OBSERVATIONS ON THE OVA

OF

ECHINOCOCCUS GRANULOSUS

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ABSTRACT

In an attempt to determine the viability of Echinococcus granulosus ova, various enzymes and other substances were tested for their hatching and activating effects.

Ova were obtained from the intestines of experimentally and naturally infected dogs. Experiments were conducted in a constant temperature water bath at 37°C, using small Wasserman tubes for the incubation of experimental solutions and ova.

Acid pepsin was found to have no effect on hatching of ova or activation of oncospheres. $NaHCO_3$ or NH_4OH at pH 10 removed shells of E. granulosus ova and the enzymes pancreatin and trypsin both caused some degree of activation of the oncospheres. Bile, bile salts and cholesterol were found to enhance the activating effect of the enzymes. Fresh sheep bile was a more effective synergist than the several bile salts tested. After preliminary experiments, a hatching-activating solution (HAS) was selected and used for the remainder of the work. The hatchability of ova incubated in HAS was high, i.e. 95-99%, and some 20-50% of hatched oncospheres were activated. Percentage activation was taken as an index of viability.

The second portion of this study was an attempt to find an effective ovicide. Several common disinfectants were tested and the effect of moist heat upon the ova was studied also.

Ova exposed to 1, 5, 10 and 20% formalin for 5, 10 and 30 minutes and for 1, 6 and 24 hours remained viable except those in the 20%, 24 hour group. In this latter case, while whole oncospheres were present but no activity was observed.

Ova exposed to 2, 5 and 10% lysol for from 5 to 24 hours, hatched normally and the oncospheres were viable. Exposure to 20% lysol for from 5 to 30 minutes reduced the rate of hatching but oncospheres within unhatched ova remained viable. Exposure to 20% lysol for 6 or 24 hours prevented hatching altogether, and, when shells of such ova were removed in sodium hypochlorite, no oncospheres were found to have survived.

Ethyl alcohol at concentrations of 50, 70 and 95%, for from 5 to 60 minutes did not interfere with the activation of oncospheres.

Ova treated with high concentrations of "Roccal", "Tide" and "Clorox" also remained viable as evidenced by the high percentage activation obtained after 5 to 30 minutes treatment.

Moist heat at 50°C for 5 minutes did not kill all oncospheres, but percentage activation was low (6.8%). Moist heat at 50°C for 10 minutes and 30 minutes gave doubtful results in as much as intact oncospheres were still present, but moist heat at 60°C for 5, 10 or 30 minutes and 100°C for 1 or 5 minutes completely prevented hatching.

When shells of these latter were removed in sodium hypochlorite solution, no oncospheres were found to have survived.

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INTRODUCTION

The importance of hydatid disease as a public health and economic problem has been recognized in many areas of the world where the major industry is sheep and cattle raising. The disease becomes widespread when slaughtering of animals for human consumption is carelessly handled, thus infecting the local dogs which serve as the main reservoir for the parasite.

Hydatid disease or echinococcosis is defined as the infection of man and a wide variety of mammals by the larval stages of Echinococcus Rudolphi, 1810 . There are several species recognized, two of which are currently considered as capable of infecting man. Echinococcus granulosus (Batch, 1786), is the single species encountered in the Middle East.

Dogs having access to discarded viscera of slaughtered animals become infected with the adult form of the worm and pass the infective ova in their stools. The frequency with which dogs are infected in any given area depends upon ease of access to infected viscera of the several intermediate hosts. The infection of man and his domestic animals in any country bears a direct relation to the rate of infection in the dog. Distribution of the dog and its close association with man, and other intermediate host animals, is the most important single factor in the dessimination of hydatid disease. Other factors of import-

ance, however, are the economic status of the people and the level of education in matters of hygiene.

Ova passed by the dog are disseminated in a variety of ways. The common and usual method of infection of food animals is by ingesting grass contaminated with dog feces. Water and fresh vegetables have been considered as vehicles for infection in humans (Rausch 1951), although more emphasis has been laid on direct contact with the dog or its environment. Close association with dogs in an endemic area is a source of danger. Infections occur frequently during childhood. Dew (1937), remarked that hydatid cysts in man may often be nearly as old as the patient harboring it. Schwabe et al. (1959) have demonstrated high susceptibility to infection in white mice 48 days of age or younger and relatively high resistance in mice 71 days of age or older. Whether similar age resistance is evidenced by man is not known.

The removal of worms from the intestinal tracts of dogs has hitherto received a good deal of attention and has constituted a major step in efforts to control the disease. However, one of the weak points in such community-wide anthelminthic control programs is the lack of a known ovicidal agent for disinfecting the hair of dogs and contaminated premises, as well as of rendering ova, which may contaminate food or water supplies, non-viable. Knowledge of suitable chemical ovicides and their conditions of use would make present

control measures more effective and safe.

Experiments described in this paper were undertaken for the purpose of evaluating the ovicidal effects of various chemicals currently used as disinfectants. Before any such attempt could be made, however, it was clear that an *in vitro* method for demonstrating the viability of *Echinococcus granulosus* eggs was necessary.

Heretofore the only available test for viability of *E. granulosus* eggs was to feed treated ova to clean susceptible natural host animals, which were then kept for long periods under controlled conditions. This was both time consuming and uneconomical because all known natural intermediate hosts of *E. granulosus* are large and expensive to maintain. In addition, in highly endemic areas it is extremely difficult to rule out accidental infections (Batham, 1957). Although mice are susceptible to infection by intraperitoneal injections of scolices (secondary echinococcosis)(Dévé, 1933, Coutelen *et al*, 1939, Schwabe *et al*, 1959), they may not readily be infected by injection of eggs (Batham, 1957). Hence the first part of these observations was primarily concerned with the development of an easy and economical *in vitro* method for hatching and activating *E. granulosus* ova and thus determining their viability. The second part was devoted to the study of ova exposed to various disinfectants, with a view of determining the ovicidal efficiency of the latter.

Life Cycle

Hydatid disease, observed clinically since Hippocrates, became well known only toward the end of the 19th century. Ancient texts frequently referred to "hydatids" or "encysted hydropsies" in sheep and cattle (Dévé, 1949). Redi (1684), Hartmann (1685) and Tyson (1691) all suspected the animal nature of these "sacs of water" (Gradwohl and Kouri, 1948, Dévé, 1949). Pallas, in 1766, even noted the similarity between hydatids and cysticerci, and described hydatids from sheep livers as "une pellicule interne parsemee de granulations" (Dévé, 1949). Goeze (1782) determined these granulations to be scolices and called the parasite Taenia viscералis socialis granulosa (Dévé, 1949). Batsch (1786) renamed it Hydatigena granulosa, in accordance with the binomial system; Gmelin, in 1790, changed it to Taenia granulosa (Wardle and Mcleod, 1952) and Rudolphi, in 1801, placed it in the newly established genus, Echinococcus.

Experimental work on E. granulosus was started by Siebold in 1853. He obtained the adult form of the parasite by feeding scolices from sheep cysts to 12 dogs and one young fox. Five of these animals were successfully infected. His results were confirmed by Kuchenmaster, von Beneden and Leuckart (Dévé 1949). Later Naunyn, in 1863, and Krabbe and Finsen, in 1863, obtained similar results by feeding cyst contents from humans to dogs (Gradwohl & Kouri,

1948).

The adult Echinococcus is remarkably small (Fig. 1). It measures from 3 to 6 mm. in length and consists of a scolex and 3 to 4 segments. The scolex is provided with 4 suckers and an eversible rostellum armed with a double row of hooks (30-36 in number). A short, slender neck is followed by a segment which, although not well differentiated, does contain developing reproductive organs. The terminal segment contains only a gravid uterus swollen with eggs (Fig. 2). The number of eggs in one gravid proglottid has been estimated to be 500-800 (Déve, 1949; Dew, 1937). The life cycle involves two hosts, the definitive host which harbors the adult parasite and the intermediate host which harbors the hydatid or larval stage. The usual habitat of the adult form is the upper part of the small intestine of a variety of Canidae, although, in heavy infections, the worms may be found covering nearly the entire mucosal surface down to the vicinity of the rectum (Fig. 3). In Naturally infected dogs worms are not often that numerous, however.

In some parts of the world feline animals also have been reported to harbor Echinococcus granulosus (Rausch, 1952), although there is considerable doubt as to whether they can attain maturity in such hosts (Wardle and McLeod, 1952; Brumpt, 1936; Déve, 1949; Southwell, 1927; Lorincs, 1933). Attempts to experimentally infect kittens by

by feeding them huge doses of scolices have been only partly successful. The degree of infection produced has been low and in none of the animals did the worms attain maturity (Berberian, 1936; Southwell, 1927). What, if any, role cats play in the epidemiology of hydatidosis is not entirely clear.

A similar abnormal host which has drawn much attention has been the fox. Matoff and Janscheff (1954), infected young foxes and found that the life span of worms was short, (25-35 days), development was abnormal, and eggs were never produced. Sweatman (1952), found no infected foxes in areas where E. granulosus occurred in wolves as commonly as in the dog. Rausch (1956) did not find E. granulosus in any foxes examined although 39 of 94 wolves, and 5 of 50 dogs from the same locality harbored the parasite. Bronzini et al. (1954) fed foxes with scolices and obtained only a few immature worms. He concluded that the fox was an unsuitable host for E. granulosus.

Foxes, however, are important hosts for E. multilocularis which differs from E. granulosus in its morphology, distribution, and host relationships. E. multilocularis occurs as an adult in foxes and dogs and as a larva in microtine rodents (Rausch, 1952), and occasionally in man (Rausch, 1956).

E. granulosus, on the other hand, occurs as an adult in dogs, wolves and coyotes and as a larva in ungulates and

man (Rausch 1952). Sheep, goats, swine, camels and horses are, perhaps, the most commonly infected ungulate animals in most areas. Rodents rarely harbor the parasite (Rausch 1954); moose, other wild ruminants, wallabies and kangaroos are of local importance in some areas. In as much as dogs do not often have access to human viscera, infection in man constitutes a dead end as far as the parasitic life cycle is concerned.

Ingestion of larval forms by the definitive host, results in the development of adult forms within the intestinal lumen. As the terminal segments become gravid they are shed and ruptured thus liberating the ova (Fig. 3 and 4). Minimum time required for eggs to appear in the excreta of host animals following infection seems to vary. Fiebiger found ova in the feces of dogs 70 days after experimental infections while Leuckart, Naunyn, Railliet and Ross observed worms to mature within about 7 weeks and ova to appear in stools in about 47 days (Dévé 1949). Lorinos (1933) found ova in the stools of dogs as early as 36 days after infection. Ova were observed in stools about 45 days after the first feeding of scolices in the present study.

The eggs consist of a <u>hexacanth embryo</u> or <u>oncosphere</u> (Fig. 6), enclosed within an <u>oncospheral membrane</u>. Both of these structures are contained within a radially striated shell composed of truncated rods joined with a cement

substance (Berberian, 1957; Silverman, 1954). When ova are collected from a proglottid, by mechanically rupturing it, there is, in addition a single layer of cells forming a vitelline covering (Fig. 7). This latter is usually lost while eggs are passing through the intestine of the host (Cameron, 1927). The eggs are slightly ovoid and measure 35 x 38 u, (Fig. 3 and 4).

When eggs are ingested by an intermediate host animal, the striated shells are lost and the liberated oncospheres then penetrate the intestinal wall and reach the portal circulation via the capillaries or lymphatics. It is believed by some that they also may migrate directly through the peritoneal cavity to abdominal organs (Dew, 1928).

Because of the arrangement of visceral circulation most oncospheres probably are carried first to the liver where most are filtered out in the capillary bed (60-70% of human cysts are found in the liver). Those that pass through or bypass the liver capillaries are carried through the systemic circulation to the lungs, which are the next most common site for the cysts. Less often oncospheres are distributed to other organs such as the spleen, kidneys, brain, bone marrow, etc.

After an oncosphere has localized within an organ it is either destroyed by the host's defence mechanism or it undergoes rapid development. A cellular mass is observed within 12 hours. The enlargement of this mass and

its vesiculation produces, in time, the mature cyst, enclosed within a fibrous adventitious capsule or "pericyst". The pericyst forms as a result of the proliferation of host connective tissue and constitutes one host response to the presence of the parasite.

The hydatid cyst or fully developed larva consists of a double walled bladder filled with clear, opalescent fluid. The fluid resembles blood serum in certain respects but its nature and composition are imperfectly understood.

The cavity of the bladder is lined with a single layer of nucleated cells, the germinal membrane or "endocyst", which is the living part of the cyst wall. Proliferation of germinal membrane cells produce so-called brood-capsules, thin walled sacs (Fig. 8) within which the scolices or invaginated tapeworm heads arise. The wall of the brood capsules resembles the germinal membrane in structure. Brood capsules are not distributed evenly over the whole germinal membrane of most cysts but tend to arise only in particular areas (Dew, 1928). Scolices are budded off from the delicate wall of brood capsules to which they remain attached by a thin stalk or peduncle, (Fig. 9). The number of scolices within a single brood capsule may range from 2 to 70, according to Devé (1949). Brood capsules frequently become detached from the germinal membrane. Some may rupture, freeing the scolices. Scolices and brood capsules floating freely in the hydatid cyst fluid constitute what is known as hydatid sand. Scolices are the source of

infection for the definitive host.

The second layer of the cyst wall is thick and tough and serves as a support for the delicate germinal membrane. It consists of concentric elastic laminae and appears to be permeable to ions and small molecules (Schwabe, 1959).

Daughter cysts, sometimes observed within hydatid cysts, are complete replicas of the original mother cyst. Daughter cyst formation is, at present, imperfectly understood.

Scolices may be set free within the host, on occasions, by rupture of cysts following a traumatic shock or by their release into the tissues during surgical intervention. Under such circumstances, each scolex has the potential to develop into another complete cyst. Experimental infection of this type (called secondary echinococcosis) may be produced by injecting hydatid sand into the tissues or body cavities of susceptible animals . In such animals cyst development may be observed.

Previous Work

Considering the important role ova play in the epidemiology of hydatid disease comparatively little attention seems to have been given to the study of this stage of the parasite's life cycle.

The first experimental work on ova of E. granulosus was that of Deve in 1908. He showed that eggs remained viable for as long as 16 days (but not for 64 days) in water, for 11 days under "dry conditions", for 2 days "in the sun" (in Normandy), and for 4 months at -1°C. Cluniess-Ross (1929), kept ova, without loss of viability, for 3 weeks in shallow water or humid sand. However, he found that eggs were destroyed after 3 weeks under "dry conditions" and in 6 weeks in deep water.

First attempts at determining the hatching conditions for taeniid ova also were made by Deve. He concluded that the action of digestive juices was not necessary for the hatching of the E. granulosus embryo (Deve 1907). This he surmized from the fact that, when mature segments of the worm were injected subcutaneously into rabbits, small cysts were present at autopsy 96 days after. He showed also with in vitro experiments, that gastric juice did not exert any dissolving or softening effect upon the shell. He postulated that hatching occurred in the alkaline environment of the jejuno-iliac region where the oncosphere escaped by rupturing its shell as does a chick (Deve 1949). This view

was later supported by Barnett (1945) who maintained that hatching took place usually in the small intestine under the influence of warmth, moisture and alkalinity. Living eggs, he noted, when injected intraperitoneally or, in fact, anywhere into the bodies of susceptible animals sometimes hatched and developed into hydatid cysts. Recently these views received further support in the work of Batham (1957), and Segarra (1959). Segarra and his associates (1959) showed that taeniid (T. taeniaformis) eggs, when introduced directly into liver tissue, were capable of giving rise to bladder worms without first being exposed to the effect of digestive juices. Batham (1957) reported moderate infections of mice following the intraperitoneal injection of eggs of E. granulosus.

Isobe (1922, 1926) systematically studied the hatching requirements of Taenia saginata eggs, and successfully obtained hatching and activation of the hexacanth embryos by treating the eggs with artificial gastric juice (acid pepsin) and intestinal juice (alkaline pancreatin). Combined action of these juices removed the striated shell but embryos were not activated until they were transferred into a solution containing 0.5% NaCl, 0.25% Na_2CO_3 and 1 drop of pig bile per ml. His conclusion was that the critical factor in the hatching medium was the concentration of ions.

Clunies-Ross (1929) demonstrated the action of

gastric juice on the ova of Taenia pisiformis and E. granulosus in vitro and in vivo. He showed that ova of both worms could pass through the stomach of guinea pigs and be recovered from the upper part of the small intestine. When however, he treated the ova with alkaline pancreatin he obtained disintegration of the shell but could not detect movement of the embryo. Penfold and his associates (1937) exposed T. saginata eggs to pepsin-pancreatin and to Na-hypochlorite. Oncospheres were liberated by both solutions but showed no activity. Taylor (1926) stated in a short note that cestode oncospheres, in general, were not liberated at 37°C by the action of gastric juice, bile or duodenal secretions, but rather that they freed themselves by their own activity, except when the embryo was enclosed in a specially developed embryophore or when the shell was "homogeneous". Gentile (1936)too, reported the results of experiments which showed that the shells of E. granulosus and T. saginata ova were not effected by digestive juices but that they freed themselves by their own activity.

Bullock (1934) reported that, when egg suspensions of Taenia taeniaeformis were introduced into the stomachs of rats and their gut contents examined 3.5 - 24 hours later, gastric juice showed no effect, while intestinal juice freed the oncospheres. He also treated eggs in vitro with aqueous and alcoholic extracts of rat pancreas, and aqueous solution of pancreatin and various other enzymes,

plus bile. He obtained no dissolution of the shell by lipases, nor by acid solutions. Strong alkaline solutions of sodium hydrate, on the other hand, caused the breakdown of the shell. This action was enhanced by the addition of orbile. Such solutions, however, he believed, either killed or impaired the vitality of the larvae.

Leonard and Leonard (1940), working on Taenia pisiformis, demonstrated that ova hatched in Tyrode's solution to which was added 1% trypsin, 0.5% bile, and sufficient $CaCO_3$ to adjust the pH to 7.3.

Silverman (1954), in designing experiments to test the viability of T. saginata eggs exposed to various environmental conditions, came face to face with the overall problem of the mechanism of hatching of taeniid ova and the evaluation of their viability in vitro. He experimented with both Taenia saginata and Taenia pisiformis and studied the roles of alkaline pancreatin, trypsin, bile salts and cholesterol in the hatching and activation of the embryo. He concluded that the hatching requirements of T. saginata differed from those of T. pisiformis. T. saginata required pretreatment with gastric juice (acid pepsin) before the intestinal juice (alkaline pancreatin and bile) could act; while T. pisiformis hatched and was activated in alkaline pancreatin alone. He described three criteria for viability, i.e. motility, appearance of oncospheres and reaction to vital stains.

Myers (1957) reported a simple technique for hatching and activating Echinococcus multilocularis. He used two solutions, the first of which contained acid pepsin and Na_3PO_4, and the second trypsin and Na_2HPO_4. The combined action of these solutions reportedly caused hatching (disintegration of the shell) and activation of the embryo within 10 minutes.

Berberian (1936) reported upon a series of experiments designed to study the effects of the digestive juices of various animals upon scolices of E. granulosus. In in vitro studies scolices were placed in digestive juices of different animals at 37°C. In in vivo experiments, kittens, rats and rabbits were fed large quantities of scolices and membranes. At intervals, animals were killed and intestines examined. He found that the gastric juice of rats, dogs, cats, sheep and cattle did not digest hydatid scolices. Human gastric juice caused incomplete digestion and acted only upon evaginated scolices. The intestinal juices of man, rats, rabbits, sheep and cattle were found to digest scolices completely, whereas the intestinal juices of dogs and cats were inactive. He ascribed natural immunity or susceptibility to adult Echinococcus primarily to the specific actions of the digestive juices, particularly of the intestinal juices. Those animals which had an intestinal juice capable of digesting scolices were on that account resistant to infec-

tion with the adult stage of E. granulosus, and those which had inactive intestinal juice were either susceptible or partially susceptible hosts.

During 1935-1936 Berberian also studied the effects of digestive juices from different animal hosts upon the ova of E. granulosus with the view of explaining host specificity to hydatid disease. These results unfortunately were not published until 1957, however. Berberian (1957) reported that the gastric juices of man, sheep, cattle, dogs and cats failed to disrupt the shells of E. granulosus ova. Intestinal juices of man, sheep and cattle, and artificial pancreatic juice, however, rapidly disrupted the shells liberating oncospheres which were actively motile. Intestinal juices of dogs and cats had very little effect. His results suggested a possible relation between susceptibility to the larval form and the action of digestive juices. Those animals which secreted intestinal juices capable of rapidly disrupting the shells of ova of E. granulosus and of activating the liberated oncospheres were susceptible to hydatid disease while those animals which produced intestinal juices relatively ineffective in disrupting the shells of the ova, or which inactivated the liberated oncospheres, were not likely to be hosts to the hydatid parasite.

MATERIALS AND METHODS

A. MATERIALS

1. Ova

Ova of E. granulosus were obtained mostly from experimentally infected, and rarely from naturally infected, dogs.

Young puppies were fed scolices and brood capsules from hydatid cysts of sheep or cattle livers obtained from the Beirut city abattoir. Puppies were housed in special cages under close supervision. Beginning thirty days after infection stools were checked every day for ova. Two methods were used for concentrating ova from stools.

Method 1: Stools were collected and suspended in tap water in conical sedimentation jars. At the end of ten minutes, after large fecal particles had settled, the supernatent mixture was transfered to another conical jar. This process was repeated several times. The last suspension was stored in distilled water at 4°C until needed.

Method 2: Infected dogs were exsanguinated after the administration of anesthetic and intestines removed and opened. Usually the entire mucosa was found to be covered with the worms (Fig. 3). Sections of intestines were placed in a large beaker and covered with tap water. Gravid proglottids, loosened by this treatment, were collected from the sediment in the beaker, washed and stored at 4°C in distilled water. When ova free of debris were needed,

gravid proglottids were picked up with a pair of fine forceps, transfered to a small dish containing water and pressed to release the eggs.

The rectums of dogs were washed separately into a beaker and stored as rectal washings. These suspensions contained ova free from proglottid tissue and vitelline membranes, and did not require further treatment before use.

Ova from naturally infected dogs were obtained by washing intestinal contents into Petri dishes and collecting gravid proglottids with forceps. Proglottids were subsequently pressed to release eggs. However, ovum suspensions collected by pressing gravid proglottids usually were not rich enough for use in experiments. Hence, more often, the proglottid suspensions were allowed to remain at room temperature for from 8-10 days, washed several times at the end of that period and resuspended in distilled water. This treatment yielded egg suspensions free from proglottid debris as in rectal washings or stools.

2. Hatching and Activating Solutions

Solutions used for hatching and activating the ova were prepared in concentrations as indicated on the tables. The following chemicals were used: Pepsin, N.F. Powder, Merck & Co., Inc., Rahway N.J.; Pancreatin , 3x USP, Mann Assayed Biochemicals, Mann Research Laboratories, New York 6, N.Y.; Trypsin 2x Cryst., 16%-50% $MgSO_4$. Trypsin activity LO, 300 BAEE, Prep.: Kunitz and Northrop, Mann Research Labora-

tories Inc. New York 6, N.Y.; Sodium glycocholate, Nutritional Biochemicals Corp., Cleveland; Cholesterol, U.S.P. Nutritional Biochemicals Corp.; Sodium Tauroglycocholate (from ox bile), British Drug Houses Ltd. Fresh bile was collected from sheep gall bladders obtained from the Beirut city abattoir, centrifuged and stored in the freezer until needed.

All hatching-activating solutions were prepared in distilled water and kept frozen when not in use.

Hatching-activating solutions suggested by Silverman (1954) and Myers (1957), also were prepared from the above ingredients according to the directions in their respective papers.

3. Disinfectants

The ovicidal potentialities of the following disinfectants and other chemicals: were tested

a) Formalin (Formaldehyde solution, GPR Hopkins and Williams, 37.41% W/W.)

b) Lysol (ordinary commercial Lysol).

c) Ethyl alcohol (diluted from 95% or absolute alcohol).

d) "Roccal" (Winthrop-Stearns, contains 10% Benzalkonium chloride).

e) "Clorox" (Clorox Co., Procter and Gamble, 5.25% by weight sodium hypochlorite)

f) "Tide" (Procter and Gamble).

B. METHODS

1. Hatching and Activation

A volume of 0.5 ml. (10 drops)of hatching and activating solution (HAS) was placed in a small Wassermann tube kept at 37°-38°C. in a constant temperature water bath. Following a few minutes, temperature equilibration, 2-3 drops of a heavy suspension of ova (approximately 200-500 ova per drop) were added and the tube was shaken gently. At 30 minute intervals 2 drops were removed with a Pasteur pipette, placed on a slide and covered with a cover slip. The cover slip was supported with broken pieces of cover glass to prevent compression of the eggs. All eggs, motile oncospheres, and non-motile oncospheres, were counted and tabulated by a systematic microscopic examination of the preparation. Percent hatched oncospheres of total eggs counted and percent activated oncospheres of total hatched oncospheres counted were computed as hatching and activation rates respectively. The activation rate was taken as an index for assessing viability.

2. Treatment with Chemicals

The chemical to be tested was prepared in double the required strength. A known quantity (10-15 drops) was placed in a tube kept in a water bath at 37°-38°C, Following temperature equilibration, an equal volume of a heavy suspension of ova was added. The tubes were shaken and the time recorded. Ova were treated for 5, 10 and 30 minutes

for most chemicals. When considered important treatment time was extended to as long as 24 hours.

At the end of treatment period, tubes were immediately removed from the water bath and filled with distilled water, effectively diluting the chemical many fold. Tubes were subsequently centrifuged at low speed for three minutes, the supernatent was pipetted off and the sediment resuspended in distilled water. This process was repeated 4 times. After the fourth washing, the tubes were returned to the water bath and HAS added.

3. Effect of Temperature

Ova suspensions (approx. 1 ml.) in Wassermann tubes were subjected to temperatures of 50°, 60°, 70° and 100°C in a constant temperature water bath for varying periods of time. At the end of the treatment period the tubes were removed to a 37°-38°C water bath and HAS added.

RESULTS : PART I

A. Experimental Infection with Echinococcus granulosus in Dogs

Fourteen dogs were fed brood capsules and scolices during these experiments (Table 1). Of these four failed to show any infection after 3-5 months. The other ten dogs showed variable degrees of infection. Two yielded only a few poorly developed worms at autopsy and three only a few mature worms. The other five dogs were heavily infected; worms covered the entire intestinal mucosa from the duodenum to the rectum (Fig. 3).

A number of dog intestines, from stray dogs collected by municipality authorities from the city of Beirut and its environs, were opened in our laboratory during the years 1958-1960. Of a total of 156 intestines examined, 17 showed varying degrees of infection with E. granulosus for a prevalence of 10.9%. The prevalence rate from the same locality reported by Berberian (1957) was 20% for the year 1936, and by Turner et al (1936), 20-25% for the years 1935-1936 (from a survey of 500 dogs). Pipkin et al (1951) reported a prevalence of 11.75-32.9% for years 1948-1949, while Witenberg (1934) found a 20% infection rate in dogs and 10% in jackals for Palestine.

B. Effects of Distilled Water and Physiologicas Saline upon Echinococcus granulosus Proglottids and Ova at 37°C.

It was considered desirable to observe the effects

TABLE 1

Experimental Infections with Echinococcus granulosus in Dogs

Dog No.	Date fed	Date killed	Worm burden*
1	June 20, 1958	November 1, 1959	-
2	November 1, 1958	March 2, 1959	++++
3	November 1, 1958	April 11, 1959	++++
4	February 27, 1959	May 8, 1959	+
5	April 25, 1959	June 25, 1959	++++
6	May 5 and 20, 1959	August 4, 1959	-
7	May 5 and 20, 1959	August, 1959	-
8	May 27 and Sept. 17, 1959	November 19, 1959	++
9	June 13 and 18, 1959	September 30, 1959	+
12	October 10 and 25, 1959	December 3, 1959	++++
13	October 10 and 25, 1959	January 22, 1960	++
14	November 16, 1959	March 16, 1960	-
15	November 24, 1959	February, 1960	++
16	April 1, 1960	May 12, 1960	++++

* Worm burden: ++++ heavy infections; ++ light infections;
+ Immature worms; - no worms.

of water and physiological saline alone upon proglottids and eggs incubated at 37°C.

Proglottid cuticles, vitelline membranes and egg shells remained intact in distilled water and physiological saline at 37°C for up to 24 hrs. (Table 2). Between 24 and 48 hours, however, proglottid cuticles and vitelline membranes broke down although egg shells remained intact. Five experiments were run for these results; ova and proglottids were obtained from four different dogs.

C. Effect of Digestive Enzymes and pH upon the Proglottids and Ova of Echinococcus granulosus

When an ovum of E. granulosus is ingested by an intermediate host animal it first passes through the stomach where it is exposed to an acid pH and the action of gastric juice. Later it passes into the alkaline environment of the small intestine. The effects of digestive enzymes and pH upon proglottids and ova of E. granulosus ovum were therefore investigated (Table 3).

A 1% pepsin solution prepared in 0.4% HCl pH 1 (artificial gastric juice) caused the dissolution of proglottid cuticles and vitelline membranes. A few egg shells ruptured, resulting in a 3.5 ± 4.6% hatching rate following 90 min. incubation at 37°C. Of the 48 free oncospheres counted in 5 experiments, only one was activated. Most eggs did not hatch, however.

A 0.4% solution of HCl had no digesting effect on

TABLE 2

Effects of Distilled Water and Physiologic Saline upon E chinococcus granulosus Proglottids and Ova:

	Observations*					
	50 minutes	60 minutes	90 minutes	24 hours	48 hours	
Distilled water + Proglottid and ovum suspension	Proglottid cuticles) Egg shells) Intact Vitelline membranes)	Proglottid cuticles) Egg shells) Intact Vitelline membranes)	Proglottid cuticles) Egg shells) Intact Vitelline membranes)	Proglottid cuticles) Egg shells) Intact Vitelline membranes) Dissolving	Egg shells) Intact Proglottid cuticles) Vitelline membranes) dissolved	
Physiologic saline + Proglottid and ovum suspension	Proglottid cuticles) Egg shells) Intact Vitelline membranes)	Proglottid cuticles) Egg shells) Intact Vitelline membranes)	Proglottid cuticles) Egg shells) Intact Vitelline membranes)	Proglottid cuticles) Egg shells) Intact Vitelline membranes) Dissolving	Egg shells) Intact Proglottid cuticles) Vitelline membranes) dissolved	

* Results of five experiments: Proglottids and ova were obtained from four different dogs.

TABLE 5

The Effects of Degistive Enzymes and pH upon the Ova of Echinococcus granulosus

	Observations at 15. min.	% Hatching			% Activation		
		30 min.	60 min.	90 min.	30 min.	60 min.	90 min.
Pepsin 1% HCl 0.4% (pH 1)	Proglottid cuticles) Dissolving Vitelline membranes) Shells) Intact	1.9± 3.2* (5)***	5.1± 5.7 (5)	3.5± 4.6 (5)	2.0 ± 4.5** (5)	0 (5)	0 (5)
HCl 0.4% (pH 1)	Proglottid cuticles) Intact Shells)	0 (4)	0 (4)	0 (4)	0 (4)	0 (4)	0 (4)
Pancreatin 1% $NaHCO_3$ 1.3% (pH 10)	Proglottid cuticles) Dissolving Vitelline membranes) Shells) Disrupting	57.5± 17.8 (5)	56.8± 15.9 (5)	57.6± 15.9 (5)	6.5 ± 4.4 (5)	15.5 ± 10.0 (5)	7.8 ± 7.6 (5)
$NaHCO_3$ 1.3% (pH 10)	Proglottid cuticles) Intact Vitelline membranes) Shells) Disrupting within) Vitelline membranes	45.6± 22.4 (7)	64.1± 33.7 (7)	71.0± 28.2 (7)	0 (7)	0 (7)	2.2 ± 4.7 (7)
NH_4OH (pH 10)	Proglottid cuticles) Intact Vitelline membranes) Shells) Disrupting within) Vitelline membranes	66.1± 13.4 (3)	69.7± 23.2 (3)	81.5± 32.2 (3)	0 (3)	0 (3)	0 (3)
Trypsin 0.4% $NaHCO_3$ 1.3%	Proglottid cuticles) Dissolving Vitteline membranes) Shells) Disrupting	7.0± 10.0 (4)	7.8± 7.7 (4)	11.0± 5.5 (4)	9.9 (4)	35.2 ± 14.1 (4)	17.5 ± 8.1 (4)

* All values are means ± standard deviation and represent % hatched of total egg counts.
** All values are means ± standard deviation and represent % activated of total hatched oncospheres.
*** Figures in parenthesis indicate number of experiments; approximately 200 to 400 ova counted in each experiment.

proglottid cuticles, vitelline membranes or egg shells during 24 hours incubation at 37°C. For longer incubation periods proglottid cuticles began to disintegrate. A total of 1110 eggs were counted in 4 experiments.

Pancreatin (1%) prepared in 1.3% $NaHCO_3$ (pH 10) caused complete dissolution of proglottid cuticles and vitelline membranes. Shells also were disrupted in this solution. They started breaking within 15 minutes of exposure and at 60 minutes to 90 minutes incubation 57.6 ± 15.9% of oncospheres were free, and 13.3 ± 10.0% were active.

Trypsin (0.4% in 1.3% $NaHCO_3$) also caused disruption of the shell but not as efficiently as pancreatin. The hatching rate in trypsin was 11.0 ± 5.5%. However, Trypsin was a better activator of oncospheres; 35.5 ± 15.5% of the hatched oncospheres became active.

The effects of $NaHCO_3$ (1.3%, pH 10) and NH_4OH (pH 10) on the shell were interesting to observe. They both removed the shell (71.1 ± 28.2% and 81.5 ± 32% hatched respectively), but left proglottid cuticles and vitelline membranes intact. Egg shell "blocks" and oncospheres could be seen floating within the confines of vitelline membranes (Fig. 10,11,12,13). Only a few (2.2 ± 4.7%) oncospheres became active, however, in $NaHCO_3$. It was difficult to detect motility because oncospheres were trapped within vitelline membranes.

D. Hatching and Activation Mechanism

When ova are observed with high magnification the hexacanth embryo is seen clearly through the shell, which consists of radially oriented rectangular blocks bound together with a cement substance (Fig. 5). Such ova exposed to the action of hatching solutions soon lose their transparency and appear increasingly darker, probably due to the process of digestion of the cement substance and the loosening of the joints between the blocks (Fig. 14 and 15). At completion of the digestive process (10-15 minutes after exposure at 37°C), the individual blocks are seen floating away, leaving the oncospheres free (Fig. 16 and 17).

Once the shell is removed and the fragile oncospheral membrane is ruptured (Fig. 23), the oncosphere becomes subject to the action of "activators". Silverman (1954), working on T. saginata, believed activation to be associated with a change in the permeability of the oncospheral membrane and direct stimulation of the hexacanth embryo. Myers (1957), working with E. multilocularis, concluded that activators operate as complexing agents in binding traces of heavy metals. He successfully used 20 different compounds, sodium phosphate and urea being among the better activators.

In the following paragraphs and in Table 4 observations on the actions of some activators and on their combined actions are recorded and discussed.

E. Comparative Effects of Various Hatching and Activating Solutions upon the Ova of Echinococcus granulosus

Table 4 summarises the results obtained in experiments testing the hatching-activating effects of various substances and their combinations on the E. granulosus ovum.

Alkaline pancreatin, as was mentioned previously, caused the disruption of the shell resulting in a 57.6 ± 15.9% hatch rate, but only a 13.3 ± 10.0% activation rate (Sol. 1). Alkaline trypsin, on the other hand, activated 35.5 ± 17.6% of hatched oncospheres although its hatch rate was poor, being only 11.0 ± 5.5% (Sol. 2). The combined action of the two enzymes resulted in 26.4 14.2% hatches with 21.7 ± 8.4% activation (Sol. 3).

Addition of cholesterol to alkaline pancreatin did not change its hatching effect, but the activation rate was raised to 19.5 ± 11.4% (Sol. 4) The addition of urea to alkaline pancreatin did not improve its actions, as both hatching and activation percentages remained low (Sol. 5). However, when fresh bile was added along with cholesterol or urea, both hatching and activating effects were enhanced (Sol. 6 & 12). Pancreatin-$NaHCO_3$-urea-bile combination gave 72.7 ± 3.4% hatches with 34.5 ± 24.9% becoming active (Sol.6). Pancreatin-$NaHCO_3$-cholesterol-bile combination resulted in 80 ± 9.1% hatches with 41.1±12.5% activation (Sol.12).

Bile added to pancreatin-$NaHCO_3$ combination (Sol. 8) was definitely a better hatching and activating medium than

TABLE 4

Comparative Effects of Various Hatching - Activating Solutions upon the Ova of Echinococcus granulosus

Sol. No.	HAS*	% Hatching			% Activation		
		30 min.	60 min.	90 min.	30 min.	60 min.	90 min.
1	$NaHCO_3$ Pancreatin	37.5 ± 17.6** (5)***	56.8 ± 15.9 (5)	57.6 ± 15.9 (5)	6.3 ± 4.4** (5)	13.3 ± 10.0 (5)	7.8 ± 7.6 (5)
2	$NaHCO_3$ Trypsin	7.0 ± 10.0 (4)	7.8 ± 7.7 (4)	11.0 ± 5.5 (4)	5.0 ± 9.9 (4)	35.2 ± 14.1 (4)	17.5 ± 8.1 (4)
3	$NaHCO_3$ Pancreatin Trypsin	12.5 ± 11.9 (5)	26.4 ± 14.2 (5)	26.0 ± 11.1 (5)	21.7 ± 8.4 (5)	15.2 ± 7.0 (5)	13.5 ± 3.4 (5)
4	$NaHCO_3$ Pancreatin Cholesterol	33.7 ± 48.7 (7)	41.1 ± 25.6 (7)	46.9 ± 30.3 (7)	12.5 ± 5.3 (7)	19.5 ± 11.4 (7)	14.9 ± 5.9 (7)
5	$NaHCO_3$ Pancreatin Urea	45.6 ± 12.2 (4)	55.5 ± 13.4 (4)	52.7 ± 22.8 (4)	0.74 ± 0.87 (4)	4.6 ± 2.0 (4)	6.7 ± 5.5 (4)
6	$NaHCO_3$ Urea Pancreatin Bile*	65.1 ± 6.5 (3)	72.7 ± 3.4 (3)	71.1 ± 2.7 (3)	13.1 ± 2.5 (3)	19.7 ± 7.3 (3)	34.5 ± 24.9 (3)
7	$NaHCO_3$ Pancreatin $Na_2HPO_4 . 12H_2O$	26.5 ± 22.2 (3)	41.4 ± 53.6 (3)	44.3 ± 79.4 (3)	9.6 ± 8.3 (3)	9.5 ± 8.8 (3)	6.0 ± 0.88 (3)
8	$NaHCO_3$ Pancreatin Bile	66.9 ± 15.3 (5)	78.7 ± 10.0 (5)	77.9 ± 9.9 (5)	19.5 ± 12.0 (5)	30.1 ± 14.2 (5)	29.7 ± 19.8 (5)
9	$NaHCO_3$ Pancreatin Na-Tauroglycocholate	65.5 ± 14.0 (6)	77.6 ± 19.5 (6)	75.8 ± 22.7 (6)	13.1 ± 6.4 (6)	18.4 ± 8.1 (6)	17.2 ± 5.8 (6)
10	$NaHCO_3$ Pancreatin Na-glycocholate	53.8 ± 37.3 (5)	64.7 ± 19.4 (5)	70.7 ± 20.7 (5)	14.5 ± 5.9 (5)	31.6 ± 14.3 (5)	32.5 ± 9.9 (5)
11	$NaHCO_3$ Pancreatin Na-Taurocholate	60.6 ± 6.9 (3)	71.3 ± 1.8 (3)	72.6 ± 3.1 (3)	12.7 ± 7.4 (3)	27.3 ± 11.5 (3)	32.5 ± 2.4 (3)
12	$NaHCO_3$ Bile Pancreatin Cholesterol	67.1 ± 15.4 (7)	70.0 ± 4.7 (7)	80.0 ± 9.1 (7)	16.8 ± 6.6 (7)	34.7 ± 8.9 (7)	41.1 ± 12.5 (7)
13	Pancreatin $NaHCO_3$ Cholesterol Na-tauroglycocholate	61.0 ± 12.8 (5)	68.0 ± 12.7 (5)	65.2 ± 8.2 (5)	13.2 ± 7.6 (5)	16.7 ± 7.8 (5)	21.7 ± 9.4 (5)
14	$NaHCO_3$ Trypsin Pancreatin Bile	70.4 ± 19.9 (5)	79.8 ± 1.9 (5)	79.8 ± 22.0 (5)	15.9 ± 12.9 (5)	32.1 ± 20.8 (5)	25.5 ± 9.9 (5)
15	$NaHCO_3$ Cholesterol Pancreatin Na-glycocholate Trypsin	48.8 ± 7.4 (3)	67.8 ± 7.9 (3)	66.2 ± 0.39 (3)	15.1 ± 8.2 (3)	26.3 ± 9.9 (3)	34.3 ± 4.2 (3)
16	$NaHCO_3$ Cholesterol Pancreatin Bile Trypsin	56.0 ± 9.4 (6)	67.1 ± 6.0 (6)	72.3 ± 7.0 (6)	18.8 ± 8.0 (6)	39.6 ± 32.2 (6)	52.0 ± 19.7 (6)
17 §	Pancreatin $NaHCO_3$ Trypsin Cholesterol NapTauroglycocholate	54.3 ± 10.2 (5)	60.3 ± 11.4 (5)	60.8 ± 14.3 (5)	10.9 ± 6.5 (5)	23.4 ± 6.4 (5)	25.7 ± 6.5 (5)
18 §§	Pepsin, NaCl, Na_2PO_4, HCl) Sol 1 — Trypsin, NaCl, NaH_2PO_4) Sol 2	0 (4)	0 (4)	0.5 ± 1.0 (4)	0 (4)	0 (4)	6.3 ± 10.2 (4)

* Hatching activating solutions (HAS) prepared in distilled water containing 1.3% $NaHCO_3$ (pH 10). Concentrations of enzymes and substances were computed by weight as follows: pancreatin 1%, trypsin 0.4%, cholesterol 0.05%, urea 1%, $Na_2HPO_4 . 12H_2O$ 0.059%, Na-tauroglycocholate, na-glycocholate, na-taurocholate 1% each.

** See Table 3 for interpretation of % hatching and % activation values.

*** Figures in parenthesis indicate number of experiments; approximately 200-500 ova counted per experiment.

* Fresh sheep bile, 2 drops per 1 ml.

§ Solution 17, according to Silverman (1954).

§§ Solution 18, according to Myers (1957).

pancreatin-$NaHCO_3$ (Sol. 1) The same increased effect on hatching and activation rates was observed when fresh bile was used with pancreatin-trypsin-$NaHCO_3$ combination (Sol.14), where 79.8 ± 1.9% ova hatched and 32.1 ± 20.8% became activated.

Bile salts tested in solutions 9, 10, 11, along with pancreatin-$NaHCO_3$, produced the same increased effect on the hatching rates but not to the same degree as when fresh bile was used. However, activation rate showed a slight increase (to 32.5%) with the addition of either Na-taurocholate or Na-glycocholate (Sol. 10 & 11). Na-tauroglycocholate, did not reveal the expected increased effect (Sol. 9); hatching was 77.6 ± 19.5% and activation only 17.2 ± 5.8%. Similarly a reduced effect was noted when Na-tauroglycocholate was added to pancreatin-$NaHCO_3$-cholesterol-combination, (Sol. 13). Only 68.0 ± 12.7% of ova were hatched in this solution with 21.7 ± 9.4% of them becoming motile. Whereas when fresh bile was substituted for bile salts in the same combination of compounds (Sol. 12) hatches were 80 ± 9.1% and activation 41.1 ± 12.5%.

Silverman (1954) obtained effective hatching and activation of T. saginata ova with a pancreatin-$NaHCO_3$-trypsin-cholesterol-Na-tauroglycocholate combination. The ova of E. granulosus tested with the same solution, (Sol. 17), resulted in a hatch of 60.8 ± 14.3% with 25.7 ± 6.5% activation. Substituting Na-glycocholate for Na-tauroglycocholate in

the same combination, (Sol. 15), hatch rate went up to 67.8 ± 7.9% and activation rate 34.3 ± 4.2%. However, the substitution of fresh bile for any bile salt (Sol. 16) resulted in 72.2 ± 7.0% hatched and 52 ± 19.7% motile oncospheres, this being the highest percentage of activation obtained in these experiments. This solution (Sol. 16) therefore was adopted as the routine hatching-activating solution (HAS) for the second part of this work.

Solution 18 was prepared according to the directions given by Myer (1957), who used the same for hatching and activating the ova of Echinococcus multilocularis. This solution consists of two parts: Sol. I, prepared by adding 0.10 g. pepsin 1:3000, 2.4 g. NaCl and 0.057 g. $Na_3PO_4.12H_2O$ to 300 ml $6.7x10^{-3}$M HCl; Sol. II, prepared by adding 1.20 g. trypsin 1:250 and 2.4 g. NaCl to 300 ml. $6.775x10^{-2}$M Na_2HPO_4 a known quantity of Sol. I and Sol. II were placed in 2 small tubes at 38°C. After a few minutes for temperature equilibration the same volume of egg suspension was added to the tube containing Sol. I. An incubation period of 5 minutes was allowed and the contents of tube with Sol. II were added on to the salt and egg suspension. Observations were carried out following 5, 15, 30, 60 and 90 minutes incubation. In 4 experiments over 2000 ova were counted, but only 4 eggs hatched and only 1 of these was observed to be active. This procedure would not appear to be very effective, therefore, for hatching or activating E. granulosus ova.

A. Resistance of Echinococcus granulosus Ova to Chemicals and Heat

In the search for an effective ovicide against the eggs of E. granulosus, several chemicals commonly used as disinfectants and germicides were tested. An attempt was made to determine the effect upon ova of heat. These results are presented in the following pages and tables.

Control experiments using HAS selected in Part I (Solution 16, Table 4) were run using ova collected from each of the experimentally and naturally infected dogs. These results, tabulated in Table 5, beside serving as controls for the chemical-treatment experiments in each instance, are interesting in themselves.

A difference was observed in hatching and activation rates of ova obtained from different portions of the intestine of the same dog. Ova from the rectum or stools of Dog 5, for example, when incubated 30-90 minutes in HAS, had a hatch rate of 72.3 ± 7.0% with 52.0 ± 19.7% activation, while ova recovered from proglottids (collected from upper part of small intestine), had a hatch rate of only 56.8 ± 19% with 20.7 ± 12% activation. Similarly, a difference in hatching and activation, was observed in experiments with ova from Dog 12. Here values were 99.5 ± 1.4% hatch and 20.0 ± 4.3% activation in ova from stools or rectum, and

TABLE 5

Comparison of Hatchability and Activation in HAS* of Ova Obtained Under Different Conditions and Ova from Different Dogs

Dog No.	Source of Ova	% Hatching			% Activation		
		30 min.	60 min.	90 min.	30 min.	60 min.	90 min.
5	Stools or Rectal Washing	58.0 ± 9.4** (6)#	67.1 ± 6.0 (6)	72.3 ± 7.0 (6)	18.8 ± 8.0** (6)	39.6 ± 32.2 (6)	52.0 ± 19.7 (6)
5	Proglottids	38.0 ± 16.4 (6)	42.5 ± 14.7 (6)	56.8 ± 19.6 (6)	14.7 ± 8.4 (6)	20.7 ± 12.0 (6)	17.4 ± 9.9 (6)
12	Stools or Rectal Washing	98.7 ± 5.9 (9)	99.5 ± 1.4 (9)	99.3 ± 0.7 (9)	7.2 ± 3.5 (9)	12.7 ± 5.1 (9)	20.0 ± 4.3 (9)
12	Proglottids	85.9 ± 12.6 (8)	95.6 ± 5.6 (8)	98.7 ± 1.3 (8)	3.8 ± 2.9 (8)	10.1 ± 3.5 (8)	12.5 ± 2.9 (8)
13	Proglottids	69.3 ± 16.7 (9)	90.6 ± 7.8 (9)	91.8 ± 9.3 (9)	4.0 ± 4.2 (9)	16.2 ± 13.6 (9)	15.3 ± 8.3 (9)
S 50§	Proglottids	96.2 ± 1.4 (4)	98.4 ± 1.2 (4)	97.6 ± 0.4 (4)	6.1 ± 1.6 (4)	13.4 ± 3.5 (4)	9.1 ± 2.9 (4)
07§	Proglottids	98.9 ± 1.5 (7)	99.8 ± 5.8 (7)	99.8 ± 0.9 (7)	7.1 ± 5.9 (7)	12.5 ± 7.1 (7)	13.7 ± 9.7 (7)
07	Proglottids acid Pepsin	92.3 ± 10.1 (17)	95.7 ± 4.4 (17)	96.3 ± 5.9 (17)	7.6 ± 7.0 (17)	15.9 ± 7.9 (17)	16.5 ± 11.0 (17)
[illegible]	Proglottid acid Pepsin	[illegible] (5)	[illegible] (5)	99.7 ± 0.5 (5)	[illegible] (5)	8.9 ± 1.9 (5)	[illegible] (5)

* Hatching activating solution: formula given Table 4, Sol. 16.

** See Table 3 for interpretation of % hatching and % activation values.

\# Figures in parenthesis indicate number of experiment; approximately 200-400 ova counted per experiment

§ S 50 and 07 were naturally infected dogs.

TABLE 5

Comparison of Hatchability and Activation in HAS* of Ova Obtained Under Different Conditions and Ova from Different Dogs

Dog No.	Source of Ova	% Hatching			% Activation		
		30 min.	60 min.	90 min.	30 min.	60 min.	90 min.
5	Stools or Rectal Washing	56.0 ± 9.4** (6)#	67.1 ± 6.0 (6)	72.3 ± 7.0 (6)	18.8 ± 8.0** (6)	39.6 ± 32.2 (6)	52.0 ± 19.7 (6)
5	Proglottids	38.0 ± 16.4 (6)	42.5 ± 14.7 (6)	56.8 ± 19.6 (6)	14.7 ± 8.4 (6)	20.7 ± 12.0 (6)	17.4 ± 9.9 (6)
12	Stools or Rectal Washing	98.7 ± 5.9 (9)	99.5 ± 1.4 (9)	99.3 ± 0.7 (9)	7.2 ± 3.5 (9)	12.7 ± 5.1 (9)	20.0 ± 4.3 (9)
12	Proglottids	85.9 ± 12.6 (8)	95.6 ± 5.6 (8)	98.7 ± 1.3 (8)	3.8 ± 2.9 (8)	10.1 ± 5.5 (8)	12.5 ± 2.9 (8)
15	Proglottids	69.3 ± 16.7 (9)	90.6 ± 7.8 (9)	91.8 ± 9.5 (9)	4.0 ± 4.2 (9)	16.2 ± 15.6 (9)	15.5 ± 8.3 (9)
S 50§	Proglottids	96.2 ± 1.4 (4)	98.4 ± 1.2 (4)	97.6 ± 0.4 (4)	6.1 ± 1.6 (4)	15.4 ± 3.5 (4)	9.1 ± 2.9 (4)
07§	Proglottids	98.9 ± 1.5 (7)	99.8 ± 5.8 (7)	99.8 ± 0.9 (7)	7.1 ± 5.9 (7)	12.5 ± 7.1 (7)	13.7 ± 9.7 (7)
07	Proglottids acid Pepsin	92.3 ± 10.1 (17)	95.7 ± 4.4 (17)	96.3 ± 5.9 (17)	7.6 ± 7.0 (17)	15.9 ± 7.9 (17)	16.5 ± 11.0 (17)
12	Proglottid acid Pepsin	[illegible] (5)	[illegible] (5)	99.7 ± 0.5 (5)	[illegible] (5)	8.9 ± 1.9 (5)	7.9 ± [illegible] (5)

* Hatching activating solution: formula given Table 4, Sol. 16.
** See Table 3 for interpretation of % hatching and % activation values.
# Figures in parenthesis indicate number of experiment; approximately 200-400 ova counted per experiment
§ S 50 and 07 were naturally infected dogs.

98.7 ± 1.3% hatch and 12.5 ± 2.9% activation in ova recovered from proglottid suspensions. These values were the highest rates obtained during a 30-90 minutes' incubation period in HAS (Table 5).

A second interesting feature of data presented in Table 5 is the difference observed in activation rate of ova obtained from different dogs. Percent activation of ova recovered from stools or rectal washings of Dog 5 and Dog 12 were respectively 52.0± 19.7% and 20.0 ± 4.3%. Similarly, percent activation in ova recovered from proglottid suspensions of Dogs 5,12,13,S13 and 07 were, respectively, 20.7±12.0% 12.5 ±2.9%, 16.2 ±13.6%, 13.4 ± 3.5% and 13.7 ± 9.7%.

During the course of chemical treatment experiments, when the supply of ova from stools or rectal washings was exhausted in any instance, it was necessary to use ova recovered from proglottids. Proglottid suspensions were treated with acid pepsin (5 minutes in 1% pepsin prepared in 0.4% HCl) at 37°C in order to break down proglottid tissues and release the ova. A comparison of hatching and activation rates, before and after pepsin treatment, of such ova from Dogs 07 and 12 showed little, if any, effect of pepsin pretreatment upon either hatching or activation. Pretreatment with pepsin caused a slight increase in percent activation (from 13.7 ± 9.7 to 16.5 ± 11.0) in ova from Dog 07, and a slight decrease in percent activation (from 12.5 ± 7.1 to 8.9 ± 1.9) in ova from Dog 12 (Table 5).

In the chemical treatment experiments the ova use were, for the most part, those recovered from rectal washings of Dog. 12. Ova from proglottid suspensions, after pepsin treatment, of dogs 12, 13, 07 and S30 also were used. Activation rates of ova from these dogs ranged from 12.5 to 20% and hatch rates from 90 to 99%.

B. Effects of Formalin upon the Viability of Echinococcus granulosus Ova

Echinococcus granulosus ova exposed to 1% formalin for 5, 10 and 30 minutes, to 5% formalin for 5 minutes, and to 10% formalin for 5 minutes did not differ in their hatching and activation rates from those of the controls. Highest activation rates obtained during 30-90 minutes incubation at 37°C in HAS for ova exposed to 1% formalin for 5, 10 and 30 minutes were, respectively, 18.4, 12.9 and 13.7% (Table 6). Hatching rates also were at a high level in the 5, 10 and 30 minute groups (they were, respectively, 97.0, 91.3 and 80.8%), and slightly lower in the 5% formalin, 5 minute group (88.8%) and 10% formalin 5 minute group (74.4%). When exposure time or concentration of formalin or both were stepped up, however, the hatching rate was reduced considerably (as in 5% formalin for 10 and 30 minutes; 10% formalin for 10, 30 and 60 minutes; 20% formalin for 5 minutes), or prevented completely (as in 10% formalin for 6 and 24 hours and 20% formalin for 10 minutes or longer.

TABLE 6

Effects of Formalin upon the Viability of Echinococcus granulosus Ova

Formalin		HAS** 30 min.		HAS 60 min.		HAS 90 min.		HAS 2 hours		HAS 30 min.		HAS 60 min.	
Concent.	Time Treated	% ± Hatched	% ± Activated	% Hatched	% Activated	% Hatched	% Activated	% Hatched	% Activated	% Hatched	% Activated	% Hatched	% Activated
1%	5 min.	89.25 (3)	7.4 #(3)	95.1 #(3)	16.7 (3)	97.0 (3)	18.4 (3)	94.2 (3)	8.7 (3)	-	-	-	-
	10 min.	81.37 (3)	9.7 (3)	89.0 (3)	7.2 (3)	91.3 (3)	12.9 (3)	91.2 (3)	11.5 (3)	-	-	-	-
	30 min.	41.91 (3)	3.6 (3)	73.4 (3)	8.9 (3)	80.8 (3)	9.6 (3)	79.8 (3)	15.7 (3)	-	-	-	-
5%	5 min.	74.19 (3)	3.5 (3)	87.2 (3)	5.5 (3)	86.4 (3)	15.8 (3)	88.8 (3)	14.6 (3)	-	-	-	-
	10 min.	29.41 (4)	2.2 (4)	54.8 (4)	4.0 (4)	73.1 (4)	6.8 (4)	77.2 (4)	4.2 (4)	-	-	-	-
	30 min.	0 (3)	0 (3)	0.7 (3)	0 (3)	0.4 (3)	0 (3)	1.5 (3)	0 (3)	61.2 (1)	26.6 (1)	-	-
10%	5 min.	31.08 (3)	4.4 (3)	55.5 (3)	4.8 (3)	75.1 (3)	4.1 (3)	74.4 (3)	15.0 (3)	-	-	-	-
	10 min.	0 (3)	0 (3)	1.9 (3)	0 (3)	10.4 (3)	0 (3)	19.1 (3)	8.0 (3)	-	-	-	-
	30 min.	[illegible] (4)	0 (4)	6.4 (4)	3.2 (4)	[illegible] (4)	3.6 (4)	[illegible] (4)	[illegible] (4)	35.7 (2)	29.9 (2)	-	-
	60 min	0.31 (2)	0 (2)	0.2 (2)	0 (2)	-	-	-	-	21.2 (2)	27.9 (2)	-	-
	6 hrs.	0 (2)	0 (2)	0 (2)	0 (2)	-	-	-	-	78.5 (2)	4.1 (2)	67.0 (2)	9.5 (2)
	24 hrs.	0	0	0	0	-	-	-	-	96.7 (1)	26.0 (1)	95.0 (1)	25.3 (1)
20%	5 min.	0.1 (2)	0 (2)	4.7 (2)	0 (2)	-	-	-	-	89.0 (2)	14.2 (2)	88.6 (2	19.9 (2)
	10 min.	0 (2)	0 (2)	0 (2)	0 (2)	-	-	-	-	44.5 (2)	8.4 (2)	51.9 (2	9.7 (2)
	30 min.	0 (2)	0 (2)	0 (2)	0 (2)	-	-	-	-	52.1 (2)	8.5 (2)	39.7 (2	8.0 (2)
	60 min.	0 (4)	0 (4)	0 (4)	0 (4)	-	-	-	-	51.5 (4)	6.0 (4)	46.4 (4	2.9 (4)
	6 hrs.	0 (2)	0 (2)	0 (2)	0 (2)	-	-	-	-	71.1 (2)	1.5 (2)	62.8 (2	0 (2)
	24 hrs.	0 (2)	0 (2)	0 (2)	0 (2)					46.1 (2)	0 (2)	56.4 (2	0 (2)

* Double line represents treatment in 1% na-hypochlorite
** Hatching activating solution; formula given in Table 4, Sol. 16.
± All values are means and represent % hatched of total egg counts.
All values are means and represent % activated of the total oncospheres hatched.
Figures in parenthesis indicate number of experiments; approx. 200-500 ova counted per experiment.

Microscopically it was not possible to tell, however, whether oncospheres within unhatched eggs, the shells of which were fixed in formalin, were living or dead in as much as they remained normal in appearance. Although such formalin-fixed ova were incubated for long periods of time in HAS (up to 18 hours) no hatching was observed. When shells were removed by treatment with 1% sodium hypochlorite solution for 3-5 minutes, however, and freed oncospheres washed four times in distilled water with subsequent incubation in HAS, it was found that a large percentage of such oncospheres were still viable. In fact, activation rates were frequently higher in these ova than in the controls. Thus in ova exposed to 10% formalin for 30 minutes, the highest activation rate was 14.8%. After removing shells in sodium hypochlorite, washing oncospheres and incubating a second time in HAS, the activation rate was increased to 29.9%. Similarly, in ova exposed to 10% formalin for 6 hours and 24 hours, there were no hatched oncospheres observed following 30-90 minutes' incubation in HAS. When shells were removed in sodium hypochlorite solution, washed and incubated in HAS, however, activation rates were respectively 9.5 and 26% (Table 6).

Approximately the same results were obtained in ova exposed to 20% formalin for 5, 10, 30 and 60 minutes 6 hours and 24 hours. There was no hatching in any of these groups of ova after initial incubation in HAS. When shells

were removed in sodium hypochlorite solution, washed and incubated in HAS, however, activation rates were, 19.9%, 9.9%, 8.5%, 6.0%, 1,5% and 0%, respectively.

It is clear from the foregoing that formalin at the various concentrations, and at the various periods of time tested, did not render all treated oncospheres non-viable; a certain percentage of active oncospheres were observed in all groups except the 20% formalin, 24 hours group.

Another interesting feature of activation rates after sodium hypochlorite treatment was their gradual decrease with increase of exposure time to 20% formalin.

C. Effects of Lysol upon the Viability of Echinococcus granulosus Ova

Exposure to 2% and 5% Lysol for 5, 10, 30 and 60 minutes did not effect hatching of ova and activation of oncospheres. Hatching and activation rates, obtained in these experiments, did not deviate much from those obtained in the controls (Table 7).

Exposure of ova to 10% Lysol for 5, 10 and 30 minutes and for 6 and 24 hours reduced hatching as exposure time was increased. The highest hatching rate after 5 minutes in 10% Lysol was 93.1% with 17.2% activated, while after 24 hours in 10% Lysol corresponding values were 47.2% and 4.0% (Table 7). In these experiments, although shells were fixed gradually as exposure time was increased, activation rates remained at a high level among hatched oncospheres.

TABLE 7

Effects of Lysol upon the Viability of Echinococcus granulosus Ova.*

Lysol		HAS 30 min.		HAS 60 min.		HAS 90 min.		HAS 2 hrs.		HAS 30 min.		HAS 60 min.	
Concent.	Time treated	% Hatched	% Activated	% Hatched	% Activated	% Hatched	% Activated	% Hatched	% Activated	% Hatched	% Activated	% Hatched	% Activated
2%	5 min.	88.8 (5)	1.1 (5)	93.33 (5)	8.44 (5)	92.3 (5)	11.8 (5)	93.6 (5)	5.8 (5)				
	10 min.	99.2 (4)	41.7 (4)	98.69 (4)	41.33 (4)	99.5 (4)	21.8 (4)	100.0 (4)	23.6 (4)				
	30 min.	97.8 (4)	9.9 (4)	98.41 (4)	16 (4)	98.3 (4)	10.0 (4)	99.1 (4)	9.2 (4)				
5%	5 min.	85.2 (5)	10.7 (5)	92.78 (5)	14.44 (5)	92.1 (5)	10.0 (5)	98.3 (5)	10.2 (5)				
	10 min.	5.1 (4)	12.8 (4)	95.7 (4)	23.68 (4)	98.1 (4)	24.6 (4)	95.5 (4)	25.9 (4)				
	30 min.	97.2 (4)	7.5 (4)	99.0 (4)	15.0 (4)	99.3 (4)	11.3 (4)	99.7 (4)	12.7 (4)				
	60 min.	97.7 (1)	2.4 (1)	97.9 (1)	11.4 (1)	98.2 (1)	12.6 (1)	-	-				
10%	5 min.	88.7 (5)	9.8 (5)	97.8 (5)	11.8 (5)	98.2 (5)	15.4 (5)	98.2 (5)	17.4 (5)				
	10 min.								12.0				
	30 min.	39.3 (4)	18.2 (4)	71.4 (4)	16.7 (4)	65.6 (4)	9.5 (4)	65.6 (4)	33.3 (4)				
	6 hrs.	36.8 (2)	3.2 (2)	41.1 (2)	6.5 (2)	46.0 (2)	17.4 (2)						
	24 hrs.	25.3 (3)	0 (3)	27.2 (3)	2.8 (3)	47.2 (3)	4.0 (3)						
20%	5 min.	7.1 (2)	0 (2)	4.1 (2)	0 (2)	-	-	-	-	52.9 (2)	22.2 (2)	22.2 (2)	50.0 (2)
	10 min.	48.2 (2)	0 (2)	57.0 (2)	12.5 (2)	-	-	-	-	60.0 (2)	33.3 (2)	50.0 (2)	33.3 (2)
	30 min.	14.0 (3)	16.7 (3)	1.1 (3)	25.0 (3)	-	-	-	-	7.4 (3)	0 (2)		
	6 hrs.	0 (2)	0 (2)	0 (2)	0 (2)	-	-	-	-	0 (2)	0 (2)		
	24 hrs.	0 (2)	0 (2)	0 (2)	0 (2)	-	-	-	-	0 (2)	0 (2)		

* Legend same as Table 6

Ova treated with 10% Lysol became dark in color and swollen (Fig. 28). Some shells cracked while others remained intact. As the exposure time was increased more ova swelled and ruptured (Fig. 27). Such changes in color, appearance and size of ova were even more obvious in ova treated with 20% Lysol.

In ova exposed to 20% lysol for 5, 10 and 30 minutes hatching was markedly suppressed and in those exposed for 6 and 24 hours it was completely prevented. Removal of shells in sodium hypochlorite solution and subsequent incubation in HAS resulted in higher percentages of activated oncospheres in some instances.

In the group of ova exposed to 20% lysol for 6 and 24 hours no oncospheres appeared to have survived when shells were removed in sodium hypochlorite.

D. Effects of Ethyl Alcohol upon the Viability of Echinococcus granulosus Ova

Exposure of E. granulosus ova to 50% ethyl alcohol for 5 and 10 minutes did not effect their hatching, although in the 30 minute group a slight reduction in hatching rate was observed (reduction from 98 to 92%). Activation rates were high in all the groups, highest rates for the 5, 10 and 30 minute groups were 22.3, 24.9, and 17.6% respectively (Table 8).

In ova exposed to 70% alcohol for 5, 10, 30 and 60 minutes, hatching rates were reduced gradually as the

TABLE 8

Effects of Ethyl Alcohol upon the Viability of *Echinococcus granulosus* Ova.*

Ethyl Alcohol		H-A Sol. 30 min.		H-A Sol. 60 min.		H-A Sol. 90 min.		H-A Sol. 2 hrs.	
Concent.	Time Treated	% Hatched	% Activated	% Hatched	% Activated	% Hatched	% Activated	% Hatched	% Activated
50%	5 min.	95.3 (4)	17.0 (4)	97.3 (4)	22.3 (4)	97.5 (4)	17.9 (4)	94.7 (4)	21.8 (4)
	10 min.	96.6 (3)	7.0 (3)	98.9 (3)	17.0 (3)	98.5 (3)	24.9 (3)	98.4 (3)	13.8 (3)
	30 min.	61.9 (3)	6.9 (3)	81.08 (3)	11.7 (3)	92.4 (3)	17.6 (3)	-	-
70%	5 min.	96.2 (3)	11.0 (3)	95.2 (3)	23.5 (3)	97.7 (3)	20.4 (3)	97.9 (3)	25.7 (3)
	10 min.	54.2 (6)	11.7 (6)	88.95 (6)	14.0 (6)	87.8 (6)	11.0 (6)	95.9 (6)	19.4 (6)
	30 min.	54.9 (4)	7.5 (4)	72.89 (4)	14.0 (4)	79.0 (4)	19.3 (4)	-	-
	60 min.	24.3 (3)	7.0 (3)	20 (3)	20.5 (3)	23.5 (3)	29.9 (3)	16.2 (3)	16.4 (3)
95%	5 min.	88.8 (3)	32.5 (3)	96.75 (3)	36.6 (3)	85.1 (3)	34.9 (3)	-	-
	10 min.	84.0 (3)	23.8 (3)	90.16 (3)	14.6 (3)	91.9 (3)	11.8 (3)	-	-
	30 min.	62.9 (2)	18.9 (2)	76.19 (2)	12.5 (2)	69.0 (2)	27.8 (2)	-	-
	60 min.	56.4 (2)	25.0 (2)	81.8 (2)	5.6 (2)	100.0 (2)	21.4 (2)	-	-

* Legend same as Table 6

exposure time was increased. Rates for the above periods, at 90 minutes incubation in HAS, were, respectively, 97.7, 87.8, 79.0, 23.5% (Table 8). This gradual decline in hatching rates suggests that shells were being fixed through prolonged contact with alcohol. The same effect was also observed in experiments with 95% alcohol.

Activation rates, however, both in 70% and 95% alcohol did not change with time of exposure. Highest activation rates after 5, 10, 30 and 60 minutes in 70% alcohol were 25.7%, 19.4%, 19.3% and 29.9% respectively, and after 5, 10, 30 and 60 minutes in 95% alcohol, 36.6%, 23.8% 27.3% and 25.0% (Table 8).

However, observations on oncospheres hatched from ova treated with 95% alcohol revealed that, with increased incubation in HAS, oncospheres were being gradually destroyed. This may be illustrated in the results of a typical experiment. The ovum count per 2 drops from a tube treated for 30 minutes with 95% alcohol before incubation in HAS was 300 ova, after 30 minutes' incubation in HAS the ovum count decreased to 35, after 60 minutes' incubation to 16, and after 90 minutes' incubation it was still 16. This effect of 95% alcohol was consistently observed and suggested the slow permeation of the egg shell by alcohol with resulting death of the oncosphere.

E. Effects of "Roccal" upon the Viability of Echinococcus granulosus Ova

"Roccal" (containing 10% Benzalkonium chloride) is commonly used as a germicide on utensils in eating establishment, milk processing plants, bakeries and canneries at a dilution of 1:5000 and as a spray on walls and floors of hospitals and for general household use at a dilution of 1:1250.

In these experiments ova were exposed to three dilutions of "Roccal", 1:1250, 1:100, and 1:50. Those exposed to 1:1250 and 1:100 dilutions for from 5 to 30 minutes hatched and were activated at rates similar to the controls (Table 9).

Ova exposed to the 1:50 dilution of "Roccal" hatched at a lesser rate, however. When such ova were treated with sodium hypochlorite solution, a high percentage of oncospheres were found to have survived (Table 9).

F. Effects of "Clorox" upon the Viability of Echinococcus granulosus Ova

A 5% solution of sodium hypochlorite was recommended by Borray (1954) for destroying the ova of E. granulosus on instruments and laboratory containers. Time of exposure was not stated in the abstract seen.

"Clorox" (containing 5.25% sodium hypochlorite) was used virtually undiluted in these experiments. That is

TABLE 9

Effects of 'Roccal' upon the Viability of *E. granulosus* Ova.*

Roccal		H - A Sol. 30 min.		H - A Sol. 60 min.		H - A Sol. 90 min.	
Concentration	Time Treated	% Hatched	% Activated	% Hatched	% Activated	% Hatched	% Activated
800 ppm	5 min.	86.9 (2)	3.2 (2)	97.9 (2)	8.5 (2)	97.3 (2)	16.1 (2)
1/1250	10 min.	97.9 (2)	42.9 (2)	99 (2)	19.4 (2)	100.0 (2)	24.2 (2)
	30 min.	84.2 (2)	5.8 (2)	94.0 (2)	7.8 (2)	95.8 (2)	12.1 (2)
1/100	5 min.	84.0 (2)	2.0 (2)	90.7 (2)	6.0 (2)	91.4 (2)	10.6 (2)
	10 min.	84.9 (2)	2 (2)	85.6 (2)	7.5 (2)	89.3 (2)	8.1 (2)
	30 min.	87.9 (2)	7.9 (2)	85.7 (2)	10.4 (2)	88.5 (2)	9.4 (2)
1/50	5 min.	15.6 (1)	4.8 (1)	14.0 (1)	2.2 (1)	16.0 (1)	2.5 (1)
	10 min.	20.0 (1)	4.4 (1)	35.1 (1)	9.3 (1)	3.1. (1)	3.3 (1)

* Legend same as Table 6

very concentrated suspensions of ova were used and the volume of "Clorox" was sufficiently in excess so that the final concentration of sodium hypochlorite approximated 5%.

The shells of ova exposed to "Clorox" dissolved completely in 3-5 minutes (Figures 18, 19 and 20), leaving the oncospheres exposed to the action of the chemical. Hatching in all experiments was 100%. Highest activation rates after 5, 10, 30 and 60 minutes exposures were, respectively, 47.3, 35.7, 26.2, and 30.8%. All values are higher than controls (Table 10).

G. Effects of a Household Detergent, "Tide", upon the Viability of Echinococcus granulosus Ova

The usual concentration of "Tide" for general household use has been estimated to be between 1% and 2%.

Ova exposed to 1% and 2% solutions of "Tide" for various periods of time did not show any deleterious effects. Hatching was normal and activation rates were even higher than those obtained in the controls (Table 11).

H. Effects of Heat upon the Viability of Echinococcus granulosus Ova

An attempt was made to determine the effect of moist heat upon oncospheres of E. granulosus (Table 12).

Exposure to 50°C for 5 minutes did not change the appearance of the ova and did not kill all the oncospheres. Following incubated in HAS for 30 minutes 81% hatched with

TABLE 10

Effects of 5% Sodium Hypochlorite upon the Viability of Echinococcus granulosus Ova.*

Na-Hypochlorite		H - A Sol. 50 min.		H - A Sol. 60 min.		H - A Sol. 90 min.	
Concentration	Time Treated	% Hatched	% Activated	% Hatched	% Activated	% Hatched	% Activated
5%	5 min.	100.0 (3)	47.4 (3)	100.0 (3)	28.8 (3)	100.0 (3)	22.2 (3)
	10 min.	100.0 (3)	10.0 (3)	100.0 (3)	35.7 (3)	100.0 (3)	24.0 (3)
	30 min.	100.0 (3)	20.5 (3)	100.0 (3)	26.2 (3)	100.0 (3)	25.0 (3)
	60 min.	100.0 (1)	26.7 (1)	100.0 (1)	26.2 (1)	100.0 (1)	30.8 (1)

* Legend same as Table 6

TABLE 11

Effects of Detergent "Tide" upon the Viability of *Echinococcus granulosus* Ova.*

Tide		H - A Sol. 30 min.		H - A Sol. 60 min.		H - A Sol. 90 min.	
Concentration	Time Treated	% Hatched	% Activated	% Hatched	% Activated	% Hatched	% Activated
1%	5 min.	95.7 (3)	11.6 (3)	100.0 (3)	28.6 (3)	100.0 (3)	25.1 (3)
	10 min.	91.1 (2)	15.0 (2)	97.3 (2)	37.9 (2)	96.5 (2)	36.2 (2)
	30 min.	51.4 (2)	57.8 (2)	41.5 (2)	58.2 (2)	49.4 (2)	22.0 (2)
2%	5 min.	92.9 (2)	12.6 (2)	99.1 (2)	19.4 (2)	99.1 (2)	27.1 (2)
	10 min.	95.8 (2)	13.2 (2)	99.1 (2)	19.4 (2)	100.0 (2)	21.9 (2)
	30 min.	95.1 (3)	53.9 (3)	94.9 (3)	73.0 (3)	100.0 (3)	52.0 (3)
	6 hrs.	100.0 (3)	43.8 (3)	100.0 (3)	60.6 (3)	100.0 (3)	65.0 (3)
	24 hrs.	100.0 (2)	30.0 (2)	100.0 (2)	28.6 (2)	100.0 (2)	9.1 (2)

* Legend same as Table 6

TABLE 12

Effects of Moist Heat upon the Viability of *Echinococcus granulosus* Ova.*

Temperature		H - A Sol. 30 min.		H - A Sol. 60 min.		H - A Sol. 90 min.		H - A Sol. 30 min.	
°C	Time Treated	% Hatched	% Activated	% Hatched	% Activated	% Hatched	% Activated	% Hatched	% Activated
50	5 min.	81 (2)	6.2 (2)	90 (2)	6.8 (2)	93.2 (2)	0 (2)		
	10 min.	81 (2)	0 (2)	85.7 (2)	0 (2)	91.6 (2)	0 (2)		
	30 min.	94 (1)	0 (1)	98.2 (1)	0 (1)	99.4 (1)	0 (1)		
60	5 min.	7.8 (2)	0 (2)	6.5 (2)	0 (2)	10.7 (2)	0 (2)	0 (2)	0 (2)
	10 min.	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (2)	0 (2)
70	5 min.	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)		
	10 min.	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)		
	30 min.	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)		
100	1 min.	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0	0
	5 min.	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0	0

* Legend same as Table 6

with 6.2% activation, for 50 minutes 90% hatched with 6.8% activation, and for 90 minutes 93.2% hatched but no active oncospheres were observed.

Ova exposed to 50°C for 10 and 30 minutes also were unchanged in appearance. In the 10 minute group, hatching was from 81% to 91.6% and in the 30 minute group, it was 94% to 99.4%. No active oncospheres were observed in either of these groups of ova.

However, in the group exposed to 50°C for 10 minutes although 2 of a total of 543 oncospheres counted appeared morphologically as though they had been activated, at the time of observation (following 30 minutes' incubation in HAS) they were not motile. Effects of 50°C are therefore equivocal.

In ova exposed to 60°C for 5 minutes, the hatching rate was low (only 6.5-10.7%). Oncospheres appeared to be of normal size and shape although no motile oncospheres were observed. Unhatched ova, which also appeared normal, when treated in sodium hypochlorite solution did not contain active oncospheres.

Ova exposed to 60°C for 10 minutes, to 70°C for 5, 10 and 30 minutes and to 100°C for 1 and 5 minutes did not hatch. Ova exposed to these temperatures appeared swollen, and the oncospheres within were structureless masses (Fig.26). When such ova were incubated in HAS, the shells as well as the oncospheres of most ova were digested. The few appar-

ently intact ova which remained in each experiment, when treated with sodium hypochlorite, completely disappeared.

DISCUSSION

A. Biology of Hatching and Activation

Evidence from these experiments indicates that the cement which binds the rectangular blocks forming the shells of eggs of E. granulosus, is not dissolved in an acid medium, even in the presence of a proteolytic enzyme such as pepsin. It is dissolved, however, under alkaline conditions ($NaHCO_3$ or NH_4OH at a pH of 10) without the aid of any proteolytic enzyme. This could lead us to assume, perhaps, that the cement substance is non-protein in nature. Enzymes such as pancreatin and trypsin, in addition to digesting proglottid tissues and vitelline membranes, appear to be important in activating the oncospheres after the removal of the shells. Through the combined actions of $NaHCO_3$ (pH 10), pancreatin and trypsin it is possible to free ova from proglottid tissues and vitelline membranes, oncospheres from their shell elements and to activate a high percentage of the latter. The further addition of cholesterol and fresh sheep bile greatly enhances the activating effect of these enzymes. Purified bile salts only partially substitute for fresh bile.

Bile and its salts are surface active agents. According to Glassman (1948), surface active agents alter energy relationships at interfaces thereby lowering surface or interfacial tensions. He points out that the interaction

of surface active agents and proteins may result in precipitation, complex formation and denaturation. Anson (1939) had previously reported that small amounts of surface active agents could have powerful denaturing effects upon proteins. Sorsenisavaya (1938) and later Haurowits (1945) have demonstrated that trypsin acts rapidly on denatured proteins but only slowly on native proteins. Perhaps bile, as a surface active agent, denatures embryonal membrane protein and thereby renders it more susceptible to trypsin by digestion.

It is also possible that bile has a direct excitatory effect upon the tissues of the oncosphere. Such an excitatory property of bile and its salts has long been recognized. Work of early workers indicated that bile had the particular characteristic of stimulating gut activity. Horall (1937) observed that bile was apparently necessary for normal intestinal peristalsis.

Various other effects of bile upon the physiology of tapeworms, particularly upon the evagination and excystation of cysticerci and cysticercoids have been reported by Malkani (1933), De Waele (1934), Edgar (1941), Smith (1954), Rothman (1958, 1959) and Read (1955). Beck (1951) observed that the addition of small quantities of dog bile to the food of male and female rats stimulated egg production in the tapeworm, Hymenolepis diminuta.

Isobe (1922), Leonard (1941) and others recognized the importance of fresh bile in the hatching of taeniid ova,

but it was Silverman (1954) who suggested its function in the activation of the oncosphere. Berberian (1957) noted that E. granulosus ova would hatch in human bile and in the intestinal juices of sheep and cattle, as well as in crude pancreatin.

Motility of the oncosphere was taken to be the criterion of viability in all of these experiments and the activation rate was considered to be an index for assessing resistance to chemical agents. However, absence of motility could not be considered as an indication of absence of life. Most oncospheres when hatched are inactive (Fig. 22, Top). Only when they are exposed to the stimulating effect of various "activators" do they become motile (Fig. 23). In no instance could all oncospheres be activated; a number always remained inactive in every experiment. Perhaps the oncospheral membranes of such ova were not influenced by activators, or, more likely, such oncospheres were perhaps insufficiently mature to be stimulated to activity (as recently suggested) in the case of Hydatigena.

This latter is evident when activation is compared in oncospheres recovered from stools and in those recovered from proglottids. The activation rate in oncospheres from stools of Dog 5, was 52 ± 19.7% and from Dog 12, 20.0 ± 4.3%; while activation in oncospheres from proglottids of the same dogs were, respectively, 17.4 ± 9.9% and 12.5 ± 2.9%. These results indicate that oncospheres recovered from proglottids,

being less mature, are activated at lower rates.

Attempts at vital staining gave further support to this view. Permeability to vital stains was poor in inactive oncospheres otherwise normal in appearance. Methylene blue or Nile blue, when added to the HAS, penetrated the living active oncospheres (Fig. 25) and activated, but dead, oncospheres. Oncospheres which were not activated did not take the stain, however, suggesting that the unaltered uncospheral membrane was not permeable to the dye.

B. Resistance to Chemicals and Heat

Hydatid disease is a major public health problem in many parts of the world. The rate of infection in Lebanon is high (Schwabe and Abu Daoud, unpublished data). In control or eradication campaigns, an efficacious procedure for the destruction of ova would be invaluable. To this end the effects of some common germicides and disinfectants upon the viability of E. granulosus ova were tested in vitro.

Formalin and Lysol at low concentrations 1 to 5% were ineffective as evidenced by the high percentage of activation obtained among hatched oncospheres. Penetration of these chemicals at low concentrations was apparently slow. At higher concentrations (10 to 20%) the shells were fixed. Oncospheres within fixed shells were activated at a high rate, however, when shells were removed in sodium hypochlorite solution.

Ova exposed to 20% formalin for various periods of

time showed a gradual reduction in percentage activation, with increase of exposure time. This could be interpreted as the result of a very slow penetration of formalin through fixed shells with consequent damage to the oncospheres.

Ova remained viable in 50% and 70% ethyl alcohol for up to 60 minutes. If there was any action of alcohol during these periods of treatment, it was to increase the activation rate rather than to damage the embryos. However, many of the ova exposed to 95% alcohol were adversely effected, although the activation rate among those that did escape damage was high.

"Roccal", at a dilution recommended for germicidal use, had no effect upon the viability or hatching of ova treated for the periods stated. At dilutions 10 times and 25 times more concentrated than that recommended "Roccal" reduced hatching but the activation rate among surviving ova remained high.

"Clorox", containing 5.25% sodium hypochlorite, completely dissolved shells in a few minutes, so that oncospheres were directly exposed to its effects. Oncospheres so treated remained viable after 5, 10, 30 and 60 minutes' exposure, as evidenced by the high rate of activation observed.

More ova hatched and were activated after exposure to 2% detergent ("Tide") than after exposure to 1%. The highest percentage activation was obtained when exposure

time was 30 minutes to 6 hours. Ova exposed 24 hours to 2% "Tide" also hatched normally and a high percentage of oncospheres were activated, but movements were poor and larvae died quickly, suggesting, perhaps, that extended exposures effected the oncospheres in some adverse way. Like bile, "Tide" is a surface active agent.

The results of experiments testing the effects of moist heat upon the viability of E. granulosus ova were encouraging. Ova exposed to moist heat at 50°C for 5 minutes were not all destroyed. Some 6.8% could be activated. Those exposed to 50°C for 10 minutes and 30 minutes also hatched normally and although oncospheres were normal in appearance, no activation was observed.

All ova exposed to 70°C for 5, 10 and 30 minutes, and to 100°C for 1 and 5 minutes, appeared swollen and abnormal. The oncospheral membranes were swollen and separated from the remainder of the oncosphere. When shells of such ova were removed in HAS or in Na-hypochlorite, the oncospheral remnants were completely dissolved.

CONCLUSIONS

The ova of E. granulosus were successfully hatched and activated in vitro at 37°C. Best results were obtained when a solution containing $NaHCO_3$ (1.3%), pancreating (1%), trypsin (0.4%), cholesterol (0.05%), and fresh sheep bile (2 drops per 1 ml) was used.

It was found that $NaHCO_3$ (1.3%; pH 10) or NH_4OH (pH 10) removed shells and pancreatin-trypsin combination, aided by cholesterol and bile, activated the free oncospheres. Activated oncospheres remained motile for from 1-3 hours. Percentage of activated oncospheres was taken as an index for assessing viability.

Bile salts, such as Na-taurocholate, Na-glycocholate and Na-tauroglycocholate, increased the activating effect of the enzymes pancreatin and trypsin, but not as effectively as fresh sheep bile. Trypsin was a better activator than pancreatin. Urea and Na_2HPO_4 did not enhance the action of pancreatin. Acid pepsin had no effect on the hatching or activation of E. granulosus ova.

Ova recovered from different dogs and ova recovered from different portions of the gut of the same dog, showed different activation rates when incubated in the hatching-activating solution (HAS) described above. Those recovered from stools or from the rectum were activated at a higher percentages than were ova recovered from proglottids collected higher up in the intestine.

The effects of several common germicides and disinfectants upon the viability of E. granulosus ova were studied. Formalin and Lysol are known to be lethal to bacteria at 2, 5, and 10% concentrations. Ova of E. granulosus remained viable at these concentrations of both chemicals for up to 24 hours. No viable oncospheres were observed in ova which were exposed to 20% formalin for 24 hours, although whole oncospheres were present. Similarly no viable oncospheres were observed in ova exposed to 20% Lysol, for from 6 to 24 hours. Here, however, there were no intact oncospheres left.

In ova exposed to 50% and 70% ethyl alcohol for from 5 to 60 minutes viability was not effected. Some ova exposed to 95% alcohol for 30 to 60 minutes were destroyed when transferred into HAS. However, among those that escaped the damaging action of 95% alcohol, the activation rate was high.

Some ova survived "Roccal" at dilution of 1:1250, 1:100 and 1:50, for from 5 to 30 minutes. Ova remained viable after 5, 10 and 30 minutes treatment in 1 and 2% "Tide", and 5% Na-hypochlorite; in fact higher percentage of oncospheres were activated after exposure to these substances.

Moist heat at 50°C for 5 minutes did not kill all oncospheres; 6.8% were activated and viable. Moist heat at 50°C for 10 minutes and 30 minutes did prevent activation

although some hatched. The viability of these oncospheres was questionable. Heat at 60°C and 70°C for 5, 10 and 30 minutes, and 100°C for 1 and 5 minutes prevented hatching. When shells were removed no oncospheres were found to have survived.

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Plate I

Fig. 1. Adult Echinococcus granulosus. (Mag. 24x)

Plate II

Fig. 2. Gravid proglottid of E. granulosus. (Mag. 32x)

Fig. 3. Dog intestine covered with adult E. granulosus (Mag. 2x)

Fig. 4. Ova of E. granulosus (Mag. 600x)

Fig. 5. Ovum of E. granulosus Note radially striated shell and central oncosphere (Mag. 1000x)

Plate III

Fig. 6. Liberated oncosphere of E. granulosus (Mag. 500x).

Fig. 7. E. granulosus Ovum showing vitelline covering (Mag. 650).

Fig. 8. E. granulosus brood capsule (Mag. 120x)

Fig. 9. E. granulosus: larval scolices attached by their peduncles to germinal membrane. (Mag. 120x)

Plate IV

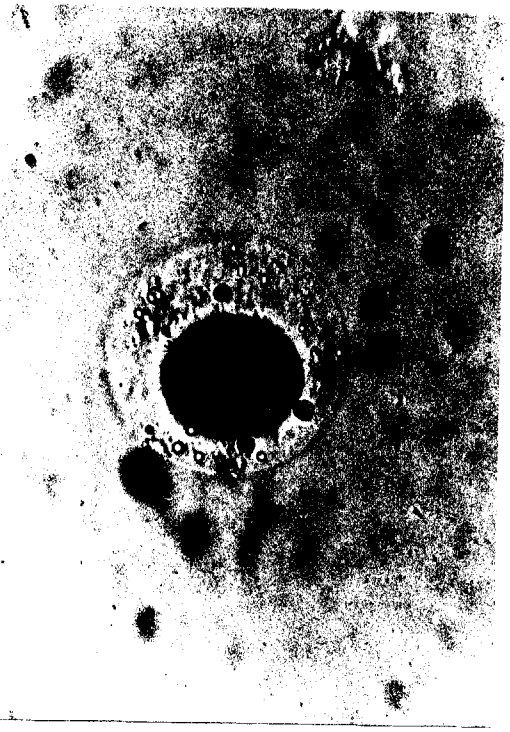

Fig. 10. E. granulosus ovum at 5 min. incubation in $NaHCO_3$ (pH 10): note intact vitelline covering. (Mag. 450x)

Fig. 11. E. granulosus ovum at 15 min. incubation in $NaHCO_3$ (pH 10): note intact vitelline covering and rupture in the shell.

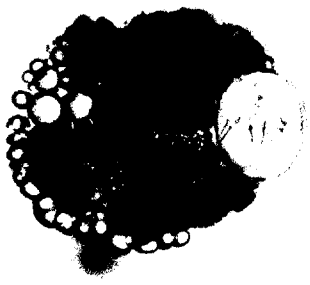

Fig. 12. E. granulosus ovum at 30 min. incubation in $NaHCO_3$ (pH 10): note liberated oncosphere and shell components within intact vitelline covering (Mag. 450x)

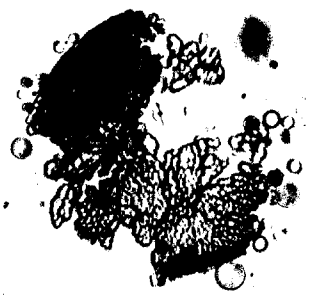

Fig. 13. E. granulosus ovum at 30 min. incubation in $NaHCO_3$ (pH 10): note disrupted shell and active oncosphere within vitelline covering (mag. 500x).

Plate V

Fig. 14. E. granulosus ovum at 5 min. incubation in hatching-activating sol. (mg. 900x).

Fig. 15. E. granulosus at 10 min. incubation in hatching-activating sol. (Mg. 900x).

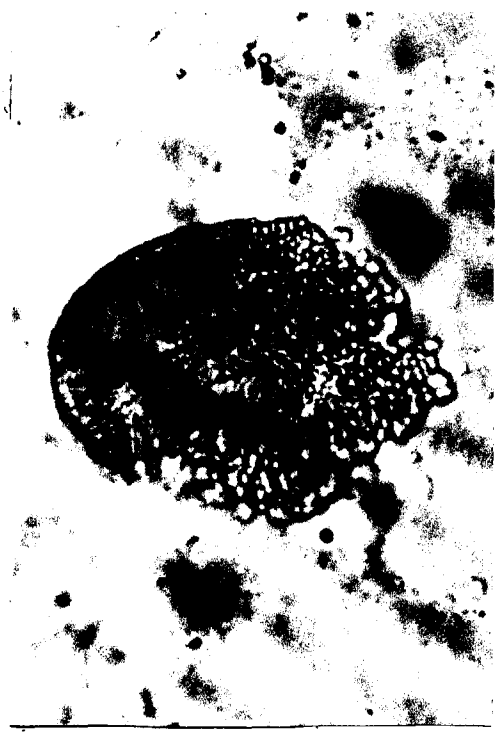

Fig. 16. E. granulosus at 15 min. incubation in hatching-activating sol. (mg. 900x).

Fig. 17. E. granulosus ovum at 30 min. incubation in hatching-activating sol: note liberated oncosphere and shell components. (mg. 600x).

Plate VI

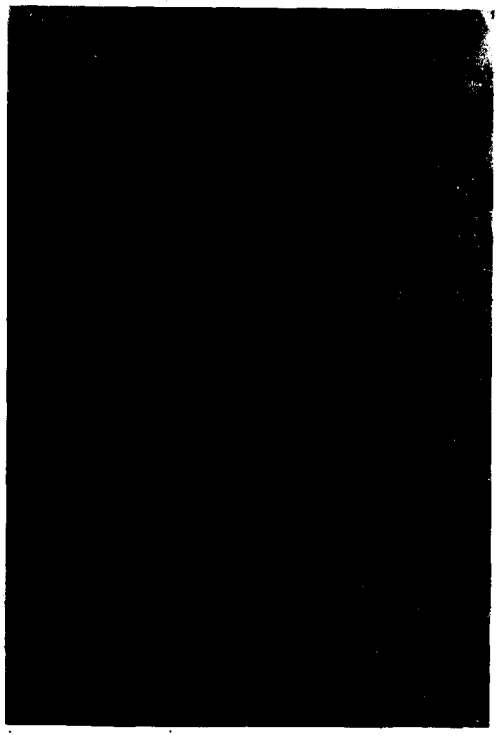

Fig. 18. E. granulosus ovum at 1 min. Incubation in "Clorox". (mg. 450x)

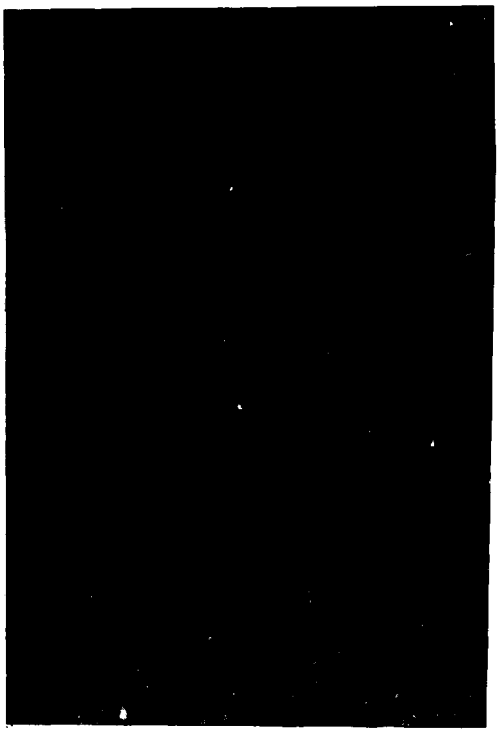

Fig. 19. E. granulosus ovum at 2 min. Incubation in "Clorox". (mag. 450x).

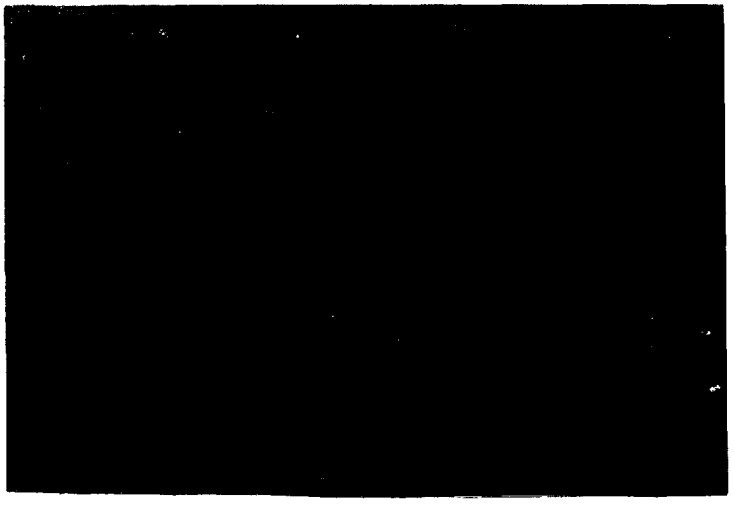

Fig. 20. E. granulosus ovum at 5 min. Incubation in "Clorox" (mg. 450x)

Plate VII

Fig. 21. Liberated oncosphere of E. granulosus in the process of activation. (mg. 250x).

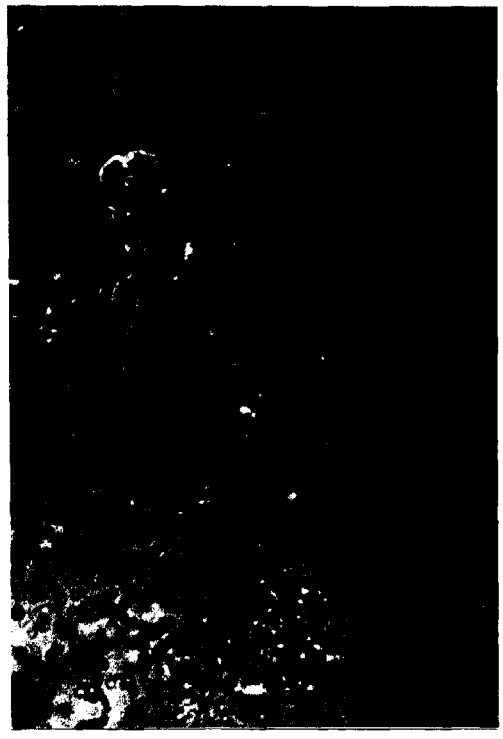

Fig. 22. Oncospheres of E. granulosus in hatching and activating sol.: note inactive oncosphere (Top) and activated oncosphere showing hook movement (bottom). (mg. 450x).

Fig. 23. Activated oncosphere of E. granulosus shewing oncospheral membrane. (mg. 450x).

Fig. 24. Activated-but-dead oncosphere of E. granulosus: note relaxed membrane and hooklets. (mag. 500x).

Plate VIII

Fig. 25. E. granulosus: activated oncosphere showing vital staining with methylene blue

Fig. 26. E. granulosus: ovum exposed to moist heat at 100°C: note oncospheral membrane and central structureless mass (mg. 1000x).

Fig. 27. E. granulosus: ovum exposed to 20% lysol showing crack in the middle, and swelling of shells. (mg. 200)

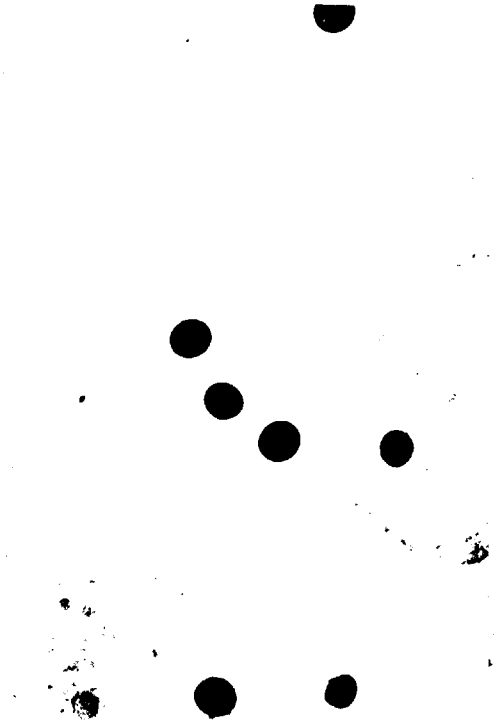

Fig. 28. E. granulosus: ova exposed to 20% Lysol: note opacity and the swollen condition of shells. (mg. 100)

Plate I

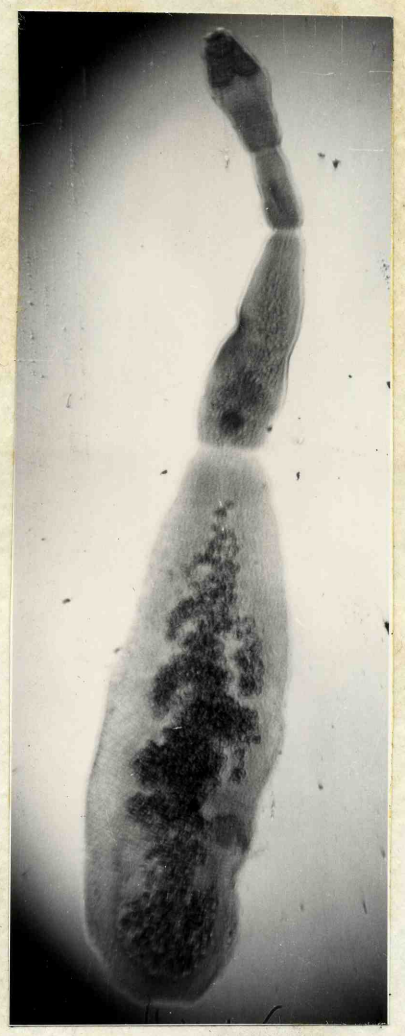

Fig. 1. Adult Echinococcus granulosus. (Mag. 24x)

Plate II

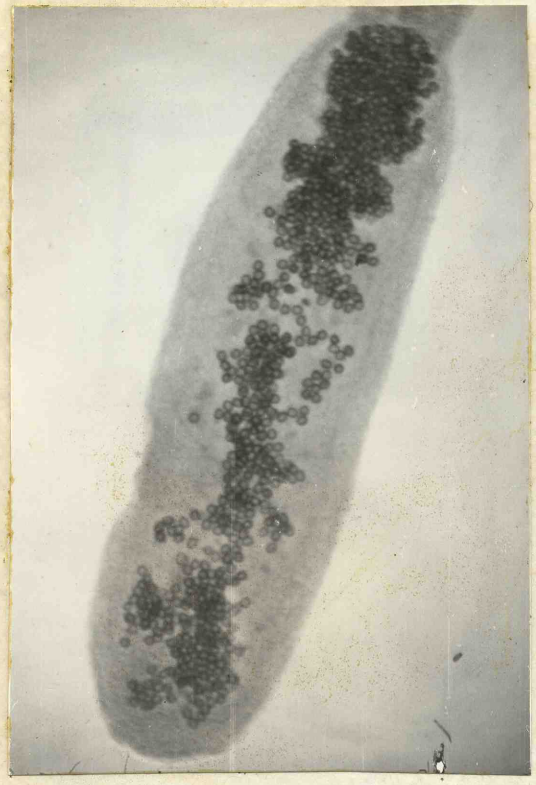

Fig. 2. Gravid proglottid of E. granulosus. (Mag. 32x)

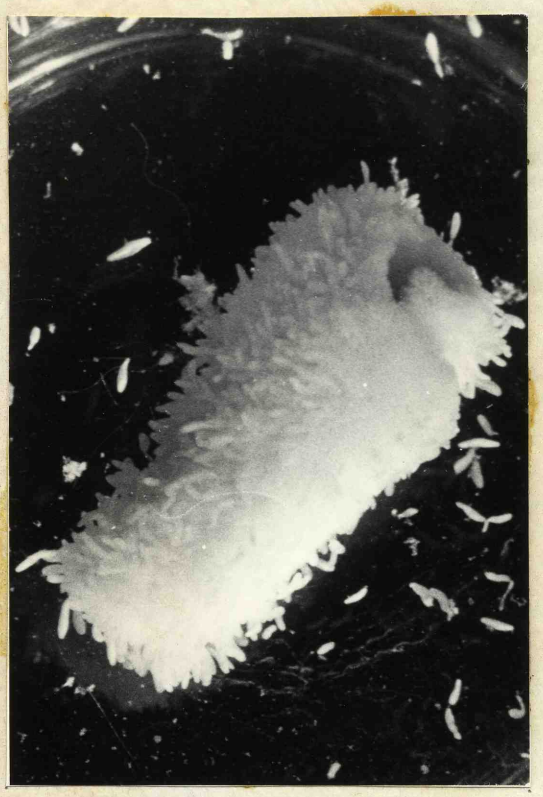

Fig. 3. Dog intestine covered with adult E. granulosus (Mag. 2x)

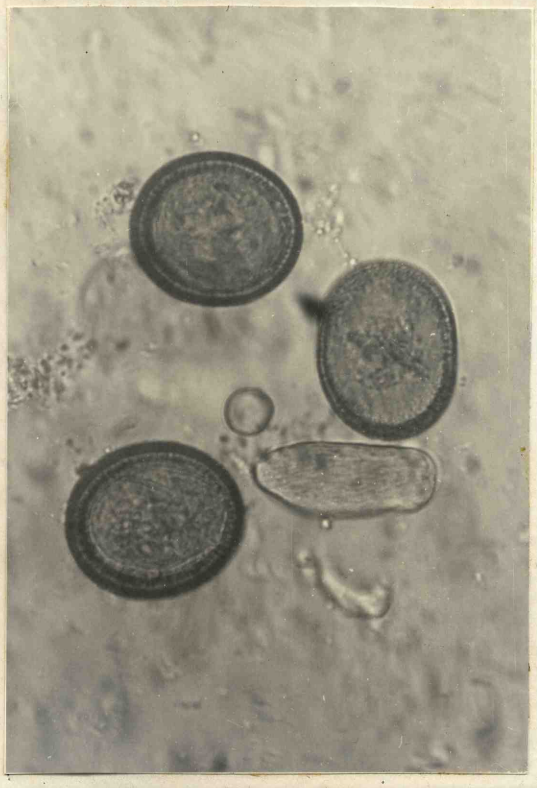

Fig. 4. Ova of E. granulosus (Mag. 600x)

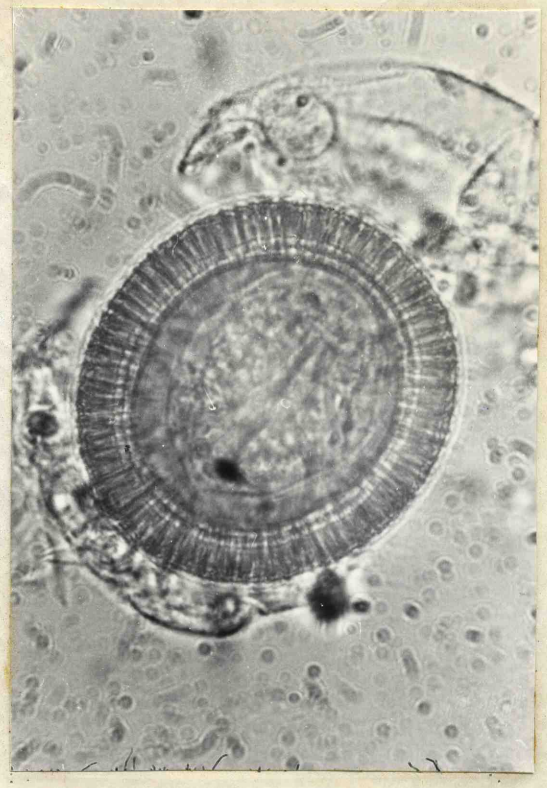

Fig. 5. Ovum of E. granulosus Note radially striated shell and central oncosphere (Mag. 1000x)

Plate III

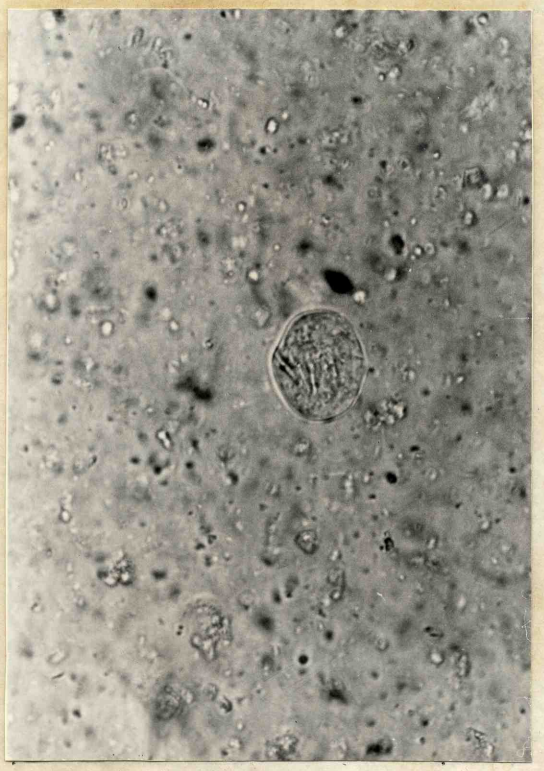

Fig. 6. Liberated oncosphere of E. granulosus (Mg. 500x).

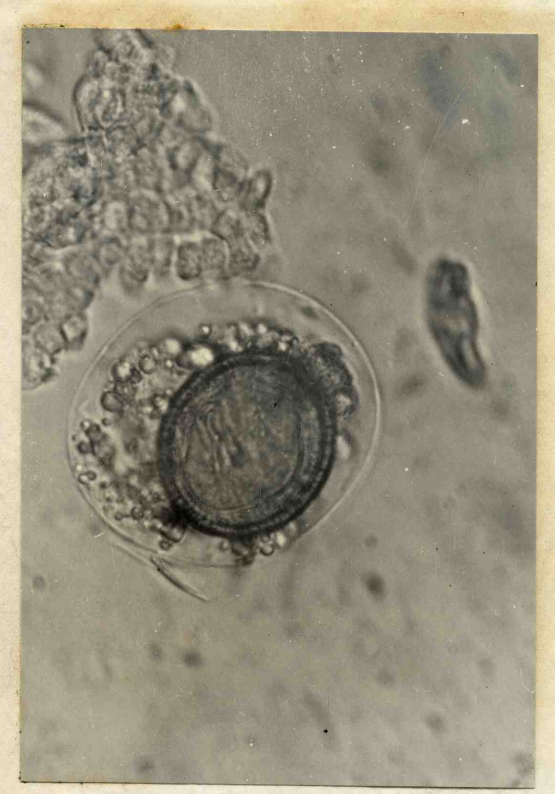

Fig. 7. E. granulosus Ovum showing vitelline covering (Mag. 650).

Fig. 8. E. granulosus brood capsule (Mag. 120x)

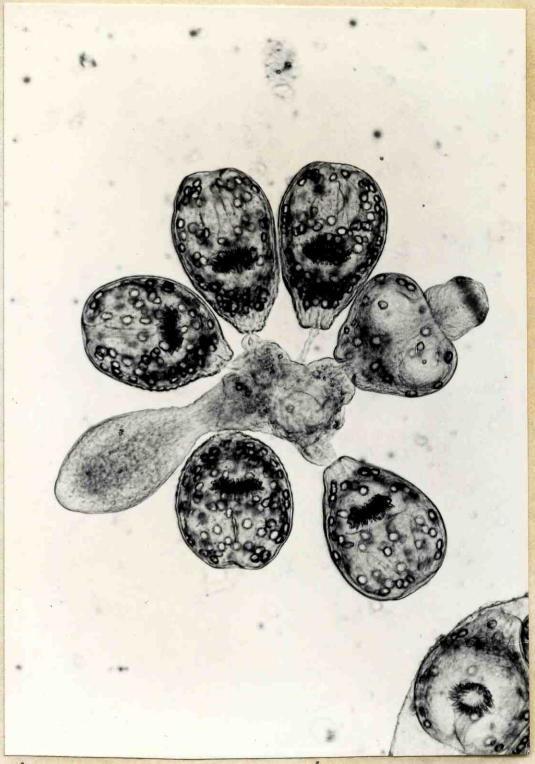

Fig. 9. E. granulosus: larval scolices attached by their peduncles to germinal membrane. (Mag. 120x)

Plate IV

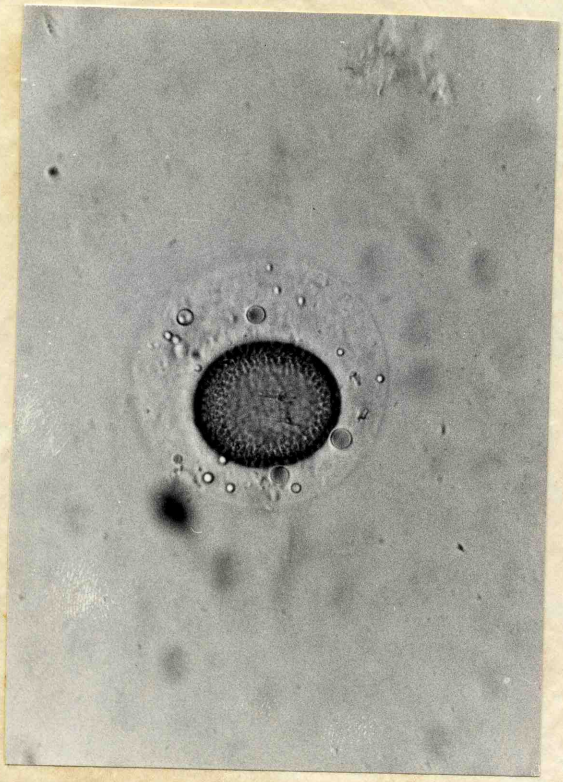

Fig. 10. E. granulosus ovum at 5 min. incubation in $NaHCO_3$ (pH 10): note intact vitelline covering. (Mag. 450x)

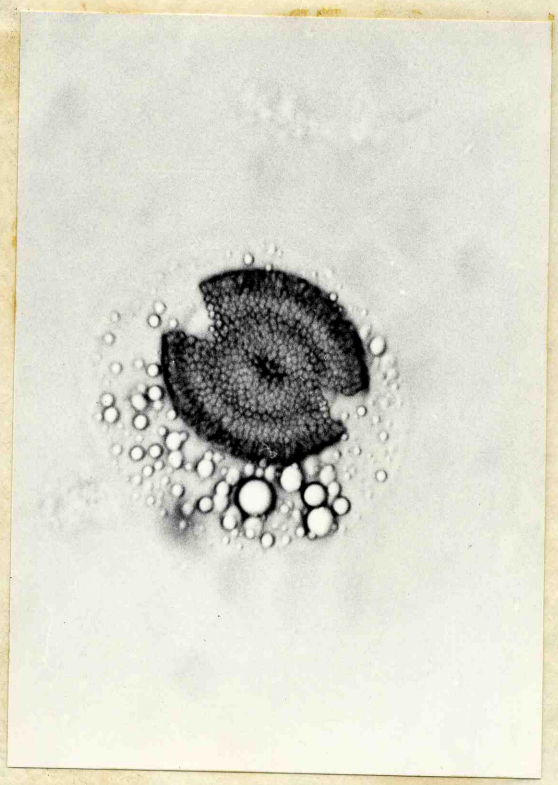

Fig. 11. E. granulosus ovum at 15 min. incubation in $NaHCO_3$ (pH 10): note intact vitelline covering and rupture in the shell.

Fig. 12. E. granulosus ovum at 30 min. incubation in $NaHCO_3$ (pH 10): note liberated oncosphere and shell components within intact vitelline covering (Mag. 450x)

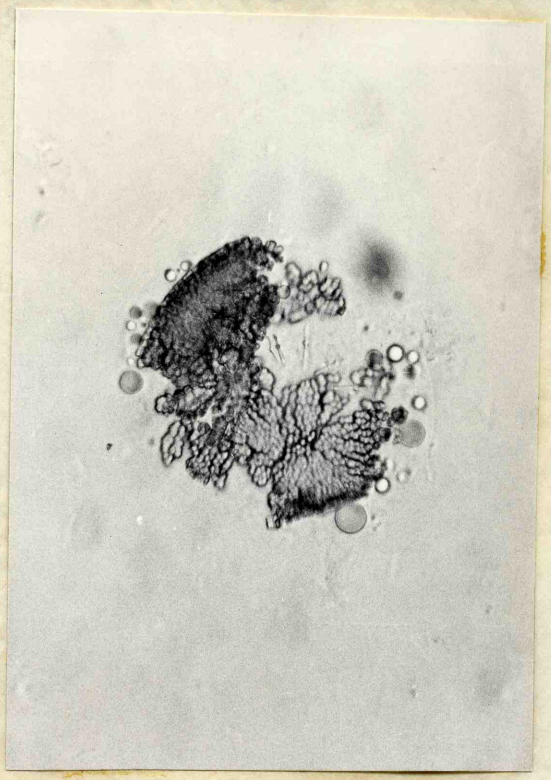

Fig. 13. E. granulosus ovum at 30 min. incubation in $NaHCO_3$ (pH 10): note disrupted shell and active oncosphere within vitelline covering (mag. 500x).

Plate V

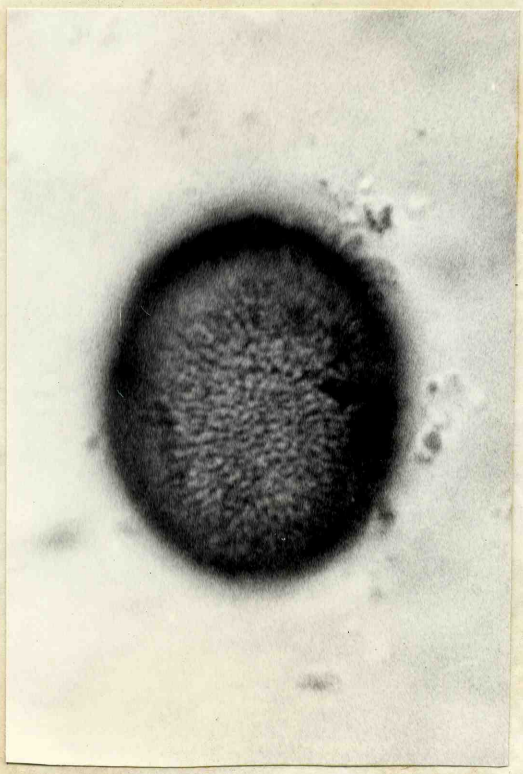

Fig. 14. E. granulosus ovum at 5 min. incubation in hatching-activating sol. (mg. 900x).

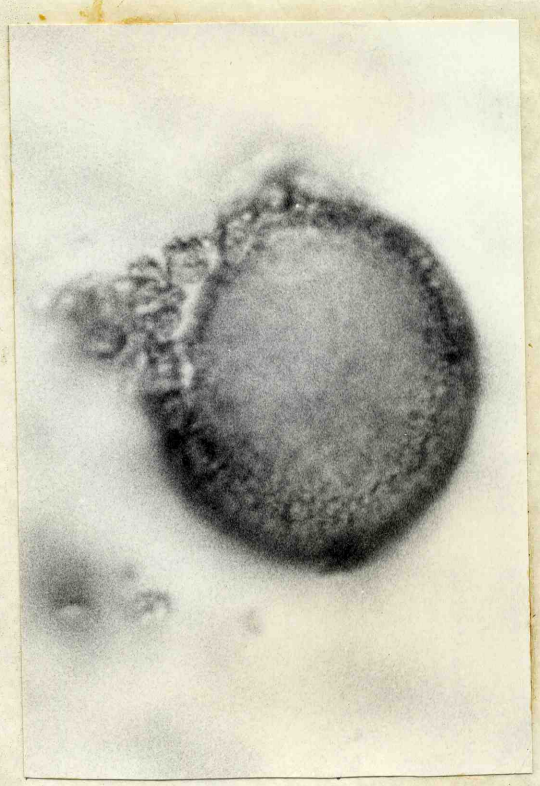

Fig. 15. E. granulosus at 10 min. incubation in hatching-activating sol. (Mg. 900x).

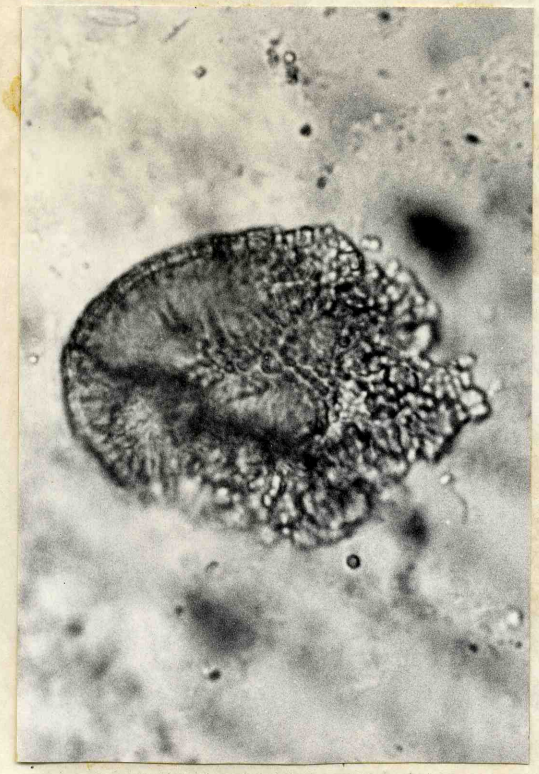

Fig. 16. E. granulosus at 15 min. incubation in hatching-activating sol. (mg. 900x).

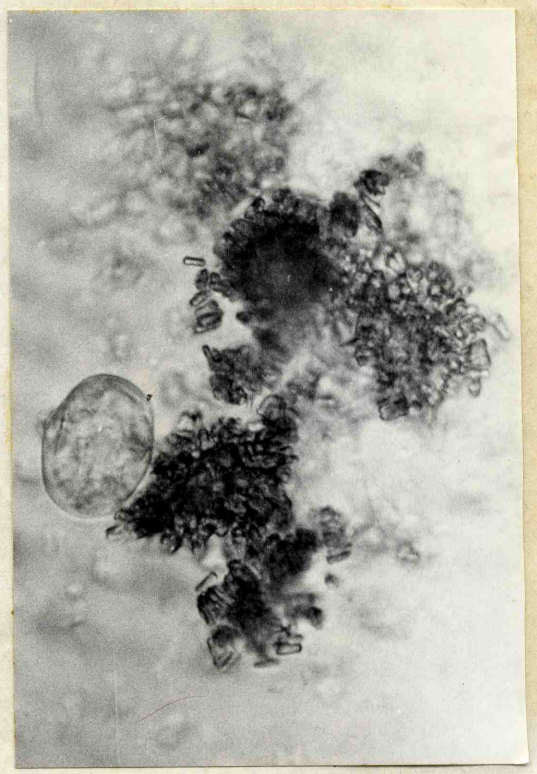

Fig. 17. E. granulosus ovum at 30 min. incubation in hatching-activating sol: note liberated oncosphere and shell components. (mg. 600x).

Plate VI

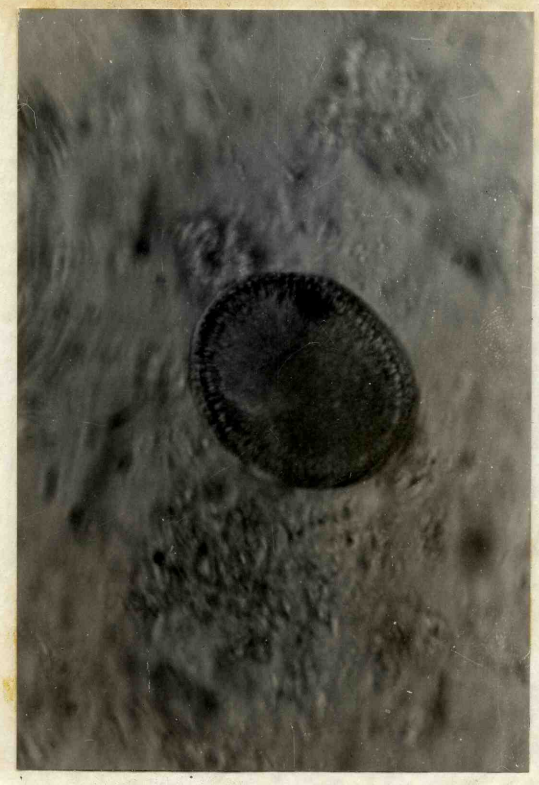

Fig. 18. E. granulosus ovum at 1 min. Incubation in "Clorox". (mg. 450x)

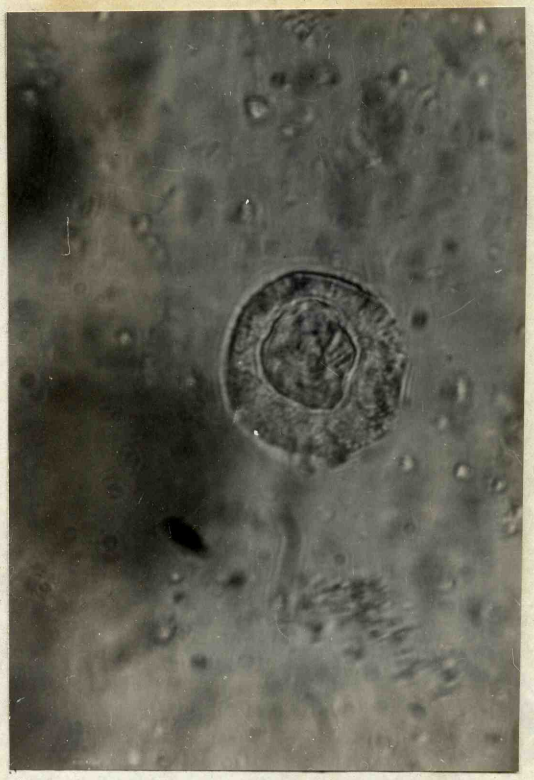

Fig. 19. E. granulosus ovum at 2 min. Incubation in "Clorox". (mag. 450x).

Fig. 20. E. granulosus ovum at 3 min. i incubation in "Clorox" (mg. 450x)

Plate VII

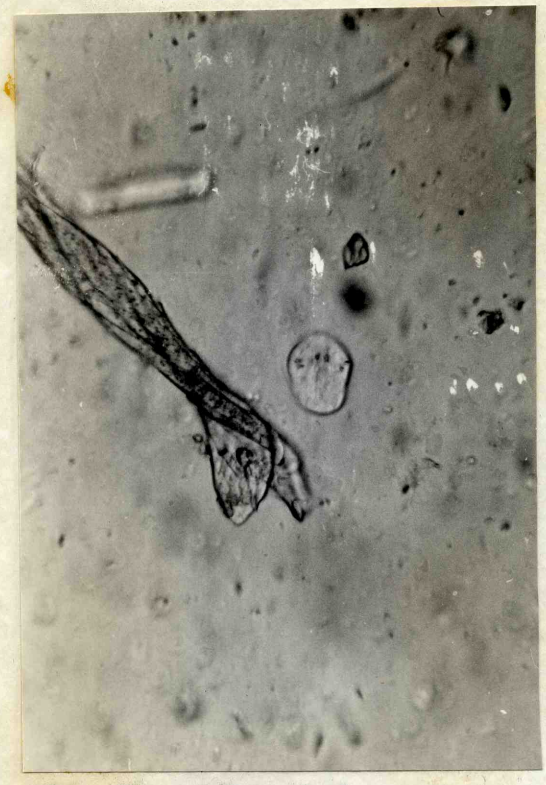

Fig. 21. Liberated oncosphere of E. granulosus in the process of activation. (mg. 250x).

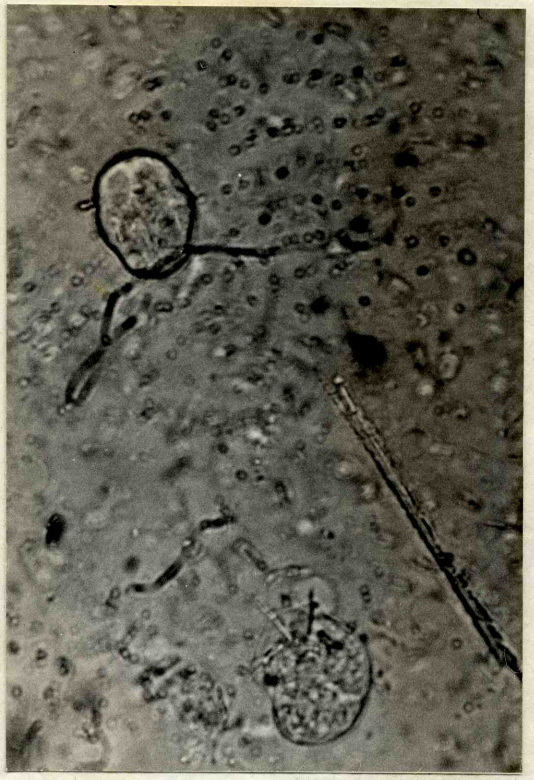

Fig. 22. Oncospheres of E. granulosus in hatching and activating sol.: note inactive oncosphere (Top) and activated oncosphere showing hook movement (bottom). (mg. 450x).

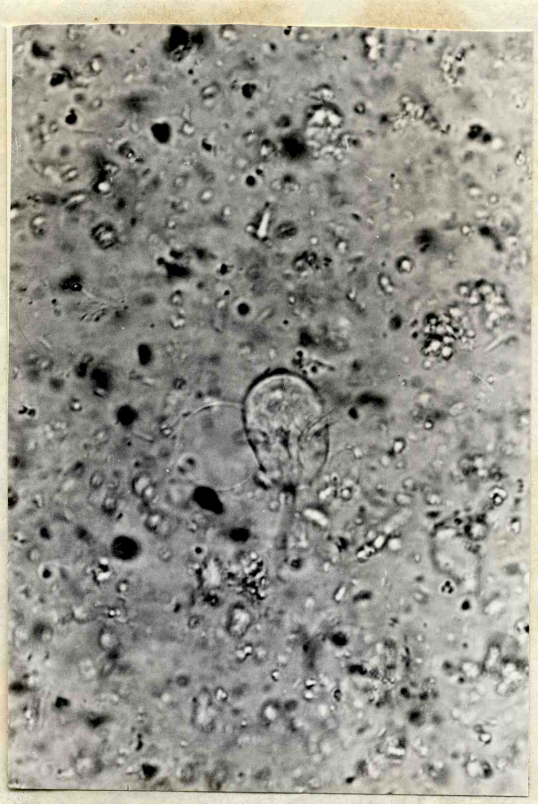

Fig. 23. Activated oncosphere of E. granulosus showing oncospheral membrane. (mg. 450x).

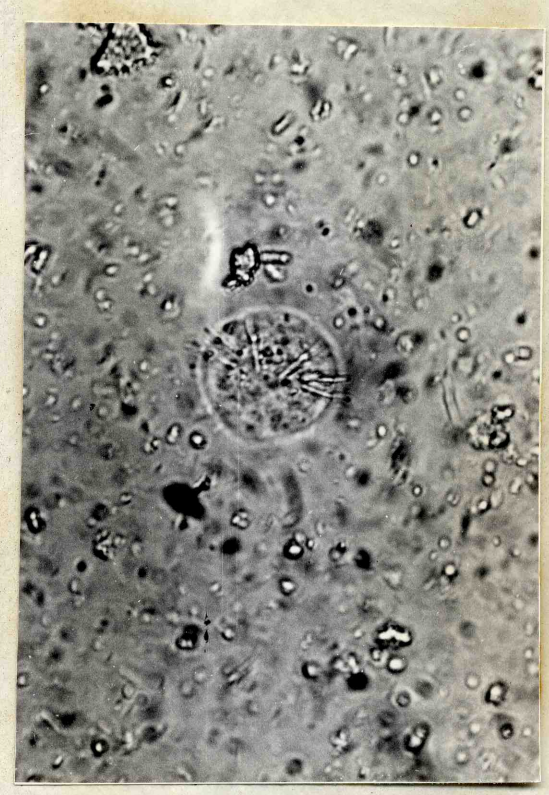

Fig. 24. Activated-but-dead oncosphere of E. granulosus: note relaxed membrane and hooklets. (mag. 500x).

Plate VIII

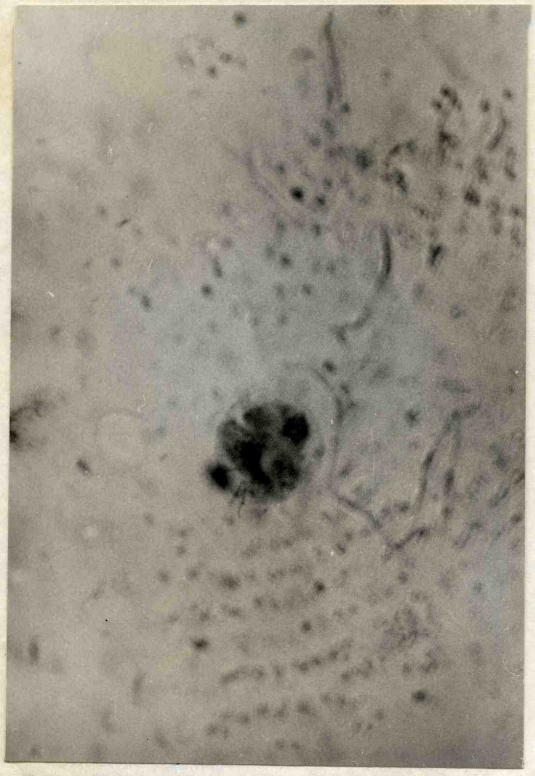

Fig. 25. E. granulosus: activated oncosphere showing vital staining with methylene blue

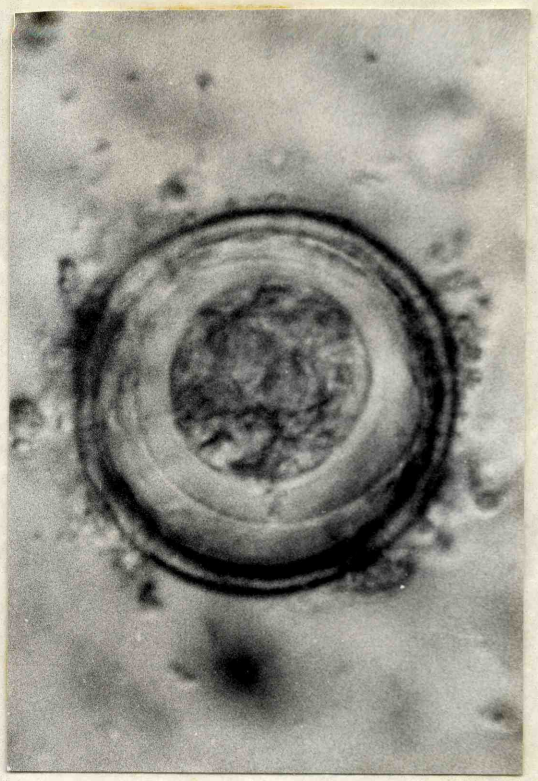

Fig. 26. E. granulosus: ovum exposed to moist heat at 100°C: note oncospheral membrane and central structureless mass (mg. 1000x).

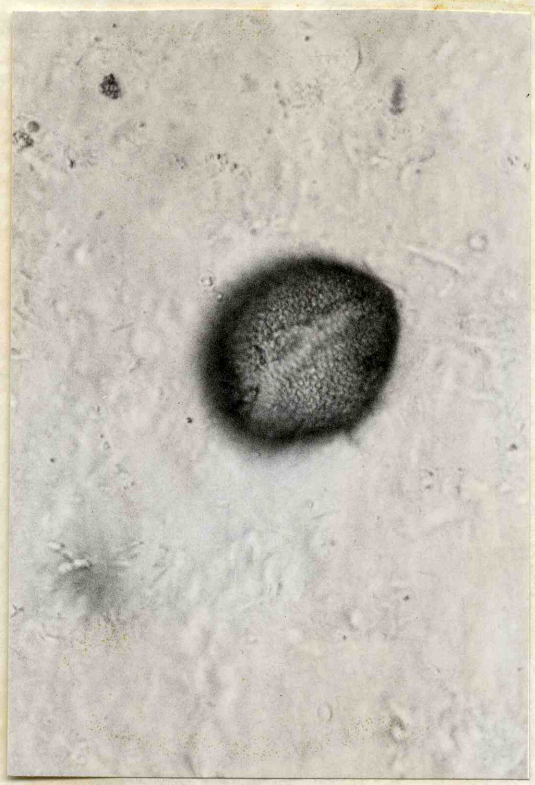

Fig. 27. E. granulosus: ovum exposed to 20% lysol showing crack in the middle, and swelling of shells. (mg. 200)

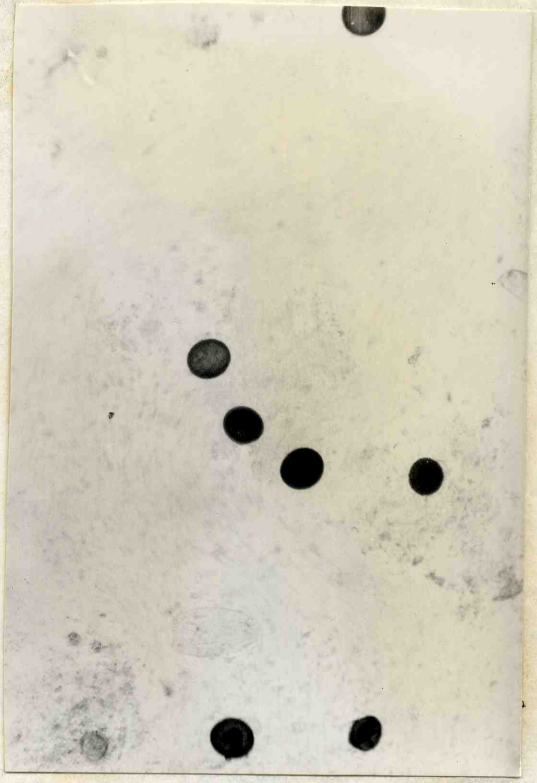

Fig. 28. E. granulosus: ova exposed to 20% Lysol: note opacity and the swelling condition of shells. (mg. 100)