The Susceptibility of Muskrats and Snowshoe Hares to Experimental Infection with a Chlamydial Agent

J. O. Iversen, J. Spalatin, C. E. O. Fraser, R. P. Hanson and D. T. Berman*

ABSTRACT

Muskrats (Ondatra zibethicus) and snowshoe hares (Lepus americanus) were exposed experimentally by various routes to a chlamydial agent (designated strain M56) originally isolated during a die-off of muskrats and snowshoe hares which occurred in Saskatchewan during 1961. Both species were susceptible to experimental infection. Whereas M56 was highly lethal for snowshoe hares (18 deaths/19 exposed), it was less virulent for muskrats (6 deaths/20 exposed).

The degree of susceptibility of muskrats to induced infections with M56 was influenced by the presence or absence of specific antibodies at the time of exposure. A febrile illness was observed in 11 of 20 muskrats. In the six that died, widespread focal necrosis was found in the liver. Following intraperitoneal or oral exposures, chronic infections were established and the agent was recovered from the brain and the small intestine up to 96 days post-infection. Specific antibodies were found in 11.8% of 127 sera of muskrats trapped from the wild in Saskatchewan, the Canadian Arctic, and Wisconsin.

In snowshoe hares, M56 induced an acute, febrile, emaciating illness, and the almost invariable fatal course was short with terminal signs of opisthotonos, convulsions, and hypoglycemia. Snowshoe hares succumbed with intravenous doses of less than ten mouse intracerebral LD_{50} of M56. The same syndrome was produced by intravenous, subcutaneous, and oral infections. M56 was found in high titers in all tissues examined. The highest titers were found in the liver and spleen which correlated with the pathology observed. M56 was recovered from female rabbit ticks (Haemaphysalis leporispalustris) engorging on experimentally infected snowshoe hares.

RÉSUMÉ

On exposa, de différentes façons, des rats musqués (Ondatra Zibethicus) et des lièvres (Lepus Americanus) à une chlamydie, désignée comme la souche M 56. Cette souche avait été isolée en 1961, lors d'une épidémie mortelle qui s'était répandue chez ces animaux en Saskatchewan. Les deux espèces s'avérèrent sensibles à l'infection expérimentale. La mortalité fut très élevée chez les lièvres (18 morts sur 19 sujets exposés) mais moins marquée chez les rats musqués (6 morts sur 20 sujets exposés). Le niveau de susceptibilité à l'infection expérimentale du rat musqué était influencé par la présence, ou l'absence, d'anticorps spécifiques au moment où l'animal était mis en contact avec M 56. Une phase fébrile fut remarquée chez 11 des 20 rats musqués. On observa de la nécrose focale intensive sur le foie des six rats musqués morts. Des infections chroniques s'établirent après une contamination intrapéritonéale ou orale, et l'on put isoler l'agent infectieux du cerveau et de l'intestin grêle de ces animaux, jusqu'à 96 jours après la contamination. Des anticorps spécifiques furent retrouvés dans 11.8% de 127 échantillons de sérum de rats musqués capturés en Saskatchewan, dans l'Arctique canadien et au Wisconsin.

Chez le lièvre, le M 56 provoqua une maladie aigüe, fébrile, rapidement débilitante et qui, presque invariablement, était mortelle à brève échéance, après des manifestations d'opisthotonos, des convulsions et de l'hypoglycémie. Les lièvres succombèrent même après des injections intraveineuses correspondant à moins de 10 fois la LD50 de M 56 intracérébrale chez la souris. Un syndrôme identique apparut après des contaminations intraveineuses sous-cutanées et orales.

De fortes concentrations de M 56 furent retrouvées dans tous les tissus examinés, dont les plus fortes dans le foie et la rate, ce qui correspond aux autres observations. Il faut ajouter que l'on put isoler M 56 d'une tique femelle de lapin (Haemaphysalis leporis-palustris) qui s'était nourrie sur un lièvre infecté.

*Department of Veterinary Science, University of Wisconsin, Madison, Wisconsin.
Present address of J. O. Iversen: Sonoma State College, Rohnert Park, Sonoma, California.
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INTRODUCTION

In 1959, according to Connell (2), the population of muskrats and snowshoe hares in north-central Saskatchewan, which he had been observing for ten years, appeared to be approaching peak densities. An unusual number of dead, sick, and malnourished muskrats were found by the winter of 1959-1960. Muskrat deaths continued to occur during the following winter. By February, 1961, sick and dead snowshoe hares were found and a decline in the hare population was obvious. During the die-off of muskrats and hares in 1961, Spalatin *et al* (28) isolated four mouse lethal agents from the blood and spleen of muskrat and snowshoe hare carcasses. On the basis of morphological and tinctorial properties and on the possession of group-specific antigen (4, 5), the isolates were considered to be members of the genus *Chlamydia* (psittacosis-lymphogranuloma-trachoma or PLT group) (22).

Using one of the chlamydial isolates (M56) a study was undertaken to determine the susceptibility of the muskrat and snowshoe hare to this agent. Host response was evaluated in terms of illness, mortality, pathology, and immunological responses. A search for natural chlamydial infections was made by a serological survey of muskrat and snowshoe hare sera.

MATERIALS AND METHODS

The M56 Agent

The agent, originally recovered from muskrat tissues, was serially passed in embryonated hens' eggs via yolk sac inoculation. By the tenth passage, the infectivity titer reached $10^{7.5}$ $CELD_{50}$/ml in seven-day chicken embryos and $10^{8.5}$ $MICLD_{50}$/ml in three week old albino mice. The seed stock was prepared from tenth passage as a 10% suspension of infected yolk sac. Tryptone broth containing 1 mg of streptomycin sulfate per ml was used as a diluent of infected yolk sac material. The seed stock was distributed into vials and maintained frozen at -60°C until used.

Experimental Animals

During 1965 and 1966, 47 muskrats were collected by live trapping from three different locations. Twenty muskrats were used in the experimental exposures to M56: five from Rochester, Alberta; 12 from Horicon Marsh, Wisconsin; and three from Tomahawk, Wisconsin. Sera were collected from 27 muskrats live-trapped in 1966 at Horicon Marsh, Wisconsin. During 1960 and 1963, sera were collected from 69 muskrats in the Canadian Arctic and preserved on paper discs by the method of Karstad *et al* (12).

Snowshoe hares were obtained from near Tomahawk, Wisconsin, and from near Rochester, Alberta, Canada. The Tomahawk population of snowshoe hares was stable, whereas the Rochester population of snowshoe hares had undergone a marked decline from 1961 to 1964 and there were fewer than 50 hares per square mile during the interval (17). The muskrats and snowshoe hares were used for experiments one to three weeks after capture. Sudden mortality similar to trap sickness, if it occurred, usually did so within the first few days of capture. Thereafter, animals, if not used sooner, were kept for many months in apparent good health (10).

Three-week old albino mice (Ha/ICR) (A. R. Schmidt Co., Inc., Madison, Wisconsin) were used for serum neutralization tests and in assays of tissues for the M56 agent.

Experimental Exposures

In muskrats the M56 agent was administered intravascularly (IV) via the dorsal vessels of the hind feet or the heart, intranasally (IN) via the external nares, intraperitoneally (IP), intramuscularly (IM), intraocularly, intracerebrally (IC), and orally.

Four routes of inoculation were used in snowshoe hares. The first was a combination of injection in the lateral ear vein and instillation into the nares (IV-IN). Since these hares were not anesthetized, most of the intranasally instilled inoculum was swallowed. The second involved injection into the lateral ear vein only (IV). The third was by feeding livers and spleens from snowshoe hares infected with M56. One hare ate 60 grams and the other ate 32 grams of tissue. The fourth route was by subcutaneous (SC) inoculation into the lateral surface of the ear.

The snowshoe hares were bled from the lateral ear vein, and the muskrats from the retro-orbital venous plexus, the dorsal vessels of the hind feet or the heart. When vigorous struggling necessitated anesthe-

sia, muskrats were injected intraperitoneally with 1.0 ml of 5% sodium pentobarbital (Nembutal, Abbott Laboratories, Chicago, Illinois).

Blood Glucose Level Determinations

Blood glucose levels were determined by two techniques: the Somogyi-Nelson technique based on the colorimetric determination of reducing substances in a protein-free filtrate (19) and by utilizing Dextrostix (Ames Laboratories, Toronto, Ontario) as colorimetric estimations (27).

Pathology

At the time of death or killing, tissues of muskrats and snowshoe hares were examined for gross lesions, and liver and spleen weights were recorded for hares. The tissues of infected muskrats and hares were fixed in 10% formalin (buffered with calcium carbonate to a pH of 7.4), embedded in paraffin, stained with hematoxylin and eosin, periodic acid Schiff's (PAS), Giemsa's or Noble's stains. Sections were examined microscopically for lesions and inclusion bodies (15).

Assay of Tissues for M56

The agent was detected by intracerebral (IC) inoculation of three-week old albino mice with a 10% suspension of tissue. The suspensions were prepared by using tryptone broth containing streptomycin sulfate and by grinding with a sterile porcelain mortar and pestle. Three or four mice were immediately inoculated with each suspension and examined daily for two weeks. The mean death times were calculated. The tissue titers were estimated by the single dilution assay of Golub (6) adapted in our laboratory for IC inoculation of three-week old mice. A standard curve (Fig. 1) was prepared by plotting log dose against survival time, and the titer of each inoculum was determined by graphic interpolation. The standard deviations were calculated by graphic rankit analysis using the rankit tables of Ipsen and Jerne (9).

Serology

The technique of Fraser and Berman (4) as modified to a microtest using volumes recommended by Sever (25) was used to detect specific complement fixing (CF) antibodies in the sera of muskrats, snowshoe hares, and a limited number of field personnel from the Wisconsin Conservation Department. A serum titer of 1:10 or greater was considered positive (5). Serum neutralization (SN) tests were performed in three-week old albino mice. A constant dilution of the seed stock of M56 containing 10^4 $MICLD_{50}$ in a 0.3 ml was mixed with 0.3 ml of the serum being tested. After incubation at 20°C for two hours, the mixtures were inoculated (0.03 ml/mouse) IC into ten mice. Mean death times were calculated and titers determined from the standard curve. The potency of the serum being tested was expressed as the number of logarithms neutralized. Neutralization of 1.5 or more logarithms was considered to be significant.

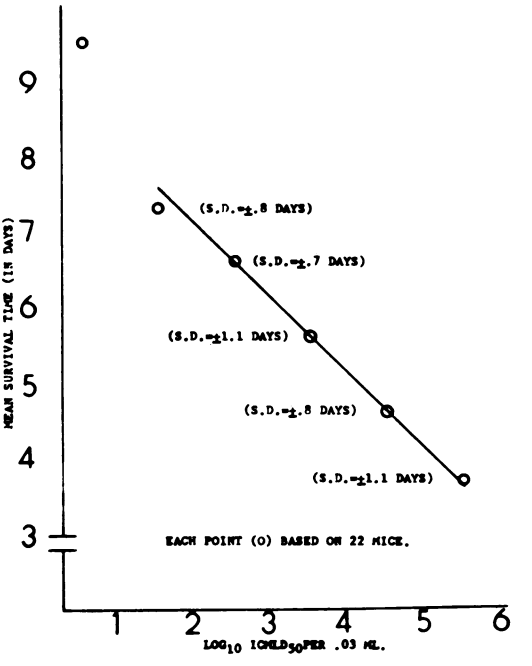

Fig. 1. Standard curve derived from a standard LD_{50} titration of M-56 in weanling mice.

RESULTS

Experimentally Exposed Muskrats

Signs of Disease — Within the first week after artificial exposure, an elevation of body temperature of 1°C or more occurred in 11 of 20 muskrats. In six, malaise, anorexia, and cachexia were observed within the first 18 days. The course of the disease fluctuated with apparent recovery followed by exacerbation. In some muskrats, erratic

TABLE I. Experimental M56 Infections in Muskrats — Mortality Ratios by Routes of Exposure

Route of inoculation	Dose ($Log_{10}MICLD_{50}$)	Time to Death (in days post-exposure)	Mortality Ratio
IV and IN	3.0 — 4.0	6, 9, and 18	3/4[a]
IN only	3.0 — 4.0	12 and 18	2/5
IC only	2.0 — 3.0	12	1/1
IP and IN	3.0 — 4.0	no deaths	0/4
Oral	6.0 — 8.0	no deaths	0/3
IM and IN	3.0 — 4.0	no deaths	0/2
Intra-ocular	2.0 — 3.0	no deaths	0/1
Total			6/20

[a]Numerator = number of muskrats that died;
Denominator = number of muskrats that were exposed.

behavior was observed (viz., unresponsiveness to stimuli, hyperexcitability, and/or uncontrolled movements). In a few animals, dyspnoe and nasal discharge and/or diarrhea were observed.

Mortality — Six of the 11 muskrats that developed signs of disease died six to 18 days after exposure (Table I). The survivors were killed 30—96 days post-exposure.

Pathology — Significant alterations were observed in the tissues of the six muskrats that died. All had widely disseminated foci of hepatic necrosis. The livers were brownish in color with irregularly circumscribed white to yellowish foci 1-4 mm in diameter on the surface and in the parenchyma. Microscopically, the foci consisted of areas of leukocytes and necrotic hepatic cells. At the periphery of the foci, the hepatic cells were swollen and frequently contained pyknotic nuclei. Glycogen could not be demonstrated in the sections of liver tissue stained with PAS. In the two muskrats inoculated via the heart, there was fibrinous pericarditis. Microscopically, the pericardium contained extensive accumulations of mononuclear cells in which plasma cells predominated. One of them also exhibited a marked fibrinous pneumonia with an accumulation of fibrin and large mononuclear cells in the alveoli. Granular inclusion bodies were present in the pneumonic lung, but were not observed in liver or pericardium.

No alterations were observed in the tissues of the muskrats that survived 30-96 days post-exposure.

Recovery of M56 from Tissues — M56 was recovered from the tissues of 13 of 14 muskrats that died or were killed following a single exposure (Table II).

Immune Response — The presence of pre-exposure antibodies influenced the outcome of the experimental infection (Table III). The sera of four of the six muskrats that

TABLE II. Recovery of M56 from Tissues of 14 Experimentally Infected Muskrats

Tissue	4 muskrats Dead 6 — 18 Days post-exposure	6 muskrats Killed 30 Days post-exposure	4 muskrats Killed 96 Days post-exposure	Totals
Brain	3/4[a]	6/6	2/4	11/14
Spleen	4/4	6/6	0/4	10/14
Liver	3/4	3/6	0/4	6/14
Small Intestine	2/4	3/6	1/4	6/14
Joints	0/1	2/6	1/4	3/11
Lungs	2/4	1/6	0/4	3/14
Blood	1/2	ND[b]	ND	1/2
Lymph nodes	1/4	ND	ND	1/4
Rectal Swab	/4	0/2	0/4	1/10
Gonads (testes or ovary)	0/2	1/6	0/4	1/12
Kidney cortex	1/4	0/6	0/4	1/14
Eye	0/4	0/6	0/4	0/14
Recoveries of M56 made from one or more tissues	4/4	6/6	3/4	13/14

[a]Numerator = number of recoveries; Denominator = number examined.
[b]ND = not done.

TABLE III. Experimental M56 Infections in Muskrats — Mortality Ratios and the Presence or Absence of Pre-exposure Antibody to M56

Route	Mortality ratios[a]	
	Presence of pre-exposure antibody	Absence of pre-exposure antibody
IC	0/4[b]	1/1
IV and IN	1/1	1/2
IN	0/1	2/2
Oral	0/2	0/1
IP and IN	0/4	—
IM and IN	0/2	—
Intraocular	0/1	—
	1/15	4/6

[a]Mortality ratio = number of deaths/number exposed.
[b]Four muskrats that survived IC challenge of 10^3 – 10^4 $MICLD_{50}$.

died by the 18th day after exposure were tested for type-specific CF antibodies. Three of them had titers of 1:10 to 1:80. Sera from 11 of the animals killed from 30 to 96 days after exposure were tested for CF or neutralizing antibodies. All were positive with CF titers from 1:40 to 1:640 or neutralization indices from 1.7 to 3.1 $\log_{10}$ $MICLD_{50}$.

Following oral exposure, CF antibodies to M56 developed in the two muskrats tested (Fig. 2).

A complex immunological response occurred following exposure of muskrats that had pre-exposure CF titers (Fig. 3). Following a combination of IM and IN challenges, the CF titers decreased and the neutralization titers increased. Both animals survived subsequent IC challenges which were followed by an increase in the CF titer.

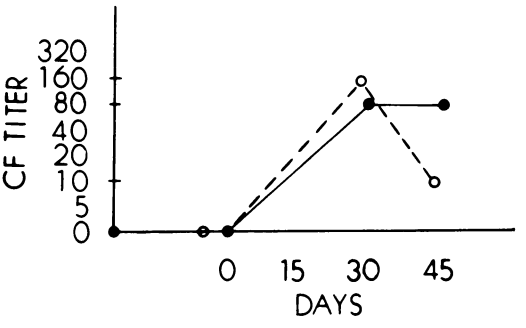

Fig. 2. Experimental M-56 infections in muskrats — production of complement fixing antibody following oral exposure. (Solid circles with solid line represent one muskrat and open circles with broken line represent a second.)

Experimentally Exposed Snowshoe Hares

Signs of Disease — The typical course of disease usually varied from five to 13 days, but two hares died 60 hours after exposure (Table IV). A biphasic febrile response was characteristic, the initial temperature rise of 0.5 to 1.0°C occurring on the second or third day and the subsequent rise of 2.3°C occurring on the fifth day. The temperature fell below normal as the infected hares became moribund. During the course of the disease, the infected hares lost an average of 114 grams, or one-eighth of their original body weight. Food consumption was reduced but the hares continued to eat throughout the course of the disease. Six hares were observed at the time of death and all six presented the same terminal features as: opisthotonos, convulsions and hypoglycemia.

Mortality — Of the 19 snowshoe hares that were exposed, 18 died (Table IV). The hare which survived had an acute illness followed by recovery and low level antibody

TABLE IV. Experimental M56 Infections in Snowshoe Hares — Mortality Ratios by Routes of Exposure

Route of inoculation	Dose ($Log_{10}MICLD_{50}$)	Time to death days (in days post-exposure)	Mortality ratio
IV and IN	1.3 — 4.5	2.5 — 13.0	5/6[a]
IV	< 1.0 — 5.3	6 — 12	9/9
SC	3.3 — 5.5	7 and 8	2/2
Oral	8.0	2 and 5	2/2
Total			18/19

[a]Numerator = number of snowshoe hares that died; Denominator = number of hares that were exposed.

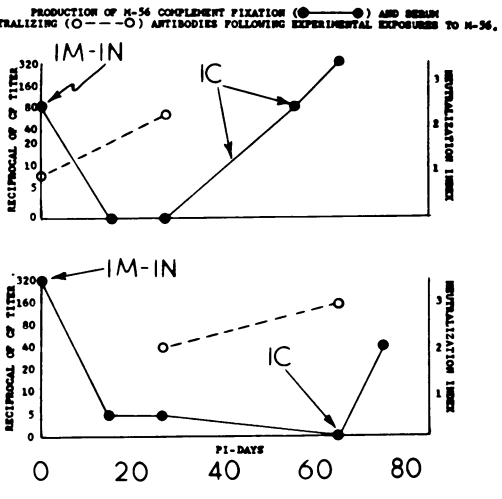

Fig. 3. Experimental M-56 infections in muskrats — serological responses following exposure of two muskrats that had pre-exposure CF titers.

formation. Pre-exposure sera were negative on CF and SN tests. The group-specific CF titer was 1:5 on day 15 and negative on day 42. The SN index was 1.7 $\log_{10}$ $MICLD_{50}$ on day 42. It succumbed when subsequently challenged intravenously with M56, 42 days after the initial experimental inoculation.

Blood Glucose Levels — The blood glucose levels of two hares were followed throughout the course of the disease and compared to the normal (Fig. 4) (7, 30). Four other hares observed at the time of death had blood sugar levels of 60 mg% or below.

TABLE V. Experimental M56 Infections in Snowshoe Hares — Weights of 14 Infected and Two Non-Infected Hares

	Mean weight (grams ± SE of mean)		
	Infected	Non-infected[a]	p[b]
Body weight (pre-inoculation)	909.±25.4	894.±25.6	0.05
Weight loss	114.±12.9	—	—
Liver weight	40.9±3.5[c]	23.6±1.0[d]	0.05
Splenic weight	6.1±0.4[c]	2.0±0.4[d]	0.01

[a]Non-infected hares were killed by dislocation of the cervical spinal column.
[b]P value, obtained from a "t" test comparing the means of the two groups.
[c]At the time of death.
[d]At the time of killing.

Pathology — Gross changes were most apparent in the liver and spleen. Infected livers were significantly enlarged (Table V), and were friable and brown with tan

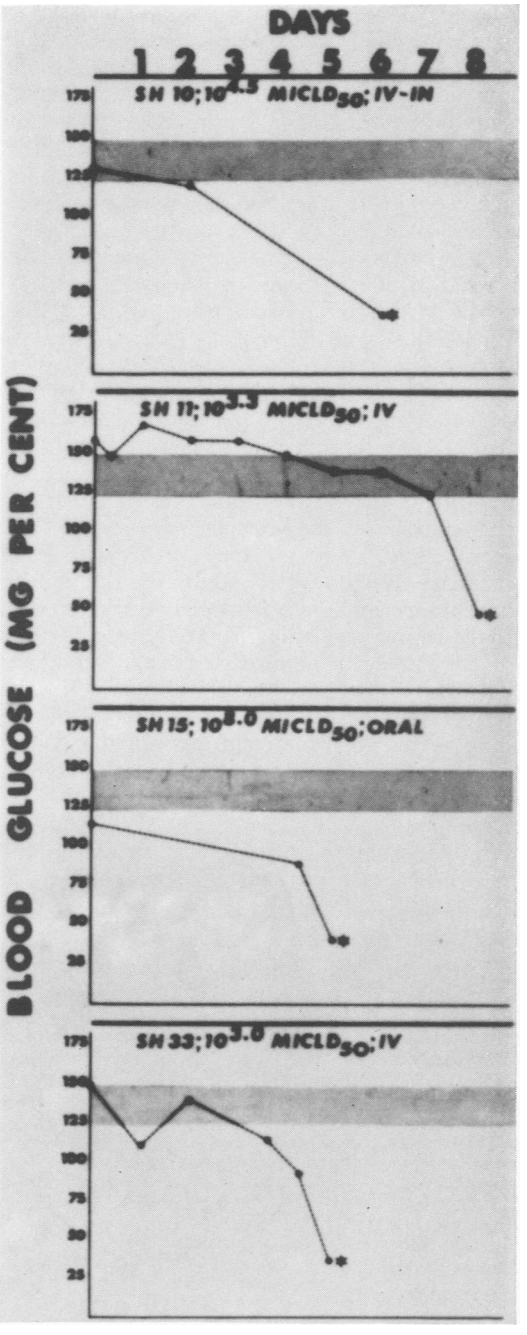

Fig. 4. Experimental M-56 infection in snowshoe hares— blood glucose levels during the course of infection (shaded areas represent normal ranges of blood glucose values for hares).
*** Opisthotony, convulsions and death.**

foci over the surface and throughout the parenchyma. Some foci had discrete borders while others were not clearly demarcated. The spleens were greatly enlarged, black and friable, and purplish pulp bulged from the cut surface. The adrenals appeared to be enlarged. The kidneys were greyish brown. The vessels on the surface of the brain was congested. The lungs were normal or slightly congested. Icterus was usually present one week after infection and yellow discoloration of the sclera, ears, toenails, subcutaneous tissues, tendons, perirenal fat, and renal pelvis was seen after ten days.

Microscopically, there was marked destruction of the liver parenchyma and necrosis was spread throughout the lobules. Hepatic cells in the necrotic regions showed varying degrees of degeneration. In PAS stained liver sections of six hares (prepared immediately after death), glycogen was either absent or greatly reduced in amount. Splenomegaly was due to an augmentation of the red and white pulp. When the course of the disease was more than seven days, there appeared to be a marked increase in the white pulp of the spleen and many splenic macrophages were seen to contain hemosiderin. The blood vessels of the cerebral meninges appeared congested. The only abnormalities noted in the lungs were accumulations of larvae of the lungworm, *Protostrongylus broughtonii*. Sections of the kidney, heart, and adrenals were not examined.

Recovery of M56 from Tissues — In an attempt to determine the distribution of M56 within the tissues early in the course of experimental infection, hares were killed at 24, 48, and 88 hours post-infection (Table VI). The agent was first recovered from the blood after 88 hours. At that time, it was also present in popliteal lymph nodes, kidney, spleen, lung, and heart.

TABLE VI. Experimental M56 Infections in Showshoes Hares — Recovery of M56 from the Tissues of Three Experimentally Infected Snowshoes Hares Killed at 24, 48, and 88 Hours Post-Infection
(Route = IV; Dose = 10^4 $MICLD_{50}$)

Tissue	Time of Sacrifice 24 hrs.	48 hrs.	88 hrs.
Blood	−[a]	−	+
Liver	+[b]	+	+
Spleen	+	+	+
Lungs	+	+	+
Heart	+	+	+
Kidneys	−	−	+
Popliteal lymph nodes	−	−	+
Testicles	−	−	−
Joints	−	−	−

[a] − = M56 not recovered.
[b] + = M56 recovered.

TABLE VII. Experimental M56 Infections in Snowshoes Hares — M56 Titers of Tissues at the Time of Death

Tissues	Number positive/ Number examined	Titers (Mean ± SE of mean)[a]
Liver	13/14	7.1 (± 0.3)
Spleen	10/11	6.7 (± 0.3)
Lymph	7/10	5.8 (± 1.1)
Kidneys	12/13	5.5 (± 1.1)
Testes	7/8	5.5 (± 0.3)
Cerebrum	12/13	5.3 (± 0.4)
Lungs	12/13	5.3 (± 0.5)
Eye (uveal tract and retina)	7/7	5.1 (± 0.5)
Thyroid	8/8	4.9 (± 0.5)

[a] Titers expressed as $\log_{10}$ $MICLD_{10}$ of M56 per gram of positive tissues.

At the time of death, M56 was recovered in high titer from all tissues examined, and the highest titers were in the liver and the spleen (Table VII). The agent was also recovered from throat and rectal swabs, bone marrow, mammary gland, uterus, pancreas, and the thyroid when they were examined.

Recovery of M56 from Ticks from Experimental Snowshoe Hares — Attempts were made to recover M56 from *Haemaphysalis leporis-palustris* ticks from two experimentally infected hares. The agent was not recovered from 12 nymphs collected on days 0, 1, 2, 3, or 4 from either hare. M56 was recovered from engorging female ticks collected from one hare on day 5 (10 $MICLD_{50}$/tick), day 6 ($10^{2.8}$ $MICLD_{50}$/tick), and day 7 ($10^{3.0}$ $MICLD_{50}$/tick) and from the other hare on day 6 ($10^{3.0}$ $MICLD_{50}$).

Serologic Survey of Muskrat, Snowshoe Hare, and Human Sera — The CF and neutralization tests detected group-specific antibodies in the sera of muskrats collected in the wild. From the 127 muskrat sera tested, 11.8% were positive by one or the other test (Table VIII). Five of these positive sera were from 89 muskrats collected in Canada and the remaining ten were from 38 muskrats trapped in Wisconsin.

No group-specific antibody was detected by the CF test in sera from 398 hares from

TABLE VIII. **Presence of Antibody Against M56 in Sera of Muskrats from Four Areas in North America**

	Wisconsin		Canada		
			Alta	Arctic	Sask
	SNT	CF	CF	SNT	SNT
1960	—[a]	—	—	1/38[b]	—
1961	—	—	—	—	2/15
1963	—	—	—	2/31	—
1965	—	6/15	0/5	—	—
1966	4/23	—	—	—	—
CF Total		6/15	0/5		
SHT Total	4/23			3/69	2/15
Total	15/127 = 11.8%				

[a] — = not done.
[b] Numerator = number of positive sera; Denominator = number of sera examined.

a declining population near Rochester, Alberta.

Three of ten sera from conservation field personnel that had contact with various wildlife species, including muskrats, were positive in the CF test.

DISCUSSION

The susceptibility of muskrats and snowshoe hares to experimentally produced infection with M56 and the similarity of the lesions in the experimentally produced and naturally occurring diseases supports the hypothesis that this member of the genus Chlamydia was an etiological factor in the widespread mortality of muskrats and snowshoe hares in Saskatchewan in 1961 (28). The response of muskrats to experimental infections with M56 was markedly different from that of snowshoe hares, and the evidence can be interpreted to mean that infections are enzootic in muskrats and epizootic in the snowshoe hares. Inapparent infection persisted in some of the experimentally infected muskrats, and the microorganisms apparently established a stable association with this host. A breakdown in such an equilibrium may result from a number of adverse conditions known to befall muskrat populations in nature, viz., starvation, crowding, intraspecific strife, and/or droughts (3). Whenever the host-parasite balance is disturbed, widespread epizootic mortality in muskrats may occur. The M56 agent may then be spread to other susceptible species such as the snowshoe hare. In hares, a short incubation period, chlamydiae in high titer in the blood, and a short course of the disease could favor a rapid spread of the agent in a population. The inability of the snowshoe hare to cope with infections with M56 suggest the agent is poorly adapted to the host and may either represent a recent introduction into the hare population or a pathogen for which the hare has failed to develop an adaptive response because of some other interfering survival mechanism.

The only previous report suggestive of an association of a chlamydial agent with a die-off of muskrats occurred in an epidemic of psittacosis in Louisiana in 1943. The index cases were a husband and wife who had processed animal pelts, chiefly muskrats (20, 21). An epizootic illness of muskrats was known to have taken place just prior to the illnesses of the index cases. In two experiments performed during the subsequent epidemiological investigation, ten muskrats were inoculated intraperitonely and intranasally with the Louisiana chlamydial agent. Although the muskrats did not die, their susceptibility to chlamydial agents remained uncertain because the pre-exposure immune status of the experimentally infected muskrats was not determined. Seven years later, it was shown by Rubin (24) that egrets in the marshes of Louisiana were infected with an agent similar to that causing a human pneumonitis episode.

A number of infectious diseases have been reported to produce mortality in muskrat populations. The lesions induced by M56 would present a problem of differential diagnosis with other diseases. Widely disseminated focal necrosis in the liver was the most consistent pathological finding in the muskrats that died on the M56 infection. In muskrat populations, hepatic lesions are also characteristic of mortality due to *Eimeria* sp. (26), *Salmonella typhimurium* (1), and *Pasteurella tularensis* (23). Liver necrosis was also found in Errington's disease along with intestinal and pulmonary hemorrhages (3). Although liver necrosis was observed in muskrats experimentally infected with M56, the hemorrhagic element of Errington's disease was not observed.

There are similarities between the disease produced by M56 and certain diseases produced in snowshoe hares by other agents. Similarities to "shock disease" (7, 8), a nontransmissible glycogenolytic con-

dition, are emaciation, liver necrosis, and terminal hypoglycemia, opisthotonos and convulsions. However, "shock disease" is accompanied by atrophy of the spleen and liver. Infections with M56 could be difficult to distinguish from two other infectious diseases reported in hares, as listeriosis (16). Jellison *et al* found *Posteurella* was isolated from three captive snowshoe hares in Newfoundland and the authors maintained some of the deaths in wild hare populations could be attributed to listeriosis (16). Jellison *et al* found *Pasteurella tularensis* among Minnesota snowshoe hares during the die-off in the 1920's (11). Similarities between M56 and these two microbes are intracellular position, stimulation of mononuclear leukocytic responses in tissues, and the production of liver necrosis. Differentiation would require cultivation and identification of the etiological agent.

There are a number of possible alternatives for transmission of M56 among muskrats and snowshoe hares in nature. The marsh habitat of muskrats is shared by numerous avian species and M56 can infect artificially exposed birds including mallard ducklings. Experimentally infected mallard ducklings and muskrats shed M56 in feces (29). Contamination of marshes with the excretions and carcasses of infected animals could provide an efficient means of transmission to marsh inhabitants. The habitats of muskrats and snowshoe hares overlap. During the summer, muskrats have been caught in traps set along snowshoe hare runs in forested areas (13) and in winter, hares will inhabit edges of marshes. Flesh-eating by snowshoe hares has often been reported during peak populations (13), and this tendency to consume a variety of flesh, especially if frozen, could provide for efficient transmission of the agent. In hares, recovery of the agent from throat and rectal swabs, feces, uterus and the mammary gland suggest other possible modes of transmission. In addition, recovery of M56 from engorging *H. leporispalustris* ticks on experimentally infected hares indicates the possibility of mechanical or biological transmission by arthropods. Meyer has postulated there may be a more widespread involvement of arthropods in the transmission of chlamydiae than previously suspected (18).

The results of the serological survey show a relatively widespread geographic distribution of chlamydiae in muskrats in nature.

Neutralizing antibody against M56 was found in the sera of two of 15 muskrats and three of 15 snowshoe hares collected during the die-off in Saskatchewan in 1961 (28). In addition to the positive sera from Saskatchewan, group-specific antibodies were detected in sera from muskrats from the Yukon and MacKenzie River deltas and from marshes in Wisconsin. Such a geographic distribution stimulates conjecture on the influence of chlamydiae on the life history of the muskrat throughout the entire range of this semiaquatic mammal.

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Book Review

DISEASES OF CAGE AND AVIARY BIRDS. *Margaret L. Petrak. Published by Lea & Febiger and the Macmillan Co. of Canada Limited, Toronto, Canada, 1969. 528 pages, 315 illustrations, clothbound. Price $35.75.*

The editor has succeeded very well in her stated objective to provide a comprehensive book on cage and aviary birds for the use of the clinician and the student. She has accomplished this by assembling thirty appropriate chapters written by twenty-five authorities. Throughout the book emphasis has been placed on finches, canaries, and budgerigars, with other species covered in a more general way.

The subjects covered are broader than those suggested by the title. The first one hundred and seventy-five pages are devoted to non-clinical subjects, such as types of cage birds, caging, environment, genetics, physiology and nutrition. Unfortunately, there is quite a variation in the quality of the information presented in the various chapters. The anatomy section presents an outstanding description of the anatomy of the budgerigars, but is limited largely to this species. Other chapters are weak; for example, the section on nutrition gives some useful background on ingredients (including good illustrations) used to form rations, but information on fundamental nutrients is weak.

The second part, consisting of over three hundred pages, headed "clinical considerations" is really made up of two sections. The first covers clinical examinations, clinical techniques, surgery and nutrition. The second deals with diseases of cage birds grouped into chapters according to the various anatomical systems of birds, plus some chapters on types of infectious agents or conditions which result in disease. Again, there is great variation in the quality of the various chapters. From a clinician's standpoint, the chapter on parasitic diseases of avian species is one of the best this reviewer has seen in a general text of this kind. If a pattern for all the chapters dealing with disease as such had been agreed upon beforehand, a much more useful book would probably have resulted.

The book is well illustrated with tables and black and white photographs. It also contains some beautiful colored photographs of a variety of caged birds (parakeets, budgerigars, finches and canaries). The index is better than average. Most of the chapters have either a list of references, or suggestions for further reading, some having both.

The veterinary practitioner or avian pathologist who is called upon to work with pet birds will find this a useful book to have on his library shelves. — *Wilson Henderson.*