in the central portion of the tumor. We have been able to demonstrate that three species of *Eusimulium* flies are concerned in the transmission of the disease. For the present and until careful comparison has been made with published descriptions and museum specimens these species have been designated as follows: Species A (probably *Eusimulium avidum,* Hoffmann), a small black fly some 2 mm in length; species B (probably *Eusimulium ochraceum,* Walker) about the same size or a little smaller with yellow thorax, the abdomen half yellow and half black and the legs mostly black; and species C (probably *Eusimulium mooseri,* Dampff), slightly larger than the others, with the thorax and most of the abdomen yellow and the legs very extensively yellow. All stages of development of *Onchocerca coecutiens* have been repeatedly observed in these flies, and the development has been traced from the time the fly just bites the infected individual and thus ingests the microfilariae from his skin, on through their passage and development in the thoracic muscles, head and proboscis. We have been fortunate in securing permanent specimens of the infective filarial form passing through and emerging from the labium of the proboscis. In this study, 4,572 flies have been dissected and examined microscopically. Other insects and particularly culicine mosquitoes are not concerned with the transmission of the disease.

The mosquito has a considerably longer proboscis than the *Eusimulium* fly and evidently inserts the proboscis deeply in sucking blood. The microfilariae of *Onchocerca,* which are not encountered naturally in the blood, but are found in the lymphatics of the skin, are not even ingested by the mosquito when it is fed on infected individuals. Possibly also the saliva of the mosquito repels the microfilariae of *Onchocerca coecutiens.*

In some of the cases in which the tumors have existed for long periods of time, disturbances of the eyes and loss of vision occur. We have been able to demonstrate microfilariae in such cases in sections of the peri-corneal conjunctiva, cornea and iris removed at operations or at autopsy. The continued presence and passage of the microfilariae through the lymphatics of the eye for long periods of time apparently give rise to an inflammatory condition and to a perivascular infiltration, in perhaps somewhat the same manner as occurs from the action of the trypanosome in the tissues of the central nervous system in sleeping sickness. In the course of time, peri-corneal, conjunctivitis, keratitis and iritis may result in such cases.

We have also investigated the blood and serum in the disease. Eosinophilia is present, and counts of eosinophiles of from 25 to 50 per cent. are usual. The serum of some of the cases of long standing gives a precipitin reaction with an aqueous or alcoholic extract of the tumors employed as antigen. However, a rabbit serum prepared by repeated intravenous inoculation of the animal with an extract of the tumors gave no such reaction.

In the clinical studies 1,383 individuals have been examined and the tumors removed in 261 cases. Some of the tumors have been hardened in Zenker's solution for histological study, while others have been used for the preparation of antigen and still others have been digested and dissected to obtain entire for study the adult male and female parasites.

The tumors (and the adult parasites within them) can be easily removed by operation under a local anesthetic, but in some cases the microfilariae continue to circulate in the body and are demonstrable in the skin and in some cases in the eye for at least several years. In order to rid the patient of the microfilariae which persist after removal of the tumors, experiments have been performed to discover a satisfactory filaricidal substance. Using a technique which gives a suspension in normal saline solution of an enormous number of motile microfilariae, it has been found that *in vitro* plasmoquinin in dilutions up to 1 to 10,000 effectively destroys the microfilaria. The motility of almost every parasite in the microscopical field ceases and they become apparently lifeless within thirty seconds; the several in the microscopical field which are not immediately killed no longer exhibit any lively movement (but may continue to bend more or less for 10 to 15 minutes) and are dead within about 20 minutes. Quinine in a solution of 1 to 5,000 produces somewhat similar results, but its lethal action is somewhat less marked while antimony compounds, neosalvarsan and mercurochrome are relatively ineffective. A serum prepared by repeatedly inoculating a rabbit with extracts of the adult filariae and embryos and with the tumors themselves has shown no filaricidal properties.

The members of the expedition, besides myself, are Dr. J. Bequaert, entomologist of the department of tropical medicine at Harvard University, Dr. M. Ochoa, parasitologist of the Board of Health of Guatemala, and Mr. B. Bennett, technician of the department of tropical medicine.

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INTRANUCLEAR INCLUSIONS IN LARYNGOTRACHEITIS OF CHICKENS

BEACH[1] has reported from this department the filtrability of the virus of laryngotracheitis in chickens. We wish in this place to record briefly the results of a histological study of spontaneous and

1 J. R. Beach, SCIENCE, 72: 633, 1930.

experimentally produced cases and the finding in both of intranuclear inclusions.

The lesions are restricted to the respiratory tract and occur first and are most pronounced in the larynx and trachea. At the outset the surface epithelial cells show various forms of cellular degeneration, but inflammatory processes of various degrees in the submucosa and other parts of the mucous membrane soon follow. The destructive process in later stages is due to the combined effect of the virus and mechanical factors produced by edema, cellular infiltration, hemorrhages, and in a few instances secondarily invading bacteria. A small number of animals show bronchitis and peribronchitis, pneumonic areas, and hemorrhages in the lungs, while involvement of the nasal passages, communicating sinuses and eyes seems to be dependent upon the point of entrance of the virus.

Certain intranuclear inclusions can be demonstrated in the epithelial cells lining the mucous membrane as well as in those of the mucous glands of the larynx and trachea in many cases. These inclusions consist of round, oval or irregularly shaped, sharply outlined, homogeneous, acidophilic masses. Usually a single inclusion occurs in a nucleus. The size may be small, but it is often so large as to occupy most of the central portion of the nucleus, which then may be considerably enlarged. The nucleoli of the affected cells are commonly attached to the nuclear membrane, and the space between the inclusion and the nuclear membrane remains entirely unstained. The inclusions resist the solvent action of acetic acid, alcohol and chloroform; they do not contain fat or iron, but give the Feulgen reaction for thymonucleic acid slightly. Silver impregnation shows small argentophilic granules inside the inclusions. The inclusions described as well as other changes in the nuclei bear a close resemblance to similar structures found in such virus diseases as herpes, varicella, virus III of rabbits and submaxillary gland disease of guinea pigs.

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IONIC EQUILIBRIA IN THE SERUM IN RELATION TO THE CRITICAL TEMPERATURE

IN our systematic researches on the critical temperature of blood serum (around 56° C.) we have so far purposely neglected the part played by the salts and we have been able to account for the observed phenomena by means of simple hypotheses which explained the facts satisfactorily without necessitating any assumption concerning ionic phenomena. However, we did not overlook this factor, neither did we underestimate its importance. We were only compelled to leave it aside in order to simplify arbitrarily a problem already so complex. We have now attempted to study this side of the question, and the purpose of this paper is to report a few preliminary results.

As it was necessary to respect as much as possible the integrity of the protein molecules, we only used distilled water as a means of disturbing the normal salt equilibrium of the serum. The first step was to study quantitatively the equilibrium: albumin-globulin, when progressive amounts of pure water were added to normal serum. The method used consisted in measuring the amount of light scattered at right angles of the incident light (more accurately, the value of $\log \frac{I_o}{I}$) as a function of the dilution. As the addition of water brings forth a precipitation of the globulins, the light scattered by the solution increases. It is known that the amount of light scattered is proportional to the number of particles (Lord Rayleigh), provided the latter are small with respect to the wave length of the light used and nearly spherical in shape. It was found that the addition of 1, and even 2 cc of water to 1 cc of serum did not determine much cloudiness, while if 3 cc were added the increase in the scattered light was very important. In other words, there is, in general, a sharp break in the curve around the point corresponding to a concentration of salts equal to 33 per cent. of the normal. If we assume that the value of the ratio $\frac{I_o}{I}$ is equal to 1 for pure serum, an addition of 200 per cent. of water will bring it to

TABLE I

LIGHT SCATTERED BY NORMAL HORSE SERUM, AFTER ADDITION OF DISTILLED WATER

Cc of water added to 1 cc of serum	Relat. concent. of salts, per cent.	Readings $= \log \frac{I}{I_o}$	
		After 1 hour	After 4 hours
Ser. undiluted	1	2.18	2.23
+ 1 cc water	0.50	2.12	2.14
2	0.33	2.06	2.08
3	0.25	1.54	1.50
4	0.20	1.30	1.30
5	0.168	1.17	1.21
6	0.143	1.15	1.16
7	0.125	1.12	1.14
8	0.115	1.12	1.14
9	0.100	1.13	1.15
10	0.091	1.15	1.17
11	0.083	1.17	1.19