

UNCLASSIFIED

AD NUMBER
AD837450
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; 1924. Other requests shall be referred to Department of the Army, Fort Detrick, MD.
AUTHORITY
SMUFD D/A ltr, 14 Feb 1972

THIS PAGE IS UNCLASSIFIED

AD837450
AD837450

TRANSLATION NO. 1442

DATE: 19/24

DDC AVAILABILITY NOTICE

Reproduction of this publication in whole or in part is prohibited. However, DDC is authorized to reproduce the publication for United States Government purposes.

STATEMENT #2 UNCLASSIFIED

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Dept. of Army, Fort Detrick, ATTN: Technical Release Branch/TID, Frederick, Maryland 21701

AUG 14 1968

Best Available Copy

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

ANNALS

of

THE PASTEUR INSTITUTE
38(8): 651-712, 1924

ETIOLOGY

OF EPIZOOTIC ENCEPHALITIS OF THE RABBIT,
IN ITS RELATIONSHIPS WITH THE EXPERIMENTAL STUDY
OF LETHARGIC ENCEPHALITIS
ENCEPHALITIZOAN CUNICULI (nov. spec.)

by C. LEVADITI, S. NICOLAU and Miss R. SCHOEN.
(Pasteur Institute.)

(With plates III and IV.)

CHAPTER I

HISTORIC GENERALITIES

The etiological problem of lethargic encephalitis seemed definitely resolved, thanks to the experimental researches of Strauss, Hirshfeld and Loewe, Mc Intosh and Turnbull, Levaditi, Harvier and Nicolau, Doerr and Schnabel, Berger, etc. (1), when, in 1922, Kling and his collaborators, Davide and Liljenquist (2), brought to light facts which, at first glance, seemed to undermine the earlier formulated conclusions. One knows that, according to Levaditi, Harvier and Nicolau, confirmed by Doerr, Schnabel and Berger, lethargic encephalitis, transmissible to the rabbit, to the guinea pig, to the mouse, and, sometimes, to catarrhinian monkeys, is due to a filterable virus, which, inoculated by cerebral method, kills the

(1) Cf. for the literature, C. LEVADITI, Ectodermoses neurotropes, 1922, Paris, Masson.

(2) KLING, DAVIDE AND LILJENQUIST. The works of these authors, published, for the most part, in the C. R. of the Biology Society are found united in the Communications of the Bacteriological Laboratory of the Swedish State, 7, 1923.

animal in five to eight days, with clinical signs and microscopic alterations of acute encephalitis. Further more, this virus, deposited on the scarified cornea of the rabbit, provokes a kerato-conjunctivitis, source sooner or later of manifestations of mortal neuraxitis (Levaditi and Harvier). Researches of crossed immunity, incited by the comparison between encephalitis and experimental herpes [Blanc (1)], and realized by Doerr and Schnabel, as well as by Levaditi, Harvier and Nicolau, had, in addition, demonstrated that the encephalic virus belongs to the same group as the herpetic germ, of which it is only, in the last analysis, a variety with eminently acute neurotropic affinities.

Now, Kling and his collaborators, on the occasion of a severe encephalic epidemic in Iapon (Sweden), undertook experiments in order to verify the statements of Levaditi, Doerr and Schnabel, etc... These experiments first of all assumed a confirmative nature. But, afterwards, the problem changed its nature. The Swedish authors succeeded in conferring the encephalitis to the rabbit, in inoculating materials of human encephalitis (neuralgia, liquid cerebrospinal, filtrated fecal materials), proceeding from mortal cases or not. Nevertheless, they themselves perceived very quickly that the experimental sickness differed notably from that studied by Levaditi, Harvier and Nicolau, Doerr and Schnabel, Berger, etc... Although the rabbits inoculated with the herpetic-encephalic virus succumbed in the few days which followed the inoculation, the animals infected with the "Swedish virus" died later on, after a few weeks, sometimes after many months; not often, there was neither death nor sickness, so to speak. The success of the experiment in these cases was only proven by the anatomical-pathological lesions which presented the neuraxe of rabbits sacrificed long after the inoculation. Besides, these lesions offered an aspect entirely different from the alterations provoked by the herpetic-encephalic germ. It was a question of, not the meningeal and parenchymatous modifications of a clearly acute nature, which is constant in true encephalitis, but of chronic lesions (meningitis with mononuclears, peri-vascular disks, and, principally, nodules with epithelioid and gigantic cells; for details, see page 661.

The "Swedish virus" produced no keratosis followed by encephalitis. Although appearing capable of traversing the filter candles, as the herpetic-encephalic germ, this virus offered several particularities allowing one to distinguish it from the other. The action of heat, in particular, showed that Kling's virus resisted temperatures that totally annihilated the pathogenic activity of the filtrable encephalic and herpes microorganism.

Another difference, no less striking, resulted from the frequency, truly extreme, of successes which the experimental tentatives of Kling and his collaborators conveyed. Whereas elsewhere the positive results were exceptional, despite a great number of inoculations, practiced with the most diverse materials, gathered up on the living body as well as on the

(1) BLANC. C. R. of the Academy of Sciences, 182, 1921, p. 725.

cadaver, the Swedish authors saw their efforts come to a head, so to speak, each time that they attempted the experiment. Instead of four to five rootstocks of herpetic-encephalic virus, isolated with great pains by their predecessors, Kling and his collaborators obtained from them a far more considerable number, with infinitely less effort.

It became evident that the hypothesis after which the "Swedish virus" was only an attenuated variety of the herpetic-encephalic germ, hypothesis formulated, in the beginning, by Levaditi and Nicolson, was no longer supportable. Too many facts, better observations, ground it into a hole. The preceding established data had thus to be interpreted from an entirely different manner: this is what Kling and his collaborators did.

For the other Swedes, all those who pretended to have in their hands the etiological agent of epidemic encephalitis were victims of a grave error. They had isolated the herpetic virus, whereas they believed to cultivate on the animal the germ of the v. Economo sickness. In effect, herpes complicates a multitude of infectious processes; why would it not add itself, in name of secondary sickness, to the lethargic encephalitis? In fact, had not Levaditi and Harvier called attention to, in the sick Hof ..., from which came their rootstock C, the presence of a facial herpes? According to all probability, affirmed the Swedish authors, Levaditi and Harvier, as well as Mc Intosh and Turnbull, Doerr, Schnabel, Berger, etc..., isolated, not the etiological agent of encephalitis, but very simply the herpetic virus, which had invaded the neuraxe by means of lesions provoked by the authentic germ of the v. Economo malady (cf. Kling and his collaborators (1)).

This authentic germ is the "Swedish virus". It alone must be considered as being the causal agent of epidemic encephalitis. This conclusion, formulated by Kling and his collaborators, thus implanted a completely new aspect to the etiological problem of the v. Economo sickness. Was it justified? The future is charged with demonstrating the contrary, as we will prove in the course of this Memoir.

We will leave aside, for the moment, the question of the etiology of epidemic encephalitis, in relation with the herpetic-encephalic virus. We will expose the actual state of the problem in a conference with "Medical days of Brussels", next June, in insisting on the arguments that authorize us to consider this virus as being the etiological agent of the v. Economo sickness(2). We will limit ourselves to the exposition of data which conducted us, little by little, to put in doubt Kling's and his collaborators' conception and, finally, to identify the experimental encephalite studied by this scientist with a spontaneous and epizootic infection of

(1) KLING, DAVIDE AND LIJENQUIST. C. R. of the Society of Biology, 40, 1921, p. 514.

(2) This conference took place in the course of the first "Medical Day", June 29; it will soon be published.

the rabbit, whose clinical and anatomic-pathological particularities had been precised by several American and English authors, and whose microbe had been recently discovered.

Mr. Kling having had the kindness of entrusting us with his "Swedish virus" in passage, we began by confirming his assertions on the subject of the principle characteristics of this virus. Here is what we ascertained in the course of our experiments:

November 16, 1922, quite a large number of rabbits had been inoculated by cerebral method, with the Swedish virus of passage, conserved in diluted glycerin. Here are the results obtained:

A. In a first serie, we used young rabbits (one month of age). Rabbit 96/V and 98/V died the 29th day; rabbit 97/V succumbed the 37th day; rabbits 96/V and 98/V were sacrificed the 61st day. No lesion of the nervous system in these animals.

B. In a second series, we used adult rabbits:

RABBIT					
LAPINS	SACRIFIÉ LE :	LÉSIONS	PASSAGE	SACRIFIÉ LE :	LÉSIONS
	- DAY -				
1° 82/V	8° jour	0	lapin 70/M	93° jour	0
2° 84/V	13° jour	0	lapin 23; lapin 30	113° jour	0
3° 85/V	20° jour	0	lapin 7/A; lapin 52	148° jour	0
4° 86/V	76° jour	0	"	"	"
5° 87/V	76° jour	+	lapin 57/E	mort le 118° jour	+++
6° 89/V	105° jour	++	"	DEAD	"
7° 91/V	131° jour	++	"	"	"
8° 94/V	131° jour	++	"	"	"
9° 88/V	131° jour	++	"	"	"

Passages were effectuated in inoculating an encephalic emulsion by corneous and intracerebral methods. Not one of these animals presented keratosis. A single one, among these rabbits, was noticeably sick: it was rabbit 57/E, who, one hundred seventeen days after the inoculation, presented signs of laziness and weakness; it died the next day.

These experiments showed that, in accordance with Kling's affirmations, the "Swedish virus" is pathogenic for the rabbit. The infection that it provokes is not manifested, in general, by any apparent clinical sign (a single one of our animals was clearly sick before succumbing). It only manifests itself by microscopic alterations(see further), which appear after a very long incubation period (seventy-six, one hundred five, one-

hundred thirty-one and one hundred eighteen days). It is interesting to notice that the passages, made with encephals exempt of microscopic lesions (animals sacrificed during the incubation period), rested negative, whereas an injection practiced with the lesioned brain of rabbit 87/V, gave a clearly positive result. It would thus seem that the virus would appear in the nevraxe at the same time as the histological modifications that it provokes.

Later on, our tentatives of passage from brain to brain stayed unfruitful. The series found themselves interrupted, despite our insistence to continue them regularly, and that, whatever was the mode of inoculation and the method of penetration. We know actually that these failures were due to the fact that we would administer to our animals virus which was conserved too long in pure sterilized glycerin. In effect, the Swedish germ has this in particular that, contrary to the true filterable virus (poliomyelitis, rabies, encephalitis, herpes, etc.), it loses quite rapidly its virulence by conservation of brain fragments in the concentrated glycerin, at the temperature of the refrigerator. Nothing surprising since, just as we will see in the course of this Memoir, the Swedish germ is not at all a filterable virus, but a Microsporidia, a more fragile protozoan than the ultra-microbes of Ectodermoses neurotrope.

Another statement struck us from the beginning of our research. Mr. Kling having made us get fragments of human encephalic brains, plunged in diluted glycerin, we inoculated these fragments in numerous rabbits by cerebral method. Not one of these animals contracted acute encephalitis; not one of them presented any more lesions of the nevraxe, at the time of a later examination practiced several months after the inoculation. Now, the same materials, injected by the scientists of Stockholm, into rabbits originating from Swedish rearing, had determined chronic encephalitis of which it was a question above.

There was in this something impressive that it was necessary at all cost to elucidate. Results so different could not depend on technical divergences, the two laboratories utilising the same procedures of inoculation. Only differences between the varieties of animals (rabbits) employed in Sweden and Paris could explain the observed deviations. That was the reason which made us determined to ask Mr. Kling to send us a lot of rabbits belonging to his rearing, so that we could simultaneously experiment on them and on rabbits of the Parisian region. He did this very amicably in July 1923. Now, it is these attempts which would end in results permitting us to resolve the problem of the nature of the Swedish encephalic virus.

In between time, we had knowledge of a complete series of works concerning a spontaneous enzootic malady of the rabbit, characterized by chronic encephalic alterations and reported in the United States and in England. The idea came to us that, seemingly, this malady, rare in the Parisian region, since, until then, in spite of the examination of several thousands of encephalic viruses, we had never happened upon it, had to be frequent in the Northern countries, and particularly in Sweden. If this hypothesis

found itself confirmed, one could suppose that chronic encephalitis observed by Kling and his collaborators, following inoculations of human materials, was none other than spontaneous encephalitis of the rabbit. This one would appear above all in animals whose brain was traumatized by experimental inoculations, such as they may be. The germ of this malady, living in the latent state in some organ (principally in young rabbits), would localize itself on the nevraxe and would produce chronic lesions each time that one would inject cerebral emulsions or others into the encephal, by themselves non-virulent. The results, so constantly positive registered by Kling and his collaborators, would explain themselves, in this case, by the intervention of a secondary spontaneous infection.

But this was only pure hypothesis, an hypothesis envisaged besides by Mr. Kling himself, who had soon isolated it on the faith of microscopic examinations of encephalic viruses of non inoculated rabbits, examinations which had ended in totally negative results. There was thus place to verify it with all the rigor that this type of research involves. This is what we did without delay.

Before undertaking the exposition of our results, it seems useful to us to review the information that was possessed (June, 1923 on the clinical and anatomical-pathological characteristics of epizootic encephalitis of the rabbit.

EPIZOOTIC ENCEPHALITIS OF THE RABBIT. -- In 1917, Bull (1), examining histological encephalitis of rabbits infected with streptococi, there discovered microscopic alterations of encephalitis, whose particularities he describes (perivascular and nodular disks); the same lesions existed in three rabbits who died of septicemia, and who had never been inoculated. Later (1922), Oliver (1) (San Francisco) observed analogous alterations in rabbits injected with variable doses of asphenamine. In a first series of experiments, the animals were intoxicated by growing quantities of asphenamine; they died around the tenth day with microscopic signs of encephalitis. In a second series of attempts, it was a question of animals who succumbed as soon as the injection was medically administered, and nevertheless they also presented alterations of the nevraxe. In these conditions, it was impossible to admit a relationship of cause and effect between the administration of the medicament and the genesis of the cerebral-medullar lesions. The force of circumstance was thus to conclude that it was a question of a spontaneous infection, not manifesting itself by any appreciable clinical symptom. In reality, around 20 p. 100 of rabbits examined in the Oliver Laboratory were contaminated. The author minutely describes the microscopic alterations of the nervous system, which consist in meningitis with mononuclears and plasmatic cells, in peri-vascular disks

(1) OLIVER. The Journal of Infectious Diseases, 30, 1922, p. 91.

(2) C. C. TROUT. The Veterinary Journal, 78, no. 6, 1922.

resembling disks that one encounters in human or experimental encephalitis, and in nodules. These last ones are constituted by a mononuclear infiltration of the cerebral parenchyma; their center is necrosed and filled with drops of fat. Not one microorganism was able to be found by Oliver, despite the variety of the utilized histological methods.

The same zootic disease of the rabbit was affirmed, in England, by C. C. Twort (2). This author inoculates rabbits with lymphadenome; these show cerebral alterations, giving evidence of a state of chronic encephalitis. Nevertheless, examination of the control animals, originating from the same rearing, ends in the same results. It is thus a question of a spontaneous infection having no relationship with lymphadenome. This infection terminated itself sometimes by death, happening after a period of weakening and of convulsions. Twort brings attention to hypothermia, muscular debility, modifications of liquid cerebrospinal (lymphocytosis), and describes the microscopic alterations already studied before by Oliver and Bull. The existence of the malady in the endemic state, in certain rearings, renders difficult the study of its experimental transmission (by contact or by inoculation). Nevertheless, certain tentatives of infection by cerebral or peritoneal method realized by Twort, seemed crowned with success.

The name spontaneous encephalic-myelitis of the rabbit is relatively ~~INEROFER TO DESIGNATE THE INFECTION STUDIED BY Bull, Oliver and Twort.~~ In effect, the central nervous system is not the only one to act up: the liver, the spleen and above all the kidney are the seats of lesions, whose characteristics were precised by Bell and Hurtzwell (1), and above all by C. C. Twort and H. E. Archer (2). The first ones confirm an interstitial lymphocytic nephritis, with nodules and atrophy of excretory tubes, in about 15 p. 100 of the rabbits examined (400 in total). Twort and Archer observe, on their part, kidney alterations, accompanied by "splenic artery" and "hepatitis", in rabbits stricken by spontaneous encephalitis. These alterations consist in lymphocytic centers, giving place to an inflammatory and degenerative nephritis, whose intensity contrasts with that of the cerebral lesions. Actually, according to Twort and Archer, the kidney can be hardly touched [cf., on the subject of urinary and sanguine modifications observed in the course of spontaneous nephritis, the work of C. C. Twort and Archer (3)].

(1) BELL and HURTZWELL. Journ. of Infect. Diseases, June 1919, p. 628 (cited according to Twort).

(2) TWORT and ARCHER. The Lancet, 1, 1923, p. 1102.

(3) The spontaneous malady of the rabbit was studied also by BONFIGLIO (Boll. e Atti delle Reale Accademia medica di Roma, 50, 1923-24). The author seems to ignore the prior works of Bull, Oliver and Twort.

* * *

There are the principle clinical and anatomic-pathological characteristics of the epizootic encephalitis of the rabbit. As we have said, the perusal of the works of Bull, Oliver, Twort and Archer, suggested to us the idea that, seemingly, between encephalitis provoked by the "Swedish virus" of Kling and this spontaneous neurasitis, there had to be close relationships, if not absolute identity. We asked our old collaborator C. C. Twort to kindly entrust to us some materials of epizootic encephalitis, in order to compare the lesions of this infection to those of the malady studied by Kling, Davide and Liljenquist. This is what he did.

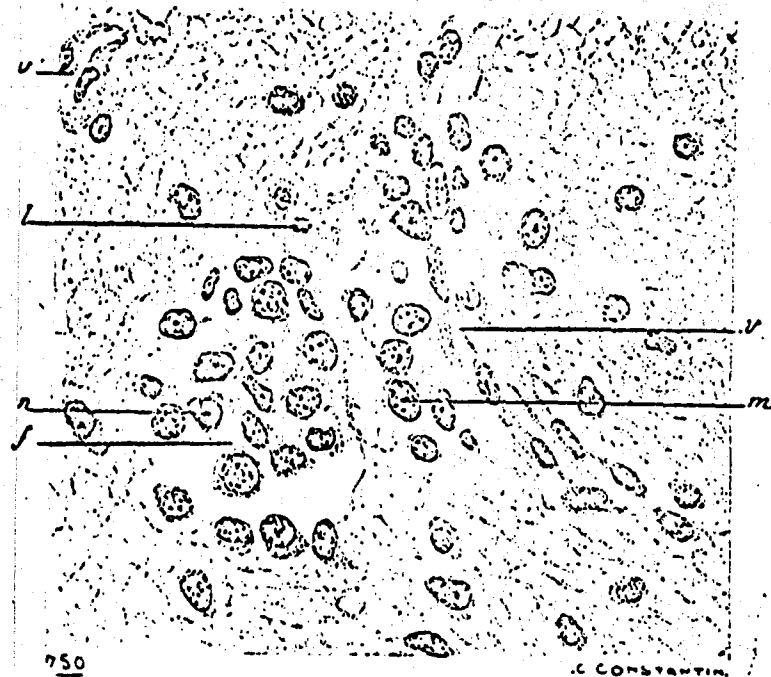


Fig. 1. Nodule in the encephal of rabbit 89/V (Kling virus).
f, nodule formed from cells of epithelioid aspect; n, nucleus;
m, mononuclears around the vessel v; l, lymphocyte; v, vessel, Hematein-eosin.

Now, these studies, finished in July 1923 and published October 20, 1923 (1), confirmed our hypothesis.

Actually, the cerebral alterations declared in the rabbits inoculated with the "Swedish virus" were certainly those described earlier by Kling and his collaborators: Meningitis with mononuclears of the cortex and of the septums, perivascular disks with lymphocytes and with plasmatic cells (on the level of the mesencephalic) and nodules, without well defined topography. These nodules are constituted by a central zone of cells of an epithelioid appearance, with voluminous nucleus, and by a peripheral zone, rich in lymphocytic mass of necrosed cellular debris in the center of the nodule. Certain ones of these nodules are situated in



Fig. 2. ___ Nodule in the encephalitis of rabbit 9/T, inoculated with the spontaneous encephalo-typhoid virus of the rabbit (Typhoid virus). ___ f, nodule; l, cell of epithelioid aspect; c, giant cell containing pigment (p); m, voluminous cell with eccentric nucleus, containing pigment; p, pigment. Hematein-eosin.

(LEVADITI and NICOLAU. C. R. of the Society of Biology, 89, 1923, p. 775. This note had to be presented to the Society of Biology in the last seance of July. Circumstances beyond our control retarded the publication of it

the neighborhood of an obstructed vessel (cf. fig. 1).

Now, alterations of the same kind were found in the encephal of two rabbits inoculated with the virus of the epizootic encephalitis, sent by C. C. Tward (fragments of encephal conserved in diluted glycerin). Here are the results furnished by these experiments:

Rabbits 9/T and 10/T. — Intra-cerebral inoculation October 6, 1922. No clinical sign apparent afterwards. Animals are sacrificed the 175th day. Histological examination of the brain shows the following lesions: chronic meningitis with mononuclears of the cortex and of the septums, peri-vascular disks under cortexes, absence of acute encephalitis, centers circumscribed (nodules) in the region of the hippocamp. These centers are constituted by a great number of mononuclears encircling a central zone, formed by epithelioid cells containing ochre pigment. The centers touch some obstructed vessels; the neurons in the neighborhood are clearly altered in their structure (cf. fig. 2 and 3). Here and there, one distinguishes giant cells.

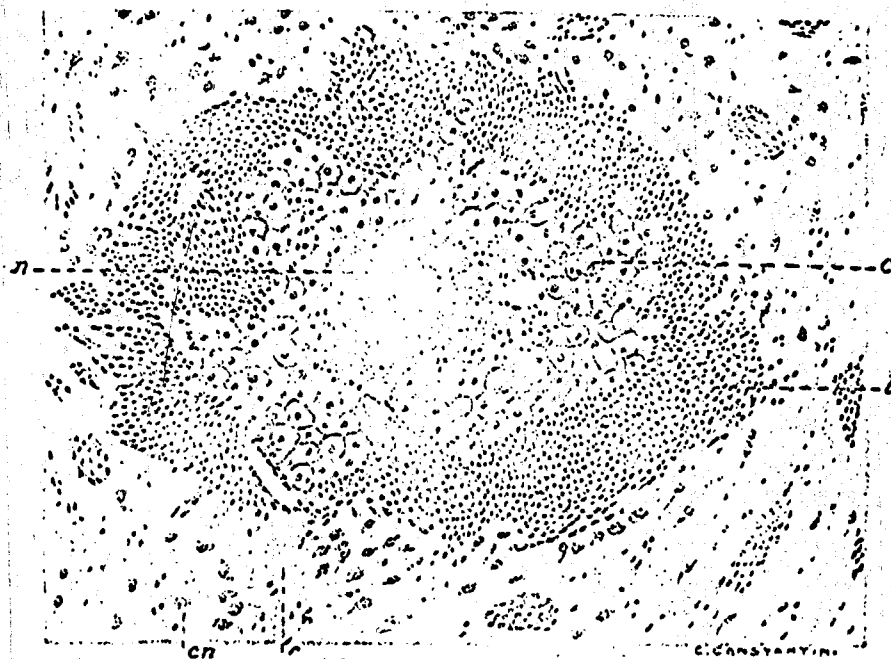


Fig. 3. — Rabbit 9/T (see fig. 2). Encephal section. — n, necrotic center of a nodule; c, cells of epithelioid aspect; l, peripheral lymphocytic zone; c(below), white substance; cn, nerve coll. Hematoxylin-eosin.

These lesions seem to be mistaken to the alterations declared in the rabbits inoculated with the encephalic Swedish virus of Kling and his collaborators. In addition, the animals who are stricken by it do not seem to react by apparent morbid disorders, thus conducting themselves as the rabbits infected with the Swedish germ.

It came out of these observations that these "encephalitis of the rabbit, provoked by viruses of diverse origins; human lethargic encephalitis, spontaneous encephalic-myelitis of the rabbit, general paralysis (1), have nothing in common with acute encephalitis which determines in the same animal, the encephalic virus of Levaditi and Harvier, of Doerr, of Berger, of Schnabel, or the diverse protozoa of hemetic germs. The clinical evolution of the malady and the characteristic of the lesions are something else entirely."

Further more, the resemblance between the histological modifications of epizootic encephalitis on one hand, of the malady provoked by the Swedish virus, on the other hand, was at such a striking point, that there was no longer room to hesitate; the same etiological agent had to find itself at the origin of these two morbid processes, considered until then as totally distinct. From there, the necessity to discover this agent. Filterable virus, or visible microorganism? In one case as in the other, experiments of crossed immunity, or precise morphological studies had to bring a convincing demonstration.

Thus we undertook researches in this path. Between times, appeared a work by Doerr and Zdansky (2) [April 1923] regarding the same question.

(1) FLAUT and RUIZER (Munch. med. Woch., 1922, no. 52, p. 1779) observed. in rabbits inoculated by testicular method, with emulsions of brain of general paralytics, lesions resembling those described above. After a long incubation (two to three months), the cerebrospinal liquid shows a marked pleiocytosis (despite a general normal state) and, in the encephal, one declares "lesions of the general paralysis" (Flaut), to know perivascular disks and inflammatory nodules. Total absence of Treponemas (our method, modified by Jahnel). One was struck by the resemblance between this experimental infection and spontaneous encephalo-myelitis of the rabbit.

Recently, JAHNEL and ILLER (Klin. Woch., 1923, no. 37, p. 1731) declared lesions resembling those of spontaneous encephalo-myelitis in rabbits inoculated with encephal originating from a case of uremia and of another case of Wilson's malady. Cf. the works of BONFIGLIO (Policlinico, Sezione pratica, 30, 1923, p. 26).

(2) DOERR and ZDANSKY. Schweizerische. med. Woch., 1923, no. 14.

The Swiss authors study the preparations of Kling (rabbits inoculated by cerebral method, with the "Swedish virus" of passage) and confirm his histopathological observations. They declare the presence of nodules (granulomata), whose periphery is constituted by lymphoidal elements, epithelioid cells and giant cells, whose center is necrosed. Doerr and Zdansky shows, further, that these nodules do not exist in the nevraxe of human subjects dead of epidemic encephalitis, and that they are not present in all the brains of rabbits inoculated with encephalic materials. Of such ones granulomata can be disclosed in the encephal of animals never having been injected with encephalic virus [cf. Neuburger (1)]. And to conclude: "It seems, up until a certain point, believable that this granulomatosis is a parasitic malady of the rabbit, completely independent of human encephalitis". It is worthy to remark that, in this work, Doerr and Zdansky do not establish any relationship between the malady of Kling and spontaneous encephalitis of the rabbit.

About a month after the presentation of our account to the Biology Society (October 20, 1923; November 17 and 24, 1923), appeared a long memoir of Flexner (2), on the etiology of epidemic encephalitis. In this memoir, the author, studying, in his turn, the "Swedish virus", makes some reserves on the subject of its encephalic origin. Actually, the cerebral lesions observed by Kling can be met in rabbits never having received human encephalic material, even never having been injected. Flexner recalls the observations, already mentioned, of Bull, Oliver, Swort, and cites in particular the declarations of his collaborator Mc Cartney, who, in about 50 p. 100 new rabbits, examined at the Rockefeller Institute, reveals some alterations of chronic nevraxitis.

The memoir of Mc Cartney (3) appeared elsewhere in January 1924. It only contains confirmative documents, that which frees us from analyzing it here in detail.

The two works that have just been cited, one before, the other after ours, were thus clearly conform to our conception concerning the etiology of encephalitis provoked by the Swedish virus. However, it is the discovery of the pathogenic agent that put an end to the discussion, in demonstrating the identity between spontaneous epizootic encephalitis and the generality of encephalitis of chronic nature, provoked experimentally in the rabbit by inoculations of varied materials, of human origin or other. A few words on the facts of this discovery.

(1) NEUBURGER. Naturforscherversammlung, Leipzig, 1922, cited according to Doerr and Zdansky.

(2) FLEXNER. Journ. of the Americ. med. Assoc., 81, 1923, pp. 1688-1735.

(3) Mc CARTNEY. Journ. of experim. Med., 39, January 1924, p. 51.

FACTS OF THE DISCOVERY OF Encenhalitozoan cuniculi, ETIOLOGICAL AGENT OF EPIZOOTIC ENCEPHALITIS OF THE RABBIT. — We have explained this history in a short note inserted in February 1924 in the Schweizerische med. Wochenschrift (1); we are reproducing it in these Annales, adding there the works since published.

In October 1923, we proposed to study, again, with the aid of further perfected methods, the histological details of cerebral lesions in rabbits inoculated with the "Swedish virus" of passage (Kling rootstock), the spontaneous encephalitis virus (rootstock Thwort) and the virus isolated from human encephalitis by Thalheimer (2), in the United States. Furthermore, we would wish to compare these lesions to those that the rabbits sent from Sweden by Mr. Kling could present, to which we had inoculated, by cerebral method, the encephal of a Parisian rabbit, supposed indemnified (See p. 574). In the course of these researches, we discovered, first in the animals belonging to the Kling serie, next in the rabbits stricken with spontaneous encephalitis; or with the Thalheimer malady, finally in the Swedish rabbits, particular elements, whose parasitic nature left no doubt. In fact, the morphology, the topographic disposition in relationship with the cerebral lesions, the coloring reactions, the mode of evolution, permitted to affirm that it was there a question of a protozoan, in particular of a Microsporidia, in strict etiological relationship with the histopathological manifestations of the infection. The fruit of our studies was recorded in a serie of Communicated notes, from November 12, 1923, at the Academy of Sciences and at the Society of Biology. Here, in a few words, is the object of these Notes:

a.(1) The microorganism, which we are calling Encenhalitozoan cuniculi is found in rabbits inoculated with "Swedish virus", Thalheimer's virus and in the animals stricken by epizootic encephalitis. It is the same everywhere and seems to belong to the group of protozoans. This parasite forms cysts containing spores, of which we give a precise description (November 12, 1923);

b.(2) Presence of cysts far from encephalic nodules; breaking out of these cysts and formation of granulomata, at the level of which the spores are engulfed by the macrophages; possibility of studying the parasite on smear preparation. We will consider the Encenhalitozoan cuniculi as a protozoan belonging to the group of Microsporidias. Here are the conclusions which unroll from this second note: "The presence of a same parasite,

(1) LEVADITI, NICOLAU and Miss SCHÖEN. C. R. of The Academy of Sciences, 177, 1923, p. 985, session of November 12.

(2) LEVADITI, NICOLAU and Miss SCHÖEN. C. R. of the Society of Biology 89, 1923, p. 984, session of November 17.

(3) LEVADITI, NICOLAU and Miss SCHÖEN. C. R. of the Society of Biology, 89, 1923, p. 1157, session of December 8.

Encephalitozoan cuniculi, in the encephala of rabbits stricken by the malady provoked by Kling's Swedish encephalic virus, of rabbits infected with the virus, called encephalic, of Thalheimer, and also of rabbits contaminated with the spontaneous epizootic encephalitis virus of Bull, Oliver and Twort, permits to identify these three maladies. Kling, Davide and Liljenquist, as well as Thalheimer, thus had worked with the spontaneous encephalitis germ of the rabbit, while they thought to have in their hands the virus of human encephalitis, which is filtersole and invisible, as we had shown it to be from 1920. May we add that Encephalitozoan cuniculi had never been found on sections of encephals of infected rabbits, by cerebral method, with the encephalitis virus of Levaditi and Harvier, or with the herpes virus" (November 17, 1923);

C.(3) Description of kidney, cerebral, hepatica and splenic lesions. Presence of Encephalitozoan cuniculi in the kidney (on smear preparations and sections). Four illustrations show the aspect of the parasite on smear preparation and its disposition in relationship with encephalic alterations, in the rabbits stricken with spontaneous encephalitis, or inoculated with Thalheimer's virus (December 8, 1923);

d.(1) Existence of Encephalitozoan cysts on the interior of epithelial cells that cover canaliculi of the renal papillas. These cysts break and the spores penetrate in the light of the canaliculi, to be poured forth outside by the urine. The examination of the urine of contaminated animals permits to verify a variable number of spores. The propagation of the malady is made by the intermediary of these spores, which, present in the urina, soil the alimentary materials and penetrate with them into the stomach and the intestine. The spontaneous contamination seems thus to affect itself by the digestive tract. Encephalitozoan is virulent for the mouse (January 7, 1924);

e.(2) Evolution of the Microsporidia in the mouse. Morphological study of spores, their presence in the peritoneal cells and in the Kupffer cells (liver) (January 26, 1924);

(1) LEVADITI, NICOLAU and Miss SCHOEN. C. R. of The Academy of Sciences, 178, 1924, p. 256, seance of January 7.

(2) LEVADITI, NICOLAU and Miss SCHOEN. C. R. of the Society of Biology, 40, 1924, p. 194, seance of January 26.

(3) LEVADITI, NICOLAU and Miss SCHOEN. C. R. of the Society of Biology, 89, 1923, p. 1157, seance of December 8.

f.(3) Coloring and histochemic reactions of Encephalitozoon spores. Comparisons between the morphology of these spores and that of the Microsporidia of the snake (Glugea darilewskyi), studied by Guyenot and Naville(4). Illustrations representing the details of structure of spores of the Microsporidia and a part of its evolutive cycle (micronucleus pansporoblasts). Receptivity of the rat and of the dog. Presence of Encephalitozoon in spontaneously contaminated rats. Study of the hereditary transmission of infection in the mouse (March 15, 1924).

There are, in resume, the facts established by us on the subject of etiological relationships between Encephalitozoon cuniculi and chronic encephalitis of the rabbit, whatever be the origin of this encephalitis, spontaneous infection or experimental inoculation.

*
* *

What was known of this parasite before the publication of our first Note at the Academy of Sciences (November 12, 1923)? A single work, relating to encephalitis provoked in the rabbit by the Kling virus, and containing a few indications on the subject of the presence of particular formations in the encephal, had appeared in April 1923; it was signed by Doerr and Zdansky (loc. cit.). These authors stained with intense stain by the Ziehl-Neelsen method the sections of brain that Kling had sent to them, and they there discovered corpuscles, whose microbial nature, far from seeming certain to them, was only, in their opinion, at the very most possible. Here is the description that they give of these corpuscles:

"Long formations of 1.5 to 3 microns, colored in red, of variable aspect, are accumulated in the epithelioid cells which occupy the center of the nodules, and above all in the necrotic zone of these nodules. Beside the egg-shaped or elongated corpuscles, one finds other ones which appear paler at the two extremities, and still other ones that are incurved. One can verify a nuclear formation be it in the center of the corpuscle, or near one of the poles."

Doerr and Zdansky verify these corpuscles in the protoplasm of epithelioid cells. Analogous formations had been found in the encephal

(3) LEVADITI, NICOLAU and Miss SCHÖEN. C. R. of the Society of Biology, 40, 1924, p. 662, séance of March 15.

(4) GUYENOT and NAVILLE. Revue de Zoologie, 30, 1922, no. 1.

of a rabbit that had been inoculated with a rootstock of encephalic virus "Basel III", by intra-cerebral method, and which had been sacrificed four months later.

Thus it was a question of corpuscular formations appearing to offer a certain structure and lying in the middle of granulomas. No cystic disposition is mentioned in this work; it is a question neither of the presence of the microorganism far from the nodules, in full cerebral substance, nor of the least morphological detail observed in smear preparation. Furthermore, the utilized methods of coloration (hematein-Eocene as first, Ziehl-Neelsen) next, showed that the formations in question were clearly of an acid-fast character. As for the interpretation that Doerr and Zdansky accorded to the corpuscles observed by them, here it is textually:

"The corpuscles described could (1) be microorganisms, opinion shared by several specialists to whom we have shown our preparations last December. But a quite particular prudence imposes itself, above all when it is a question of declarations concerning the central nervous system, and principally when one utilizes the methods of coloration which put in evidence fatty elements (acid-fast characters). Besides, we have not had the occasion to realize all of the desirable control researches and to utilize procedures of coloration permitting to formulate a clear opinion. The important thing is to know if the granulomas and the described corpuscles (it is still necessary to know if these last ones are parasites, falls as such um Parasiten handeln sollte) are in relationship with the etiology of encephalitis."

It results from it that if Doerr and Zdansky, saw on Kling's sections the more or less altered spores of Encaphalitozoan cuniculi, they gave but one inexact description of their tinctorial affinities, since they talk of acid-fast character, which is contrary to reality. Furthermore, they did not affirm, with all of the certitude desirable in a similar occurrence, their parasitic nature, still less the characteristics which make of them protozoans belonging to the group of Microsporidias.

It is only on December 27, 1923, more than a month after the publication of our Note to the Academy of Sciences, and when our three first communications had already appeared, that Doerr and Zdansky (1), returning to the question, confirm the microbic nature of formations observed in April 1923. This time, they describe the cysts, of which they give an illustration, and realizing that the acid-fast character, mentioned in their first work, is nothing less than certain. Being given that all this was demonstrated by our former works, one asks himself for what reason

(1) Underlined in the text.

(1) DOERR and ZDANSKY. Schweizerische med. Woch., No. 52, 27th of December 1923, p. 1189.

Doerr and Zdansky pretend to describe, in this second Memoir, a "new parasite". (Hauts konnten wir über einen NEUEN PARASITEN berichten, etc.).

• •

Our researches were nearly finished, when we found, in a recent publication of Mc Cartney (loc. cit.), a bibliographical indication of higher interest. It concerns a work of J. Wright and E. Craighead (1), appeared in July 1922 and concerning the study of an infectious driving paralysis of young rabbits. These authors tried to transmit infantile paralysis to rabbits, without success elsewhere, and, in the course of these tentatives, observe a spontaneous infection manifesting itself by drowsiness, tremblings, paralysis, and terminating itself often by death. The nervous system of these young animals show inflammatory and necrotic lesions, in the neighborhood of which one declares corpuscles having the aspect of illustrated elements. These corpuscles contain one or two light vesicles, are 4 microns in length and 1.5 microns in width, are colored by the Gram and have a relative acid-fast character. Further more, Wright and Craighead declare the same formations in the renal cells, in the light of canaliculus of the kidney and also in the urine of infected animals. The authors conclude that it concerns, in the species, a microorganism belonging, very probably, to the group of protozoans, in etiological relationship with the spontaneous malady of young rabbits, malady of which the propagation would take place by the intermediary of the urine.

The comparison of microphotographies that illustrate the work of Wright and Craighead and of our preparations show a striking resemblance between Encephalitozoan cuniculi and the microorganism observed by the American authors. Everything brings one to believe that enzootic paralysis of young rabbits is only a particular form of spontaneous encephalic-myelitis, studied by Bull, Oliver and Twort, and that the etiological agent is the same in the two morbid processes.

If, in the future, the hypothesis of the identity between the paralysis of the young rabbits and spontaneous enzootic encephalitis found itself confirmed, we would regret that at the Encephalitozoan cuniculi denomination, proposed by us to designate the etiological agent of enzootic encephalitis, the names of Wright and Craighead could not be added. The Microsporidia that provokes encephalic-myelitis of the rabbit had to be called Encephalitozoan cuniculi (nov. spec.) [Wright and Craighead].

(1) WRIGHT and CRAIGHEAD. Journ. of experim. Med., 36, 1922, p. 185.

CHAPTER II

EXPERIMENTAL STUDY

I. ENCEPHALITIZOAN CUNICULI IN RELATIONSHIP WITH THE "SWEDISH VIRUS".

General remarks. — Experimental study of the etiological role of Encephalitozoan cuniculi implies the two following reserves:

1 The existence of a epizootic infection in the rabbit, infection whose frequency seems to vary following the regions and the rearings, fact that in every tentative of transmission by inoculation one must keep in mind the possibility of a spontaneous contamination of supposedly fresh animals. Luckily our stocks of rabbits, originating from the Farisian region have shown themselves to be exempt from epizootic encephalitis except for a few very rare exceptions. In fact, on nearly 700 encephals examined on smear preparation and on sections since the discovery of encephalitozoan, we have only met three of them showing characteristic lesions, as well as parasites. It results that the causes of error in the interpretation of our results are reduced to the strictest minimum. It is not of the same results in experiments on the mouse, animal in which the cerebral Microsporidiosis is infinitely more frequent, as we will see later on.

2 The evolution of spontaneous encephalitis, or of chronic encephalitis provoked experimentally, is of the slowest (Kling and his collaborators). Slow also is the cerebral development of the Encephalitozoan. It is generally necessary at least one and one half months to two months in order that the encephalic alterations become appreciable and that the parasite can be disclosed on the smear preparation and on the sections. It follows that, if one practices inoculations on a great number of rabbits, it is necessary to keep track only of the results furnished by the animals that survived beyond fifty to sixty days.

We would conform strictly to these indications in the interpretation of our experimental data.

The Protocol I (see Annex) shows that, among the rabbits inoculated with Kling's Swedish virus (rootstock Henriksson, seventh generation + rootstock Karl, I E, second generation, of October 6, 1922, conserved in diluted glycerin), two presented intense lesions of the brain and also parasites disclosable on sections. One of these animals was sacrificed one hundred five days after the inoculation; the other had been examined the one hundred fourteenth day.

The character of microscopic modifications and the morphology of Encephalitozoon correspond to the particularities of the same lesions and parasites declared in the rabbits stricken with spontaneous encephalitis (cf. Plate III, fig. 2 and 7).

II. Encephalitozoon cuniculi IN RELATIONSHIP WITH C. C. TWORT'S SPONTANEOUS ENCEPHALIC-MYELITIS OF THE RABBIT, ENGLISH ROOTSTOCK). — The first glycerined virus sent by Mr. Twort served at the intra-cerebral inoculation of rabbits 9/T and 10/T, of which the clinical and anatomical-pathological observation was exposed page 662. In both of them, Encephalitozoon was found on the level of encephalic nodules.

Further more, in December 1923, C. C. Twort was kind enough to confer upon us in London one of his spontaneously contaminated rabbits. The brain of this animal (discreet lesions) was inoculated, in the fresh state, in 6 rabbits, sacrificed or dead from the forty-second to the one hundred second day, 5 offered parasites. These last mentioned were present, now in the encephal, now in the kidney. Two rabbits had a parasited nevraze, while three others showed cysts in the renal epitheliums.

The English virus thus seems virulent for the rabbits of the Parisian region (1).

III. Encephalitozoon cuniculi IN RELATIONSHIP WITH THALHIMER'S VIRUS (2). Thalhimer (Milwaukee) inoculates rabbits with materials originating from human encephalitis cases (liquid cerebrospinal), and declares some lesions of the nevraze resembling alterations of epizootic encephalitis (meningitis, peri-vascular and nodule disks). The author however was persuaded, like Kling and his collaborators, to have experimentally transmitted lethargic human encephalitis to the rabbit.

(1) Concerning this, we want to attest that C. C. TWORT saw Encephalitozoon cuniculi before the first publications of DOERR and ZILANSKY and of LEVALITI and his collaborators. In fact, at the time of our voyage to London, in December 1923, C. C. TWORT showed us sections of encephal containing parasitic cysts. Convinced of the filterability of the spontaneous encephalic virus, C. C. TWORT had considered these cysts as a secondary infection, without etiological relationship with the maledy.

(2) THALHIMER. Archives of Neurology and Psychiatry, 5, 1921, p.113; 6, p. 286.

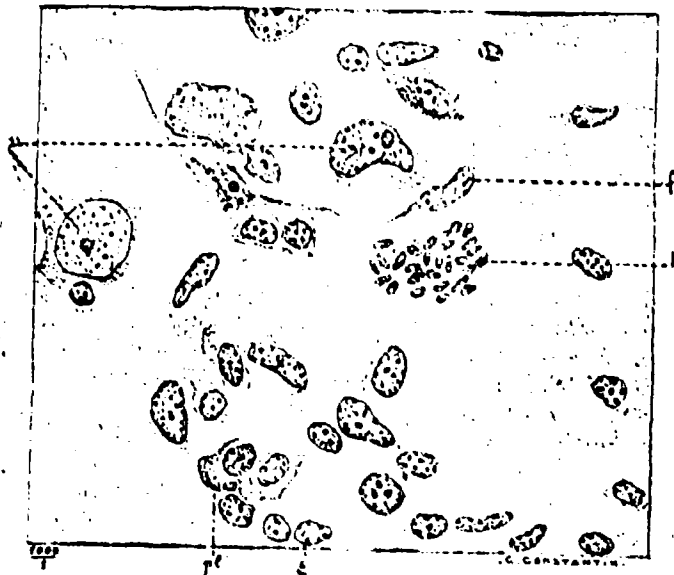


Fig. 4. — Cerebral nodule in rabbit 6/Y. Thalhimer Virus.
n, nerve cells; pl, plasmatic cells; f, fusiform cell; k, cyst containing
Encephalitozoon. Giemsa coloration.

Mr. Thalhimer was kind enough to send us several samples of his virus, conserved in diluted glycerin. The inoculation of this virus in the rabbit furnished us with results consigned to Protocol III. One sees there that 2 rabbits, 6/Y and 56/V, inoculated by cerebral method with rootstock Thalhimer 400 — 4 — 2, were sacrificed the one hundred fifty-seventh day. Both of them presented cerebral and mesocephalic alterations, consisting in meningitis with mononuclears, peri-vascular disks and nodules containing cariolyzed polynuclears. Typical parasites were distinguished in the encephalon of the rabbit 6/Y (cf. fig. 4).

It results from it that Thalhimer, like Kling and his collaborators, believes to have transmitted to his rabbits human encephalic virus, while actually he was in the presence of the epizootic encephalic germ.

IV. — Encephalitozoon cuniculi, ETIOLOGICAL AGENT OF EPIZOOTIC ENCEPHALITE OF THE RABBIT, PARTICULAR ROOTSTOCK.

A. — INOCULATION BY INTRA-CEREBRAL METHOD

a) Our first experience of transmission was made with Parisien virus on rabbits of Swedish origin (Protocol IV), besides, completely unknown to us. In fact, persuaded since July 1923 that the encephalitis provoked by Kling and his collaborators was due to the localisation of the spontaneous encephalic virus on the neuraxe, still unknown at this epoch, localization facilitated by a traumatism of the nervous system, we proceeded in the following manner:

From the stock of 12 young Swedish rabbits sent by Mr. Kling, we selected 10 of them, which we inoculated, by cerebral method, from fragments of encephal of a Parisien rabbit supposed fresh and that had just been sacrificed (rabbit 30/V). We had thought thus to realize this traumatism of the brain, destined to make the tension center. The 10 rabbits were kept alive until the beginning of October 1923, and it is then only that we examined the brain of rabbit 30/V, considered indemn. Now, we discovered there, not only the characteristic lesions of spontaneous encephalitis, but still Encephalitozoon cuniculi cysts (see fig. 5 and 6).

This examination showed to us thus, afterwards, that in reality the 10 Swedish rabbits had been inoculated, not with a normal emulsion of encephal, but with a suspension of neuraxe containing the germ of spontaneous encephalitis, Parisien rabbit.

The result of this first experiment is recorded in Protocol IV. One sees there that, among the 10 inoculated rabbits, dead or sacrificed from the eighty-third to the two hundred forty-eighth day, two only were exempt from cerebral lesions (rabbits 31/V and 39/V, sacrificed the eighty-third and the two hundred forty-eighth day). They seem to have escaped from the infection until then. A third rabbit (rabbit 32/V, died the eighty-third



Fig. 5. — Rabbit 30/V, stricken with spontaneous encephalitis, Parisien rabbit. Encephal section. m, mononuclear; n, polynuclear; beginning of reaction around an Encephalitozoon cyst (m); on the wall of the cyst, a cell with flattened nucleus. H&E method.

day), offered encephalic alterations, without detectable parasites on the sections. On the other hand, in the 7 other animals, we observed lesions and Encephalitozoon, be it in the brain, or in the kidney, or in the two organs at the same time. There was thus infection in 70 p. 100 of the cases. Here are the results obtained, following the examined organs:

Brain: 7 positive cases on 10 70 p. 100
Kidney: 4 positive cases on 7 58 p. 100

One must note that, sometimes, one can declare cerebral alterations noticeable, without being able to notice ENCEPHALITOZOAN on the sections. That is understandable, if one takes into account the two following considerations:

First, the microorganisms being deposited by groups, it is necessary to examine quite a large number of preparations before discovering a cyst, or parasitized nodules. It is possible thus that such examinations rest negative, despite the real presence of the microbe in the encephal.

In the second place, the nodular alterations being the expression of a defense reaction with regard to the encephalic parasite, one conceives that at a given moment these reactions end in the more or less total destruction of the germ. This is what happens quite frequently in the kidney, as we will see in the course of this Memoir.

b) In a second serie of experiments (Protocol V), an emulsion prepared

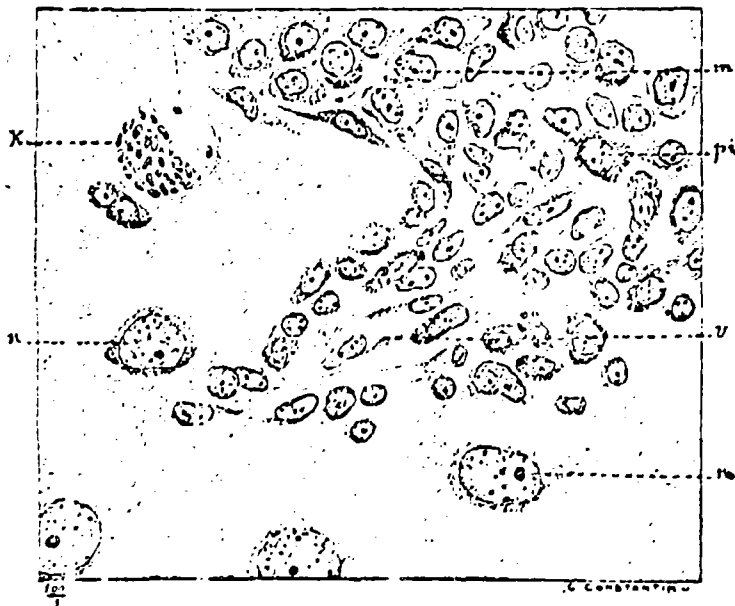


Fig. 6. — Cerebral nodule in Rabbit 30 V (see fig. 5).
Spontaneous encephalitis.
mo, big mononuclears; n, ves-
sels; pi, pigmentary cell;
n, nerve cell; k, cyst
containing Encephalitozoon.
Eosin-orange-blue of Unna.

with the encephals of rabbits 34/V, 35/V and 41/V, containing Encephalitozoens, was inoculated into 5 rabbits, by cerebral method or by peritoneal method. These ones are dead or were sacrificed from the seventy-fifth to the one hundred forty-seventh day. In 3 of them, the Encephalitozoen was found either in the brain, or in the kidney, or still yet in the two organs simultaneously. The frequency of the positive inoculations was from 60 p. 100.

c) The encephals of rabbits 36/V, 76/U and 40/V, enclosing parasites, served to prepare an emulsion that was injected in the brain of four fresh rabbits; these were dead or were sacrificed from the 76th to the 132nd day. In three of them, we noticed Encephalitozoen in the brain or in the kidney. The percentage of positive inoculations was from 75 p. 100 (See Protocol VI).

d) The results were less favorable in a fourth series of attempts (inoculation in 6 rabbits of a mixture made with the brains of rabbits 13/V and 37/V, both of them infected). Among these (dead or sacrificed from the 110th to the 125th day), only one proved to be contaminated [rabbit 93; presence of Encephalitozoen in the kidney (See Protocol VII)]. Percentage of positive inoculations: 16 p. 100.

These attempts show that intra-cerebral inoculation of encephalic emulsions containing Encephalitozoen curiculi confers the disease to the rabbit, little matters the race of animals utilized (Swedish rabbits or rabbits of the Parisian region). The frequency of positive results can vary from one serie to the other. In three of our experiments, this frequency oscillated between 60 and 75 p. 100, but, in a fourth trial, 16 p. 100 only of inoculated rabbits showed themselves to be parasited. These deviations are attributable, on the one hand, to the richness in germ of the injected material, on the other hand, to the more or less pronounced receptivity of the animals in experiment.

It is interesting to state that despite the exclusively intra-cerebral inoculation of the virus, this one can localize itself in the kidney without the encephal being apparently parasited.

It was impossible for us to precise the reasons of this preference of the germ for the kidney or for the brain, but the fact rests no less incontestable: in certain rabbits, one of these organs shows itself more apt than the other to attract the Encephalitozoen and to react by more or less pronounced histological alterations.

e) Positive results were obtained by inoculation of parasited renal emulsions in the encephal of fresh rabbits. Protocol VIII shows that, among the four animals infected in this manner, with a virus originating from rabbits 36/V, 76/U and 40/V, and that are dead, or were sacrificed

from the 71st to the 132nd day, three contracted the malady (75 p. 100). The Encephalitozoon was discovered in the urine, in the kidney and in the encephal. In addition, in the experiment that is the object of Protocol IX, one of two rabbits inoculated in the brain with a parasited renal emulsion, originating from rabbit 37/V (examined, one the 75th, the other the 112th day), was contaminated (presence of germs in the kidney). An analogous result was registered in the course of trials consigned in Protocols X and XI. These results from it that at the example of the encephal, the kidney can serve for the transmission of the infection by intra-cerebral inoculation. The frequency of positive results seemed to equal that which one observes when one utilises the emulsions of parasited brains in intracranial injection.

B. __ INOCULATION IN THE SCIATIC NERVE.

A kidney emulsion of rabbit 42/V, containing numerous Encephalitozoon, was inoculated in the sciatic nerve of rabbits 35/A and 36/A. The first of those animals died the 103rd day; the encephal and the kidney were parasited (1) (see Protocol XI bis). The second animal was sacrificed the 116th day; absence of Encephalitozoon in the brain and in the kidney.

This experiment shows that it is possible to transmit encephalitis epizootic to the rabbit by inoculation of virus in the sciatic nerve.

C. __ INOCULATION BY INTRA-VEINOUS METHOD

The intra-veinous method seems to lend itself to the experimental transmission of epizootic encephalitis. An experiment, resumed in Protocol XII, shows that the four rabbits injected in the marginal vein of the ear, with a kidney emulsion originating from rabbit 42/V (presence of Encephalitozoon) and that died, or were sacrificed from the 53rd to the 111th day, were contaminated. In two of these animals, Encephalitozoon was distinguished only in the encephal, while in the two others parasites were declared in the brain or in the kidney.

This trial proves that the infection is transmissible by inoculation of virus in the circulatory stream (see Protocol XII).

(1) Absence of parasites and of lesions in the inoculated sciatic nerve.

D. ___ INOCULATION BY INTRA-TESTICULAR METHOD

We inoculated in two rabbits, by intra-testicular method, an emulsion of encephal and of kidney originating from rabbit 71/B, containing some Encephalitozoan (Protocol XIII). The first of these animals died the 5th day, the second succumbed the 5th day. In one, as in the other, we found parasites in the kidney. Nevertheless, the examination of the testicles, practiced on sections as well as on smear preparations, revealed neither appreciable alterations, nor Encephalitozoan.

This experiment shows that microsporidian infection is transmissible by inoculation of virus in the testicular tissue; it seems to overcome the kidney, without localizing to begin with on the seminal gland.

E. ___ INFECTIOUSNESS OF THE PERITONEAL LIQUID.

In a certain number of our rabbits, contaminated experimentally, we verified a quite marked ascites. The examination of the peritoneal liquid brought to light rare lymphocytes, but it was impossible for us to find Encephalitozoan spores there. The presence of the ascites explains itself by the existence of renal lesions, so frequent in the course of epizootic encephalitis.

Ascites liquid was injected, by cerebral method, in a rabbit (Protocol XIV). This rabbit (89/B) was sacrificed the 132th day, without being visibly parasited.

The peritoneal liquid does not seem to close in the microsporidian germs, discernable on smear preparation, or by inoculation in fresh animals.

F. ___ INFECTION BY CONTACT

It was interesting to establish if the rabbits placed in the same cage as the experimentally infected animals were susceptible to contracting epizootic encephalitis. Two experiments of this kind were realised (cf. Protocols XV and XVI).

In the first one, we put into contact two Swedish rabbits 41/V and 42/V, with the great series of 10 animals having received, by cerebral method, the virus of spontaneous encephalitis, Parisian rootstock (cf. Protocol IV). These two rabbits lived in contact with the others during 165 and 147 days. They were then sacrificed. One of them (rabbit 41/V) showed neither lesions, nor Encephalitozoan. The other (rabbit 42/V)

offered intense alterations of the brain and of the kidney, with the presence of quite a large number of parasites. These same parasites had been passed in the urine.

In a second series of trials, two fresh animals were placed in the same cage as the contaminated rabbits 35/V, 37/V and 42/V. The first of these rabbits died the 60th day, without lesions or parasites. The second one was sacrificed the 119th day. Its brain as well as its kidney presented obvious alterations and some Encephalitozoan.

These results from it that the rabbits that live during a quite prolonged time (119 to 147 days) in contact with the animal carriers of Encephalitozoan contract the infection. The latter finishes by localising itself on the encephal and on the kidney (elimination of the germ by urinary secretion).

G. __ INFECTIOUSNESS OF THE URINE.

We verified, many times over, the presence of spores of Encephalitozoan in the urine, gathered together in vivo, by pressure on the bladder, or post mortem, by vesical puncture. The smear preparations, made with calot obtained by centrifugation of the urinary secretion, showed plate epithelial cells, granulous cylinders, absolutely typical leucocytes and spores. The experiment permitted to establish that these spores, present in the urine, were capable of germinating and of conferring encephalitis to fresh rabbits (cf. Protocol XVII).

In one of these experiments, some urine, gathered up post mortem in two rabbits whose kidneys were parasitized, was inoculated, by cerebral method, into rabbit 91. This urine contained spores. This rabbit was sacrificed the 123rd day; its brain as well as its kidney contained Encephalitozoans; these organs were obviously lesioned, besides.

One can transmit the infection in administering the urine, not only by cerebral method, but quite simply per os. Thus, in one of our trials, urine collected in vivo in rabbit 42/V [presence of parasites in the kidney (1) and in the urinary secretion] was administered two times, by the stamachic probe, to rabbit 190 (see Protocol XVIII). The animal was sacrificed the 103rd day. Its kidney, as well as its brain were strongly altered and contained Encephalitozoans.

These trials show that the urine of experimentally infected animals, or spontaneously contaminated, can enclose Encephalitozoan spores; moreover, it is virulent when it is administered to fresh rabbits, either by intra-cerebral method, or by gastric method.

(1) The kidney was examined later, when the rabbit was sacrificed.

Such results are very favorable to the hypothesis of natural transmission of epizootic encephalitis by gastro-intestinal method. The germ, multiplying in the kidney, eliminates itself by renal canaliculus (see Chapter III), invades the urine and thus contaminates the foods. The spores, deposited on these foods, are swallowed at the same time as they are, then, by a still imprecise mechanism, succeed in crossing the barrier that opposes them the bucho-pharyngeal and gastro-intestinal mucous membranes. Do they germinate in the intestine itself? Are they englobed by the leucocytes that transport them elsewhere? So many problems that rest to be solved.

H. — IS THE VIRUS OF SPONTANEOUS ENCEPHALITIS A FILTERABLE VIRUS?

In the beginning of this Memoir we saw that, according to Kling and his collaborators, the so called "Swedish" virus would be capable of traversing the filter candles in porcelain. In the species, it would be a question of a filterable virus, similar to the ultra-virus of encephalitis (Lavediti and Harvier), of the herpes (Luger and Lauda), of the poliomyelitis (Landsteiner and Lavediti), etc. On his part, C. C. Twort, basing himself on simple analogies, envisaged, he himself, the virus of epizootic encephalo-myelitis as a germ belonging to the group of invisible and filterable microorganisms.

Despite the microsporidian nature of the etiological agent, demonstrated by our observation, we experimentally researched to see if this agent was capable of traversing the Chamberland candles nos. I and III. The dimensions of the Encephalitozoan spores render very little probable their filterability. It is possible however that the Microsporidia comprises, in the course of its evolutive cycle, unsuspecting forms, small enough to cross through the filters. What does the experiment show on this subject?

We prepared a cerebral emulsion rich in Encephalitozoan, that we first centrifuged, then filtered under pressure through a Chamberland candle no. III (sterile filtrate, see Protocol XIX). The filtrate was inoculated by intra-cranial method, into four rabbits, that were sacrificed from the 84th to the 128th day. Not one of them presented lesions or Encephalitozoans.

In a second series of experiments, the filtrate (Chamberland candle no. I), prepared from two encephals containing parasites, was administered, by intra-cerebral method, to 7 rabbits. These died or were sacrificed from the 102nd to the 111th day. The result was similar to the preceding: total absence of microscopic alterations and of parasites, in the brain as well as in the kidney (cf. Protocol XX).

One can conclude from these different researches that the Encerhalitozoan guniculi is not composed of visible forms, capable of passing through filter cordles.

I. VIRULENCE OF THE Encerhalitozoan guniculi
For ANIMAL SPECIES OTHER THAN THE RABBIT.

1 Guinea pig. — Our experiments on the guinea pig are too few to permit definitive conclusions (see Protocols XXI, XXII AND XXIII). In animals infected by cerebral or peritoneal method, and who survived from fifteen to forty-one days, we found neither lesions, nor parasites in the kidney or brain. It was the same thing in guinea pigs sacrificed the 168th day; however, in one of these last animals, it seemed to us that a microsporidian was detectable on the smear preparation kidney.

2 DOG. — A dog was inoculated, by intra-cranial method, with a cerebral emulsion of mouse containing numerous Encerhalitozoans. The animal succumbed the 22nd day. One verified, at the necropsy, an intense congestion of the meninges and of the brain. Quite a few spores were discernable on the smear preparations of encephalitis (see Protocol XXIV).

3 HONKEY. — A cerebral emulsion of mouse, rich in Encerhalitozoan, was injected in the brain of a Macaca cynomolgus. The animal, sacrificed the 32nd day, showed neither microscopic alterations, nor cysts or microsporidian spores (see Protocol XXV).

4 RAT. — Control researches assured us, first of all, that rats originating from the same rearing of the Pasteur Institute that furnished our rats do not seem subject to a spontaneous infection by the Encerhalitozoan guniculi (1). In fact, the examination of the brain of twelve fresh rats showed negative, on the smear preparation as well as on the sections.

In a first series of experiments (see Protocol XXVI), four rats were inoculated by peritoneal method, with an emulsion of rabbit brain containing quite a few Encerhalitozoan spores (rabbit 42/V). These animals died from the 22nd to the 67th day. Two proved to be contaminated, namely: rat 1, dead the 22nd day (presence of microsporidian spores in the peritoneal cells (see page 699), and rat 2, dead the 56th day (parasites on

(1) It is the same in the guinea pig (nine negative results on nine examinations).

smear preparation of encephal).

Same result in a second series of trials. This time, we injected in the peritoneal cavity of four rats a kidney emulsion originating from the same rabbit 42/V (presence of Encephalitozoan). The animals died from the 31st to the 4th day. Rat 1 showed typical parasites in the liver; Rat 2 offered, on smear preparation, spores localized in the encephal (see Protocol XXVII).

The rat is thus susceptible to contract the infection by injection of virus in the peritoneal cavity.

5 MUSE. — In the course of our researches on the transmission of spontaneous encephalitis to the mouse, we examined sections of brain of a mouse originating from the rearing of the Pasteur Institute, and which had never been inoculated. We there declared the presence of encephalitis lesions and of typical microsporidian cysts. Later on, we sacrificed 7 mice having lived in contact of our contaminated animals and 7 others originating directly from the Pasteur rearing. The first series comprised 5 parasitized mice; 3 of the animals of the second serie offered Encephalitozoons localized in the encephal.

It resulted from these first examinations that the mouse is subject to enzootic spontaneous encephalitis, provoked by a Microsporidia offering all of the characteristics of Encephalitozoan cuniculi. Between time, appeared a work by Cowdry and Nicholson (1) ending up in the same conclusions as ours. The authors find, in 25 mice on 141 examined at the Rockefeller Institute, chronic encephalitis lesions resembling the alterations described in the rabbit by Bull, Oliver and C. C. Twort. Moreover, they there discover parasites (spores of 1.8 to 2 microns long by 0.5 to 0.8 microns wide; cysts) that they compare to the Encephalitozoan cuniculi.

These declarations show that the mouse is frequently infected by a virus of apparently the same nature as the etiological agent of enzootic encephalomyelitis of the rabbit. In what proportion? All depends, very logically, of the rearings which furnish the animals, and also from the precise moment where the mice of the same origin are examined. After our investigations, on 37 animals of normal appearance, 26 presented encephalitozoa in the brain, as well as more or less pronounced lesions, be it a percentage of 70 0/0.

One conceives that the frequency of the spontaneous malady in the mouse renders difficult, if not impossible, experimental study of the infection on this species of animal. Also, we are embarrassed to formulate

(1) COWDREY and NICHOLSON. Journal of the Americ. med. Assoc., 82, February 4, 1924, p. 545.

no matter how precise it be on the subject of results furnished by numerous trials undertaken on the mouse, in the goal of elucidating diverse problems, such as, for example, the filterability of the virus, its methods of penetration, the mode of contagion, etc. Let us say, simply, that the inoculation of the most varied virulent materials (brain, kidney, urine, peritoneal liquid, dried out virus, virus conserved in glycerin, etc.), practiced on 92 mice, furnished 56 clearly positive results, let us say on a percentage of 60.8 p. 100.

In order to precise if the infection is hereditarily transmissible in the mouse, we examined the encephal of a great number of descendants aged from two to thirty days, issues of injected mothers and who had lived in contact with the mothers. All of the examinations stayed negative (smear preparation and sections, see Protocol XXVIII). A single small mouse, fifteen days of age, on the 37 animals in observation, was parasited, among its brothers and sisters belonging to the same litter. It is strongly probable that this little mouse infected himself in contact with the mother.

These declarations, as does the absence of Encephalitozoon in the ovary and the testicle of rabbits and of mice stricken with spontaneous encephalo-myelitis, renders hardly believable the hereditary transmission of the infection(1).

Conclusions.

The ensemble of data exposed in this chapter shows that the Encephalitozoon cuniculi can be present, either in the encephal, or in the kidney, or still yet simultaneously in these two organs, in rabbits inoculated with the viruses of Kling and of Thalhimer, or experimentally infected with the epizootic encephalo-myelitis virus. The brain seems more frequently parasited than the kidney (66 p. 100 in place of 33 p. 100).

In addition, the infection is transmissible to the rat, to the dog and to the mouse. This last animal species is subject to an epizootic

(1) We did not discover parasites in the placenta and the embryos of a rabbit stricken by epizootic encephalitis (experimental inoculation). Since the editing of this Memoir, we examined more closely the question of the contaminations of descendants issues of parasited procreators (mouse). Certain litters are infected, while others can be indem. We will return to this subject later on.

encephalitis provoked by a microsporidia resembling an Encephalitozoon cuniculi, malady that seemed to transmit itself by contact and which does not seem hereditary.

In the rabbit, the virus eliminates itself by urinary secretion. Spontaneous contamination effectuates itself by the intermediary of foods which contaminate the urine, rich in microsporidian spores. Penetration of the germs in the organism seems to operate through the nasal-pharyngeal and gastro-intestinal mucous membrane.

CHAPTER III

MORPHOLOGICAL STUDY OF THE ENCEPHALITOZOAN CUNICULI

The morphological characteristics of the Encephalitozoon cuniculi were defined on the smear preparation and on the sections.

1. SMEAR PREPARATION METHOD. Technique: Fixation of dried out smear preparations, by Bouin-Brazill liquid, from twenty minutes to two hours, water bath; minutes in absolute alcohol, water bath. Coloration;

- a) Cresyl G (1 p. 100) for ten minutes, water bath;
- b) Eosin (1 p. 100* for twenty to thirty minutes, water bath;
- c) Ethia polychrome of Hanna (1/10) for fifteen to twenty minutes; water bath. Differentiation by absolute alcohol added to essence of clove. Absolute alcohol, xylol, mounting in balsam.

a) Examination in the fresh state: One takes up a small fragment of cerebral skin, that one breaks up between the slide and the glass cover, after addition of a few drops of dirty isotonic water. The examination permits to discover microsporidian spores, refractive corpuscles, oval or lightly pear-shaped, without structural details. These spores are immobile and are not colored by the blue of the methylene(vital coloration) (1).

(1) It was impossible for us to provoke the departure of the germ from the filament, in making the diluted acids react on the fresh preparations. Besides, the observation is rendered difficult by the result of the opacity of the medium (cerebral or renal emulsion).

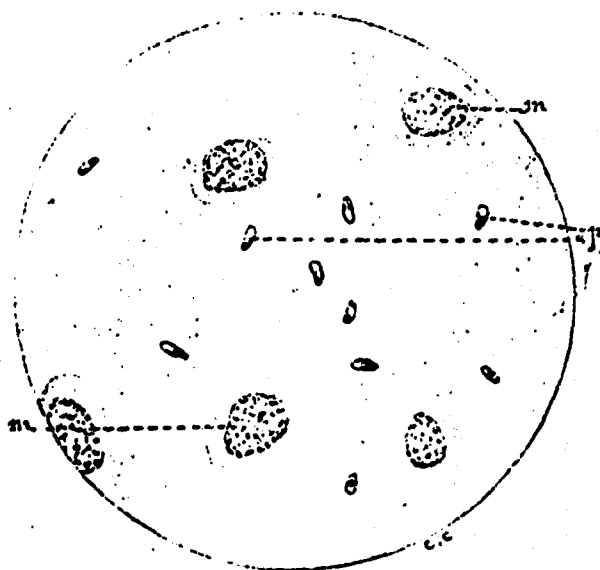


Fig. 6. Smear preparation of rabbit brain stricken with spontaneous encephalitis. m, mononuclear cells; n, Encarnalitozon uniculi. Eosin-orange-blue polychrome of Unna. Bulk: 1/1000.

b) Examination after coloration: Certain smear preparations of brain have the appearance of a rich culture of microsporidies.

One meets with, on each microscopic field, 2-5 to 20 isolated spores, or deposited by groups. The aspect of these spores is the following: the corpuscle is delimited by a membrane, contains a biconcave disk of chromatin, deposited transversally, situated nearer one of the two poles and separating the two polar vacuoles. These vacuoles are of unequal dimensions; the great vacuole is situated near the the least drawn out extremity of the spore. In this vacuole one distinguishes a grain of chromatin, appearing attached to a thin filament (see. fig. 6 and 7; Plate IV, fig. 9).

Dimensions: longitudinal = 2.5 microns; transversal = 0.5 microns by 1 micron.

c) Coloring and histo-chemical reactions: The microsporidian spores, arrived at a state of maturity, do not color on smear preparation (brain of mouse or of rabbit), by the Trenchrome of Laveran, or by the prolonged Giemsa, after fixation in absolute alcohol. Only the young forms (sporoblasts) color in a deep violet by these procedures. The envelope of the

spore seemed impermeable to the basic coloring materials, as well as to iron hematoxylin. The previous fixation of the Bouin-Brazil smear preparations modifies the permeability of this membrane and renders the spore colorable by the orange-blue polychrome eosin of Unna or by LeMann. With this last technique, the spore appeared tinted in bright red, on the blue background of the preparation (analogy with the Negri bodies) (see Plate IV, fig. 9).

On the example of Guyenot and Neville (1) (study of the microsporidia of the snake, Glucos donilewskii), we modified the permeability of the spores' membrane, in treating the smear preparation (before all fixation) by the normal sodium carbonate, pure chlorhydric acid and sulfuric acid at 5 p. 1,000 (two to four minutes). This preliminary treatment facilitates the colorability of the spores by iron hematoxylin and the precision of certain structural details. Let us add that the coloration methods of the bacterial spores (mordant action with chromic acid, coloration by carbol fuchsin) stay without effects on the spores of the Encephalitozoon. These spores are not acid-resistant.

d) Details of structure: The spores (smear preparation of mouse brain), treated first of all with chlorhydric acid, colored next by iron-hematoxylin, show the structural details represented by figure 8. The spore closes in one or two chromatic granulations (nuclei), situated in the vacuole corresponding to the posterior pole (d, e, i). The most frequent aspect is the one drawn in i. In c, one sees a spore strangled by the medium, as if it divided itself transversally. In f, a median disk separates the two polar vacuoles. In h, the chromatin is deposited right against the wall of the spore, eccentrically.



Fig. 7. Smear preparation of parasitized mouse brain. Encephalitozoon isolated or disposed in mass. Coloration with eosin-orange-blue of Unna.

(1) GUYENOT and NEVILLE. Swiss Review of Zoology, 30, no. 1, 1922.

Figure 9 shows spores colored by the safranin. In a, the two nuclei seem to be tied together by a thin filament of chromatin; in b, the two nuclear formations are polar.

2. SECTION METHODS. Technique: Fixation of the tissues by the Bouin-Brdil liquid, paraffin sections. Methods employed: Mann; iron-haematoxylin; Safranin-micro-indigo-carmin; Twort; Giemsa prolonged (forty-eight hours); Blue polychrome of Unna. This last method comprises a few details.

- a) Coloration to orange G (1 p. 100), during twenty minutes; water bath;
- b) Coloration to eosin (1 p. 100), thirty to sixty minutes; water bath;
- c) Blue polychrome of Unna (pure), twenty minutes; same differentiation and mounting as for the smear preparation.

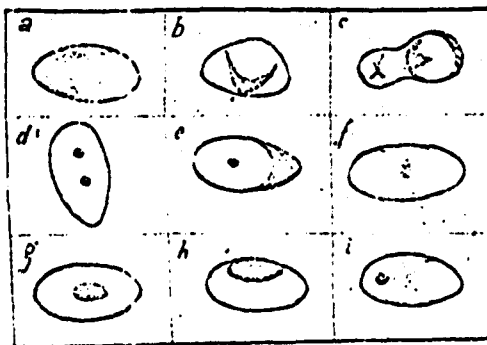


Fig. 8. Encerhalitozoon cuniculi spores. Smear preparation treated first of all with chlorhydric acid, next colored with iron haematoxylin.

We will study the morphology of the Encerhalitozoon cuniculi first of all in the rabbit, then in the rat and the mouse.

a) RABBIT. THE parasite could not be distinguished, up until now, except in the encephal and in the kidney.

ENCEPHAL (Brain, central and mesocephal nuclei). The parasite exists in variable quantity in the encephal, either at the level of the cortical nuclei and the under-cortical, or, more rarely, far from these nuclei (see Fig. 10). In this last case, one declares it in the interior of the cysts of variable dimension, being able to attain sometimes the diameter of a big pyramidal cell (20 to 30 microns). These cysts, constituted by a thin membrane, are spherical or ovoid; one or two flattened nuclei are molded on their wall. The cyst can enclose an incalculable number of parasites doubled one on top of the other. The spore is oval, pear-shaped or needle-shaped (cf. Plate III, fig. 6, 7 and 8; Plate IV, fig. 4; in the text, fig. 11).

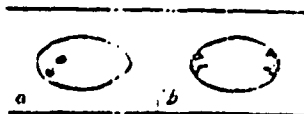
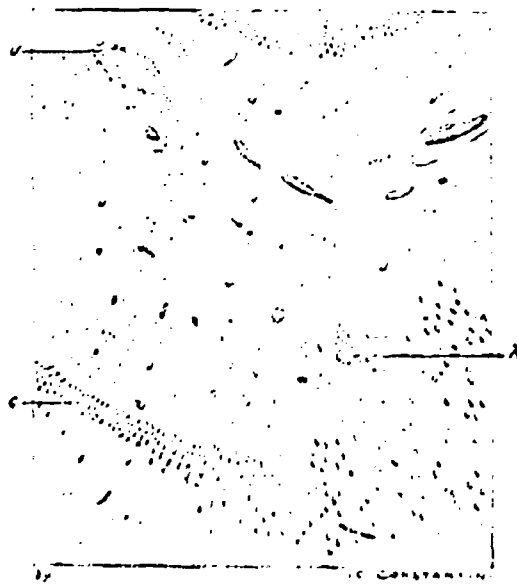


Fig. 9. Some spores, same coloration (cf. fig. 8).

On the level of analysis, or in their immediate neighborhood, the Engelskianers could be, among the same object as in the isolated eyes. However, the forms then undergo relative modifications, which takes for the fact that they are insufficient to identify. Following



_____ 2017 (10/10/17). _____
 _____ 2017 (10/10/17). _____

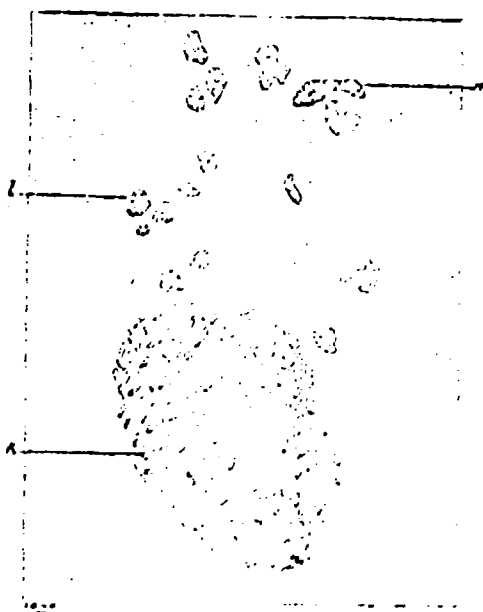


Fig. 11. -- E. coli 36/V, inoculated June 27, 1941,
1. 1st method, with an excysted embryo from rabbit 36/V, striated
with a, numerous small illis, large infectious. Gravidized the embryo
1. 2. -- 2, cyst containing numerous large infectious oocysts. Beginning
1. 3. -- 3, around the cyst. 1, multinuclear; 2, binuclear. 11th method.

122. IV. The alterations in the skin as well as in the internal organs were caused by the action of the melanin substance and its derivatives at the site of the degenerative and inflammatory. In the cortical zone, the lesion under the aspect of a thick, light-colored band (cf. fig. 12). The lesion is also restrained by an interstitial inflammatory tissue, constituted by lymphocytes, big mononuclear cells, and, so often, by a chondroid detritus of increased elements.

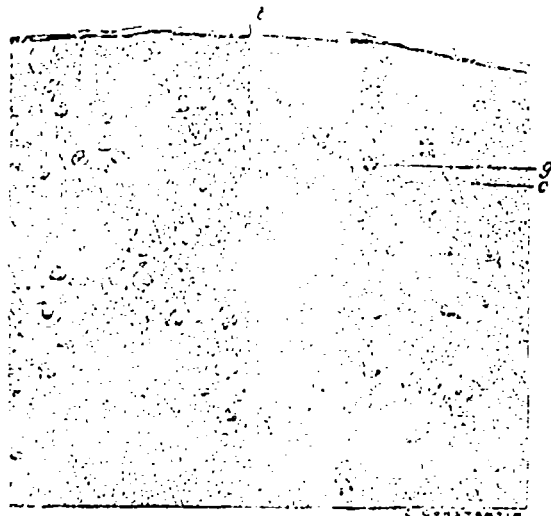


Fig. 12. Section 122.V (cf. fig. 11). Section 122.V.
1, skin; 2, cortical zone; 3, cortical layer; 4, dermis.

In the level of the papilla, the lesion consists of a thick (papular) zone, in which the cells are similar to the cells that are observed in the epidermis. In the center of these papules the straight papules are, either totally or partially, or even by dilated. In the last case, their light contains a dense, granular substance, constituted by a pile of epithelial cells that are separated and of white polymorphic globules, in a state of decay. Granulated vessels are found in these papules.

A melanin can there be discovered easily, above all when one uses the method, or still the ordinary procedure of Unna procedure (1).

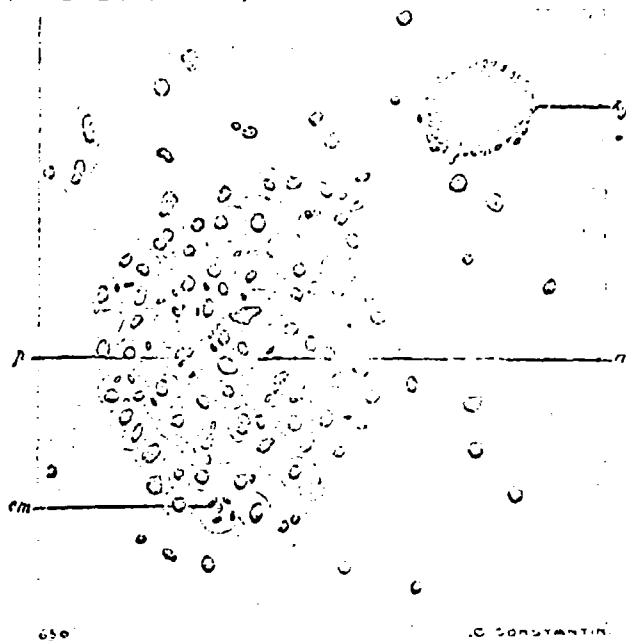
(2. Section 122.V (cf. fig. 11) in using Curtis's procedure (conf. method, p. 122.V).

It multiplies rapidly exclusively in the epithelial cells which cover the renal tubules. One there variously sized cysts of variable size, containing 2 to 4 spores, or several groups of ten of spores (cf. fig. 13). In the



FIG. 13. Paratyphlocyba, isolated November 22, 1942, with the Paratyphlocyba Paratyphlocyba; intra-epithelial cysts; line the lower edge: Paratyphlocyba Paratyphlocyba; Paratyphlocyba Paratyphlocyba; Paratyphlocyba Paratyphlocyba. All of the epithelial cells (K, L) are paratized. In the Paratyphlocyba cysts (K, L) and its contents were spread in the light of the microscope. (Gross method).

beginning, the isolated epithelial cells were absolutely normal; the proto-plasma colorless; the nucleus, if still, reflected near the periphery of the cell a vertical line of chromatin and a nucleolus in perfect state. After on, the cyst, containing beyond measure, spores and the spores spread in the light



Page No.: 1, Total Number of pages: 1, Date of completion: 10/10/2023

3. The tumor center can be put in evidence in the center of the nodules, showing the cellular debris and homogeneous debris which constitutes the central necrotic mass shown. Metastases, their aspect seems to be the same. The enlarged involvement of the lymphatic, blood vessels, and the blood-colored in the interlobular connective tissue, they often show the presence of one or more cells in the inter-lobular lymphatic.

... of it now, and divided the others, the other others seemed to not
... of it. In the middle of the ... in the ... the ...
... the ... the ... the ... the ...
... the ... the ... the ... the ...
... in the last ... in the ... the ...
... and, however, ... of those ... seem
... altered, ... the liver, the ... and the lung.

The spleen sometimes presents a myeloid transformation of the most acute or a, with myelocytes and metamyelocytes. The splenic sinusoids often in great numbers contain an excess of eosinophils. In the liver and the lung, are distinct nodules of lymphocyte nodules; it is the same thing in the muscularis. But, still again, all of these lesions are devoid of parasites.

5) KODIE. — The mouse, rabbit, can present not only intra-cerebral cysts, but also perivascular disks and genuine nodules. These last mentioned are constituted by a pile of cells of an epithelioid aspect, with abundant protoplasm, colorable in greenish blue by Leffmann. (cf. fig. 1, 15 and 16). These cells enclose typical spores and have the aspect of

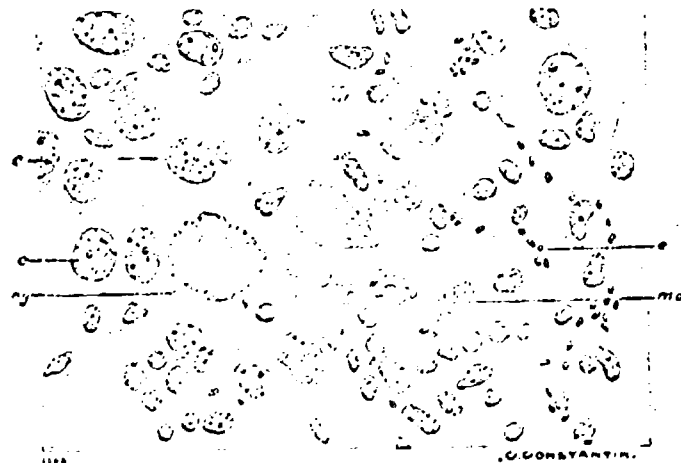


FIG. 15. — Brain of a mouse, spontaneous encephalitis, acute stage. — Greenish blue, brain corn; a, nerve cells of normal aspect; b, cyst enclosing in numerous Leffmann spores; inflammatory reaction in the brain corn, with c, mononuclear cells, and d, free or aggregated spores. Leffmann method.

In sum, the study of the morphology of the set of Brachypteryx cinctus, or one of its relatives, with the morphology of the set of Brachypteryx cinctus, is a very important part of the study of the set of Brachypteryx cinctus.

1. The first step in the process is to identify the problem or issue that needs to be addressed. This involves gathering information and understanding the context of the problem.

[illegible][illegible]

EVOLUTION OF THE DISSEMINATION COEFFICIENT

1. IN THE MUSEUM. — An oval, pale specimen, poor in detail, which was included in the peritremes of several slides. One of these slides, eight days after the inoculation, the epithelial lining of the peritreme of peritremes included in the slide-epithelial cells. The peritremes of peritremes is rejected to the epithelium, the peritremes of the peritremes filled with a protozoan. The peritremes is present in the peritremes of the peritremes, included in the peritremes of the peritremes.

2. In this NLT infected by peritonitis caused, we discovered, on smear preparation of peritoneum, vol. numerous ciliated elements, containing filum or nitro stored (fig. 19); other elements 1 collar (fig. 17, at the top on up to the left) often a median retractor towards the territory and a pseudo-protect containing a chain in control of direction. These are constituted by two granulitins of character, up, arising tied by a filament, they are surrounded by a capsule.

(1) 50 M.M.. Journal of the American Medical Association, Chicago, 1916.

Albert's records for August 1951, sent to Tanager, Vol. 21(2). The Tanager scientists observed Two immatures in rabbits incubated with the "G-13" virus" of 1951, and in 1952 and 1953 at the same place. One of the group of immatures, called immature and verified this date. One of the photographs, which recalls the pre-epidemic phase previously described by us.

As for Illing, Little and Little's (1), they do not at all agree with our way of viewing things, especially with the relative value in our devastation. The same idea is repeated in the "World's" collection and runs on as a small volume of or perhaps 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 8

- [illegible]

[illegible][illegible]

1942-43, 1944.

• • •

Protocol 1. 222, 223

1. James Earl Ray (DOB: 1928-05-01) - John Edgar Hoover (DOB: 1918-01-30),
FBI Director, 1962-1972

	89 V	87 H	42 Ac
1000 au. 80 jours	S. 105 jours	M. 115 jours	S. 114 jours
Gripes et infections	Posit.	Posit.	Posit.
Parasites (1. parasites	Posit.	"	Posit.

2. 1916

5. 1916

10. The no. of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1100, 1110, 1120, 1130, 1140, 1150, 1160, 1170, 1180, 1190, 1200, 1210, 1220, 1230, 1240, 1250, 1260, 1270, 1280, 1290, 1300, 1310, 1320, 1330, 1340, 1350, 1360, 1370, 1380, 1390, 1400, 1410, 1420, 1430, 1440, 1450, 1460, 1470, 1480, 1490, 1500, 1510, 1520, 1530, 1540, 1550, 1560, 1570, 1580, 1590, 1600, 1610, 1620, 1630, 1640, 1650, 1660, 1670, 1680, 1690, 1700, 1710, 1720, 1730, 1740, 1750, 1760, 1770, 1780, 1790, 1800, 1810, 1820, 1830, 1840, 1850, 1860, 1870, 1880, 1890, 1900, 1910, 1920, 1930, 1940, 1950, 1960, 1970, 1980, 1990, 2000, 2010, 2020, 2030, 2040, 2050, 2060, 2070, 2080, 2090, 2100, 2110, 2120, 2130, 2140, 2150, 2160, 2170, 2180, 2190, 2200, 2210, 2220, 2230, 2240, 2250, 2260, 2270, 2280, 2290, 2300, 2310, 2320, 2330, 2340, 2350, 2360, 2370, 2380, 2390, 2400, 2410, 2420, 2430, 2440, 2450, 2460, 2470, 2480, 2490, 2500, 2510, 2520, 2530, 2540, 2550, 2560, 2570, 2580, 2590, 2600, 2610, 2620, 2630, 2640, 2650, 2660, 2670, 2680, 2690, 2700, 2710, 2720, 2730, 2740, 2750, 2760, 2770, 2780, 2790, 2800, 2810, 2820, 2830, 2840, 2850, 2860, 2870, 2880, 2890, 2900, 2910, 2920, 2930, 2940, 2950, 2960, 2970, 2980, 2990, 3000, 3010, 3020, 3030, 3040, 3050, 3060, 3070, 3080, 3090, 3100, 3110, 3120, 3130, 3140, 3150, 3160, 3170, 3180, 3190, 3200, 3210, 3220, 3230, 3240, 3250, 3260, 3270, 3280, 3290, 3300, 3310, 3320, 3330, 3340, 3350, 3360, 3370, 3380, 3390, 3400, 3410, 3420, 3430, 3440, 3450, 3460, 3470, 3480, 3490, 3500, 3510, 3520, 3530, 3540, 3550, 3560, 3570, 3580, 3590, 3600, 3610, 3620, 3630, 3640, 3650, 3660, 3670, 3680, 3690, 3700, 3710, 3720, 3730, 3740, 3750, 3760, 3770, 3780, 3790, 3800, 3810, 3820, 3830, 3840, 3850, 3860, 3870, 3880, 3890, 3900, 3910, 3920, 3930, 3940, 3950, 3960, 3970, 3980, 3990, 4000, 4010, 4020, 4030, 4040, 4050, 4060, 4070, 4080, 4090, 4100, 4110, 4120, 4130, 4140, 4150, 4160, 4170, 4180, 4190, 4200, 4210, 4220, 4230, 4240, 4250, 4260, 4270, 4280, 4290, 4300, 4310, 4320, 4330, 4340, 4350, 4360, 4370, 4380, 4390, 4400, 4410, 4420, 4430, 4440, 4450, 4460, 4470, 4480, 4490, 4500, 4510, 4520, 4530, 4540, 4550, 4560, 4570, 4580, 4590, 4600, 4610, 4620, 4630, 4640, 4650, 4660, 4670, 4680, 4690, 4700, 4710, 4720, 4730, 4740, 4750, 4760, 4770, 4780, 4790, 4800, 4810, 4820, 4830, 4840, 4850, 4860, 4870, 4880, 4890, 4900, 4910, 4920, 4930, 4940, 4950, 4960, 4970, 4980, 4990, 5000, 5010, 5020, 5030, 5040, 5050, 5060, 5070, 5080, 5090, 5100, 5110, 5120, 5130, 5140, 5150, 5160, 5170, 5180, 5190, 5200, 5210, 5220, 5230, 5240, 5250, 5260, 5270, 5280, 5290, 5300, 5310, 5320, 5330, 5340, 5350, 5360, 5370, 5380, 5390, 5400, 5410, 5420, 5430, 5440, 5450, 5460, 5470, 5480, 5490, 5500, 5510, 5520, 5530, 5540, 5550, 5560, 5570, 5580, 5590, 5600, 5610, 5620, 5630, 5640, 5650, 5660, 5670, 5680, 5690, 5700, 5710, 5720, 5730, 5740, 5750, 5760, 5770, 5780, 5790, 5800, 5810, 5820, 5830, 5840, 5850, 5860, 5870, 5880, 5890, 5900, 5910, 5920, 5930, 5940, 5950, 5960, 5970, 5980, 5990, 6000, 6010, 6020, 6030, 6040, 6050, 6060, 6070, 6080, 6090, 6100, 6110, 6120, 6130, 6140, 6150, 6160, 6170, 6180, 6190, 6200, 6210, 6220, 6230, 6240, 6250, 6260, 6270, 6280, 6290, 6300, 6310, 6320, 6330, 6340, 6350, 6360, 6370, 6380, 6390, 6400, 6410, 6420, 6430, 6440, 6450, 6460, 6470, 6480, 6490, 6500, 6510, 6520, 6530, 6540, 6550, 6560, 6570, 6580, 6590, 6600, 6610, 6620, 6630, 6640, 6650, 6660, 6670, 6680, 6690, 6700, 6710, 6720, 6730, 6740, 6750, 6760, 6770, 6780, 6790, 6800, 6810, 6820, 6830, 6840, 6850, 6860, 6870, 6880, 6890, 6900, 6910, 6920, 6930, 6940, 6950, 6960, 6970, 6980, 6990,

Protocole II. — Virus Tard.

Virus cérébral du lapin 100, inoculé dans le cerveau des lapins 12. Résultat négatif.

N° des lapins	100	101	102	103	104
Mort ou sacrifié	M. 65 j.	S. 102 j.	S. 102 j.	S. 102 j.	S. 102 j.
Frattus	Cerv. Posit.	0	0	Posit.	0
Couper	Rein. 0	0	Posit.	Posit.	0
Couper	Lés. 0	0	Posit.	Posit.	0
Couper	Par. 0	0	0	0	0
Couper	Lés. 0	0	Posit.	Posit.	Posit.
Couper	Par. 0	0	Posit.	Posit.	Posit.

Protocole III. — Virus Thalmine.

Virus thalmine 100-1-2, passé deux fois sur lapin, inoculé dans le cerveau des lapins.

Mort ou sacrifié	S. 107 jours	S. 107 jours
Numéro des lapins	36/V	36/V
Frattus	Lés. 0	Posit.
Couper	Cerv. 0	Posit.
Couper	Par. 0	Posit.
Couper	Meso. Lés. 0	Posit.

Protocole IV.

Virus cérébral 36/V (Posteur), inoculé dans le cerveau des lapins.

Numéro des lapins	31/V	32/V	33/V	34/V	35/V
Mort ou sacrifié	S. 83 j.	M. 83 j.	S. 105 j.	S. 105 j.	S. 110 j.
Frattus	Cerv. 0	0	0	0	0
Couper	Rein. 0	0	0	0	0
Couper	Lés. 0	Posit.	Posit.	Posit.	Posit.
Couper	Par. 0	0	Posit.	Posit.	Posit.
Couper	Lés. 0	0	Posit.	Posit.	Posit.
Couper	Par. 0	0	Posit.	Posit.	0
Numéro des lapins	36/U	36/V	36/V	36/V	36/V
Mort ou sacrifié	S. 118 j.	S. 118 j.	S. 118 j.	S. 118 j.	S. 118 j.
Frattus	Cerv. 0	0	Posit.	0	0
Couper	Rein. 0	0	Posit.	0	0
Couper	Lés. 0	Posit.	Posit.	Posit.	0
Couper	Par. 0	Posit.	Posit.	Posit.	0
Couper	Lés. 0	Posit.	Posit.	Posit.	Posit.
Couper	Par. 0	0	Posit.	Posit.	0

Protocole V.

Virus cérébral des lapins 35 V, 36 V et 41 V, inoculé dans le péritoine et le cerveau des lapins :

		DANS LE PÉRITOINE			DANS LE CERVEAU ET LE PÉRITOINE	
		7 B	9 B	10 B	2 B	4 B
I	Nombre des lapins	5	5	5	5	5
	Mort ou sacrifié	5.115 j.	M. 75 j.	S. 147 j.	M. 125 j.	M. 80 j.
II	Cerveau	Posit.	0	Posit.	0	0
	Protus (Rein)	0	Posit.	Posit.	0	0
III	Coupes (Lés.)	Posit.	0	Posit.	0	0
	ceru. (Par.)	0	0	Posit.	0	0
IV	Coupes (Lés.)	0	Posit.	Posit.	0	0
	rein. (Par.)	0	Posit.	Posit.	0	0

Protocole VI.

Virus cérébral des lapins 35 V, 36 V et 41 V, inoculé dans le cerveau des lapins :

		31 B	74 B	76 B	77 B
		M. 70 j.	M. 52 j.	M. 105 j.	S. 100 j.
I	Nombre des lapins	5	5	5	5
	Mort ou sacrifié	M. 70 j.	M. 52 j.	M. 105 j.	S. 100 j.
II	Cerveau	0	0	0	Posit.
	Protus (Rein)	Posit.	0	Posit.	0
III	Coupes (Lés.)	0	0	Posit.	0
	ceru. (Par.)	0	0	Posit.	0
IV	Coupes (Lés.)	0	0	Posit.	Posit.
	rein. (Par.)	0	0	Posit.	0

Protocole VII.

Virus cérébral des lapins 35 V et 37 V, inoculé dans le cerveau des lapins :

I	Nombre des la-						
	pins	5	32	77	94	95	99
	Mort ou sacrifié .	M. 110 j.	S. 115 j.	S. 115 j.	S. 115 j.	M. 125 j.	S. 125 j.
	Cerveau	0	0	0	0	0	0
	Protus (Rein)	0	0	0	0	Posit.	0
	Coupes (Lés.)	Légère.	0	0	0	0	0
	ceru. (Par.)	0	0	0	0	0	0
	Coupes (Lés.)	0	0	0	0	Posit.	0
	rein. (Par.)	0	0	0	0	Posit.	0

Protocole VIII.

Virus rénal des lapins 36/V, 70/V et 40/V, inoculé dans le cerveau des lapins :

Numéro des lapins	71 B	70 B	36 B	91 B
Mort ou sacrifié	M. 71 j.	M. 81 j.	S. 132 j.	M. 107 j.
Frottis { Cerv.	Posit.	Posit.	0	0
{ Rein	Posit.	0	Posit.	0
Coupages { Lés.	0	0	Légères.	0
{ cerv. { Par.	0	0	0	0
Coupages { Lés.	0	0	Posit.	0
{ rein. { Par.	0	0	Posit.	0

Protocole IX.

Virus rénal du lapin 27/V, inoculé dans le cerveau des lapins :

Numéro des lapins	95 77/12	97
Mort ou sacrifié	M. 75 jours	S. 112 jours
Frottis { Cerv.	0	0
{ Rein	0	0
Coupages { Lés.	0	Légères.
{ cerv. { Par.	0	0
Coupages { Lés.	0	0
{ rein. { Par.	Posit.	0

Protocole X.

Virus rénal des lapins 33/V et 37/V, inoculé dans le cerveau des lapins :

Numéro des lapins	80	81
Mort ou sacrifié	M. 55 jours	S. 125 jours
Frottis { Cerv.	0	Posit.
{ Rein	0	0
Coupages { Lés.	0	Posit.
{ cerv. { Par.	0	Posit.
Coupages { Lés.	0	0
{ rein. { Par.	0	0

Protocole XI.

Virus rénal du lapin 42/V, inoculé dans le cerveau des lapins :

Numéro des lapins	305	308
Mort ou sacrifié	M. 61 jours	M. 55 jours
Frottis { Cerv.	0	0
{ Rein	0	Posit.
Coupages { Lés.	0	Posit.
{ cerv. { Par.	0	0
Coupages { Lés.	0	Posit.
{ rein. { Par.	0	Posit.

Protocole XI bis.

Virus rénal du lapin 41/1, inoculé dans le nerf sciatique des lapins :

Numéro des lapins	41/1	50/1
Mort ou sacrifié	M. 15 jours	S. 115 jours
Frottis } Cerveau	Posit.	0
} Rein	Posit.	0
Coupes } Lés.	Posit.	0
} ceru. / Par.	Posit.	0
Coupes } Lés.	Posit.	0
} rein. / Par.	Posit.	0
Nerf sciatique	0	

Protocole XII

Virus rénal du lapin 41/1, inoculé dans les reins des lapins :

Numéro des lapins	41/1	51/1	52/1	53/1
Mort ou sacrifié	M. 3 j.	S. 111 j.	S. 111 j.	S. 111 j.
Frottis } Cerveau	0	Posit.	Posit.	Posit.
} Rein	Posit.	Posit.	0	0
Coupes } Lés.	0	Posit.	Posit.	Posit.
} ceru. / Par.	0	Posit.	Posit.	0
Coupes } Lés.	0	Posit.	Posit.	Posit.
} rein. / Par.	Posit.	0	0	0

Protocole XIII.

Virus cérébral et rénal du lapin 51/1, inoculé dans les testicules des lapins :

Numéro des lapins	51/1	52/1
Mort ou sacrifié	M. 57 jours	M. 56 jours
Frottis } Cerveau	0	0
} Rein	Posit.	Posit.
Coupes } Lés.	0	0
} ceru. / Par.	0	0
Coupes } Lés.	0	Posit.
} rein. / Par.	0	Posit.

Protocole XIV.

Liquide péritonéal du lapin 40/V, inoculé dans le cerveau du lapin :

Numéro du lapin	40/V
Mort ou sacrifié	S. 132 jours
Frottis } Cerveau	0
} Rein	0
Coupes } Lés.	0
} ceru. / Par.	0
Coupes } Lés.	0
} rein. / Par.	0

Protocole XV.

<i>Infection par cohabitation avec les lapins infectés du protocole IV. Lapins</i>		
Culte :		
Inoculé :		
Numéro des lapins	31/V	42/V
Mort ou sacrifié	S. 117 jours	S. 105 jours
Coupe } Lés.	0	Posit.
Cerv. } Par.	0	Posit.
Coupe } Lés.	"	Posit.
Rein. } Par.	"	Posit.

Protocole XVI.

<i>Infection par cohabitation, avec les lapins infectés du protocole IV. Lapins</i>		
Culte :		
Inoculé :		
Numéro des lapins	50	51
Mort ou sacrifié	M. 60 jours	S. 119 jours
Coupe } Cerv.	0	Posit.
Coupe } Rein.	0	Posit.
Coupe } Lés.	0	Posit.
Cerv. } Par.	0	Posit.
Coupe } Lés.	0	Posit.
Rein. } Par.	0	Posit.

Protocole XVII.

Urine des lapins 33/V et 37/V, inoculée dans le cerveau du lapin :

Numéro du lapin	91
Mort ou sacrifié	S. 125 jours
Coupe } Cerv.	0
Coupe } Rein.	0
Coupe } Lés.	Posit.
Cerv. } Par.	Posit.
Coupe } Lés.	Posit.
Rein. } Par.	Posit.

Protocole XVIII.

Urine du lapin 42/V, administrée « per os » :

Numéro du lapin	150
Mort ou sacrifié	S. 105 jours
Coupe } Cerv.	0
Coupe } Rein.	Posit.
Coupe } Lés.	Posit.
Cerv. } Par.	Posit.
Coupe } Lés.	Posit.
Rein. } Par.	Posit.

Protocole XIX.

Emulsion cérébrale filtrée des lapins 55/V, 75/U et 86/V, inoculée dans le cerveau des lapins :

Numéro des lapins	72 H	75 U	86 V	86 D
Mort ou sacrifié	S. 81 j.	S. 125 j.	S. 81 j.	S. 128 j.
Proitus } Cerve	0	0	0	0
} Rein	0	0	0	0
Coupes } Cerve	0	0	0	0
} Rein	0	0	0	0
Coupes } Cerve	0	0	0	0
} Rein	0	0	0	0

Protocole XX.

Emulsion cérébrale filtrée des lapins 55/V et 87/V, inoculée dans le cerveau des lapins :

Numéro des lapins	72	75	87	81
Mort ou sacrifié	S. 111 j.	S. 111 j.	S. 111 j.	M. 137 j.
Proitus } Cerve	0	0	0	0
} Rein	0	0	0	0
Coupes } Cerve	0	0	0	0
} Rein	0	0	0	0
Coupes } Cerve	0	0	0	0
} Rein	0	0	0	0

Numéro des lapins	82	79	877
Mort ou sacrifié	S. 111 j.	M. 102 j.	S. 111 j.
Proitus } Cerve	0	0	0
} Rein	0	0	0
Coupes } Cerve	0	0	0
} Rein	0	0	0
Coupes } Cerve	0	0	0
} Rein	0	0	0

Protocole XXI.

Emulsion filtrée

Emulsion cérébrale du lapin 42/V, inoculée dans le cerveau des cobayes :

Numéro des cobayes	21 A	22 A
Mort ou sacrifié	M. 26 jours	M. 45 jours
Proitus } Cerve	0	0
} Rein	0	0
Coupes } Cerve	0	0
} Rein	0	0
Coupes } Cerve	0	0
} Rein	0	0

ENCEPHALITE ÉPIDÉMIQUE DU LAPIN

709

Protocole XXII.

Émulsion cérébrale du lapin 42/V, inoculée dans le péritoine des cobayes :

Numéro des cobayes	48/A	49/A
Mort ou sacrifié	M. 41 jours	S. 105 jours
Frottis { Cerv.	0	0
{ Rein	0	Posit. (races apores).
Coupes { Lés.	0	0
{ Cerv. { Par.	0	0
Coupes { Lés.	0	0
{ Rein. { Par.	0	0

Protocole XXIII.

Émulsion rénale du lapin 42/V, inoculée dans le péritoine des cobayes :

Numéro des cobayes	50/A	52/A
Mort ou sacrifié	M. 41 jours	S. 105 jours
Frottis { Cerv.	0	0
{ Rein	0	0
Coupes { Lés.	0	0
{ Cerv. { Par.	0	0
Coupes { Lés.	0	0
{ Rein. { Par.	0	0

Protocole XXIV.

Émulsion cérébrale souris 4 (série V), inoculée dans le cerveau du chien :

Numéro du chien	2
Mort ou sacrifié	M. 22 jours
Frottis { Cerv.	Posit.
{ Rein	0
Coupes { Lés.	0
{ Cerv. { Par.	0

Protocole XXV.

Émulsion cérébrale souris 4 (série V), inoculée dans le cerveau du singe :

Singe	Mac. cynomolgus.
Mort ou sacrifié	S. 32 jours
Frottis cerv.	0
Coupes { Lés.	0
{ Cerv. { Par.	0

Protocole XXVI.

Emulsion de l'azote du lapin 42 N, inoculée dans le péritoine des rats :

Numéro des rats	R 1	R 2	R 3	R 4
Mort ou sacrifié	N. 22 j.	M. 36 j.	M. 60 j.	M. 67 j.
Étiologie	Par.	"	"	"
Étiologie	Par.	Post.	0	0
Coupes à l'ess.	0	0	0	0
Coupes à l'ess.	0	0	0	0

Protocole XXVII.

Emulsion stérile du lapin 42 N, inoculée dans le péritoine des rats :

Numéro des rats	R 1	R 2	R 3	R 4
Mort ou sacrifié	M. 42 j.	M. 42 j.	M. 42 j.	M. 42 j.
Étiologie	Par.	0	0	0
Coupes à l'ess.	0	0	0	0
Coupes à l'ess.	0	0	0	0
Coupes à l'ess.	0	0	0	0

Protocole XXVIII.

Transmission héréditaire de l'infécondité chez la souris :

Petite de mère n° 1. Petits morts : cerveau mère = 0.

Série n° 1 : 6 petits de 8 jours. Tous négatifs.

Série n° 2 : 6 petits de 10 jours. Tous négatifs.

Total : 12 petits, 0 négatifs.

Série n° 3 : 6 petits de 10 jours. Tous négatifs.

Série n° 4 : 6 petits âgés de 10 jours. Tous négatifs.

- A : 6 petits — de 1 jour. Tous négatifs.
- B : 6 petits — de 2 jours. Tous négatifs.
- C : 6 petits — de 4 jours. Tous négatifs.
- D : 6 petits — de 6 jours. Tous négatifs et 1 positif.
- E : 1 petit âgé de 1 jour. Négatif.
- F : 1 petit — de 2 jours. Négatif.
- G : 1 petit — de 4 jours. Négatif.
- H : 1 petit — de 6 jours. Négatif.
- I : 1 petit — de 10 jours. Négatif.

Total : 51 petits âgés de 1 heure à 10 jours. 50 négatifs et 1 positif.

1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 39. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50. 51. 52. 53. 54. 55. 56. 57. 58. 59. 60. 61. 62. 63. 64. 65. 66. 67. 68. 69. 70. 71. 72. 73. 74. 75. 76. 77. 78. 79. 80. 81. 82. 83. 84. 85. 86. 87. 88. 89. 90. 91. 92. 93. 94. 95. 96. 97. 98. 99. 100. 101. 102. 103. 104. 105. 106. 107. 108. 109. 110. 111. 112. 113. 114. 115. 116. 117. 118. 119. 120. 121. 122. 123. 124. 125. 126. 127. 128. 129. 130. 131. 132. 133. 134. 135. 136. 137. 138. 139. 140. 141. 142. 143. 144. 145. 146. 147. 148. 149. 150. 151. 152. 153. 154. 155. 156. 157. 158. 159. 160. 161. 162. 163. 164. 165. 166. 167. 168. 169. 170. 171. 172. 173. 174. 175. 176. 177. 178. 179. 180. 181. 182. 183. 184. 185. 186. 187. 188. 189. 190. 191. 192. 193. 194. 195. 196. 197. 198. 199. 200. 201. 202. 203. 204. 205. 206. 207. 208. 209. 210. 211. 212. 213. 214. 215. 216. 217. 218. 219. 220. 221. 222. 223. 224. 225. 226. 227. 228. 229. 230. 231. 232. 233. 234. 235. 236. 237. 238. 239. 240. 241. 242. 243. 244. 245. 246. 247. 248. 249. 250. 251. 252. 253. 254. 255. 256. 257. 258. 259. 260. 261. 262. 263. 264. 265. 266. 267. 268. 269. 270. 271. 272. 273. 274. 275. 276. 277. 278. 279. 280. 281. 282. 283. 284. 285. 286. 287. 288. 289. 290. 291. 292. 293. 294. 295. 296. 297. 298. 299. 300. 301. 302. 303. 304. 305. 306. 307. 308. 309. 310. 311. 312. 313. 314. 315. 316. 317. 318. 319. 320. 321. 322. 323. 324. 325. 326. 327. 328. 329. 330. 331. 332. 333. 334. 335. 336. 337. 338. 339. 340. 341. 342. 343. 344. 345. 346. 347. 348. 349. 350. 351. 352. 353. 354. 355. 356. 357. 358. 359. 360. 361. 362. 363. 364. 365. 366. 367. 368. 369. 370. 371. 372. 373. 374. 375. 376. 377. 378. 379. 380. 381. 382. 383. 384. 385. 386. 387. 388. 389. 390. 391. 392. 393. 394. 395. 396. 397. 398. 399. 400. 401. 402. 403. 404. 405. 406. 407. 408. 409. 410. 411. 412. 413. 414. 415. 416. 417. 418. 419. 420. 421. 422. 423. 424. 425. 426. 427. 428. 429. 430. 431. 432. 433. 434. 435. 436. 437. 438. 439. 440. 441. 442. 443. 444. 445. 446. 447. 448. 449. 450. 451. 452. 453. 454. 455. 456. 457. 458. 459. 460. 461. 462. 463. 464. 465. 466. 467. 468. 469. 470. 471. 472. 473. 474. 475. 476. 477. 478. 479. 480. 481. 482. 483. 484. 485. 486. 487. 488. 489. 490. 491. 492. 493. 494. 495. 496. 497. 498. 499. 500. 501. 502. 503. 504. 505. 506. 507. 508. 509. 510. 511. 512. 513. 514. 515. 516. 517. 518. 519. 520. 521. 522. 523. 524. 525. 526. 527. 528. 529. 530. 531. 532. 533. 534. 535. 536. 537. 538. 539. 540. 541. 542. 543. 544. 545. 546. 547. 548. 549. 550. 551. 552. 553. 554. 555. 556. 557. 558. 559. 560. 561. 562. 563. 564. 565. 566. 567. 568. 569. 570. 571. 572. 573. 574. 575. 576. 577. 578. 579. 580. 581. 582. 583. 584. 585. 586. 587. 588. 589. 590. 591. 592. 593. 594. 595. 596. 597. 598. 599. 600. 601. 602. 603. 604. 605. 606. 607. 608. 609. 610. 611. 612. 613. 614. 615. 616. 617. 618. 619. 620. 621. 622. 623. 624. 625. 626. 627. 628. 629. 630. 631. 632. 633. 634. 635. 636. 637. 638. 639. 640. 641. 642. 643. 644. 645. 646. 647. 648. 649. 650. 651. 652. 653. 654. 655. 656. 657. 658. 659. 660. 661. 662. 663. 664. 665. 666. 667. 668. 669. 670. 671. 672. 673. 674. 675. 676. 677. 678. 679. 680. 681. 682. 683. 684. 685. 686. 687. 688. 689. 690. 691. 692. 693. 694. 695. 696. 697. 698. 699. 700. 701. 702. 703. 704. 705. 706. 707. 708. 709. 710. 711. 712. 713. 714. 715. 716. 717. 718. 719. 720. 721. 722. 723. 724. 725. 726. 727. 728. 729. 730. 731. 732. 733. 734. 735. 736. 737. 738. 739. 740. 741. 742. 743. 744. 745. 746. 747. 748. 749. 750. 751. 752. 753. 754. 755. 756. 757. 758. 759. 760. 761. 762. 763. 764. 765. 766. 767. 768. 769. 770. 771. 772. 773. 774. 775. 776. 777. 778. 779. 780. 781. 782. 783. 784. 785. 786. 787. 788. 789. 790. 791. 792. 793. 794. 795. 796. 797. 798. 799. 800. 801. 802. 803. 804. 805. 806. 807. 808. 809. 810. 811. 812. 813. 814. 815. 816. 817. 818. 819. 820. 821. 822. 823. 824. 825. 826. 827. 828. 829. 830. 831. 832. 833. 834. 835. 836. 837. 838. 839. 840. 84

[illegible]

On 10/10/1961, the following information was received from the New York City Police Department, New York City:

Ans. 1. Samuel Taylor Coleridge was born on 21 October 1796 at the English town of Northampton. He was the youngest child of Samuel and Elizabeth Coleridge. He was educated at St John's College, Cambridge.

Witness.
of the delivery of the above mentioned sum of \$100,000.00.
There are three, namely the said [redacted] and [redacted]. In
this matter, I ever acted as [redacted].

Only a few days before the end of the year. 1944: 1944/2.

[illegible][illegible][illegible]

110. 1. 1. 1971 (1971-1972) 1. 1. 1971 (1971-1972)
1. 1. 1971 (1971-1972) 1. 1. 1971 (1971-1972)
 610 1. 1. 1971 (1971-1972) 1. 1. 1971 (1971-1972)

~~CONFIDENTIAL~~

~~CONFIDENTIAL~~

1. The first of these is the fact that the United States is a democratic country, and that the United States is a democratic country. This is a fact that is well known to all people who are interested in the United States. The second of these is the fact that the United States is a democratic country, and that the United States is a democratic country. This is a fact that is well known to all people who are interested in the United States. The third of these is the fact that the United States is a democratic country, and that the United States is a democratic country. This is a fact that is well known to all people who are interested in the United States.

1-18. A. _____ ID: _____ of _____, 1967. The _____
_____ _____
_____ _____
_____ _____
B. _____
C. _____
D. _____

1. The first step is to identify the problem or question that needs to be answered. This involves understanding the context and the specific requirements of the task.

1. The first of these is the fact that the Commission has not yet received any information from the Government of the United States regarding the results of its investigation of the activities of the American Friends Service Committee in the Philippines. It is therefore requested that the Commission be kept advised of any developments in this regard.

[illegible]

100-10768-100

Journal of Management Education 30(6)p.789-804

NOTES:

See above right in the
... ..
... ..

... ..

FIG. 9.

... ..

... ..

... ..

... ..

... ..

FIG. 10.

... ..

... ..

... ..

... ..

... ..