ENCEPHALITOZOOON CUNICULI AS A KIDNEY PARASITE IN THE RABBIT.

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PLATES 1 TO 3.

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The parasite to be described is already well known since it has figured largely in the controversial literature on experimental encephalitis in rabbits. In this Department it has been under more or less desultory observation since 1918 when it was first seen by Ten-Broeck in sections of the kidneys of rabbits used for experimental purposes. The earliest reference to it in print is that of Wright and Craighead (1) who in 1922 found it in the central nervous system, the kidneys, and urine of rabbits suffering from motor paralysis. They believed it represented "probably an intermediate stage in the life history of some protozoan parasite." In 1923 Levaditi, Nicolau, and Schoen (2,3) found it in the central nervous system of rabbits used by them in a comparative study of the virus of encephalitis obtained from four different sources, and somewhat later (4-6) in the kidneys of three rabbits inoculated with virus from a spontaneous case. Doerr and Zdansky (7, 8) also found a protozoan-like parasite in the brains of 8 out of 224 rabbits examined. They hesitated to express themselves as to its relation with encephalitis, but thought it identical with the parasite which they had seen in the original preparations of Kling (9). Recently Oliver (10) has restudied the brain sections of rabbits described in an earlier paper (11) and has found a parasite which he believes to be the same as that of Wright and Craighead and of Levaditi and his associates. Still more recently Goodpasture (12) has described this parasite in the brain and kidneys of rabbits suffering from spontaneous encephalitis.
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The rabbits used in this study belonged to the stock raised in the animal houses of this Department. Since October, 1922, in a total of 163 autopsies 45 cases have been found. 37 of these rabbits were found dead and the remaining 8 were sick when killed. The disease appears to be more or less endemic with an increase in the number of cases in the early summer. During December of 1923 and the following January there was a noticeable increase in cases over the preceding year. A study of the breeding records has shown that, as a rule, all the individuals of a litter are not invaded to the extent of showing symptoms, and only once was the parasite found in fatal cases from two successive litters from the same doe.

No characteristic symptoms indicating the presence of the parasite have been observed, but the animals have all been small and weakly. The parasite has been found in young rabbits only. The majority were from 4 to 6 weeks old; the youngest, 15 days, and the oldest, 3 months. In 49 per cent of our cases the parasitism was associated with a demonstrable coccidiosis of varying severity, and in three cases with salivation or "slobber.s."

When the material collected was passed in review in the preparation of the manuscript it seemed too meager to present. The recent review by Da Fano (13) of the entire literature bearing on experimental encephalitis in rabbits and the protozoon associated with it made it seem desirable to publish at this time the accumulated observations, since they and the inferences drawn may stimulate those having the proper material to solve some of the problems bearing not only on the parasite but on both brain and kidney lesions found in rabbits.

The Parasite.

The kidneys of the invaded rabbit are as a rule though not invariably enlarged symmetrically. The surface is still smooth. All fat has disappeared and the tissue has a slightly congested and edematous appearance. Rabbits of the same stock when over 6 weeks old frequently had the surface of both kidneys uniformly beset with minute depressions of a bluish black color. When the cut surface of the freshly removed kidney is gently scraped, the parasites may be readily detected. Often portions of tubules are in the scrapings in which
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The parasites are still within the host cells. They appear as either pale or more highly refractive bodies of rod-like outline. The ends are rounded. In the fresh condition they are 2.5 to 3 μ long and 1.5 to 2 μ broad.

The parasite may be stained in films prepared by scraping the cut surface. It is to a certain degree Gram-positive (Fig. 1), but not acid-fast. By Goodpasture's method (14) it is stained bright red (Fig. 2). It is also readily stained with eosin and methylene blue. The most prominent form presented by the parasite in fixed and stained material was that of a blunt rod-like body with rounded ends, occurring either in loose or dense groups within epithelial cells, or else singly in the debris collecting within the affected tubules (Fig. 3). In sections stained with eosin and methylene blue it was deeply stained and sharply outlined. Whenever a clear view of an individual was obtainable it showed a narrow, transverse, deeply stained mid-piece, holding together two coccus-like end-pieces. These were feebly stained except in the periphery. It is thus roughly in form of a figure of eight. This form, which for convenience may be considered the ripe or spore form, gives way in some kidneys to shorter forms, rather feebly stained, roundish or oval, without the characteristic mid-piece and with what may be called the chromatin slightly accentuated at one end. These are also found in groups within vacuoles of epithelial cells.

The habitat of the kidney parasite is the epithelial cell. In our cases the collecting tubules near the tip of the papilla and even the epithelium covering the papilla were most frequently invaded. Next came the loops of Henle. Rarely the parasite appeared in the convoluted tubules; in one case in an epithelial cell of Bowman's capsule. The number of rod-like bodies in an epithelial cell varies considerably. They usually appear in a cell vacuole and the cell may not be enlarged or it may be so distended and distorted as to fill the lumen of the tubule. The number may thus vary from 5 or 6 to 50 or more in a section which may not include the entire cell (Fig. 3). The cell membrane ruptures after a time and the rod-like bodies are thrown into the tubule. Here they may be seen scattered through the substance of casts or molds of a more or less homogeneous, hyaline appearance. The outward passage of the rod-like bodies has been demonstrated in
living rabbits by Wright and Craighead and by Levaditi and his associates. In a few cases we have found the parasite in stained films of the sediment of urine collected at autopsy and centrifuged.

If the forms described were the only ones in the life cycle of this organism one might be tempted to regard it as a bacterium so far non-cultivable. In favor of this view is the variable number of individuals within host cells, suggesting an indefinite multiplication by fission. Moreover we now know of one bacterium, *Bacillus abortus*, which though non-motile multiplies within the epithelial cells of the bovine chorion and greatly distends them. There are, however, indications that the spore-like bodies in the kidneys arise in a different way. In a rabbit about 15 days old in which the kidneys were quite heavily invaded, some of the epithelial cells contained each a roundish mass of granular cytoplasm with a limiting membrane. The cytoplasm was permeated with minute deeply staining bodies. These "pansporoblasts" measured 12.6 to 14.4μ by 16.2 to 19.8μ (Fig. 4).

These same bodies are probably genetically related to certain forms found in the lumina of the loops of Henle and more rarely in convoluted tubules. They were roundish balls with a homogeneous, slightly stained stroma, within which were regularly distributed stained bodies permeating the entire mass, rather vaguely outlined, and in general resembling undeveloped forms of the spores already described (Fig. 5). These balls were on the average 10 to 12μ in diameter, some much larger, some smaller. They were either free of chromatin, indicative of remains of a nucleus, or else some clumps of chromatin were attached to the periphery. In some tubules they were numerous enough to fill and dilate them. These balls are evidently pansporoblasts prematurely set free by disintegration of the host cell. These bodies are probably the same as those described by Veratti and Sala (15) and by Da Fano (16) in the brains of rabbits.

**The Parasite Cycle.**

From what we know at present we may assume that the stainable, rod-like bodies in the kidneys are spores, in virtue of their refringence and non-mobility. Leaving the body of the host in the urine, they are ingested by the young rabbit and after undergoing certain changes
they actively penetrate the mucosa of the intestinal tract and are carried to different parts of the body. Only those reaching the kidneys are in a position to survive. It has already been stated that the same parasite has been found by a number of investigators in the central nervous system, and by Goodpasture in the lungs. More recently Cowdry and Nicholson (17) have found it in the central nervous system of mice. The writers have so far failed to find it in organs other than the kidneys and the brain but the search has not been at all thorough. There is more or less parallelism between the parasite under discussion and the coccidium inhabiting the mouse’s kidney, Kloissiella muris.1 This sporozoan has not been found in organs other than the kidneys. It develops the gametes, oocysts, and spores in the convoluted tubules and the spores pass out in the urine. The hypothesis suggested places the organism definitely as a kidney parasite and thereby classes the parasites found in the brain as aberrant and of no significance in the life cycle. This simple cycle may be greatly modified and extended should an intermediate host be found for the sexual cycle.

Concerning that part of the cycle which is passed in the rabbit’s kidney—probably the vegetative or multiplicative phase—there are certain observations which make probable several asexual generations of the parasite while in the kidney. In some rabbits the heavy invasion of the epithelium of certain tubules, involving every cell of the portion to be seen in the section, indicates that the spore entering the kidney by way of the circulation may have multiplied in the cortical tubules and after discharge the progeny may have entered and multiplied in the tubules farther down. In this way two or even three generations could be cared for, the last invading the cells of the mouths of the collecting tubules. This interpretation harmonizes with the positive results obtained after serial inoculations by several workers with encephalitic or herpetic virus. The evidence is, to be sure, weak for all the rabbits used may have been spontaneously affected, although this supposition itself can scarcely be regarded as true for all the cases reported. The multiplication of the vegetative

1 A closely related coccidium was found by Pianese, in 1901, in the kidneys of guinea pigs. In 1914, it was rediscovered by Seidelin, and again in 1916 by Pettit in France and Louise Pearce in America.
phase is paralleled by the frequently observed serial passages of malarial parasites after the injection of blood.

A further phase of the problem which remains under debate for the time being is the actual host relation of the described parasite. Is it a true parasite of the rabbit, one whose existence is not possible in the absence of the rabbit, or is it an aberrant form entering the mammal from an insect host and finding accidentally a favorable nidus? In the latter case the rabbit is not a necessary host and the stages passed in the rabbit are redundant asexual phases. This aspect of the problem is supported by the successful development of the parasite in different tissues of the rabbit, in the mouse (17), and even in a dog (18). On the other hand the wide geographical distribution of the parasite, both in Europe and this country, favors the view that we are dealing with an old genuine parasite of the rabbit. Whatever the status of the parasite may prove to be it is, for a protozoon, remarkably cosmopolitan, both as regards host species and tissues.

Pathology.

The injury inflicted by any protozoan parasite depends upon the extent to which it multiplies asexually in the host and this in turn on the number of introduced spores. A few epithelial cells destroyed are easily replaced as the mitoses frequently found in the kidney indicate. If, however, a heavy invasion takes place within a short period followed by an active asexual multiplication, the simultaneous destruction of many host cells may lead to serious lesions.

In the affected kidney the lesions manifestly referable to the parasite are an interference with the normal excretory functions by a plugging (a) of the limbs of Henle's loop and (b) of the branches of collecting tubules. In the loops, a single parasitized cell when expanded by many spores fills the lumen entirely. The breaking down of such cells and the accumulation of the roundish balls of immature sporoblasts described above in the lumina produce plugs which lead to fusiform distension of the tubules and evident stagnation of the contents (Fig. 5). In later stages the tubule is buried in a dense mass of cells not polynuclear, and probably largely lymphocytic, which are in various stages of disintegration. These cell masses represent zones
around the former tubule as well as dense masses which have entered the lumina. The cause for this cellular reaction may be the setting free of body-foreign protein in the degenerating, partly developed parasites enclosed in the pansporoblasts prematurely shed, or it may be due to the loss of the epithelial lining.

A natural sequel of these conditions is an interference with the normal outflow of urine from the convoluted tubules and the descending limbs of Henle's loops. Dilatation of these tubes is a common condition of the involved kidneys, especially in the later stages of the parasitism. In some cases this distension, frequently associated with hyaline casts, is very conspicuous both as to the abnormal diameter of the tubules and the areas involved.

There are other lesions frequently met with in the animals that came under observation, notably in the cortex. These consisted of areas of lymphoid cell infiltration probably interstitial in origin. Since the young rabbit may pass through several diseases, such lesions cannot be referred to the protozoan parasite without further proof. One of the diseases in the flock studied was coccidiosis. The denudation of the intestinal villi due to the invasion of several crops of merozoites opens the circulation to individual merozoites which, as is well known, enter the portal circulation and later set up a coccidiosis of the intrahepatic bile ducts. Merozoites may be carried to other organs of the body where their fate is unknown. Macrogametes have been found in the rabbit's brain tissue. It is our opinion that the numerous bluish black pits seen on the surface of the adult rabbit's kidney may be the result of emboli due to the invasion of the circulation by stages of coccidia from the intestinal tract, which, however, do not develop farther. These pits are the bases of interstitial lesions of fan-shaped outline, in which more or less dilated tubules are embedded in a proliferated interstitial tissue.

The above statements are supported by conditions found in the brains of our rabbits. Here throughout the pia, the cerebral cortex, the basal ganglia, cerebellum, and medulla were found small collections of cells, probably of lymphocyte type. These were either directly associated with a capillary which they sheathed and in some cases filled and thrombosed, or else they were not so associated. Some of the latter if traced in serial sections might perhaps have fallen into
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the first group. These lesions it seems to the writers are not due to the kidney parasite for this is rarely present in them. On the other hand this parasite may be found within nerve cells which are filled and distended with the spore-like bodies and without a trace of reaction about them (Fig. 6). The spores may fill several adjacent cells (Fig. 7) or else break away when the latter are overdistended, for one of the groups of spores in the cortex measured 48 μ in diameter (Fig. 8). Such a group would contain at least several thousand spores.

This total absence of host reaction about the infected cells corresponds with conditions in the kidney and is characteristic of many protozoan invasions. It supports the contention that the focal cell accumulations are not due to them and may perhaps be accounted for by a dissemination of the trophozoites and other stages of coccidia. Cowdry and Nicholson (19) likewise consider the possibility that the lymphocytic foci may arise in more than one way.

Goodpasture (12) refers to the presence of the parasite in the lesions described. This association may be a coincidence or it may be due to the fact that bodies in the circulation would be strained out more readily when there are vascular lesions in existence. Complete demonstration of one or the other view cannot be brought with rabbits infected with both parasites.

The general effect upon the rabbit of a large invasion of kidney parasites would be to reduce permanently the effective secreting tissue. In the rabbit population studied adult breeders dying from time to time were autopsied, and chronic nephritis associated with hypertrophy of the heart demonstrated in several cases. In one, decompensation had caused extensive changes in the liver and effusion into the large serous cavities. The literature dealing with spontaneous nephritis in rabbits is now fairly voluminous. This has been gone over with some care with the hope of perhaps harmonizing some of the conflicting data and viewpoints by tracing lesions from those of the kidney parasite as a starting point. This has not been successful and must be left to future observers who will take into consideration the effects of all infectious diseases of young rabbits. Such effects are still to be unraveled and this can only be done safely by studying at different ages populations which carry only one of the several infections.
Of the many papers on this subject only one is cited as bearing on the early incidence of the disease. Twort and Archer (20) found 10 to 20 per cent of certain stocks of rabbits affected with a spontaneous encephalomyelitis and a spontaneous nephritis. They assume that both conditions may possibly be due to a single virus "almost certainly a filter passer." The disease was more easily transmitted by cage infection than by artificial inoculation. It is highly probable that the writers had the protozoan nephritis in their group. The reason that the parasite has been so often overlooked is due to the fact that the invasion is one of the early weeks of life and when the nephritis comes under observation the parasites have been discharged. In the central nervous system, however, such discharge is impossible and the parasites persist intact since a cellular reaction is absent, at least from many invaded areas.

In our group, exposure to the parasite began evidently at birth and may be called a nest infection. This is also true of coccidiosis. Just what would happen to a population which had never been subject to this disease if exposed when 2 months old or even older is well worth study on the part of those who happen to have the necessary conditions at hand. The early invasion may perhaps explain Goodpasture's experiment, who placed two young white and two young gray rabbits with an infected mother. After 2 weeks the white rabbits became ill, the gray rabbits remained well. The obvious inference to be drawn is that the white were already infected when exposed.

The extensive discussion of this subject by Levaditi (21) and his associates did not come into our hands until after the completion of this manuscript. Among the new features of the parasite described is the pansporoblast in the inoculated rat. The facts presented do not lead the writers to modify any views already expressed.

SUMMARY.

A spontaneous epidemic of nephritis among young rabbits associated with a protozoan parasite has been observed in a certain breeding stock since 1918. It is regarded as a nest infection. The parasite is tentatively classed among the microsporidia and the stages encountered are regarded as vegetative which may perhaps pass through several generations in the same host. It is a parasite of
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the epithelial cell, provoking no immediate host reactions. These are supposed to follow injury such as destruction of the epithelium and denudation and plugging of the tubules. The localizations in the brain are also without cell reaction, except under special conditions, and the many cell foci present are attributed to coccidia and perhaps bacteria and other intestinal parasites. The kidneys are looked upon as the normal habitat and the brain parasites as aberrant and outside the normal cycle unless it can be shown that the spores discharged into the circulation may again multiply in some organ like the kidneys in direct communication with the exterior.

It is highly probable that many reported cases of nephritis among older rabbits used in experiments had their origin in an early invasion of the parasite described.

BIBLIOGRAPHY.

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EXPLANATION OF PLATES.

PLATE 1.

Fig. 1. Film of kidney tissue of a rabbit. Parasite in an epithelial cell. Gram stain. \(\times 1,000\).
Fig. 2. Film of kidney tissue of the same rabbit. Parasites escaped from a ruptured cell. Goodpasture's stain. \(\times 1,000\).
Fig. 3. Tangential section through the kidney of a rabbit. Parasite in epithelial cells of collecting tubules and of Henle's loop.

PLATE 2.

Fig. 4. Transverse section through kidney of a young rabbit. "Pansporoblasts" in epithelial cells of collecting tubules. \(\times 840\).
Fig. 5. Longitudinal section through kidney of a rabbit. Henle's loops distended and blocked by "pansporoblasts." In the upper right hand corner the epithelial cells of the loop still \textit{in situ} are involved. \(\times 750\).

PLATE 3.

Fig. 6. Transverse section through the cerebral cortex of a rabbit. Two groups of spores, \(a\) and \(b\). \(\times 78\).
Fig. 7. \(a\) of Fig. 6. \(\times 750\).
Fig. 8. \(b\) of Fig. 6. \(\times 750\).
(Smith and Florence: *Encephalitozoon cuniculi*)
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