

Effect of Ovine Hydatid Cyst Fluid on the Cardiovascular and Respiratory Systems in Sheep (1)

by M. TABATABAI, M. H. ISMAILI, M. SAMI, R. FARDIN and R. KADIVAR

*Departments of Physiology and Surgery, School of Medicine,
Pahlavi University, Shiraz, Iran.*

Summary.

Rupture of the hydatid cyst in man brings about mild to severe toxic reactions including death. The present study was undertaken to investigate some of the responses resulting from administration of the ovine hydatid fluid to the sheep, which, like man, is an intermediate host of the *Echinococcus granulosus*.

In 50 sodium pentobarbital-anesthetized sheep, the arterial blood pressure (A.B.P.), central venous pressure (C.V.P.), respiration and electrocardiogram were recorded.

Intravenous administration of 5-10 ml hydatid fluid brought about moderate to severe fall in A.B.P. and rapid respiration with or without transient apnea or permanent respiratory cessation in 80 percent of the animals. Fifty percent of the sheep died of circulatory and respiratory failure after the first injection of the hydatid fluid. Boiled hydatid fluid did not lose its potency to evoke the above responses.

Pretreatment of the animals with atropine sulfate, 0,5 mg/kg subcutaneously, did not block the reactions.

Administration of the antihistamine chlorpheniramine, 4 mg/kg intravenously, caused partial prevention of the reactions in 6 out of 10 responsive sheep.

The cardiovascular and respiratory responses to ovine hydatid fluid may be due to antigen-antibody reactions or some toxic component of the fluid.

Résumé.

Effet du fluide hydatique ovin sur les systèmes cardio-vasculaire et respiratoire des moutons.

La rupture du kyste hydatique chez l'homme provoque une réaction toxique d'une intensité modérée à très sévère, parfois même mortelle. La présente étude a pour but d'éta-

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blir la réaction des ovins à l'administration intraveineuse de fluide hydatique provenant d'animaux de même espèce et comme l'homme, hôtes intermédiaires d'*Echinococcus granulosus*.

Chez les moutons anesthésiés avec 50 mg de pentobarbital sodique, la tension artérielle, la pression veineuse centrale, la respiration et l'électrocardiogramme ont été enregistrés.

L'administration intraveineuse de 5-10 cc fluide hydatique a entraîné un effondrement de la tension artérielle et l'accélération du rythme respiratoire, avec ou sans apnée temporaire, ou un arrêt complet respiratoire dans 80 % des cas. Après la première injection de la substance mentionnée, 50 % des animaux sont morts à la suite d'une défaillance cardiaque ou respiratoire. L'ébullition du liquide hydatique a entraîné la perte de son efficacité.

Un pré-traitement des animaux avec injection sous-cutanée de 0,5 mg/kg de sulfate d'atropine n'a pas non plus bloqué les réactions déjà décrites.

Une prévention partielle des troubles observés a été reconnue chez 6 d'entre les 10 animaux injectés par voie intraveineuse d'un produit antihistaminique (chlorphéniramine, 4 mg/kg).

Des réponses respiratoires autant que la défaillance cardiaque peuvent être en relation avec l'effet des composants toxiques. De même l'action antigène-anticorps des produits ci-dessus mentionnés, peut également être incriminée.

Introduction

Injection of the sheep hydatid cyst fluid into dogs and cats has been reported to produce shock and respiratory changes such as apnea and/or shallow rapid breathing (Rocha e Silva and Grana, 1945 ; Tabatabai et al., 1973). Rupture of the hydatid cyst in man may also cause toxic manifestations like wheals, erythema, fever, dyspnea, cyanosis, vomiting, diarrhea, circulatory shock and sudden death (Di Bello and Menendez, 1963 ; Brown 1963 ; Hankins, Dutz and Kohout, 1968).

Dogs and cats are the definitive hosts of the *Echinococcus granulosus*. It is more valuable to study the effects of hydatid cyst fluid in the intermediate hosts of *Echinococcus granulosus* such as sheep, in whom the cyst may develop.

In this investigation, the sheep respiratory and cardiovascular responses to ovine hydatid fluid administration were studied. The effect of the parasympatholytic agent atropine and the antihistaminic substance chlorpheniramine on the blockade of the responses were searched.

Materials and Methods

Fifty sheep, weighing 20-39 kg, were anesthetized by intravenous injection of sodium pentobarbital, 25 mg/kg. Respiration, arterial blood pressure (A.B.P.), central venous pressure (C.V.P.), electrocardiogram (EKG), or intermittently, instantaneous heart rate (cardiotachogram) were recorded by a Grass Polygraph. All the intravenous injections were administered through the catheter used for measurement of C.V.P.

Each animal could be tested four times, each test 24-48 hours apart. The vessels and the order in which these vessels were used during the consecutive tests were as follows:

Right femoral artery and vein, left femoral artery and vein, right axillary artery and vein, and left axillary artery and vein.

At the beginning of each test and prior to administration of various agents, the baseline A.B.P., respiration, and EKG were checked to ensure that the animal had recovered from the previous operation and testing.

At the end of the last test, the animals were killed and the liver and lungs were grossly examined for the presence of hydatid cyst.

Preparation of the Hydatid Cyst Fluid.

The infected livers and lungs of freshly slaughtered sheep were obtained from the city slaughterhouse. Under sterile conditions, the cysts were punctured by 20-gauge needle and the hydatid fluid was withdrawn into syringes. The fluid was then centrifuged for 20 minutes with 500 G. The supernatant fluid was separated and examined microscopically to see if it is free of scolices. If not, the fluid was re-centrifuged for another 10 minutes and 500 G. The fluids obtained from liver cysts and lung cysts were used either separately or as a mixed pool.

Pretreatment with atropine or Chlorpheniramine.

Four sheep which developed cardiovascular and respiratory responses to hydatid fluid administration during the first test, were pretreated with atropine sulfate 0.5 mg/kg subcutaneously 20 minutes prior to hydatid fluid injection during the second test.

Likewise, ten other responsive sheep were pretreated with the antihistaminic substance chlorpheniramine, 4 mg/kg intravenously, 3 to 10 minutes prior to hydatid fluid administration during the second or third test.

Protein-denatured Hydatid Fluid.

Hydatid fluid, heated at 70 °C for 20 minutes or boiled for 5 minutes, was administered to 5 responsive sheep in order to study the effect of hydatid fluid containing denatured protein.

Controlled Ventilation.

In order to determine whether the blood pressure response to hydatid fluid is of primary nature or secondary to respiratory change, 4 sheep which developed cardiovascular and pulmonary reactions to hydatid fluid administration during the first or second test, were put on positive pressure breathing during subsequent test. The muscle relaxant succinyl choline was given by intravenous drop in a dose sufficient to paralyze the respiratory muscles while the animals were being ventilated.

Results

a) Cardiovascular Responses.

Five to ten seconds after injection of 5-10 ml hydatid fluid, the arterial blood pressure dropped severely (*fig. 1 A and 1 B*). This period was considered as the latency of the response. The blood pressure fall appeared in 80 percent of the sheep after administration of the hydatid fluid for the first time. Subsequent injections of equal volumes of the same hydatid fluid during the same test elicited little or no hypotensive response, however 24 hours or more later, during the second test, a hypotensive reaction equal to or greater than the initial one would develop.

Concomitant with the arterial blood pressure diminution were elevation of the central venous pressure (*fig. 1 A*), and commonly increase in the heart rate.

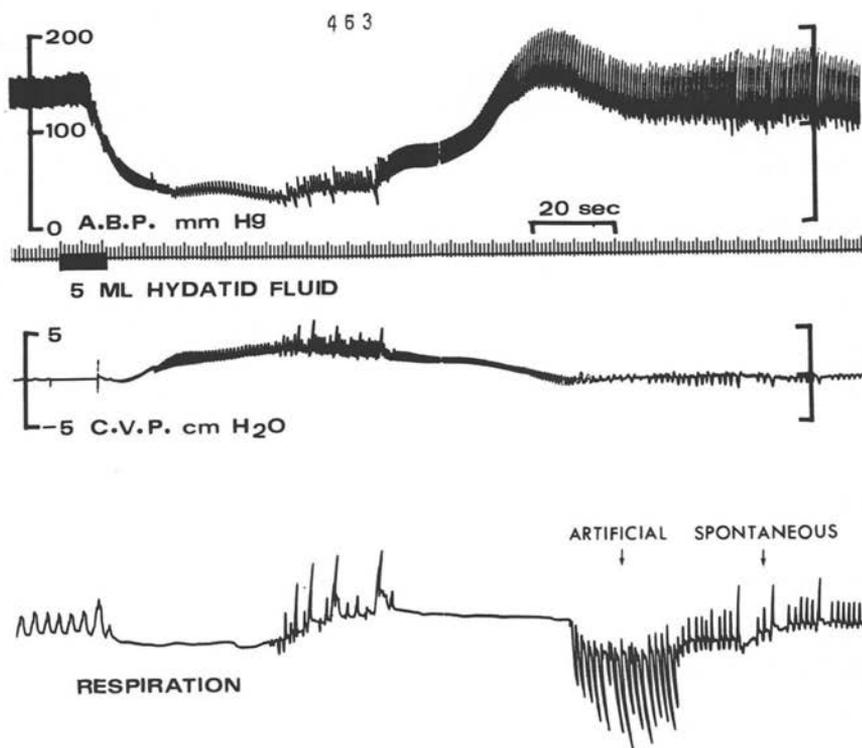


FIG. 1 A. — Circulatory and respiratory responses to intravenous injection of 5 ml ovine hydatid fluid. The arterial blood pressure (A.B.P.) dropped precipitously and the central venous pressure (C.V.P.) increased concomitantly. Respiration stopped initially, but started again only to be followed by another period of respiratory cessation. Artificial respiration by rhythmic compression of the chest wall resuscitated the animal and spontaneous breathing started again.

The electrocardiographic changes, besides the tachycardia, included occasional premature atrial and ventricular beats, depression of the ST segment and flattening or inversion of the T wave. All these changes were transient.

The cardiovascular changes reverted to normal within 20 seconds to 5 minutes, although, sometimes it took as long as 30 minutes for the arterial blood pressure to return to the pre-injection level.

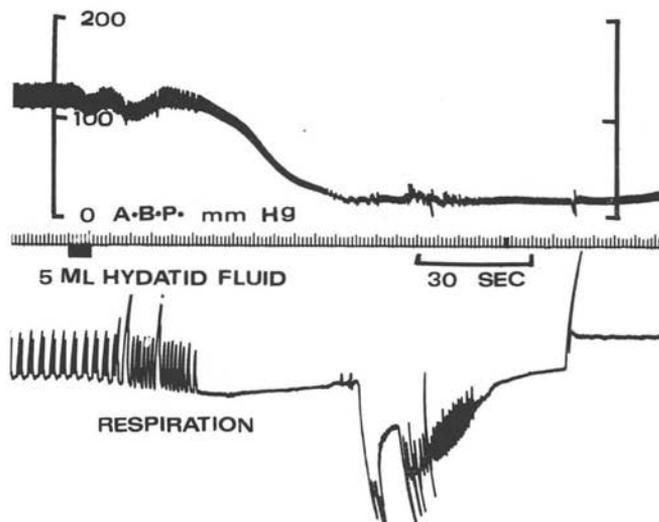


FIG. 1 B. — Fatal hypotension and respiratory cessation after administration of 5 ml hydatid fluid. Note that respiration initially became rapid, then stopped, then restarted spontaneously and finally stopped permanently. Artificial respiration (not shown in the picture) failed to restore spontaneous breathing. A.B.P. = arterial blood pressure.

Those sheep which did not show cardiovascular responses to hydatid fluid administration during the first test developed the aforementioned responses to the same hydrating fluid 48 hours or more later during the second or third test.

b) Respiratory Responses.

Simultaneous with the blood pressure changes, were respiratory changes which included rapid respiration, with or without transient respiratory cessation, the latter appearing either before (*fig. 1 A*) or after (*fig. 1 B*) the phase of rapid respiration. In some cases, respiration permanently stopped following the tachypneic period (*fig. 1 B*). In such instances artificial respiration by pressing upon the chest occasionally resulted in reappearance of spontaneous breathing (*fig. 1 A*),

although it often failed (*fig. 1 B*). Sometimes, respiration became irregular and gasping after hydatid fluid injection. The phase of rapid respiration usually lasted for 15 to 20 minutes before normal respiration was restored.

Twenty five sheep (50 %) died 2-10 minutes after the first injection of hydatid fluid during the first test apparently due to circulatory and/or respiratory failure. None of the sheep, either those which died or those which survived fluid injection, had hydatid cyst of the liver or lungs as noticed on *post-mortem* examination.

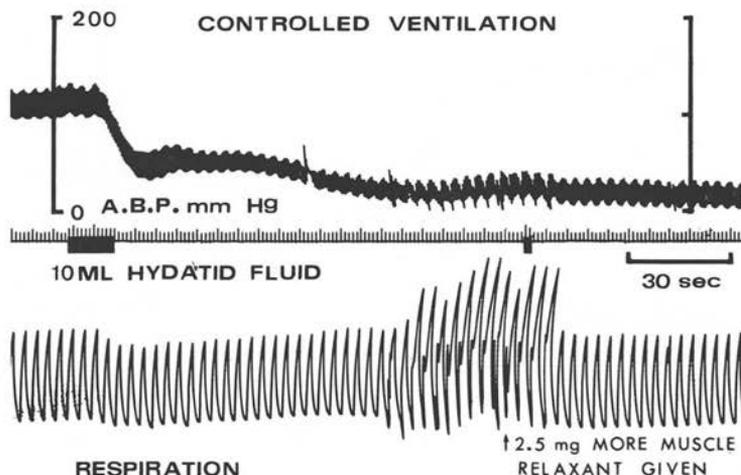


FIG. 2. — Arterial blood pressure (A.B.P.) fall after injection of 10 ml hydatid fluid in a sheep under controlled ventilation.

EFFECT OF PRETREATMENT WITH ATROPINE: All the atropine-pretreated animals developed hypotension and respiratory changes after injection of the hydatid fluid.

EFFECT OF PRETREATMENT WITH CHLORPHENIRAMINE: Three sheep developed full cardiovascular and respiratory responses as if, no antihistamine had been given. One sheep initially did not show any response to hydatid fluid, but ten minutes later, manifested responses identical to those of the first test. In 6 of the antihistamine-pretreated sheep, the degree of arterial blood pressure fall was one third to half of that observed during the first test.

EFFECT OF CONTROLLED VENTILATION: Following injection of the hydatid fluid, full cardiovascular responses developed in all the animals of this group (*fig. 2*), confirming that the latter are due to direct effect of the hydatid fluid on the cardiovascular system or its regulating mechanisms.

DENATURED PROTEIN-HYDATID FLUID: Hydatid fluid heated for 20 minutes at 70 °C or boiled for 5 minutes did not lose its potency to evoke cardiovascular and respiratory responses (fig. 3).

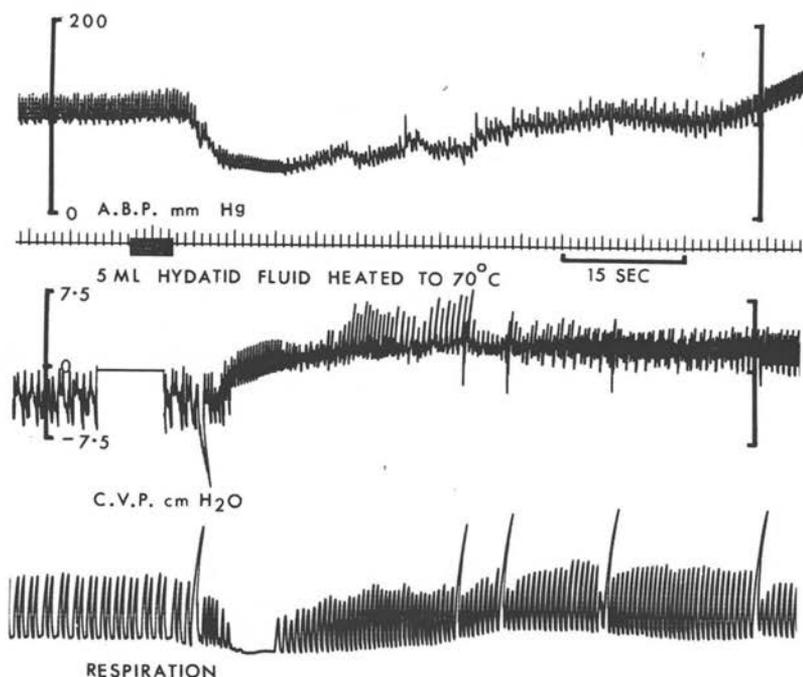


FIG. 3. — Arterial blood pressure (A.B.P.) diminution, central venous pressure (C.V.P.) elevation and respiratory stimulation after administration of hydatid fluid heated at 70 °C for 20 minutes.

Discussion

The high mortality rate in sheep (50 percent) from the ovine hydatid fluid injection is striking when compared with the reported 2 percent in dogs (Tabatabai et al., 1973), and none in cats (Tabatabai, Etemadi, and Cohanin, 1973).

Mechanical blockade of the blood vessels does not seem to be responsible for the findings reported under results, since the fluid injected was scolex-free.

The parasympatholytic agent atropine did not block the responses to hydatid fluid administration, therefore the possibility that the cyst fluid has acetylcholine or other parasympathomimetic agents may be safely ruled out.

Hydatid fluid has been shown to contain histamine (Grana and Rocha e Silva, 1945), and the findings reported here may be due to the histamine content of the

fluid. However, the antihistamine chlorpheniramine given in the dose 4 mg/kg intravenously did not completely prevent the reactions to the cyst fluid injection. One may tend to rule out the role of histamine in the mediation of the responses on the basis of the above evidence; though conceivable, the possibility remains that the amount of the antihistamine given was not sufficient to block the actions of histamine. This possibility is more substantiated if the hydatid fluid causes local release of histamine from histamine-containing cells such as mast cells in the respiratory and cardiovascular systems. The reason we did not use a larger dose of antihistamine is that chlorpheniramine has fatal hypotensive effect in sheep if given in greater quantities.

Antigen-antibody reactions between the hydatid fluid antigens and sheep antibodies may explain the cardiopulmonary responses. The fact that non-responsive sheep could become reactive to the hydatid fluid 48 hours or more after initial exposure to the fluid, gives support to this possibility. That hydatid fluid has antigenic substances is well documented. It contains proteins (Kagan and Norman, 1963 *a*, 1963 *b*), lipoproteins (Chordi and Kagan, 1965), polysaccharides (Agosin, 1957; Kilejian, Saure and Schwabe, 1962), and some end-products of carbohydrate and protein metabolism (Agosin, 1957). The tissue extracts of hydatid cyst have specific antigenic property too (Kagan and Agosin, 1968).

It is important to determine whether the hydatid fluid antigens are of parasite or host origin. Using immunoelectrophoretic and gel-diffusion techniques, hydatid fluids from different host species have been tested (Kagan and Norman, 1961; 1963 *a*, 1963 *b*; Norman, Kagan and Chordi, 1964; Chordi and Kagan, 1965; Norman and Kagan, 1966). Hydatid fluid globulins have been identified with host serum globulins and hydatid fluid albumin with host serum albumin (Kagan and Norman, 1963 *a*). Analysis of sheep hydatid fluid has shown that 10 of the 19 bands developed by the reaction of sheep hydatid cyst fluid and its homologous antiserum could be identified with sheep serum components (Chordi and Kagan, 1965).

If the responses to hydatid fluid are indeed due to antigen-antibody reactions, then the question is how the antibodies were formed in sheep in the first place. If the sheep had hydatid disease, the answer was clear, but none of the animals used in this study had either liver or lung hydatid cyst as observed during post-mortem examination. To be sure, hydatid cyst may occur anywhere in the body, but the common sites are liver and lungs and the fact that hydatid cyst did not exist in the livers and lungs of our animals is a strong evidence that the sheep did not have hydatid disease.

Another mechanism which may be responsible for the formation of antibodies in sheep is the response to the antigenic components of the parasites living in the sheep intestines. If this is the case, we must assume that the hydatid fluid and the intestinal parasites of sheep have one or more common antigenic compounds.

Finally, it is possible that some yet unidentified toxic substance may be the cause of the reactions elicited by administration of hydatid fluid to the sheep.

Bibliographie

- AGOSIN (M.), 1957. — Studies on the metabolism of *Echinococcus granulosus*. II. Some observation on the carbohydrate metabolism of hydatid cyst scolices. *Exp. Parasit.* 6, 586-593.
- BROWN (H. W.), 1963. — Chapter on: Diseases caused by metazoa, In P. B. Beeson and W. McDermott « Cecil and Loeb Textbook of Medicine », 421-423. *W.B. Saunders Company*, Publ., Phyladelphia and London.
- CHORDI (A.) et KAGAN (I. G.), 1965. — Identification and Characterization of antigenic components of sheep hydatid fluid by immunoelectrophoresis. *J. Parasit.*, 51, 63-71.
- DI BELLO (R.) et MENENDEZ (H.), 1963. — Intracardiac rupture of hydatid cyst of the heart. A study based on three personal observations and 101 cases in the world literature. *Circulation*, 27, 366-374.
- GRANA (A.), ROCHA E SILVA (M.), 1945. — Effect of hydatid fluid on histamine content of rabbit blood. *Am. J. Physiol.* 143, 314-323.
- HANKINS (J.), DUTZ (W.), et KOHOUT (E.), 1968. — Surgical treatment of ruptured and unruptured hydatid cyst of the lung. *Ann. Surg.*, 167, 336-341.
- KAGAN (I. G.), et NORMAN (L.), 1961. — Antigenic analysis of *Echinococcus antigens* by agar diffusion techniques. *Am. J. Trop. Med. Hyg.*, 10, 727-734.
- et AGOSIN (M.), 1968. — Echinococcus antigens. *Bull. Wld. Hlth. Org.*, 39, 13-24.
- et NORMANN (L.), 1963 a. — The isolation and characterization of two host antigens in hydatid fluid of *Echinococcus granulosus*. *Am. J. Trop. Med. Hyg.*, 12, 346-357.
- et —, 1963 b. — Analysis of helminth antigens (*Echinococcus granulosus* and *Schistosoma mansoni*) by agar-gel methods. *Ann. N.Y. Acad. Sci.*, 113, 130-153.
- KILEJIAN (A.), SAURE (K.) et SCHWABE (C. W.), 1962. — Host-parasite relationships in echinococcosis. VIII. Infra red spectra and chemical composition of the hydatid cyst. *Exp. Parasit.*, 12, 377-392.
- NORMAN (L.) et KAGAN (I. G.), 1966. — Preparation and evaluation of antigens for use in the serological diagnosis of human hydatid disease. I. Identification and partial purification of the reactive elements in *Echinococcus granulosus* antigen prepared from sheep hydatid fluid. *J. Immunol.*, 96, 814-821.
- , — et CHORDI (A.), 1964. — Further studies on the analysis of sheep hydatid fluid by agar gel methods. *American J. Trop. Med. Hyg.*, 13, 816-821.
- ROCHA E SILVA (M.) et GRANA (A.), 1945. — Schock produced in dogs by hydatid fluid. *Am. J. Physiol.*, 143, 306-313.
- TABATABAI (M.), BOROOMAND (K.) et GETTNER (S.), 1973. — Respiratory and cardiovascular responses resulting from intravenous injection of sheep hydatid cyst fluid to dogs. *Experimental Parasitology*, 34, 12-21.
- , ETEMADI (A. A.), COHANIM (N.), 1973. — Shock induced in cats by intravenous injection of ovine hydatid cyst fluid. *Pahlavi Med. J.*, 4, 398-416.
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