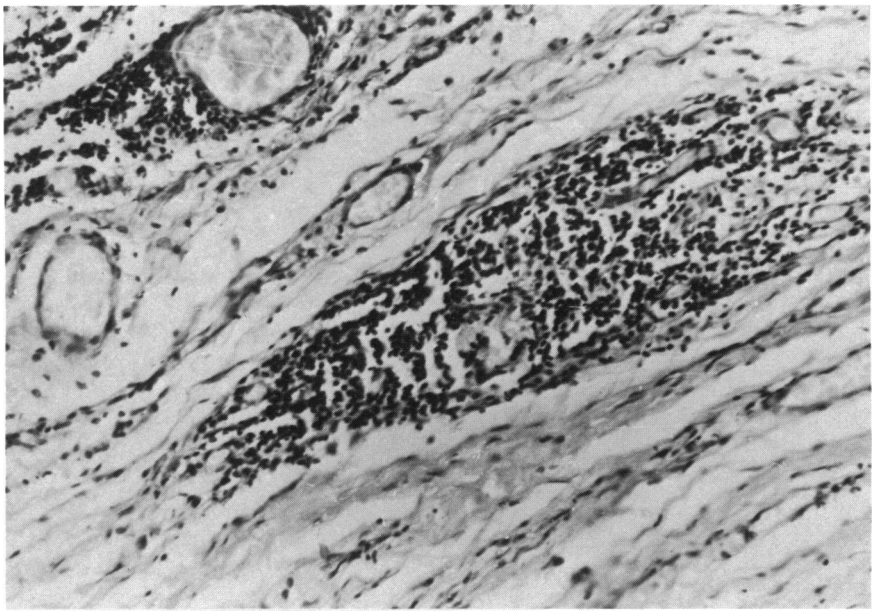

Fig. 5. Mostly perivascular lymph and plasma cell infiltration within the interstitial layers adjacent to the fibrous capsule around the hydatid cyst. Haematoxylin–eosin stain. Magnification, 12.5×25.

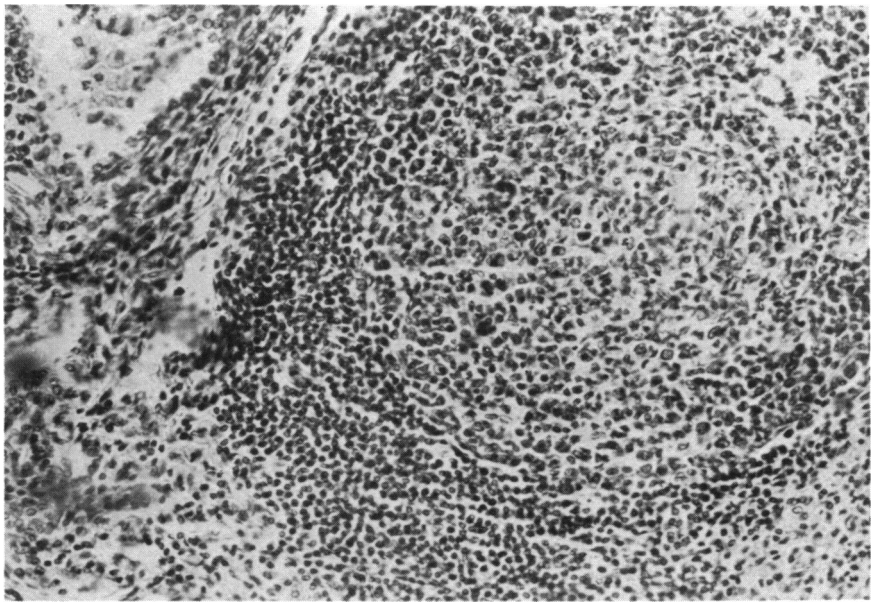

Fig. 6. Lymphoid follicles with germinal centre. Infiltration of lymph and plasma cells in the interstitial septa. Haematoxylin–eosin stain. Magnification, 12.5×10.

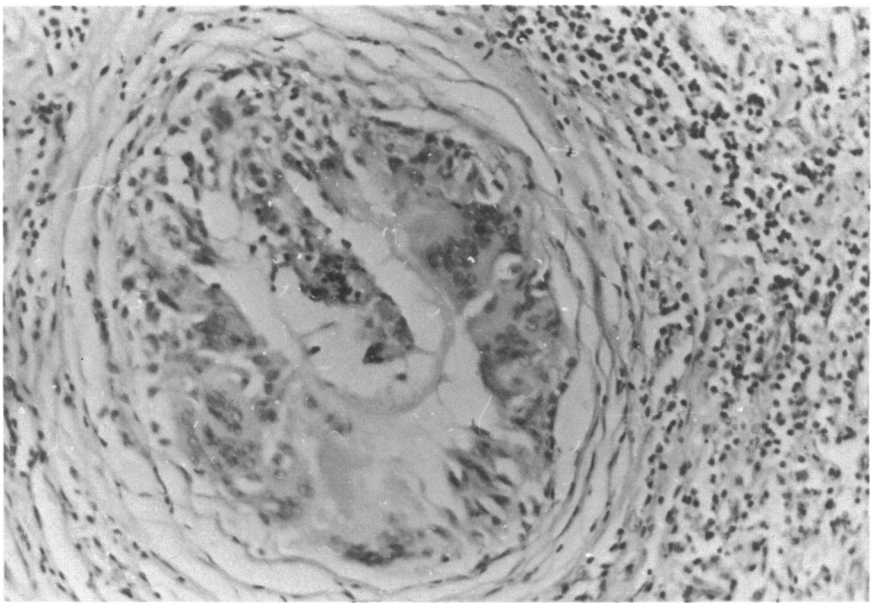

Fig. 7. A smaller hydatid cyst surrounded by corpus alienum-type giant cells. Infiltration of lymph and plasma cells next to the fibrous capsule. Haematoxylin–eosin stain. Magnification, 12.5×25.

Table 4. Comparison of geometric mean titres (GMT) of antibody response measured by the complement fixation (CF), latex agglutination (LA), bentonite flocculation (BF), and passive haemagglutination (PHA) tests in patients with pulmonary hydatid cysts in relation to the type of hydatid fluid

Type of hydatid fluid	No. examined	CF		LA		PHA		BF		
		% positive	GMT	% positive	GMT	% positive	GMT	No. examined	% positive	GMT
Clear	63	49.2	1:5	63.5	1:14	65.1	1:270	59	61.0	1:8
Turbid	13	84.6	1:10	84.6	1:16	69.2	1:288	13	76.9	1:13
Suppurative	8	87.5	1:25	87.5	1:54	75.0	1:803	6	100.0	1:92

Table 5. Comparative antibody response measured by the complement fixation (CF), latex agglutination (LA), bentonite flocculation (BF), and passive haemagglutination (PHA) tests in patients with pulmonary hydatid cysts in relation to the size of cysts (2–5 cm, 6–10 cm, or >10 cm) and the type of hydatid fluid

Type of hydatid fluid		CF			LA			PHA			BF		
		2–5 cm	6–10 cm	>10 cm	2–5 cm	6–10 cm	>10 cm	2–5 cm	6–10 cm	>10 cm	2–5 cm	6–10 cm	>10 cm
Clear	% positive	31.8	53.8	53.8	52.4	65.4	76.9	52.4	65.4	76.9	52.6	62.2	76.9
	GMT	1:3	1:6	1:5	1:4	1:12	1:9	1:44	1:118	1:190	1:4	1:14	1:8
Turbid	% positive	75.0	100.0	×	75.0	100.0	×	62.5	75.0	×	66.7	100.0	×
	GMT	1:7	1:28	—	1:9	1:25	—	1:200	1:1200	—	1:8	1:56	—
Suppurative	% positive	100.0	66.7	×	100.0	66.7	×	66.7	66.7	×	100.0	100.0	×
	GMT	1:20	1:58	—	1:67	1:400	—	1:463	1:10960	—	1:20	1:360	—

× = insufficient number of cases.

Table 6. Relation between type of morphological and immunological change in pulmonary echinococcosis and antibody titre

Changes	Case no.									
	1	2	3	4	5	6	7	8	9	10
Fibrosis of connective tissue capsule (Fig. 1)	+					+			+	+
Chronic proliferative and suppurative inflammatory changes in fibrous capsules (Fig. 2)		+		+			+	+		
Suppuration of the cyst (Fig. 3)			+							
Chronic proliferative and suppurative inflammatory changes in the adjacent pulmonary tissue				+						
Presence of small cysts next to the fibrous capsule (Fig. 4, 7)				+						+
Infiltration of lymphocytes and plasma cells in the fibrous capsule, with lymph nodules (in outermost layer) without germinal centres					+					
Infiltration of perivascular lymphocytes and plasma cells in the interstitial portion of the lung adjacent to the fibrous capsule (Fig. 5)						+	+			
Interstitial pulmonary fibrosis and plasma cell infiltration, with lymph nodules without germinal centres							+	+		
Interstitial pulmonary fibrosis, with abundant lymph nodules with germinal centres adjacent to dilated bronchioles (Fig. 6)									+	+
CF test	(−)	10	(−)	10	40	80	20	(−)	20	80
LA test	(−)	80	(−)	40	40	2 560	10	(−)	20	640
BF test	(−)	40	(−)	40	40	1 280	5	(−)	20	320
PHA test	400	25 600	200	6 400	6 400	819 200	1 600	400	800	51 200

(Reciprocals of antibody titres)

Type of hydatid fluid

For those cases with cysts full of clear hydatid fluid, the antibody response varied from 49.2% for the CF test to 65.1% for the PHA test and was lower than this in cases with turbid or suppurative hydatid fluid (Table 4). The differences in the immune response were statistically significant only between those with clear and those with suppurative hydatid fluid. The geometric mean titres were lowest in those with clear fluid. They increased for those with turbid fluid and were highest for those with suppurative cysts. Immediate hypersensitivity in the ID test for the three types of cyst was observed in 82.7%, 70%, and 100% of cases, respectively, while delayed hypersensitivity occurred in 51.9%, 50%, and 60%, respectively.

Size of cysts and type of hydatid fluid

The cysts 2–5 cm in diameter and with clear fluid showed the lowest immune response in the four serological tests, i.e., the lowest geometric mean titres and percentage of positive results (Table 5). These indices increased in value when the hydatid fluid was suppurative and when the cyst diameter increased; particularly characteristic were the changes in the geometric mean titres, which reached very high values.

Parasitological examination

Clear hydatid fluid was found in 38 of the 42 pulmonary cysts examined; one was full of purulent fluid and the other three had ruptured before surgery. Both separate protoscolices and brood capsules were found in all of these 38 cysts. The protoscolices had retained their viability.

Immunomorphological studies

The material studied was divided into two groups according to the results obtained. The first group (cases 1–4) showed inflammatory changes in the fibrous capsule. The second group (cases 5–10) showed immunomorphological changes in the connective tissue capsule (Table 6).

DISCUSSION

The number of hydatid cysts does not appear to be related to immunoreactivity in lung echinococcosis. If the cysts are intact, this may affect the sensitivity of the serological tests, and particularly in the case of multiple lung echinococcosis. Hydatid cyst size does not affect significantly the immunoreactivity in pulmonary hydatid disease either; we did not find any relationship between the geometric mean titres and cyst size. In the ID test also, it seems that the number of cysts and their size are not related to skin reactivity.

The changes within the hydatid cyst itself, which affect its content and the adjacent lung tissue, may influence the immunoreactivity in pulmonary echinococcosis. The patients with clear hydatid fluid, in whom the cyst walls were intact, were characterized by a very weak antibody response. The sensitivity of the serological tests was lowest when clear hydatid fluid was combined with the smallest (2–5 cm) cyst size. In contrast, a very strong antibody response was observed when the cysts were full of turbid or suppurative hydatid fluid and had damaged walls.

When hydatid cysts are surrounded by a thick fibrous capsule the diffusion of antigen into lung tissue is restricted and no immunomorphological changes occur in the connective-tissue capsule or in the lung tissue surrounding it. In such cases the serological results are also negative (Table 6).

In cases with suppurative changes of the fibrous capsule that also involve the adjacent lung tissue, the serological tests may be positive, showing relatively high antibody levels. This is explained by diffusion of antigen through the fibrous capsule which does not have a thick membrane (Table 6, cases 2 and 7). Death of the hydatid cyst (suppurative necrotic destruction) stops the antigenic stimulation and the serological tests may be either negative or positive with low titres (Table 6, cases 3 and 8).

When there are smaller peripheral cysts the antigenic stimulation may be increased because of partial resorbtion of the hydatid membranes by the "corpus alienum"-type giant cells. This would explain the high titres in case 10 (Table 6). The higher titres in case 6 are due to the multiple nature of the hydatid disease.

In three of the cases in which there was pulmonary sclerosis together with immunomorphological changes we did not find a correlation with the serological findings, which were lower (Table 6, cases 7, 8 and 9). The immunomorphological changes in these cases may have been induced by the nonspecific antigens contained in the secretions retained within the dilated bronchioles.

Our data concerning the pathological changes within the fibrous capsule reconfirm the conclusions of Kagan (*6*) and Kagan et al. (*7*) about the possible reduction in antigenic stimulation as a result of

thickening of the connective-tissue capsule. The weak immune response in the case of smaller cysts that were not so well separated from the host's tissue may be explained, on the one hand by the smaller (sub-immunogenic) amounts of antigen that leak out through the cystic wall (*7*), or, on the other hand, by the gradual appearance of a low-zone immune tolerance (*5, 10*).

The suggestions that the weaker response is related to the infertility of pulmonary hydatid cysts (*7*) or their short period of development and rapid death (*3*) have not been confirmed by our study. All of the pulmonary hydatid cysts examined, except ruptured and suppurative cysts, were found to be fertile and to contain viable protoscolices. It must be accepted therefore that the hydatid fluid has not lost its antigenicity. The changes in hydatid cysts that are accompanied by the loss of wall integrity as a result of aging or degeneration (*17*), lead to the leakage of a larger quantity of antigen than in the case of intact hydatid cysts and to the immunomorphological changes we have found. It seems that a change in the character of the hydatid fluid from clear to turbid or suppurative does not, at least at first, significantly affect its antigenic properties. A comparatively high antibody response in recently broken and in infected cysts was observed by Yarsabal et al. (*18*). However, it cannot be excluded that with time the molecular structure of hydatid fluid itself changes, reducing its immunogenicity.

RÉSUMÉ

LA RÉACTIVITÉ IMMUNITAIRE DANS L'ÉCHINOCOCCOSE PULMONAIRE

2. ÉVALUATION DE LA RÉPONSE ANTICORPS

On a étudié le rôle joué par certains facteurs dans la détermination de la réponse anticorps dans l'échinococcose pulmonaire. Cinq méthodes d'immunodiagnostic ont été utilisées — fixation du complément, agglutination au latex, floculation à la bentonite, hémagglutination passive et intradermo-réaction — et des examens parasitologiques et anatomo-pathologiques ont été réalisés. Le nombre de kystes hydatiques n'a qu'une faible influence sur l'aspect qualitatif de la réponse immunitaire, mais son effet quantitatif est considérable. La réponse immunitaire ne varie pas sensiblement en fonction de la taille des kystes hydatiques, mais est affectée par des modifications de l'intérieur des kystes et du tissu pulmonaire environnant. On a trouvé la plus forte proportion de résultats négatifs et de titres d'anticorps relativement bas chez les malades présentant des kystes emplis de liquide hydatique limpide. Chez ces malades, la taille du kyste semble jouer un rôle — plus elle est réduite, plus la production d'anticorps est faible. La réponse immunitaire est d'autre part faible ou totalement absente lorsque le kyste hydatique possède une capsule fibreuse épaisse. Lorsqu'il y a suppuration dans le liquide hydatique et/ou dans la capsule fibreuse, et lorsqu'il y a inflammation du tissu pulmonaire environnant, on peut observer à un stade précoce une réponse immunitaire facilement décelable, quelquefois même avec une forte production d'anticorps, mais en général cette réponse diminue par la suite, et peut disparaître complètement. Une hypothèse pour l'explication de la réactivité immunologique dans la maladie hydatique pulmonaire fait l'objet d'une discussion.

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