more closely than was the case immediately after isolation. But we did not observe a reversion to normal bacterial morphology as was observed in the case of the L_1 strains.

We were unable to verify the presence of fine non-bacterial filaments such as described by Klieneberger. No elements inconsistent with bacterial morphology were visible in the colonies of our strains.

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Diagnosis of Echinococcal (Hydatid) Disease in Man by Intradermal Reaction to Rabbit *Cysticercus* Antigen.

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A skin test originally described by Casoni[1] and since modified by several investigators is generally considered to be the most reliable single guide to the presence or absence of suspected hydatid infection in patients.[2] The test is usually performed by injecting intradermally 0.1 to 0.5 cc of carbolized fluid obtained from a fertile hydatid cyst of either sheep or man. This fluid contains an antigen which maintains its potency for months. Both immediate and delayed skin reactions, consisting of wheals with pseudopods and surrounding erythema, have been described. The necessary hydatid fluid may be readily obtained in certain world areas, such as Southern Australia, where echinococcal disease is common in both man and animal. However, throughout North America the supply of cysts is small due to the rareness of the disease, and the clinician is often confronted with a diagnostic dilemma when the antigen is unavailable.

In the present communication observations are reported concerning an antigen which may be procured readily from cysticerci occurring in rabbits. Tests made thus far indicate that patients infected with echinococcus, or those who have recently had cysts removed, will give positive skin reactions with the antigen, whereas essentially

1 Casoni, T., *Folia Clinica Chem. Micros.*, 1911-1912, **4**, 5.

2 For reviews of the literature, consult Taliaferro, W. H., *The Immunology of Parasitic Infections*, 1929, The Century Co., New York; and Culbertson, J. T., *Arch. Path.*, 1938, **25**, 85, 256.

negative results are obtained in individuals who have never harbored the parasite. Cysticerci obtained from the rabbit are those of *Tænia pisiformis*, a cestode which is found naturally in that animal. Among stock laboratory rabbits the incidence of infection varies from 25 to 50%, being higher in the older animals.

Preparation of Antigen: The abdomens of healthy rabbits are open with sterile precautions and searched for cysts. When these are found they are removed to Petri dishes where the adventitial envelope, which is composed of rabbit tissue, is teased away with sterile dissecting needles. From 5 to 7 of the cysts are triturated in a sterile mortar, thus destroying the contained scolices, and are then extracted with 3 to 4 cc of sterile saline for 2 hours at 37°C. The resulting suspension is centrifuged to remove all heavy particles and portions are cultured aërobically and anaërobically to determine sterility. The fluid finally obtained is opalescent, but no sediment appears on standing.

Relationship of Rabbit Cysticercus Antigen to Specific Hydatid Antigen: Taxonically the rabbit cysticercus is closely related to the echinococcus since both are cestodes of the genus Taenia. Furthermore, an antigenic relationship between these species has recently been demonstrated by Outeirino.[3] In order to study this antigenic relationship several procedures were carried out. Blood serum from a patient who had recently had an echinococcal cyst removed was found to give a delayed precipitin-reaction with the rabbit antigen as well as with the fluid removed from the hydatid cyst itself. Furthermore, both antigens partially fixed alexin in the presence of the aforementioned serum. Finally, rabbits which proved to be infected with *Tænia pisiformis* all gave a skin reaction of the delayed type not only with the rabbit antigen but also with the human echinococcal cyst fluid. The positive reactions appeared in 24 to 48 hours and consisted of erythematous, indurated areas from 1 to 2 cm in diameter. Rabbits in whom autopsy failed to disclose the parasite invariably had negative skin tests.

Skin Tests in Known Cases of Hydatid Disease and in Normal Subjects: Four patients known to be suffering from hydatid disease were skin tested with both the rabbit and the human cyst antigens. Normal saline and rabbit serum diluted 1-10 were used as controls. Intradermal injections were made on the volar surfaces of both forearms, using 0.1 cc of each test material. All of the patients exhibited a prompt and vigorous reaction to both antigens, whereas the saline and rabbit serum controls were uniformly negative.

[3] Outeirino, J., *Ann de méd.*, 1935, **38**, 493.

The positive reactions were characterized by wheals which began to form in less than 5 minutes and reached their maximal size in from 15 to 30 minutes. The wheals varied from 2 to 4 cm in diameter, with large pseudopods and usually some itching. A surrounding zone of erythema from 5 to 10 cm in diameter was noted. Fading of the reactions was complete in about 12 hours although residual induration persisted for from 24 to 36 hours. The reaction to the rabbit antigen was invariably more marked than that with the echinococcal cyst fluid, probably due to the fact that the former represented a more concentrated antigen.

As a control group, 10 normal men and 10 normal women were also skin tested. In several of these individuals both antigens produced wheals up to 1 cm in diameter with some erythema, but no pseudopods or subjective itching appeared. These slight reactions faded promptly, leaving no induration.

It must be noted that skin reactions resulting from the intradermal injection of antigenic extracts obtained from members of the genus *Tænia* are fundamentally group-specific rather than species-specific. Therefore, in the diagnosis of hydatid disease by the methods described in this paper, a careful stool examination is essential in order to exclude positive skin reactions due to infection with intestinal cestodes such as *Tænia saginata.*

Summary. An extract of cysticerci of the common rabbit cestode, *Tænia pisiformis,* has been used as an antigen for skin testing in cases of echinococcal infection. An antigenic relationship between *Tænia pisiformis* and *Tænia echinococcus* is suggested by the precipitin-reaction, the alexin fixation, and the skin reaction in rabbits harboring *Tænia pisiformis.*

Four patients known to have hydatid disease gave immediate positive skin reactions with the rabbit antigen. Twenty normal subjects used as controls exhibited at most but slight reactions, and were considered negative.