part of the superior olive; the ventral group lies adjacent to the ventromedial part of the superior olive; the intermediate cell group lies between the dorsal and ventral cell groups and medial to the superior olive; while the dorsal cell group lies between the nucleus of VIth nerve medially and the superior olive laterally.

In normal material stained with Mallory's phosphotungstic haematoxylin, fibers can be seen coursing dorsally along the medial and lateral sides of the superior olive and then medially toward the mid-line. In all probability these represent fibers from the cells of the superior olive. It was impossible to trace these fibers with certainty to the vestibular nerve by this method. However, chromatolytic changes could be demonstrated in the superior olive of the opposite side following the auditory nerve section; therefore, it seems plausible to assume that fibers of the superior olive course with fibers of the vestibular nerve in the alligator exactly as Rasmussen has described in the cat.

Summary. 1. When the entire facial nerve of the alligator was transected intracranially, there were chromatolytic changes in the facial nucleus, but none in either superior olive. 2. When both the facial and auditory nerves were sectioned, chromatolytic changes were particularly noticeable in the contralateral superior olive. This indicates that the olivocochlear bundle which Rasmussen has described in the cat is also present in the alligator.

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A Factor in Serum of Human Beings and Animals that Destroys *T. vaginalis.* (24086)

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When a pure culture of *Trichomonas vaginalis* (Donne 1836) was injected into the anterior chamber of eyes of rabbits, the parasites disappeared rapidly and cultures of the aqueous humor became negative for parasites after 5 to 30 minutes(1). Since aqueous humor itself has been shown not to be trichomonicidal (unpublished), it was thought that the parasites were probably cleared from the anterior chamber through canals of Schlemm and eventually reached the blood stream. To learn more about the fate of the parasites in the blood stream, pure cultures of *T. vaginalis* (8-10 million motile organisms) were injected into the veins of 2 rabbits and cultures of the blood and various organs taken. Cultures for *T. vaginalis* taken within 10 to 15 minutes after inoculation showed that the bloods were sterile and that virtually all organs were sterile. Although the reticulo-endothelial system probably was active in ridding blood of many organisms, the possibility was considered that a large number of parasites were killed by the blood itself. Experiments demonstrated that human and animal serums have a remarkable ability to destroy *T. vaginalis* and that this lethal activity is lost when serum is heated at 56° C for 30 minutes.

Materials and methods. Organisms. Two strains of *T. vaginalis* isolated from 2 patients with vaginal trichomoniasis were employed; they reacted similarly in all studies. At first, 2-day cultures grown on thioglycolate horse serum medium with antibiotics (T.H.A.)(2) and containing about 2 million organisms/ml were used. In later experiments, cultures were

concentrated by centrifugation so that 1 ml contained approximately 4 million parasites.

Serums. A total of 54 fresh serums from unselected human beings and from various normal animals were tested for trichomonicidal activity. These included 18 human serums, and serums from 10 guinea pigs, 5 rats, one pool from 4 hamsters, 4 serums from 2 dogs, and 16 serums from 9 rabbits. Controls consisted of the same serums heated in water bath for 30 minutes at 56°C. *Importance of pH on trichomonicidal activity of serum.* Early in this work it became apparent that pH of a serum was an important factor in the tests to be described. pH of a serum rises when stored over-night at 4°C; simultaneously the disintegrating effect of serum for the parasite is reduced. To prevent loss of CO_2 with consequent rise in pH approximately 4 ml of blood were distributed into 10 x 1.2 ml tubes and the tubes immediately plugged with rubber corks. After remaining at room temperature 1 to 2 hours, they were placed in refrigerator at 4°C over-night. The clot was rimmed and the air over clotted blood was replaced by 5% CO_2 in nitrogen gas. The tubes were immediately closed with rubber corks and centrifuged at 1500 rpm for 20 minutes. The serums obtained from the clots were kept in ice water bath while being tested. Surplus serum was stored at 4°C after replacing air over serum with CO_2. In this fashion most of the disintegrating activity of serum for *T. vaginalis* was maintained after storage for 2 weeks at 4°C. The pH of mixtures of equal parts of culture and serum was approximately 7.2.

Results. Lethal effect of fresh serum on T. vaginalis. Fresh rabbit serum and the same serum heated to 56°C for 30 minutes were diluted 1:2 and 1:4 with thioglycolate medium (without horse serum and antibiotics added). To 1 ml of each tube, 0.05 ml of thioglycolate medium and 0.05 ml of the culture were added. After incubating all tubes in water bath at 37°C for 30 minutes or 1 hour, 0.5 ml or 1 ml of the contents of each tube was cultured in T.H.A. Cultures of 1:2 and 1:4 dilutions of fresh serum and the parasites were negative for *T. vaginalis,* whereas cultures of heated serums and parasites, and culture control were in every case positive within 24 hours. This experiment was repeated with serums from 2 other rabbits and one dog with similar results and demonstrated that fresh serum killed *T. vaginalis* while controls with heated serum had no such effect.

Morphologic changes in parasite when exposed to serum. Three variations of direct, warm stage, microscopic examination of mixtures of the parasite and serum were used. *First,* 2 drops of chilled serum and 2 drops of culture were mixed directly on a slide; *second,* equal volumes of culture and chilled serum were mixed in a tube and 4 drops of this mixture immediately transferred to a slide; *third,* equal volumes of cultures and chilled serum were mixed in a tube, incubated at 37°C in water bath and 4 drops transferred to a slide after intervals of 1 to 5 minutes or longer. Controls with "inactivated" serum were made. The 3 variations of technic gave essentially the same results with 50 different human and animal serums tested.

Microscopic examination of the mixtures of serum and cultures revealed that the parasites first lost their motility, then suddenly appeared indistinct and flattened out, forming floating shadows or ghosts. The shadows gradually disintegrated and in 5 minutes or less virtually disappeared, leaving only a few indistinct forms and granular debris on the slide. The progressive transformation from the motile organisms to complete dissolution appeared identical for all serums tested, but the time required for destruction of the parasites varied according to the species from which the serum was obtained. Thus, the parasite was usually completely lysed within 3 minutes in dog and rat serum whereas it usually required 4 or 5 minutes for the parasite to be lysed by human, rabbit, guinea pig, and pooled hamsters' serums. In general the potency of a serum appeared to be inversely proportional to the time required for disintegration of the parasite. These destructive effects were not observed in controls. In the preparations of serum that had been heated to 56°C for 30 minutes plus parasites, the organisms remained motile for 15 to 20 minutes, at which time the preparation began to dry and the parasites became distorted without forming shadows or ghosts. Several of the

heated serums agglutinated the organisms slightly but such agglutination did not interfere with motility of the parasite. The preparation of parasites diluted with thioglycolate medium gave results similar to preparations of heated serums and parasites except that agglutination did not occur.

Discussion. Nuttall(3) and Buchner(4) were the first to demonstrate that normal blood and serum were bactericidal for certain bacteria. Nuttall(3) observed that the bactericidal property of normal serum was destroyed by heating at 56°C for 30 minutes. For many years thereafter "complement," a term used by Ehrlich(5) for the heat labile substance together with natural antibody, was considered the only bactericidal substance in normal serum. Recently, however, Pillemer and his associates(6) have demonstrated in normal human serum and other serums a substance which they named properdin. In the presence of complement and Mg++, properdin participates in bactericidal, virus-neutralization and hemolytic activities. Properdin, like the factor in serum which destroys *T. vaginalis,* is also thermolabile. Feldman (7) has reported a toxoplasmal antibody activator in the serum-properdin system. Recently Borsos and Warren (Am. Soc. Tropical Med. 1957) described 2 factors in serum of adult chickens; the first was thermolabile and lysed *Trypanosoma cruzi,* the second was thermostabile and agglutinated this parasite.

This paper is limited to the demonstration that normal serum from human beings and various animals kills and disintegrates *T. vaginalis,* and that this ability is lost after heating at 56°C for 30 minutes. In current studies attempts are being made to determine the nature of the active substance and its effect on other parasites.

Summary. Human serums and serums from several species of animals have the capacity to kill and disintegrate *T. vaginalis* with great rapidity. This activity of serum is destroyed by exposure of the serum to 56°C for 30 minutes.

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Influence of Inorganic Ions on Fluoride Retention in the Rat.* (24087)

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Previous work has indicated that rats retain less fluoride in their carcass from a fluoride drinking water than from a chemically pure sodium fluoride solution when equal quantities of fluoride are given(1). These differences were thought to be due to mineral content of natural water, indicating that inorganic ions interfere with either fluoride absorption or skeletal retention. There is evidence for this assumption by Weddle and Muhler(2), and the theoretical studies of Feldman, Morken, and Hodge(3). The present study investigated several different inorganic ions, constituents of natural water supplies, for their influence on fluoride retention. Calcium, magnesium, iron, and phosphorus were chosen for investigation. These were evaluated for their ability to affect fluoride retention by comparing retention in the carcass of rats receiving fluoride from an aqueous solution containing the different inorganic ions with retention from a mineral-free water containing only fluoride.

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