reports and the other pages to reports of officers and business matters. Many of the papers report details of local plants, animals, insects and minerals, but a very large number are concerned with matters of general scientific interest, both theoretical and practical. The cost of publication, except for the years 1921–33, has been borne by state appropriation.

The academy had accumulated about 20,000 volumes of scientific magazines and books, largely by exchanges. Six years ago the academy allocated this library to three of the state schools. These institutions are cataloging and integrating the exchange journals into their libraries. By a system of mutual loaning, all books and periodicals are available to every one of the academy members from any one of the cooperating institutional libraries.

The annual meetings are held in odd years alternately at the State University at Lawrence and the State College at Manhattan and on even years at the various smaller colleges. The meetings are usually held a week after Easter and last from Thursday evening through Saturday afternoon. The evening meetings are given over to special lectures and the afternoons and parts of the forenoons to sectional meetings. The following sciences hold sectional meetings: Chemistry, physics, botany, zoology, entomology, psychology, medical sciences—bacteriology, physiology and anatomy and the Junior Academy.

In 1935, 150 papers were presented in the different sections and in 1936 meetings in a smaller college there were 120 papers. At the end of the 1936 session there were 430 members.

The Junior Academy has a number of very active clubs and holds an enthusiastic meeting on Friday afternoon. The organization of new clubs is being actively pushed by the academy. A special committee is handling this phase of the work.

A special committee has been appointed to study out the best plan of encouraging research work by the use of the research award of the American Association for the Advancement of Science. They are also finding ways and means to add to the amount of the award.

The committee on conservation of plants and animals is setting aside natural preserves in various parts of the state. It already has some "state parks" to its credit.

Another committee is working out a plan by which all the scientists of the state shall have some means of cooperating for the welfare of the state. It is hoped to create some kind of a super-council, a Kansas Association for the Advancement of Science, to include: The Kansas Engineering Society, the Kansas Horticultural Society, the Kansas Medical Association, the Kansas Dental Association, the Kansas Home Economics Association, the Kansas Mathematical Society (partly concerned with teaching), the Kansas Geological Society (partly commercial) and any others who are carrying on research or investigations. The Kansas delegate to the American Association for the Advancement of Science meeting is anxious to receive any and all suggestions for getting more cooperation among state scientists. It is believed that if the scientists of a state act as an aggregate they could add much to the efficiency of state governments and that they could often be of great benefit to each other and their respective societies.

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*Delegate from Kansas Academy of Science*

# SPECIAL ARTICLES

## TOXOPLASMA AND OBLIGATE INTRACELLULAR PARASITISM

TOXOPLASMA have been described as the causative agents of various pathologic conditions in birds and mammals, including man, in various parts of the world, but hitherto almost unnoticed in North America. The accidental isolation of toxoplasma early in 1935[1] in the course of experimental work with viruses has led to a study of these parasites by methods and procedures commonly used in virus work. The purpose of this communication is to call attention to some of the more striking results which were thus obtained, particularly their apparent obligate intracellular parasitism, and to indicate that as a result of this property these highly organized parasites (about $6–7 \times 3–4\ \mu$) have many features in common with certain ultramicroscopic viruses. Many of the problems encountered in the study of virus diseases, such as cultivation, pathogenesis, immunity, etc., are, to a great extent, influenced by the obligate intracellular "parasitism" which is an outstanding characteristic of the filtrable viruses.

In the course of tests for virus in guinea pig brains, two mice, injected intracerebrally, exhibited signs of encephalomyelitis nine days later, which proved not to be due to the virus under investigation. The disease was readily transmissible in series by intracerebral injection[2] of mice and was shown by extensive subsequent studies to be caused by a parasite which, in

[1] Toxoplasma were observed in guinea pigs in Mexico (H. Mooser, *J. Inf. Dis.*, 44: 186, 1929) and in birds of the Syracuse, N. Y., region, also in English sparrows kept in the laboratory and in canaries (R. D. Manwell and C. Herman, *J. Parasitol.*, 21: 415, 1935).

[2] All such operations were done with the aid of ether anesthesia.

morphology and in the wide range of hosts for which it proved pathogenic, corresponded closely to the as yet ill-defined group of Protozoa called *Toxoplasma*. Numerous investigations extending over the past two years have been made possible by proper preservation of the parasite, which, after many trials, consisted of storing an infected mouse brain in Tyrode's solution in the refrigerator for 14 days and then passaging it by intracerebral injection of mice. Fifty brain-to-brain passages have now been accomplished with the strain obtained from one of the original mice, and thirty-six with that from the other.

Our studies on the biology of these strains revealed that multiplication was possible only within living cells. The process consisted of penetration of the susceptible cell by the semilunar or piriform-shaped parasite and subsequent division by longitudinal fission. In the first two mouse passages after isolation both strains multiplied until they filled and distended the cells, leading usually to the ultimate extrusion of the nucleus and leaving structures consisting sometimes of hundreds of the parasites surrounded by the cell membrane and referred to by some as "cysts" or "pseudocysts." With the third intracerebral passage a spontaneous change occurred in one of the strains, whereby the invaded cells disintegrated when the total number of parasites in them was still quite small. This change was accompanied by a marked increase in pathogenicity that did not occur with the second strain until after about the twenty-fifth passage. In keeping with this observed intracellular parasitism, it was not surprising that they failed to multiply in media which did not contain living, susceptible tissue. Defibrinated blood did not support their growth. They could be cultivated, however, when minced chick embryo suspended in Tyrode's solution (the Li-Rivers medium, in which many viruses have been cultivated) was used. In this medium two series of six successful subcultures were carried out without loss of pathogenicity. The multiplication was found to occur within the cells. Concentrated, cell-free, Tyrode's solution extracts of chick embryo, inoculated for control, contained no living parasites even in the first culture, 4 days after incubation at 37° C.

The pathogenicity of our strains was studied in a number of different hosts. They produced fatal infections in mice, guinea pigs, rabbits and newly hatched and full-grown chickens; in *rhesus* monkeys a non-fatal disease was induced. Mice succumbed to infection when the parasites were given directly into the brain, into the peritoneal cavity, under the skin and even after instillation without trauma by way of the nose or mouth. With the increased pathogenicity acquired by passage, intracerebral injection in mice caused death within three to five days. The brain was always involved in mice, regardless of the route of inoculation, but pathological studies revealed that the distribution of the lesions was characteristically different after intracerebral and after peripheral inoculation. In the former instance the parasites were distributed by way of the cerebrospinal fluid and the lesions were situated chiefly periventricularly, at the base of the brain, and dorsally about the midbrain, while after peripheral inoculation the lesions were in the cerebral blood vessels and in the nerve cells surrounding them. The growth of the toxoplasma through the vessels, parasitizing cells of almost all the coats, and the subsequent perivascular nerve cell involvement were clearly apparent. Next to the brain the lungs were most constantly affected, while vascular organs, such as the kidney and liver, rarely showed any appreciable lesions, even though the parasites could be shown by animal inoculation to be present in the blood and all the organs.

It is not known how toxoplasmic infection is transmitted in nature. While an insect vector has been suspected, none has been identified or demonstrated. Our own experiments on contact infection among mice were entirely negative, until small numbers of starved animals were allowed to feed on others recently dead of the experimental disease. This suggests that at least one method of natural dissemination may be by means of the eating of toxoplasma-contaminated tissues.

Intracerebral inoculation in guinea pigs and rabbits led to fatal infection within four to six days, but the attack was chiefly on the meninges (dorsal as well as ventral). The distribution of nerve-cell lesions was directly beneath the meninges and almost not at all along the ventricles. Intracutaneous injection of the unchanged strain (*i.e.*, before the capacity of early disintegration of the parasitized cell was acquired) induced local skin lesions associated with systemic disease, but the rabbits always survived. After the spontaneous change occurred, more marked hemorrhagic and necrotic skin lesions ensued associated with systemic disease which invariably led to death of the animal within 8 to 12 days. A study of the pathogenesis of the latter disease revealed that the parasites were distributed to all the organs by way of the blood, but here again variations in the relative vulnerability of the viscera were apparent. As contrasted with the mouse, the rabbit brain showed little or no change, while the liver, spleen, adrenals, intestines and lungs were the seat of multiple focal lesions which in the gross, and even under the low power of the microscope, were almost indistinguishable in appearance and localization from the lesions of generalized vaccinia and certain other viruses. Under greater magnification one could see that the focal necrotic lesions were

due (1) to a growth through the vessels, *i.e.*, intracytoplasmic multiplication of the parasites and ultimate distintegration of the cells constituting their walls, with secondary involvement of the parenchymal cells by the parasites, and (2) to thrombosis of involved blood vessels with secondary necrosis of parenchymal cells without preliminary parasitization. The kidneys were again, strangely enough, only irregularly affected, and even then only the interstitial vessels seemed to suffer while the glomeruli and tubules appeared almost uninvolved. The destruction of cells either directly by the parasites or indirectly by thrombosis of blood vessels whose walls were parasitized comprised the outstanding pathological changes, while inflammation was either absent or, in certain instances, a late manifestation.

Intracerebral inoculation in *rhesus* monkeys was followed only by a febrile disease. Intracutaneous injection gave rise to a local lesion associated, as after other forms of peripheral inoculation, with systemic disease and the presence of the parasites in the circulating blood, as demonstrated by mouse inoculation.

The toxoplasma also appear to offer an opportunity for direct investigation of certain as yet obscure problems in immunity of obligate intracellular parasites. It has been possible to show, for example, that *rhesus* monkeys recovering from an infection with toxoplasma are immune to reinoculation and that the serum of such monkeys contains antibodies which may be termed "neutralizing" or "protective." The "neutralization" or "protection" tests were performed in the same manner as with viruses, *i.e.*, by mixing *in vitro* the serum with a tissue suspension or exudate containing the parasites and injecting the mixture intracerebrally or intraperitoneally in mice or intracutaneously in rabbits. The latter proved to be the method of choice, since the parasite suspension as well as a number of different sera could all be titrated quantitatively on the back of one rabbit. It was interesting, however, that rabbits which recovered from the non-fatal disease induced by the intracutaneous injection of the "unchanged" toxoplasma developed a solid tissue immunity resisting the constantly fatal intracerebral injection of the same strain, as well as inoculations with the highly pathogenic and fatal changed strain, but, as a rule, had no demonstrable protective humoral antibodies. In some rabbits only sufficient antibody to protect against a single skin infective dose was present. Similar observations are not uncommon with certain viruses.

Preliminary studies on the protective antibody in convalescent monkey sera revealed that it apparently had no effect on the toxoplasma *in vitro*. No agglutination or disintegration of the parasites could be observed in mixtures which proved innocuous on animal inoculation. Centrifugation of such mixtures after incubation for several hours *in vitro* and separation of the parasites from the serum showed that they had retained their infectivity. Further studies now in progress on the nature of this protective antibody, as well as the solid tissue immunity unassociated with such antibodies, are expected to yield data of interest to the understanding of similar phenomena with other obligate intracellular parasites.

The rabbit skin protection test may, perhaps, also prove useful in the diagnosis of infection with toxoplasma. The present evidence that they cause disease in man is rather tenuous and has been questioned by many competent parasitologists. The reason for this uncertainty is that the diagnosis has been based either entirely on morphological grounds without tests for pathogenicity or only on animal inoculation. In a recent study[3] on glandular fever (infectious mononucleosis) doubt arose as to whether the toxoplasma which were isolated were derived from the patients' blood or from the experimental animals (rabbits) which might have been spontaneously infected. The protection test just described might aid in elucidating this problem.

The work just outlined will be described in detail in a future communication. The aim of the present report is primarily to call attention to the existence of toxoplasma in North America and to point out their obligate intracellular parasitism, a study of which reveals many features in common with certain of the filtrable viruses, particularly as regards pathogenesis, cultivation, immunity and other host-parasite relationships.

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## HEPARIN AND THE FORMATION OF WHITE THROMBI

IN a recent preliminary communication Murray, Jaques, Perrett and Best[1] reported that the incidence of thrombus formation after mechanical or chemical injury to veins was appreciably decreased when a solution of purified heparin was administered to the dogs before and for adequate periods after the injury. In animals which did not receive heparin the injury was followed by the appearance of typical thrombi. Areas exhibiting the structure of thrombi could be observed in many of the histological sections made through the obstructing mass. Since the veins from the heparinized animals were in many cases free of obstruction,

[3] J. O. W. Bland, *Lancet*, 2: 521, 1930; *Brit. Jour. Exp. Path.*, 12: 311, 1931.

[1] D. W. G. Murray, L. B. Jaques, T. S. Perrett and C. H. Best, *Can. Med. Assoc. Jour.*, 35: 621, 1936.