In the first 6 months, 24 serial passages in rabbits and 22 in irregularly alternating rabbits and mice have been carried out by intracerebral or combined intracerebral and intraperitoneal injection of infected brain emulsion. Filtrates of emulsions passed through Berkefeld "N" filters proved non-infective. Of 148 rabbits inoculated, 131, and of 105 mice, all but 2, succumbed, the majority in from 5 to 9 days. Of 98 infant mice inoculated intracerebrally, 52 or four-fifths of the non-cannibalized succumbed, the majority in from 2 to 3 weeks. In most of the animals, symptoms did not appear until the last day of life. These included sluggishness, pareses, tremors, convulsions and respiratory difficulties. A rise in temperature beginning on the second or third day was noted in the rabbits.

In every instance, there was a severe disseminated encephalomyelitis marked by focal inflammation and necrosis. The exudate included lymphocytes, plasma cells, mononuclear leucocytes and fewer neutrophiles and eosinophiles. Granulomas like those in the infant's brain were often observed. Focal inflammatory lesions were less frequently encountered in the lungs, striated muscles, heart, spleen and liver. Parasites identical with those in the human case were found in large numbers in the lesions. Attempts to cultivate the Protozoan on a variety of media free of living cells failed.

In addition to rabbits and mice, chicks from 1 to 11 days old and guinea pigs were inoculated intracerebrally. These species proved susceptible as evidenced by the development of typical histologic lesions containing parasites. A rhesus monkey injected intracerebrally and subcutaneously remained well and its temperature continued normal. The susceptibility of this species to this strain of Toxoplasma is being investigated further.

Six rabbits which did not succumb to an initial inoculation were re-inoculated intracerebrally from 1 to 3 times within 3 weeks to 3 months and all proved to be immune. Eight control rabbits and 3 mice injected with the same material by the same route succumbed. Four mice which did not succumb to an initial inoculation were also re-inoculated intracerebrally and intraperitoneally within 2½ to 4½ months. All survived, while 2 rabbits and 11 mice used as controls succumbed.

That the microorganism isolated from the human case is a Toxoplasma is indicated by the following: (1) Its morphology corresponds to that of Toxoplasma of animal origin. (2) The course of the disease and the lesions produced in the animals inoculated with it are very similar to those noted in the same species by inoculation of a Toxoplasma of animal origin. (3) The susceptibility of the rabbit, mouse, guinea pig and chick to this Toxoplasma corresponds to the wide host range of Toxoplasma of animal origin. (4) Convincing evidence of the nature of the microorganism was obtained by cross-immunity experiments. Toxoplasma from a guinea pig passaged through mice was kindly furnished us by Sabin and Olitsky.[4] The 6 rabbits and 4 mice, noted above to be immune to the human strain of Toxoplasma, were re-inoculated respectively intracerebrally, and intracerebrally and intraperitoneally with the Sabin-Olitsky strain using infected mouse or rabbit brain emulsion. All 10 animals proved to be immune. Seven control rabbits and 6 control mice succumbed. Conversely, 2 rabbits immunized against the Sabin-Olitsky strain proved to be immune to the human strain, while two controls succumbed. Working with the same strains of Toxoplasma, Sabin and Olitsky, using other methods, have confirmed this cross-immunity and will report their results in the near future. The Protozoan found in the infant might be called *Toxoplasma hominis,* with the reservation that it may later prove to be identical with one or all of the animal strains.

Four other cases[1,2,5,6] (respectively from New York City, Chicago, Prague and Rio de Janeiro), very similar clinically and pathologically to the present case, have been shown by two of us to constitute a distinct disease entity marked by encephalomyelitis. In one of these reports,[6] the author mentioned focal inflammatory lesions in the heart, striated muscles and subcutaneous tissue as well. The lesions in each infant contained a parasite morphologically indistinguishable from that in the present case. There is little doubt then that they too were cases of toxoplasmic encephalomyelitis, although experimental evidence is lacking. That there may be other forms of human toxoplasmosis is very probable.

In conclusion, toxoplasmosis has been demonstrated in man. It has been shown to occur as a characteristic disease of young infants involving the central nervous system. The first experimental transmission of a human toxoplasmosis to animals is described.

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THE LOCALIZATION OF MINERALS IN ANIMAL TISSUES BY THE ELECTRON MICROSCOPE[1]

IN recent years there have appeared a number of articles on the electron microscope with reference to its use in biological investigations. Most of these papers

[4] A. B. Sabin and P. K. Olitsky, SCIENCE, 85: 336, 1937.
[5] J. Jankû, *Casopis lekarew ceskyck,* 62: 1021, 1923.
[6] C. M. Torres, *C. R. Soc. de Biol.,* 97: 1778, 1927.

[1] Aided by grants from the Rockefeller Foundation and the Josiah Macy, Jr., Foundation.

have been confined to the possibilities of securing tremendously high magnification. In these studies the absorption of parts of an electron beam by the substance traversed gave rise to profile pictures, as it were, of bacteria, colloidal suspensions and in several instances epidermal cells. Another use of this new tool was in the examination of surfaces, particularly of metals, by means of the pictures resulting from thermionic excitation of electrons at the metallic surface. It occurred to us, about four years ago, that this latter adaptation of the electron microscope would be particularly useful in localizing minerals in sections of animal tissues.

It has been appreciated for some time that gentle and careful ashing of sections of biological tissues gives a remarkably faithful picture of the topographic distribution of minerals in such materials. Cells and their parts can be recognized with little difficulty. If material is prepared by a modification of the Altmann-Gersh[2] frozen dehydration method there is little chance that there is any perceptible shift in the cellular location of the inorganic constituents. Since most of the inorganic elements in tissues, particularly Na, K, Ca and Mg, are excited to thermionic emission of electrons at more or less specific temperatures, we expected to be able to differentiate between these various elements and localize them in cells. With this information at hand it seemed advisable to ash sections *in vacuo* on the surface of a barium and strontium coated cathode in the electron microscope.

After many experiments, both with apparatus and method, we have been able to secure pictures in which cellular structure in striated muscle, gastric mucous membrane, nerve and in other tissues can be clearly made out. So far the emission pictures which have been obtained have been due to magnesium and calcium only. It has been possible to localize these elements definitely in the contraction bands of frozen and dehydrated muscle. Epithelial cells of the mucous membrane of the stomach and intestinal tract show extensive concentration of magnesium and calcium in the free borders of the cells.

These results are being published in detail elsewhere, and the experiments are being continued.

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EXPERIMENTAL PROLIFERATIVE ARTHRITIS IN MICE PRODUCED BY FILTRABLE, PLEUROPNEUMONIA-LIKE MICROORGANISMS

A PROGRESSIVE, proliferative polyarthritis bearing a clinical and pathological resemblance to human rheumatoid arthritis was produced experimentally in mice with a filtrable, pleuropneumonia-like microorganism which was recently isolated from the brain of a normal mouse.[1] Arthritis can be produced in practically 100 per cent. of mice when 0.5 cc of a 24-hour culture is injected intravenously or 1 cc intraperitoneally. Swelling of the joints may appear as early as 4 to 5 days. The arthritis is migratory, new joints becoming involved while others recede. Fusiform swellings of isolated digits, seen so often in human rheumatoid arthritis, occur frequently in these mice. The process is progressive and chronic in one or more joints, leading often to ankylosis, especially in the knees. By the method of "blind passage"[1] the microorganism has been cultivated from chronically affected joints as late as 70 days after intravenous inoculation. Tests revealed that the microorganism does not multiply in the brain, viscera, pleura or peritoneum. Excepting the arthritis, the mice appear in good health and not one of 150 with joint involvement has as yet died of the infection. Pathological changes are limited to the joints, and, as in the human disease, consist chiefly of proliferation in the synovial membrane, the capsule, the perichondrium of the articular cartilage, combined with a synchronous proliferation of the connective tissue and probably endosteum of the epiphyseal marrow directly below the joint cartilage. Intracutaneous, subcutaneous, intramuscular or intrathoracic injection or nasal instillation with or without ether anesthesia induced neither arthritis nor any local or systemic disease. Rabbits and guinea-pigs developed neither arthritis, fever or other signs of disease after inoculation with large amounts of culture.

A pleuropneumonia-like microorganism recently isolated from toxoplasma-infected mouse tissues[1] differs from the strain just described, in that it can also multiply in the brain as well as in the serous surfaces of the peritoneum, pleura and pericardium with the production of a characteristic exotoxin which has a special affinity for the cerebellum and can give rise to chronic choreiform signs when it does not prove fatal. When the rapid toxic death which follows intravenous injection of the culture was prevented either by the use of older mice (at least 2 months of age) or by injection of the centrifuged microorganisms, about 30 per cent. developed an arthritis similar to that produced by the other strain. The two strains are not serologically identical but possess a common antigen. Both strains have been found to pass through 500 mμ but not 396 mμ gradocol membranes (kindly supplied by Dr. J. H. Bauer), while on one occasion it was possible, by preliminary filtration through a 584 mμ membrane, to obtain a positive filtrate through a 322 mμ membrane; this suggests that the size of the smallest

[1] A. B. Sabin, SCIENCE, 88: 575, 1938.

[2] I. Gersh, *Anat. Rec.*, 53: 309, 1932.