EPIZOOTIOLOGICAL FACTORS IN THREE OUTBREAKS

OF PSEUDOTUBERCULOSIS IN BRITISH COLUMBIA CANARIES

by

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ABSTRACT

Three naturally-occurring epizootics of <u>Pasteurella</u>
<u>pseudotuberculosis</u> in canaries were studied. Cultural details
and gross histopathological lesions were described for birds
from two of the aviaries. Epizootiological observations were
made in all three cases following visits to the premises and
recovery of data on management, first clinical signs, and
mortalities.

A reasonably complete study was made of early and
current literature concerning pseudotuberculosis infections
and incidence in birds and mammals, both feral and domesticated.
Although this disease has been commonly reported in canaries
in Europe from 1884 onwards, the epizootics herein reported are,
as far as the author is aware, the first bacteriologically con-
firmed canary infections to be reported on the North American
Continent.

Because of the high mortalities encountered, and
because of the known potential of the causative organism to
produce human disease, the epizootiological considerations were
extended to include experimental studies on the faecal excretion
rate of viable <u>Pasteurella</u> <u>pseudotuberculosis</u> by naturally and
artificially infected canaries.

With the evolution of suitable culturing techniques, the recovery of viable organisms of the inoculation strain from experimentally infected canaries was performed with ease. This allowed counts to be carried out on total daily faecal samples from twenty inoculated and four control birds for a three-week period following oral inoculation. It was found that in the group of twenty inoculated birds which suffered five (20%) mortalities, some sixteen (80%), including those birds that died, excreted organisms for periods ranging from three up to nineteen days. Peak amplitudes for the estimated faecal counts of Pasteurella pseudotuberculosis per day varied from Log 4.3 up to Log 8.1. The four dead birds showed gross lesions typical of naturally-occurring pseudotuberculosis, and yielded recovery cultures from various organs. The remaining twelve shedder birds all ceased shedding viable P. pseudotuberculosis by the twentieth day following the conclusion of oral inoculations (24th experimental day), and although none yielded a positive culture from various organs, there were slight-to-marked splenic lesions in all but two when they were autopsied on the twenty-fifth experimental day. Four birds which never shed detectable numbers of viable P. pseudotuberculosis were found to have no visible gross lesions when sacrificed and autopsied. Four non-inoculated control birds also failed to shed detectable numbers of viable P. pseudotuberculosis.

Attempts were made in the laboratory to allow naturally and experimentally infected canaries to transmit the infection to healthy contact birds. These attempts were unsuccessful, and it was concluded from this and from direct observations on the natural epizootics that predisposing factors other than the presence of the organism (such as climatic or poor-management stress, or gastro-intestinal irritation) are required at times for the disease to become epizootic in canaries. From the faecal excretion rates of viable P. pseudotuberculosis measured in experimentally infected birds, and from epizootiological observations, it was concluded that canaries infect each other (rather than the infection coming from a common source) and that they are potential spreaders of infection (or of the infecting organism) to other species in contact, including man. Gross and histopathological observations of experimentally infected birds correlated with their faecal counts, and gross pathological observations on naturally-infected birds, indicate that lesions in the bowel wall, in particular caecal abscesses, are the lesions which when present predispose to a high potential of infectivity in the shed faeces.

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

Specific bacterial disease due to infection with
Pasteurella pseudotuberculosis has been known since 1883 when
it was first isolated in the guinea pig by Malassez and Vignal.
Subsequently reported isolations indicate that this pathogen is
world-wide in its distribution, and has a wide host spectrum,
(Meyer 1958) (Stafseth 1959) (Burrows 1963). The principal
reservoirs appear to be wild rodents and birds, (Meyer 1958)
(Wilson and Miles 1955). In many cases the organism causes
progressive disease characterized by pseudotubercle formation
and high mortalities in the population involved.

pseudotuberculosis infections have economic importance
in some domestic animals e.g. sheep, mink and chinchillas; in
birds, e.g. chickens, turkeys and pheasants; and in laboratory
animals, in particular the guinea pig, (See Literature Review
and Appendix). Only a few generalized fatal cases of P. pseudo-
tuberculosis infection were reported in man until it was dis-
covered that this organism is the cause of a non-fatal mesenteric
lymphadenitis. This condition is common in Europe, and it has
been recognized in California (Goldman 1957) and Alberta (Hnatko
and Rodin 1962). In the last fifteen years of the nineteenth

century, epizootics due to this infection were reported frequently in canaries in Europe. Though recent reports are lacking, infections in canaries may still be common in Europe; however, the cases herein presented appear to be the first confirmed reports for North America to be published or cited.

This study was occasioned by the appearance of canary pseudotuberculosis in epizootic form involving three well-separated aviaries in 1958. Two occurred on the British Columbia lower mainland, and one on Vancouver Island.

Preliminary attempts to isolate the causative organism from the faecal material of infected canaries were unsuccessful. In view of the opinion of at least one authority on the oral route of transmission (Meyer 1958), and in view of the high mortality noted in canaries, this seemed surprising. With more study on techniques, isolates were secured with ease from droppings of canaries artificially infected by the oral route. This allowed quantitative determinations to be made on the basis of plate counts of diluted faecal material.

Since completing the above determinations, a human case of mesenteric lymphadenitis in Britain has been linked with the presence of P. pseudotuberculosis in the faeces of a canary owned by the patient (Daniels 1961). Early in 1963 a confirmed outbreak of canary pseudotuberculosis occurred in a Vancouver aviary following the introduction of a male breeding

bird which was imported from Europe. In July, 1962, a report was submitted from Maryland (Clark and Locke 1962) describing a major epizootic affecting common grackles in an icterid roost with a normal capacity of about one million birds. Recently an epizootic of guinea pig pseudotuberculosis occurred on a farm in the Fraser River delta area of British Columbia (Stovell 1963). These current incidents help to illustrate the widespread occurrence and varied forms of disease caused by P. pseudotuberculosis.

Since it has been established (Knapp 1954, 1958) that the organism under study causes the abscess-forming reticulocytic lymphadenitis of the mesentery (ARLM) described by Masshoff and Dölle (1953), the matter becomes of great clinico-pathological significance in human medicine. Mair et al. (1960) quoted the figures of Aird (1945) for the Royal Hospital for Sick Children, Edinburgh, and their own figures for the Royal Infirmary, Leicester, also involving children. These figures show that of 120 clinically suspected appendicitis cases in Edinburgh (1944) and 93 such cases at Leicester (part of 1959), some thirty-seven (30.8%) and twenty (21.5%) respectively proved to be suffering from "non specific" or "acute" mesenteric adenitis. While in retrospect it can be noted that many possible causes have been suggested (but not proven) such cases occurring in the future must, in the light of new knowledge, be considered for differential laboratory diagnosis which includes pseudotuberculosis.

From time to time it is to be expected that natural epizootics in peak-cycle populations of wild rodents and birds will be reflected by periodic increases in the human case rate. There appears to be little chance of an improvement being made in this uncontrolled situation, unless the epizootiologist and epidemiologist join forces to establish the important connecting links between man and the creatures of his environment.

REVIEW OF THE LITERATURE CONCERNING
PSEUDOTUBERCULOSIS IN BIRDS[1]

Because of the apparent absence of North American
reports on pseudotuberculosis in canaries, and in fact the
rarity of such reports on other birds as well, a preliminary
search of the literature was made. It was noticed that Stafseth
(in Beister and Schwarte 1959) gave an excellent description of
the disease which was based entirely upon European work except
for Beaudette's (1940) contribution on the blackbird case in
New Jersey in 1939. Recently, Clark and Locke (1962) in des-
cribing the major epizootic in grackles, have given no other
specific references to pseudotuberculosis in North American
birds.

It became apparent that there was a dearth of
literature reports on pseudotuberculosis in any North American
animals or birds, with the probable exception of guinea pigs
(which would presumably show in laboratory annual reports) and
possibly chinchillas. One large question remains. Are diagnoses
in birds and mammals being made but not reported or is there in
fact less pseudotuberculosis in North America than in Europe?

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1 For pseudotuberculosis in mammals and man, see Appendix.

Various references contained herein appear for the first time. These references involve canaries, finches, chinchillas, cattle (in BC), chickens, and beaver (in Alberta). In order to better understand the probable factors involved in answering this question, it was necessary for the disease reports to be studied in detail, covering as many species as possible so that a true picture of epizootiological possibilities could be obtained. The following pages carry tabular and descriptive details of pseudotuberculosis, reports noted in the literature.

Canaries and Other Cage Birds

Canaries

The first report of Pseudotuberculosis in canaries (Zurn 1884) was followed by at least fifteen others. France, Germany, and other European countries including Scandinavia were affected. Most reports appeared in the next thirty years up to 1914. Possibly the dearth of reports thereafter was due to the condition being considered too commonplace to report in the literature.

The presence of canary isolates in lists of strains studied by various workers over the intervening years suggests that the incidence of infection continues. (Beck and Huck 1925; Lerche 1927; Pallaske 1933; Thal 1954; Keymer 1959). Only one probable case was reported previously in North America, and this

was not confirmed bacteriologically. This incident occurred in Pennsylvania in birds recently brought in from Washington, D.C. (Kinyoun 1906). Three outbreaks occurred in British Columbia in one year (Stovell 1959 -- herein reported in detail). A fourth outbreak occurred in 1963 in Vancouver, following introduction to the aviary of an imported European bird, (Stovell 1963).

In view of imports of canaries from Europe especially, and the presence of infection in other species, it seems surprising that more cases of pseudotuberculosis have not been diagnosed and reported. Other diseases such as canary pox have been introduced to British Columbia by imported birds (Stovell and Simon 1960).

One case of pseudotuberculosis occurring in finches is considered in this section (Bryner 1906).

As noted in Table I there is little information given on the aviary history, or on factors suggesting the mode of transmission in many of the literature reports. It seems that extremely high mortality rates were common, although not invariably encountered. Transmission appears to have occurred from bird to bird, either directly or through contaminated food, water, or appliances. The initiation of an epizootic can evidently be due to transfers of infected birds, contact with rodents, or inoculation of accidentally infected material. During the period discussed, spontaneous outbreaks were also recognized in chickens,

YEAR	AUTHOR	HISTORY OF EPIZOOTIC OR DISEASE INCIDENCE	EPIZOOTIOLOGICAL TRANSMISSION FACTORS ASSOCIATED
1889	Rieck (1) Dresden	May 1888. 1 decomposed bird. Then 3 females and young bird. Last of 200.	No observations
1889	Rieck (2)	60 canaries of unstated number died in 14 days	No observations
1903	Wasielewski & Hoffman Berlin	1 bird of 3 inoculated with tissue from yellow bunting in malaria studies. Died day 11 PI. Resulting colony disease worse in Apr & May.	a) import with exp. yellow buntings from Holland for malaria studies. b) rats? Former colony thus destroyed
1905	Pfaff Prague	4 birds examined which came from a dealer	Cross contact infection at dealer's premises?
1906	Kinyoun Wash, DC	Several birds from dealer were affected	Contact at dealer's premises?
1906	Bryner Germany or Switzerland	60 tiger or butterfly finches and Japanese titmice. 1 cage spread to 3. 22 dead in 1 mo.	Lateral spread from cage to cage
1908	Meissner & Schern ex Magdeburg	'Typical' disease (losses not stated) affecting 800 birds	Shipped in from Magdeburg
1908	Zwick Stuttgart	a) Sept 1904. 27/28 females died. 8 day period. b) Males 14 days later. 41/42 in 14 – 16 days. c) 6 females, 4 males purchased in Dec & placed in clean cages in adjoining rooms d) In 3-4 days surviving female (a) placed with new females. All died but new males.	Apparently brought in by caretaker who lost own birds previously. 500 – 600 birds said to have died around Stuttgart during this period
1914	Zeiss Giessen	July 1912, 44 birds of unstated number died in three weeks	
1959	Stovell BC	5 month period Feb to June 58, 3 major separate epizootics in aviaries (reported herein) 90 – 100 % mortalities	2 cases rodent contact known Dead rodents in flight cage in one case evidence of severe field mouse population drop at this time

TABLE   I   HISTORY AND EPIZOOTIOLOGY OF PASTEURELLA PSEUDOTUBERCULOSIS
OUTBREAKS IN CANARIES AS REPORTED BY VARIOUS AUTHORS

and toward the end of the period, in turkeys. Possibly the incidence in domesticated birds during that period suggests either a greater degree of exposure or maintenance of a lower standard of sanitation than is now the average in poultry and aviary husbandry.

Clinically, there appear to have been few distinguishing features of pseudotuberculosis reported in canaries. This is probably due in part to the small size of the birds and the difficulty in handling them. (Table II).

The post-mortem lesions recorded are consistent though fragmentary. It appears that in birds the presence of typical 'millet-sized nodules' in liver and spleen is pathognomonic. Where the last few surviving birds of an outbreak are the only specimens examined, the diagnosis may be more difficult. For purposes of comparison, some of the findings from epizootics in canaries in British Columbia are recorded in Table III.

No descriptions of the histopathology of this condition in canaries were found in the literature. Pallaske (1933) has described the lesions noted in one canary which was infected parenterally.

The problem of differential diagnosis of pseudotuberculosis is not serious in cage birds, in contrast to the

YEAR	AUTHOR	CLINICAL OBSERVATIONS	Lung	Liver	Spleen	Bowel	Other
			PATHOLOGY NOTED IN SPONTANEOUS DISEASE IN CANARIES				
1889	Rieck Dresden	Quiet, Anorexia Diarrhoea		Grey-yellow necrotic spots		Catarrhal enteritis	Discolored skin,neck breast,abd
1903	Wasielewski & Hoffman Berlin	Infections followed use of contaminated inoculum. No observations.		If disease three days clinically: As spleen but less abundant Nodules often bump up from surface*	Swollen Many yellow nodules		Died 11 days PI. Yellow spots on & in breast muscle
1903	Pfaff Prague	Anorexia, diarrhoea sleepiness		4 birds yellowish-white spots		Enteritis	
1906	Bryner Switzerland	No observations Finches	Some had lesions	Positive for lesions		Some had pancreatic lesions	
1906	Kinyoun Wash, DC	No observations Did not see birds alive	In some Catarrhal exudate upper air passages	Yellowish nodules projecting from surface*		Some had enteritis	
1908	Meissner & Schern ex Magdeburg	No observations		Raised nodules*			Some had Diphtheric membrane
1908	Zwick Stuttgart	No observations		Grey-yellow raised nodules			
1914	Zeiss Giessen	No observations			Hypertrophy	Haem Enteritis	Cardiac Haem

* Findings in birds examined in Vancouver Area

TABLE II CLINICAL OBSERVATIONS AND PATHOLOGICAL LESIONS NOTED BY VARIOUS AUTHORS IN CANARIES SPONTANEOUSLY INFECTED WITH PASTEURELLA PSEUDOTUBERCULOSIS

YEAR	AUTHOR	CLINICAL OBSERVATIONS	PATHOLOGY NOTED IN SPONTANEOUS DISEASE IN CANARIES				
			Lung	Liver	Spleen	Bowel	Other
1958	The author Vancouver 2 out of 3 outbreaks occurring **within reach of laboratory**	Depressed Ruffled No singing Hop around at times Usually continue eating Usually utter hoarse chirps Diarrhoea in young birds	Very occasional caseous areas or nodules	Quite frequent yellow raised millet-sized nodules No hypertrophy usually	Almost all cases numerous yellowish nodules often giving extreme hypertrophy	Enteritis in young birds Some had caecal nodules or abscesses	Breast muscle very occas thymus area Caseous material occasion-ally

TABLE III CLINICAL OBSERVATIONS AND PATHOLOGICAL LESIONS NOTED BY THE AUTHOR
IN CANARIES FROM SPONTANEOUS EPIZOOTICS OF PASTEURELLA PSEUDOTUBERCULOSIS
INFECTION IN BRITISH COLUMBIA

case with the disease in rodents. Generally, the clinical picture is of little help; some birds are depressed and do not eat, others may be active and will continue to eat or attempt to eat until shortly before death. Many, but not all birds develop diarrhoea late in the course of the disease. On autopsy it is necessary but not difficult to differentiate pseudotuberculosis from other diseases characterised by caseating granulomatous or necrotizing focal lesions; such as listeriosis, enterohepatitis,vibrionic hepatitis, and others. In addition, care has to be taken to differentiate various types of catarrhal enteritis such as salmonellosis and coccidiosis.

These minor difficulties were evidently encountered by early reporters of pseudotuberculosis in canaries; however, apart from some difficulty in naming the condition, the trail was by no means lost at any stage. Malassez and Vignal (1884) named the condition "zoogleic tuberculosis" in the first discovery in the guinea pig. Several reporters apparently gave no name to the condition (Zurn 1884) (Rieck 1889) (Wasielewski and Hoffman 1903) (Pfaff 1905) (Zwick 1908). In 1894 Preisz declared the causative organizm in birds to be the same as the P. pseudotuberculosis of guinea pigs and rodents. 'Infectious necrosis' was the term used by Meissner and Schern in 1908. In 1914 Ziess classed his causative organism along with those of a number of earlier authors, in the 'haemorrhagic septicaemia group', and Beck and Huck (1925) referred to the disease in canaries and

turkeys as 'paracholera'.

Lerche, who worked with both avian and mammalian strains, and Truche and Bauche (1929) who worked with turkeys, were all of the opinion that the disease should be named 'parapest' because of its close similarity to _Pasteurella pestis_. Because of possible confusion with the disease 'fowl pest', Lerche did not actually use the name 'parapest', but rather called his disease 'paracholera' after Beck and Huck. In succeeding years the disease gradually became referred to as 'pseudotuberculosis'.

Budgerigars (Shell Parakeet)

No reports involving budgerigars were found in the literature. A personal communication with a pathologist who was at that time in a hospital in London, England (Colbeck 1940) disclosed that a significant number of deaths occurred in a budgerigar aviary owned by one of the hospital staff members. The lesions in the birds were classical for pseudotuberculosis and the causative organism was isolated and identified. No visit was made to the aviary, so we have no observations on epizootiology or transmission.[1] Further observations on the

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1 The owner stated that she suspected the deaths of the birds to be partly a result of their "being frightened" by owls which swooped over the flight cages frequently. Pseudotuberculosis in owls in Sweden has been reported by Thal (1961).

possible susceptibility of budgerigars will be found in a later section dealing with pathogenicity for experimental animals.

Finches and Other Captive Hard-Billed Birds

One outbreak of pseudotuberculosis in finches has already been covered in part by Bryner (1906). No other references to the disease in finches have been found in the literature for the next half-century. In 1954, Thal listed finches as the source of two of his avian strains. Later, in 1959, Keymer stated that pseudotuberculosis is probably the commonest bacterial agent to cause disease among captive hardbilled birds (especially canaries) in Great Britain. He goes on to state that he has also diagnosed the condition in a Cuban finch (Tiaris canora) and in a wild snow bunting (Plectrophenax nivalis). Langford in 1960 diagnosed the condition in a single bull finch in British Columbia. According to the owner, the bird died suddenly with no previous signs of illness.

As can be noted in Figure 7 (Key) finches were present in one canary epizootic in B.C. However they remained unaffected.

Free-living or Free-ranging Birds

In general, the infection is thought to be common in wild birds at least in some areas such as the British Isles (Keymer 1959). Some authors (Hutyra, Marek, and Manninger 1946) consider that the infection is present in many countries, and its occurrence is so common in nature that its introduction is not necessary for the disease to occur. These authors incriminate infected faeces of birds, in causing spread of the disease.

Pigeons and Doves

As can be noted in Table IV, the infection has been reported frequently in both sporadic and epizootic form. Quite possibly pigeons and doves may be the most important avian species involved in natural epizootics leading to subsequent infection in domesticated species and man.

Of particular interest would be any findings pertaining to the relationship, in various areas, between wild birds and wild rodent epizootics. In the area of England covered by Clapham (1953) detectable infection was apparently confined to birds during the study period.

Recently Paterson and Cook (1962) reported on 'repeated re-introduction of pseudotuberculosis to a large guinea pig colony by use of greenfeed contaminated with the excreta of infected wood pigeons, (Columba palambus)'.

YEAR	AUTHOR	CIRCUMSTANCES	PATHOLOGY	AUTHOR'S CONCULSIONS
1916	Dolfen Hannover	Dead 3 yr old carrier pigeon	Typical nodules in spleen liver lungs	Similar organism isolated by Pfaff (1905) & Meissner & Schern (1908) from canaries
1916	Meder* Germany	7 pigeon deaths in recent flock add'ns	as above	as above
1928	Beck Leipzig	1 pigeon	Miliary necrotic lesions in spleen and liver	Identical to 1 canary and 5 turkey strains isolated at Leipzig 1923-24
1933	Pallaske Leipzig	Not stated		Worked with 2 strains from spontaneous infections
1934	Les Bouyries France	20 sick. Flock of 30. 10 deaths. Pronounced lameness, anorexia.Die 3-8 days after clinic-al onset	2 birds autopsied 1 hepatic & splenic nodules; 1 nodules in duodenum	
1951	VanDorssen	2 diseased pigeons organism isolated		Suggested organism closely related to Shigella pfaffi
1953	Clapham Hampshire England	3 month epizootic in stock doves. 1 wood pigeon of 17 examined found infected	Small caseating nodules in liver & spleen. Both organs slightly enlarged	Did not seem to be a rodent epizootic in the area at that time
1950 to 1954	Marthedal & Villing Denmark	7 outbreaks recorded in pigeons	Original paper not obtained	
1962	Patterson & Cook Southern England	Flocks of wood pigeons Columba palambus found grossly affected and excreting numerous P. pseudo organisms	Not stated whether deaths recorded	Repeatedly infected guinea pig colonies by faecal contamina-tion of green feed

* Cited by Dolfen 1916

TABLE   IV   PARTIAL FINDINGS OF VARIOUS AUTHORS RELATING TO SPONTANEOUS PASTEURELLA PSEUDOTUBERCULOSIS INFECTION IN PIGEONS & DOVES

Pheasants and Other Galliformes

There are several reports of infection in or work carried out on strains from species of this group, particularly in the pheasant (Hutyra et al.) (Truche and Bauche 1933; Tottire Hippoliti 1942; Karlson 1945; Clapham 1953; Thal 1954). Hutyra et al. have classed the disease reported by Klein (1921) as pseudotuberculosis. Truche and Bauche, in France, noted that three or four deaths occurred in a pheasant flock over an eight-day period. Small grey spots were noted on the liver surface and the bacterium was isolated from liver, blood, and bone marrow. Both Karlson and Thal are cited here because they each worked on partridge strains. Clapham isolated the organism from a pheasant, a partridge, and a Bobwhite quail.

Blackbirds and Grackles

A complete description of a case of pseudotuberculosis in a blackbird has been contributed by Beaudette (1940). This report was accompanied by an excellent review of the infection in birds. Beaudette noted that apart from the canary episode reported by Kinyoun (1906) (Table I) (unconfirmed bacteriologically, but doubtless pseudotuberculosis -- PLS), his case in the blackbird was the first reported in any bird in the United States. The bird was thin, lame, and suffering from diarrhoea. The liver was found to contain one yellow nodule and a number of grey ones.

Clark and Locke (1962) reported on two outbreaks of pseudotuberculosis occurring in purple grackles (Quiscalus quiscula) in Maryland in 1959 and 1960. The second event was a major epizootic occurring during early spring in an icterid roost which held up to one million birds during the winter months. Isolations of Pasteurella pseudotuberculosis were made from dead and sick birds (shot). Gross and histopathological lesions were well described. The liver, spleen, lungs, breast and other skeletal muscles were involved with white or yellow pseudotubercles. The mortality was calculated to be high, and grackle carcasses were found in large numbers along roads and around buildings up to ½ mile away from the roost. Various factors of late, winter stress were thought to contribute to such epizootics.

Other Free-living Species of Birds

Although the information is by no means complete, Table V has been prepared by listing some of the reports which can be found in the literature, involving among other species buntings, larks, waxwings, jackdaws, rooks and swans.

Apparently a wide spectrum of avian hosts can be affected by the nodular lesions of P. pseudotuberculosis, and one is tempted to suspect that many cases of this disease must occur which are not investigated, identified or reported.

YEAR	AUTHOR	LOCATION	SPECIES AFFECTED AND DETAILS	PATHOLOGY
1954	Marthedal & Villing	Denmark	Snow Bunting	
1959	Keymer	England	Snow Bunting	
1903	Wasielewski & Hoffman	Germany	Yellow Buntings from Holland	Not noticed apparently
1953	Clapham	Hampshire England	One lark found dead	Liver showed signs close network of caseating fibers. Spleen showed two bulging nodules of pinhead size
1956	Marthedal & Villing	Denmark	Waxwings	
1953	Clapham	Hampshire England	Jackdaw and Rook	
1935	Truche	Normandy France	Swan -- 2 died	Enteritis; nodules in liver and spleen Cardiac haemorrhage Renal congestion
1937	Urbain & Nouvel	France	Toucans -- 2 varieties In captivity for some years	
1954		Sweden	Paradise Birds	Strains only mentioned

TABLE   V   PSEUDOTUBERCULOSIS IN VARIOUS WILD BIRDS   PARTIAL DATA OF
SEVERAL AUTHORS

Domesticated Fowl Used for Human Food

As will be noted in Table VI, a number of outbreaks of pseudotuberculosis have occurred in chickens in various European countries, and one incident is recorded here involving a flock of chickens in Alberta.

Severe outbreaks of pseudotuberculosis have occurred in turkeys in Oregon, and the condition has also been reported in California (Table VII). The condition was common in Europe in the early part of the present century, and it may still occur there, but perhaps is reported less. The findings of the various authors suggest that under certain adverse conditions, turkeys must be highly susceptible to P. pseudotuberculosis. Perhaps the current sanitary level in turkey management is less likely to predispose to epizootics even if the organism should be there, due, for example, to the presence of commensal or wild rodents.

There have been at least two reported cases of pseudotuberculosis in ducklings. Boquet (1937) attributed one of these to Truche and Bauche (1930). Another case was reported by Plasaj (1929). It seems that the disease is probably not an economic problem in this species.

YEAR	AUTHOR	CIRCUMSTANCES & INVESTIGATION	PATHOLOGY	AUTHOR'S COMMENTS
1885	Nocard France			Same organism as Malassez & Vignal's Bacillus
1897	Woronoff & Sineff Germany			
1926	Tottire Hippolito Italy			
1933	Truche & Bauche France	Flock of 50. Several vaguely sick. 8 dead one morning and two the next.	Exudate in nares Yellow gelatinous material in pericardial sac. Peritrachealoedema Nodules in kidneys Slight enteritis	Non-pathogenic for fowls IV. Considered P.pseudo required a predisposing infection
1937	Truche & Isnarde Aisne region France	Slow moving infection flock of 57. 11 deaths in 11 months. 12 pullets died at age 3 mos.	Nodules in intestine & mesentery noted by owner. He submitted feet to lab. Custom- ary in France	P. pseudo isolated from bone marrow
1939	Schafer Konisberg Germany	INfections in young chickens		
1947	Karlson Sweden	Worked on one chicken strain of a total of 13 avian source isolates		
1948	Mira Spain			
1925	-- Denmark	Importation of live poultry species banned due partly to pasteurella infections		

TABLE VI   PARTIAL LIST OF AVAILABLE REFERENCES IN THE
            LITERATURE CONCERNING PSEUDOTUBERCULOSIS IN
            CHICKENS

YEAR	AUTHOR	CIRCUMSTANCES & INVESTIGATION	PATHOLOGY	AUTHOR'S COMMENTS
1954	Marthedal Denmark	'Sporadic chronic or subacute cases still noted in fowl'		Some cases of Pasteurella were Pseudotuberculosis
1960	Stovell & Avery	40 chickens died in flock of 4-5 month old birds	One bird examined. Miliary lesions in liver and hypertrophy of spleen	Typical of P.pseudo bacto and by animal inoculation (canaries and guinea pigs) initially fermented sucrose

TABLE VI

page 2

PARTIAL LIST OF AVAILABLE REFERENCES IN THE
LITERATURE CONCERNING PSEUDOTUBERCULOSIS IN
CHICKENS

YEAR	AUTHOR	CIRCUMSTANCES & INVESTIGATION	PATHOLOGY	AUTHOR'S COMMENTS
1924	Krage & Weisgerber Germany	Outbreak in 14 turkeys. Chickens, pigeons and geese in contact were not affected	Catarrhal enteritis. Enlarged spleen. Haem on peritoneum.	No tubercles seen Isolated from two turkeys with no gross lesions
1925	Beck & Huck Leipzig	Five strains isolated in 1923		Reported on bacto similar to canary strain in all respects
1926 1927	Lerche Leipzig	Outbreak in turkeys. Worked on total of 35 strains		Called Paracholera after Beck & Huck Perhaps better called Parapest
1928	Haupt Germany	Worked on rodent, canary and turkey strains		Found no difference in strains
1929	Truche & Bauche France	First case in turkeys in France. Several flocks. Av Mort 14 - 16%	Catarrhal enteritis of duodenum. Degen. of liver & kidneys Miliary foci of necrosis in liver often, spleen occas., lung negative	Organism could be called B. parapestis and disease called Parapest
1942	Stephan	Worked on one turkey strain		
1927	Karlson Sweden	Worked on ten turkey strains of 30 avian isolates		Felt that disease came directly or indirectly from rodents and required predisposing factors
1944	Rosenwald & Dickinson Oregon, USA	Ten flocks of turkeys infected in Oregon in 20 month period Fall 1940. 13250 birds involved Also 500 deaths of 2700 poults	23 dead birds from 10 flocks examined. 122 deaths in mkt wt birds. Most caseation nodules in liver, at times in spleen. Panc and int submucosa showed 'greyish infiltration'	15 isolates made. Most cases occurred in rainy season & birds drank puddles. Liver best organ for isolation but spleen and heart blood too

TABLE VII   PARTIAL LIST OF AVAILABLE REFERENCES IN THE LITERATURE CONCERNING PSEUDOTUBERCULOSIS IN TURKEYS

YEAR	AUTHOR	CIRCUMSTANCES & INVESTIGATION	PATHOLOGY	AUTHOR'S COMMENTS
1954	Mathey & Siddle Calif USA	Sporadic case in eight-month old tom turkey	Abdominal ascites shrunken cirrhotic liver Pale heart muscle	Culture from heart and liver
1954	Thal	Sweden	Of 186 strains studied 19 of 32 avian strains were of turkey origin	

TABLE VII

page 2

PARTIAL LIST OF AVAILABLE REFERENCES IN THE LITERATURE CONCERNING PSEUDOTUBERCULOSIS IN TURKEYS

24

DESCRIPTIONS OF THREE EPIZOOTICS OF PSEUDOTUBERCULOSIS IN BRITISH COLUMBIA CANARIES

Three canary epizootics of pseudotuberculosis occurred in 1958 in B.C. These are described and discussed in terms of the recording of natural conditions and events, the epizootilogical deductions that were drawn, and the laboratory supportive work as far as it concerned diagnosis.

History and Recorded Observations

AVIARY No. 1

Date of Epizootic

Late December to early April (balance of stock in aviary destroyed April 3, 1958).

Location of Aviary

The building faced south in a well-drained, sheltered lot in a spacious residential area in south central Vancouver. There were many trees and garden lots close by, including one immediately adjacent to the back of the aviary which was used for growing vegetables. This lot was fertilised once yearly in the fall with cow manure from a dairy farm in Richmond.

Design and Condition of Building

The aviary was a single-storey, permanent, double (two-flight) aviary constructed of timber and wire and completely roofed with cedar shingles. There was fine gravel on the concrete floor in the flights, a central entrance and service passageway gave access. The flights were open to the front and part of the sides (Figs. 1 and 2).

Operational Details and Management

Feeding of a commercial canary seed mixture was carried out with the use of two wooden floor troughs which were replenished daily. In mid-winter the caretaker mixed cod liver oil emulsion with dampened seed to a porridge consistency and offered this in flat dishes on the broad ledges of the flights. For most of the year, chickweed from the vegetable plot was fed once or twice weekly to the birds, being pushed into the flight cage wire so that the birds could pull it.

Water was supplied in a galvanised trough and replenished daily. During the breeding season, extra water dishes were frequently placed on the flight ledges.

Because of the full cover and sheltered area, no extra weather protection (such as glass screens) was used in the winter. When health conditions were normal, the nest boxes were placed in the flight. These were attached to the beams and

Figure 1   Aviary No 1   Southerly aspect
           with entrance to service
           passageway.

Views on this and the following pagees
show the aviary involved in the first
epizootic of pseudotuberculosis in
canaries. This building combined flights
for both canaries and budgerigars.

Figure 1

posts close to the ceiling of the flight, thus being sheltered
by the overhang.

Rodent control was carried out by occasionally setting
spring traps and by covering with tin any holes which were
gnawed through the back of the flight. It was stated by the
owner that there had been no rodent problem until the fall of
the year when the outbreak commenced in December.

Stock in Aviary at Commencement of Losses

The west flight contained approximately fifty budger-
igars, which were managed in much the same way as the canaries,
and which suffered no losses or noticeable disease, (Figure 3).

The east flight contained twenty-nine canaries in
late December, 1957. These were mostly hatched in 1956 and 1957.
Approximately twenty birds of the older stock were given away
to the Stanley Park aviary in early fall.

Record of Mortalities

As will be noted in Table VIII, in a total of twenty-
nine birds, ten deaths occurred over an approximately three-month
period. The mortality rate climbed to the level of about one
death per four days by mid-March. The remaining nineteen birds
were destroyed with CO_2 at the beginning of April, and sixteen
of these were found to have advanced lesions of pseudotuberculosis
mainly involving the spleen (Fig. 4).

Figure 2   Aviary No 1. Flight used for
            canaries.

            Note the gravel floor and
            spacious quarters for approx-
            imately thirty birds. The
            elevated feeder at centre was
            installed later on the suggest-
            ion of the author.

Figure 3   Aviary No 1. West flight
            housing budgerigars which
            remained healthy.

            Note nest boxes, hanging
            roosts, and placement of
            feed and water dishes similar
            to those used in the canary
            flight.
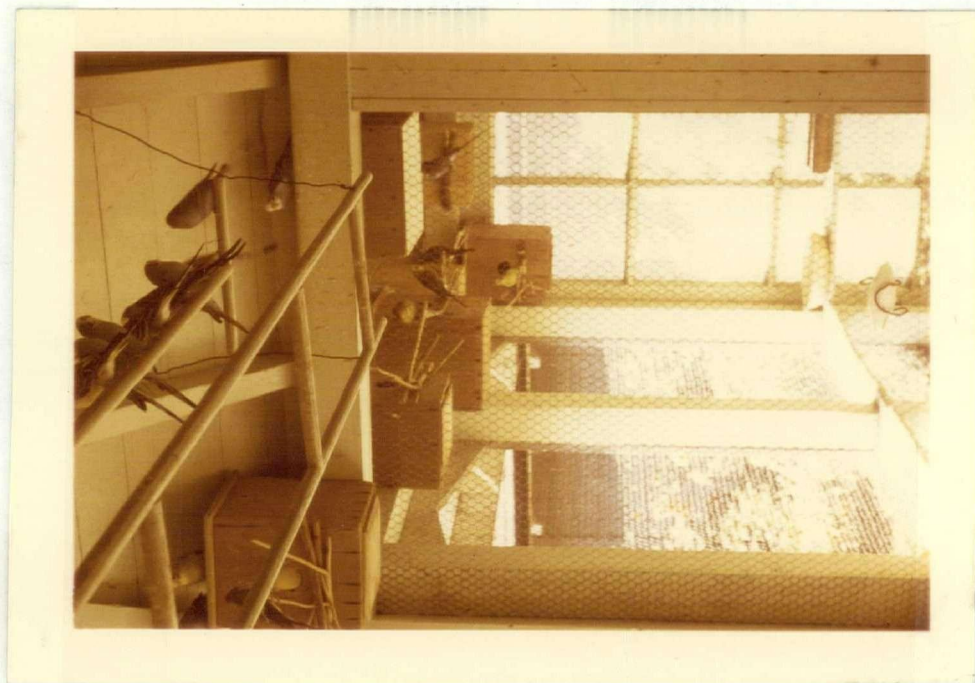
Figure 3.

Figure 2.

Figure 4   Aviary No 1. Some internal
            organs of infected canaries.
            These birds were sacrificed
            sixteen days after mortalit-
            ies became heavy.

One normal and three affected spleens
(middle row) can be compared for size
with hearts(top row) and gonads (bottom
row) for two male and two female birds.
Note the presence of characteristic yellow
nodules on the spleens. This lesion,which
is virtually pathognomonic, was noted
predominantly in the spleens in this
outbreak. Fourteen of sixteen birds
sacrificed showed this involvement.
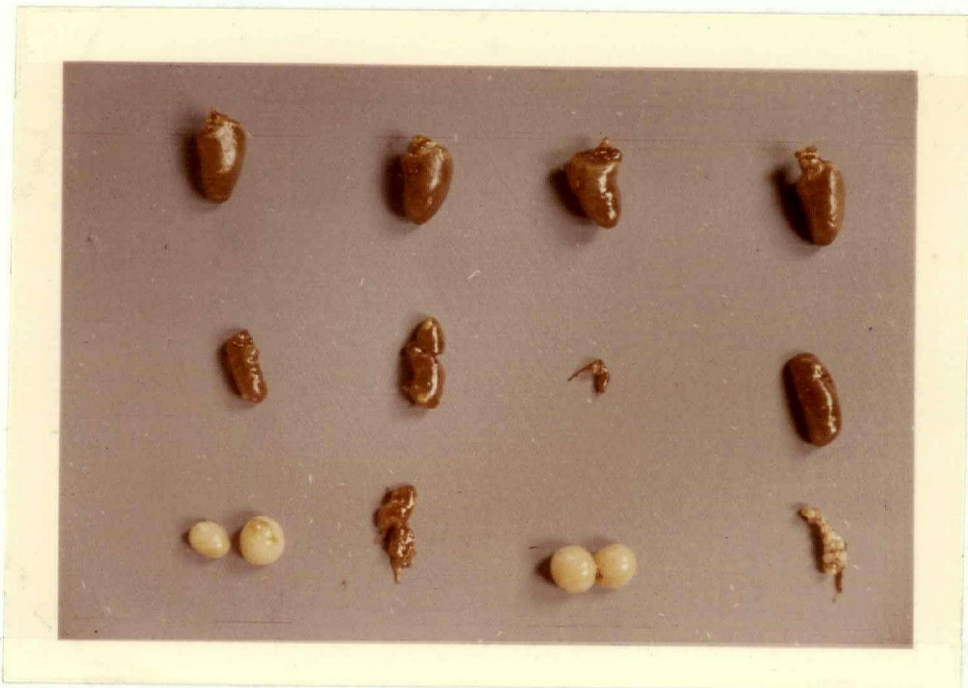
Figure 4.

BIRD NO.	SEX	DATE OF DEATH	DATE KILLED CO2	CLINICAL APPEARANCE TO CARETAKER	PRESENCE OF LESIONS			P.PSEUDO ISOLATED
					SPLEEN	LIVER	OTHERS	
Not designated, but four birds died from Xmas to early March				Individual birds that died usually sick 1 - 2 days. Gradually whole group depressed. Singing stopped.	Not examined			
1	F	14 March		Depressed, rough 1 - 2 days	pos	pos	caecal buds yellow nodules	isolated
2,3,4	2M 1F	16 17 March		Depressed, rough 1 - 2 days	3 pos	1F pos	3 neg	not attempted
5	F	29 March		Depressed, rough 1 - 2 days	pos	pos	neg	not attempted
6,7,8,9	2F 2M		1 April	Slightly dull	3 pos 1M neg	4 neg	4 neg	not attempted
10-25	8M 8F	1 died en route to lab	3 April	Slightly dull	14 pos 2M NEG	3 pos 2M 1F	16 neg	not attmpeted

TABLE VIII RECORD OF NATURAL LOSSES, BIRDS DESTROYED FOR AUTOPSY, AND
POSTMORTEM RESULTS FOR THE EPIZOOTIC OF PASTEURELLA PSEUDO
TUBERCULOSIS INFECTION IN AVIARY NUMBER 1

Discussion of Epizootiological Aspects

(a) Sanitation was excellent; more birds could have been housed in this flight without overcrowding.

(b) The feeding regimen was adequate and unvarying, although one remote and one less remote possibility of infection through this source should be considered. These possibilities were: contamination of canary seed with rodent droppings before it left the mill; and secondly, the contamination of chickweed from wild rodents or birds, or from the cow manure used for fertilising the plot.

(c) There had been no additions to the flock for the last eighteen months, except for natural increases. The previous fall, a group of about twenty adult birds was transferred to a local zoo aviary. No losses occurred in the transferred birds during a three-month isolation period at the zoo. No subsequent trouble developed in these or contact birds at the zoo during 1958.

(d) During the fall, many field mice were seen around the garden in the vicinity of the aviary. Several penetrations were made into the flight by mice. About two weeks before the first canary death occurred, two field mice were found dead one morning in the canary flight cage. Following the laboratory diagnosis, the caretaker was asked to live-trap or poison rodents for examination. Shortly thereafter he reported that there were

no longer any signs of mice in the area. Although no mice were examined, the above factors raised the suspicion of the canary disease being a reflection of an epizootic in mice. Contamination of canary seed could have occurred leading to oral transmission of the organism.

AVIARY No. 2

Date of Epizootic

Late April to July 1958, a period of approximately ten weeks.

Location of Aviary

The aviary was situated in the sheltered garden of a spacious rural residential lot at Qualicum Beach on the east coast of Vancouver Island. This was a luxury summer cottage with year-round caretaker residence.

Design and Condition of Building

This aviary (Fig. 5) was similar in style and construction materials to tbe building involved in outbreak No. 1. The canaries were housed in a flight which formed a horseshoe shape about the service alleyway. The floor had been concreted several years earlier to control rodent entry. Number 1 and Number 2 aviaries belonged to the same owner but were situated about seventy miles apart on opposite sides of the Strait of Georgia.

Figure 5   Aviary No. 2. General
            aspect of building. The
            stock was comprised
            entirely of canaries.

Figure 6   Aviary No. 2 Close up view
            of flight cage. This shows
            feeding trough(above) and
            water dishes on the floor.

As illustrated, many birds prefer to
feed from the floor on seeds scattered
by the birds above. This seems to be
representative of passerine behaviour
under natural conditions where a few
individuals attack the heads of grasses
and grains, while others pick up the
fallen seeds. Thus exposure to fecal
material by ingestion is increased.

Figure 5.

Figure 6.

Operational Details and Management

These were essentially the same for the two aviaries; in fact the caretaker at the No. 1 premises had earlier been in charge at the No. 2 premises. He had built both aviaries and later re-floored them with concrete.

Stock in Aviary at Commencement of Losses

There was a total of sixty-eight canaries involved. These were comprised of forty-eight adult birds and twenty young birds hatched during the period of the epizootic. About the middle of May, twelve of the more mature young birds were selected and transferred to aviary No. 1. In the meantime, following the destruction of the birds in the No. 1 Aviary on April 3, the premises had been thoroughly cleaned and disinfected. This, with the addition of rodent control which was also employed, would seem to eliminate the possibility that these transferred birds became infected from the Vancouver environment.

There were no budgerigars or other captive birds on the premises of the No. 2 aviary. However, there were crows in the area which came around the flight cage. During the previous year California quail had been numerous in the area also.

Record of Mortalities

No exact records were kept by the caretaker. However, since the disease, with or without complications of secondary infections, caused the death of most of the birds, it was not hard to calculate the percentage mortality. By July 10, deaths had ceased and there were two birds (3.6%) left of the fifty-six adult and young which had become sick. Thus the mortality was 96.4%. At ohe point the caretaker moved many of the sick birds to some old pheasant pens at the opposite end of the garden, thinking that he might modify the ravages of the infection in the remaining birds. Deaths of birds continued unabated in both locations.

Of the twelve young birds which were moved to the Vancouver (No. 1) aviary, nine (75%) survived. Shortly after their arrival, the caretaker noticed that two birds were depressed and a third became unusually tame. One of the former and the latter died after about seventeen days, and these were placed in the freezer. Finally, in August the author was again contacted. The two frozen birds were autopsied and the third previously sick bird which was still not vigorous was sacrificed for autopsy. The two dead birds showed typical lesions of pseudotuberculosis and a pure culture of B. psuedotuberculosis was isolated. The third showed no gross pathological lesions, and a culture of pooled tissues was negative. The nine birds remaining gave no

further trouble, and from these birds the aviary population
was built up to twenty birds in about two seasons. Now, five
years after the clean-up, there has been no visible recurrence
of disease.

Discussion of Epizootiological Aspects

(a) Sanitation was good and there was no overcrowding
in this aviary.

(b) There is no reason to suspect the feed of being
contaminated prior to dispensing. There is a possible exception
to this again in the green feed collected from the vegetable
garden, which might have been contaminated by the faeces of wild
rodents or birds.

(c) There had been no additons to the birds other
than natural increase for five years.

(d) The area surrounding the garden was the haunt of
various species of rodents, galliform birds, and others suscep-
tible to pseudotuberculosis. There were crows around, which
would settle on the cage and thus doubtless at times contaminate
the wire fence with faeces. Field mice were seen at times in the
aviary surrounds.

(e) There is the remote possibility that the owner or
one of his family might have mechanically transferred the infection

from the Vancouver aviary, however, it was not the habit of
these people to actually enter the aviary at either location.

AVIARY No. 3

Date of Epizootic

Mid-June to late August, a period of about eleven
weeks.

Location of Aviary

The aviary was located on a sheltered south slope in
North Burnaby municipality of the greater Vancouver area, situated
about twelve miles from the No. 1 aviary. The aviary building was
surrounded by brush and brambles.

Design and Condition of Building

The building was a lightly constructed converted
chicken house which had been previously used for housing dogs
(all of which were reported to have died). It was a single-
storey unlined wooden house of shiplap construction with a tar
paper exterior. Half of the south wall of the building could
be opened down outside and the space was covered with wire in
some places, in other places with glass. Except for an entrance
doorway along the south side, the east end of the building was
taken up with three semi-outside flight cages. There were many

cages spaced out in bays inside the building, two large inside flights, and a number of small cages hung in the poorly ventilated space above the window on the south side. A few cages, containing ten to twelve birds, were hung on the back of the building (Fig. 7).

Operational Details and Management

The aviary was unsanitary, overcrowded and in most parts, poorly ventilated. Rodent control was quite inadequate, and wild or commensal rodents were encouraged by a large pile of feed sweepings on the ground outside, close to the entrance doorway. Watering dishes were removed from cages, emptied, rinsed together in a pail, filled and distributed at random. Feed dishes were removed, emptied, refilled with new feed, and replaced at random. Breeding was carried out in a row of cages next to the first inside flight at the east end of the building. For the general layout of cages and groups of birds, see Fig. 7 .

Stock in Aviary at Commencement of Losses

There was a total stock of 300 birds at the middle of June. These were made up of seventy-five native Lennets, Weavers, Cockatiels, Budgerigars, and Finches (species not recorded). The balance of 225 birds were either canaries, mules[1],

---

[1] Canary cross with (a) Red Sisken, or (b) the F1 or F2 (backcross)

Key to Figure 7

Reconstructed Line Drawing of Aviary Involved in
Epizootic Number 3

1. Flight "MF" containing thirty mules and factors when the
   epizootic began. The initial losses started in this
   flight about ten days before extension of the disease
   to other locations as marked* .

2. Metal strip along the inside of the baseboard along the
   north wall which covered rodent holes. The termination
   of this strip at a point opposite the "MF" flight had the
   effect of funneling the rodent entries through the back
   of the building along a line marked with V's.

3. Point of ingress of rodents which then followed a pathway
   through the MF flight and under or over the area where
   breeding pairs were kept, to the inside of the aviary.

4. Counter area where most of the breeding cages were
   located.

5. Semi-outside flights which contained respectively:

       (a) Lennets and two cockatiels
       (b) Weavers and finches
       (c) Budgerigars, approximately sixteen in number.

6. Small cages for individual birds, suspended in overheated
   space above the windows and against the roof on south
   front.

7. Flight "O" which contained old birds at the beginning
   of the epizootic. These were serviced with fixed seed
   and water containers, and perhaps for this reason remained
   free of infection until younger exposed birds were placed
   in the flight. Four of the birds from this flight were
   transferred to the laboratory as part of group I after
   losses commenced in this flight.

8. Location of a large dump of seeds and sweepings from
   the aviary.
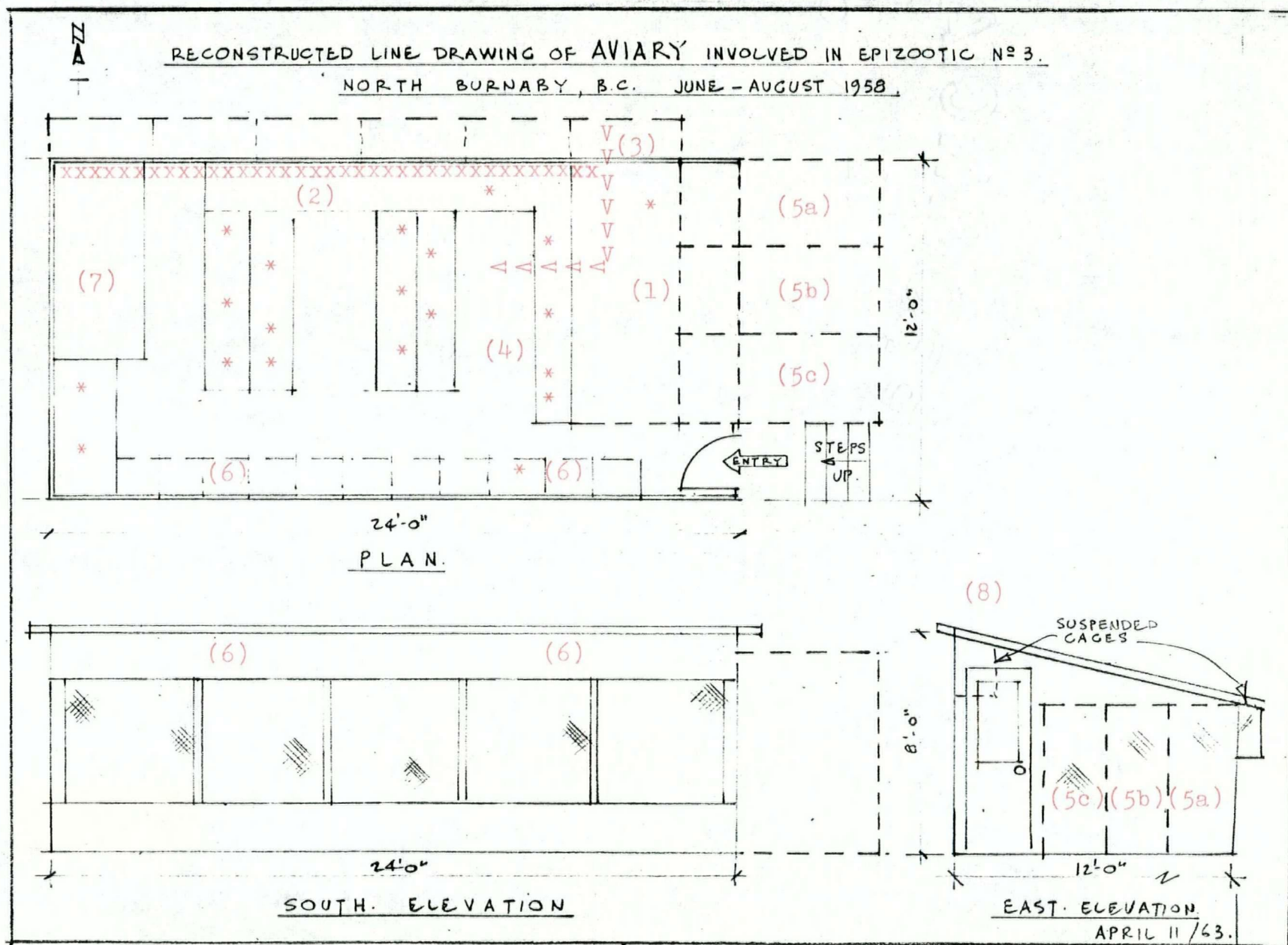
Figure 7. Aviary No. 3 housing canaries and other cage birds.

or red factors[1]. Many of the birds were fledglings of the breeding season in progress.

The aviary had been stocked three months prior to the outbreak, from other premises (belonging to the same owner) where no subsequent disease trouble arose.

Record of Mortalities

Morbidity, according to the owner, was 100% among the 225 canaries and canary cross birds (mules and red factors). Except for the modifying effects involving the thirty-five birds removed to the laboratory, the mortality was also 100%. The budgerigars, cockatiels, native lennets, weavers and finches were not affected to all appearances, and no mortalities occurred.

There was an initial loss of two or three birds around mid-June. The epizootic came to a peak at the end of June and seventy-five birds were lost between June 26 and 30. By July 15, when news was next received by the writer, the losses totalled 165 (73.3%) of the 225 canary and canary cross birds. At this time twenty live birds (designated as Group I, Experiment A) were removed to the laboratory. On a revisit July 29, ten more deaths had occurred, and fiteen more birds (designated as Group

---

1 Canary crossed with F3 mule (backcross)

II, Experiment A) were removed to the laboratory. Fifteen fledglings remained at the aviary. These were removed to the outside by the owner in hopes of effecting a modification in the disease course. On September 8, the owner informed us that the last of the birds died around the end of August. Thus mortality on the premises was 100 per cent of the 190 susceptible birds which were left there. These figures are summarised in Table IX.

Of the thirty-five birds transported to the laboratory, thirty-four died eventually, either of confirmed pseudotuberculosis directly or of its apparent after-effects.

Discussion of Epizootiological Aspects

Four main factors appear to be significant if one assumes that rodent contact could have been involved in starting the canary epizootic. There are: the size of the rodent population; the form of dispersal of the birds and rodent contact with them; the poor sanitation practices with regard to food and water; and the general stress on the birds due to breeding activity, poor ventilation, and actual overheating of many parts of the building, particularly during the month of July. An important potential factor was the feeding of paprika to birds in the flight where the first death occurred. The first two of these factors may be summarised and broken down as follows:

(a) Observed presence of large number of mice in and

TIME PERIOD	NUMBER OF NEW LOSSES	CUMULATIVE TOTAL LOSSES AT AVIARY	BALANCE IN AVIARY*	REMOVED TO LABORATORY**
June 12 - 15 (circa)	3	3	222	
June 16 - 24	0	3	222	
June 26 - 30	75	78	147	
July 1 - 15	87	165	60	
July 15		165	40	20 (Group I)
July 16 - 29	10	175	30	
July 29		175	15	15 (Group II)
July 30 - August 31 (circa)	15	190	0	
TOTAL	190	190	0/225	35

* Of the 225 canary and canary-cross birds present which appears to have been the only group susceptible to the degree of exposure which occurred

** See Experiment A

TABLE IX   RECORD OF MORTALITIES OCCURRING IN NO. 3 EPIZOOTIC OF SPONTANEOUS PASTEURELLA PSEUDOTUBERCULOSIS INFECTION IN CANARIES

around the premises. This would have been predictable in light
of:

> (i) The pile of floor and cage sweepings with
>   discards from feed containers a few feet
>   from the entrance.
>
> (ii) The presence of uncovered food in bags
>   and cans on the floor.
>
> (iii) The ample cover for rodent breeding right
>   against the back wall of the inside of the
>   building. This fact was confirmed when
>   the building was viewed after removal of
>   the cages.

(b) Form of dispersal of the birds and rodent contact.
It will be noted in the accompanying chart that the unaffected
species of birds were housed in the semi-outside flights at the
east end of the building. There were thirty selected mules and
factors which were in the inside flight adjacent to their cage,
(Flight MF, Figure 7). These birds were being fed a gastro-
intestinal irritant, paprika (supposedly to improve the colour
for show purposes). This inside flight by chance straddled the
route of ingress of the rodents to the house. A baseboard metal
strip along the rest of the back of the building restricted the
rodents' entry to this point. The epizootic first made its
appearance in these birds.

An additional factor not previously mentioned was the
presence of about six or eight loose birds flying in the aviary,
which could have spread the infection through their droppings.
Their presence was noted on the July 15 inspection.

Summary of Epizootiological Considerations for the Three
Affected Aviaries

The main epizootiological factors to be considered
in light of the histories of these aviaries are felt by the
author to be:

(1) The presence of significant numbers of rodents,
especially field mice, and in particular any evidence of sickness,
or of their entry into flight cages where canary deaths subse-
quently occurred.

(2) The presence of other wild species known to be
susceptible to psuedotuberculosis which came around or came in
contact with the flight cages. Wild birds are an example.

(3) The development of generalised stress in the
presence of specific stress mechanisms (e.g. feeding of paprika -
a bowel irritant) which would act as a predisposing factor to the
occurrence of frank disease in the presence of the causative
organism.

It is apparent that rodents, even dead ones, were
in close contact with the canaries in aviary No. 1. Also
reasonable is the assumption that contact was equally close in
the MF flight of Aviary No. 3 (where deaths commenced). In the
case of Aviary No. 2, the evidence of mice is more circumstantial
than actual. The environs evidently harbored a considerable range

of wildlife including avian species known to be susceptible to pseudotuberculosis, such as galliformes and crows. It seems reasonable to attribute some importance to the winter period (Aviary No. 1) the reproductive period (Aviary No. 2) and the over-heating and poor ventilation (Aviary No. 3), when considering general factors of stress. One specific stress factor, the feeding of paprika, also occurred in Aviary No. 3.

Laboratory Diagnosis of Pseudotuberculosis

Bacteriological Diagnosis

Introduction

Meyer (1959) states that the term 'pseudotuberculosis' must be reserved strictly for infections caused by Pasteurella pseudotuberculosis. In spite of a total of at least ten synonyms which are still quoted by various authors (Meyer 1959; Bergey 1957; Stafseth 1959; Zinsser 1957), the actual identification of the organism now known universally as P. pseudotuberculosis is not considered difficult by most bacteriologists.

The difficulties of differentiation of this organism from Pasteurella pestis and Pasteurella multocida are overcome by adequate biochemical characterisation, inoculation of experimental animals including the albino rat; and demonstration of motile qualities for the pseudotuberculosis organism. This

author has experienced few aberrations while identifying avian strains. However, this is not the case with all those of mammalian origin; for example, those from British Columbia chinchillas.[1]

In recent years, various leading authorities (Meyer 1962; Murray 1962) have expressed the view that the Pasteurellas are inadequately designated, and that some familial re-grouping may be desirable for certain members, including P. pestis and P. pseudotuberculosis.

Bergey (1957) makes a positive contribution to this problem by listing a separate species designated Pasteurella pfaffi (Hadley 1918) which has been considered for many years to be identical to P. pseudotuberculosis (Haupt 1934). In light of recent advances in bacterial genetics, the separate designation for a single strain of an organism studied thirteen years after its isolation (Pfaff 1905) appears unjustified.

Primary Isolation

Routine methods were used throughout with caseous material or ground saline emulsion of tissues being plated onto blood agar and sometimes MacConkey's plates. Typical

---

1 This experience has also been encountered in Denmark with some chinchilla strains which are active sucrose fermenters (Knox 1963).

P. pseudotuberculosis colonies were picked with the use of a hand lens and placed in beef heart infusion or Robertson's meat mash and incubated at 37°C for biochemical identification or at 22°C for demonstration of motility.[1]

Identification of Pasteurella Pseudotuberculosis

(a) Colonial morphology

(i) On nutrient and blood agar. The appearance of the colonies was followed from 24 to 72 hours. During this period, the appearance progressed from low convex to umbonate; from smooth and shiny to granular (sometimes coarsely so); from being completely translucent to grayish yellow and opaque; and from having entire edges to showing effusive edges with radial striations stretching back to the umbonate centre.

(ii) On MacConkey agar plates. On this medium, the colonies were more inclined to show granularity at twenty-four hours; were more effusive at the edges; and were inclined to lyse after 72 hours.

---

1 Current practice is to completely avoid incubation at 37°C and recent isolations have been carried out at room temperature.

(b) Staining characteristics

(i) Gram's stain. The cocco-bacilli, the short
rods, and the few filaments were uniformly Gram-negative,
variably bi-polar.

(ii) Wayson's Plague Stain. The bacillus
uniformly showed marked bipolarity with characteristic banding
in the filamatous forms. Swelling (perhaps due to use of the
methyl hydrate fixative) was common with many of the cocco-
bacillary forms so that the result of the staining was to give a
hamburger-like appearance.

(iii) Acid-Fast Staining. A slight degree of
acid fastness could be demonstrated in all the canary isolates
with the use of Cook's acid fast method (see Appendix) and this
quality was further accentuated with the use of Macchiavello's
rickettsia stain.

(c) Motility

Nine strains and two substrains of P. pseudotuber-
culosis were tested for motility of 25 per cent or better by
repeated subculturing in beef heart infusion broth at room
temperature (20 to 22°C). These included seven type-strains
received from Dr. W. Knapp of Tubingen, Germany; three strains
isolated from two canary epizootics in British Columbia (Aviaries
No. 2 and No. 3); and one chinchilla isolate from British Columbia.

Eight strains were motile after two transfers in beef heart infusion broth. One other required four passages. The two remaining strains were not motile after seven passages; however, it was noted that they were both inclined to roughness, and since both were substrains, the results were considered satisfactory.

(d) Growth characteristics in broth

Fresh isolates gave a uniform turbidity for one or two days in beef heart infusion broth, and to a lesser extent in Robertson's meat mash. Following this, the broth usually cleared, leaving a ring at the surface and a heavy flocculent sediment. Isolates from two of the three aviary epizootics yielded clearly motile forms at 22°C (see above).

(e) Biochemical alignment

The strains isolated from canaries aligned well with the reports in the literature for both carbohydrate fermentations and for other biochemical tests (see appendix for list of results and accompanying key).

(f) Serological typing

Isolates from the No. 1 and No. 2 aviaries were submitted to Dr. W. Knapp at Tubingen, Germany, and were reported to belong to Type I of P. pseudotuberculosis.

Pathogenicity for Experimental Animals

Guinea pigs and canaries were found to be susceptible to infection with the isolated strains of P. pseudotuberculosis, the latter by oral as well as parenteral routes. A budgerigar which was dosed orally was found dead with typical gross lesions twelve days later. White mice were found to be comparatively resistant, and albino rats completely resistant, to the strains isolated. In some cases, as when dosed with ground-up canary spleens, the mice died in twenty-four hours or less, presumably due to the carry-over of endotoxin. One budgerigar dosed parenterally died in the same way. Six-week-old chickens were found to be almost completely resistant to both oral and parenteral inoculations of strains pathogenic for canaries and guinea pigs.

In one case out of three, a guinea pig was successfully infected with intraperitoneal inoculation of canary faecal material, and was killed and cultured at fourteen days postinoculation. The same faecal material failed to yield the strain by culturing; however, after this occurrence, significant improvements were made in the technique for the culture of canary faecal material.

Pathology Diagnosis

Gross Lesions

A total of twenty-five autopsies were conducted on birds from the No. 1 Aviary epizootic. Six of these were natural deaths, the remaining nineteen were destroyed with CO_2. Of thirteen females and twelve male birds, three destroyed males only showed absence of gross lesions of pseudotuberculosis. Total organs affected of birds with lesions were: spleen - 22 (100%); liver - 4 (18%); and caecal abscesses - 1 (4.5%). The pathognomonic lesion appeared to be the classical pin-point to millet-sized yellowish tubercles in the spleen. In some cases the liver was similarly affected. The presence of a primary detectable bowel lesion, which appeared in gross, suggested that other microscopic bowel lesions may occur, thus serving as a portal of entry for spread to the spleen. The presence of liver nodules suggested a sequel to a bacteremic phase.

For Aviary No. 2, only two birds were examined. These birds showed the presence of typical pseudotuberculosis in both spleen and liver.

The gross pathological lesions recorded for birds transferred to the laboratory from Aviary No. 3 are listed in Tables XI and XII.

Several birds with splenic tubercles and one with
liver tubercles were examined culturally in making the initial
diagnosis. For the birds transferred to the laboratory, more
observations were recorded with the aim of correlating gross
pathological lesions with ability to culture P. pseudotuberculosis,
(Table XII).

Histopathological Changes

The structure of the pseudotubercle was studied with
the aid of paraffin sections stained with haematoxylin and eosin.

Of those spleens and livers which were examined from
field cases, all showed a marked granulomatous response. Mono-
nucleic macrophages predominated in the cellular mass. Scattered
about were numerous small areas of caseation necrosis, eosino-
philic in tone, and with areas of blue smudging probably due to
degenerating masses of bacteria. The cellular response was more
diffuse about the necrotic foci in the spleen than in the liver.
In the latter organ, intense cellular cuffing could be noted,
(Figs. 8,9,10,11). Kupffer cells were absent, doubtless due
to their cytometamorphosis to form larger macrophages. The
liver cords were completely disrupted with marked vacuolar de-
generation of the hepatic cells. The appearance of the macro-
phages suggested intense bacteriophagia although clearly defined
microorganisms were not visible in the way noted in the experi-
mental disease (Fig. 56). Extensive haemorrhages were noted in

H & E   150X

Figure 8   Aviary No. 1. Spleen from
            spontaneous case of canary
            pseudotuberculosis.

            This low power photomicro-
            graph shows necrotic foci
            surrounded by a diffuse
            mass of macrophages.

H & E   500 X

Figure 9   Aviary No.1. Spleen from
            the case illustrated in
            Figure 8. More details of
            the macrophages can be
            seen under high power.
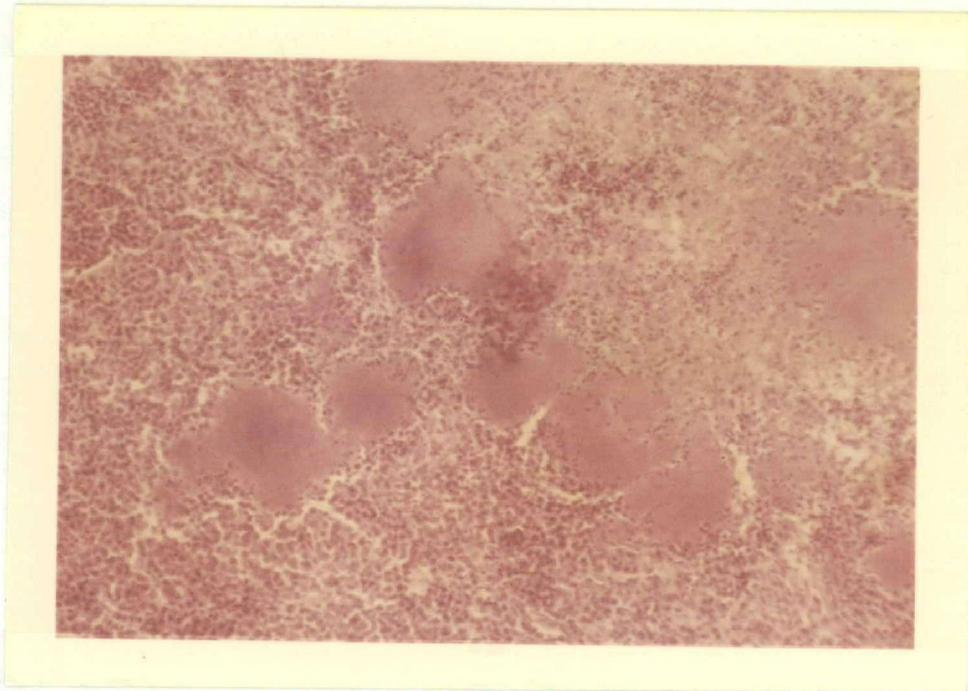
Figure 8.
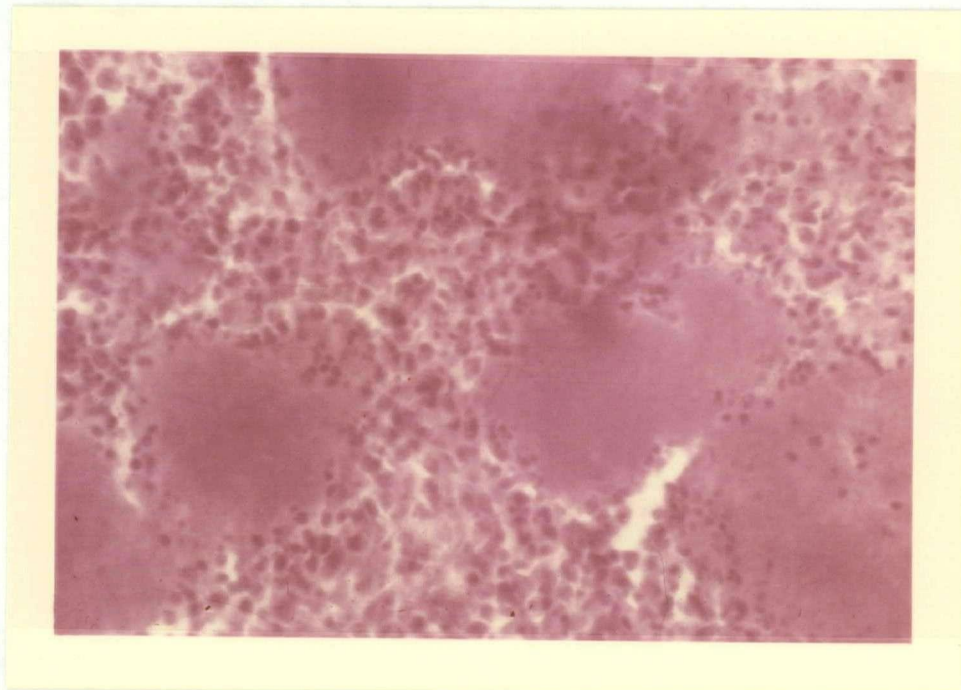
Figure 9

H & E   500X

Figure 10   Aviary No. 3. Liver from
             spontaneous case of canary
             pseudotuberculosis. This
             photomicrograph shows a
             discrete pseudotubercle.

H & E   500X

Figure 11   Aviary No. 3. An aggregation
             of pseudotubercles in the
             liver illustrated in Figure
             10. In these photomicrographs
             it can be noted that the cellular
             reaction is denser than in the
             spleen (Figures 9 and 10), and
             more inclined to cuff the necrotic
             area closely. There is a diffuse
             degeneration of hepatic cells.
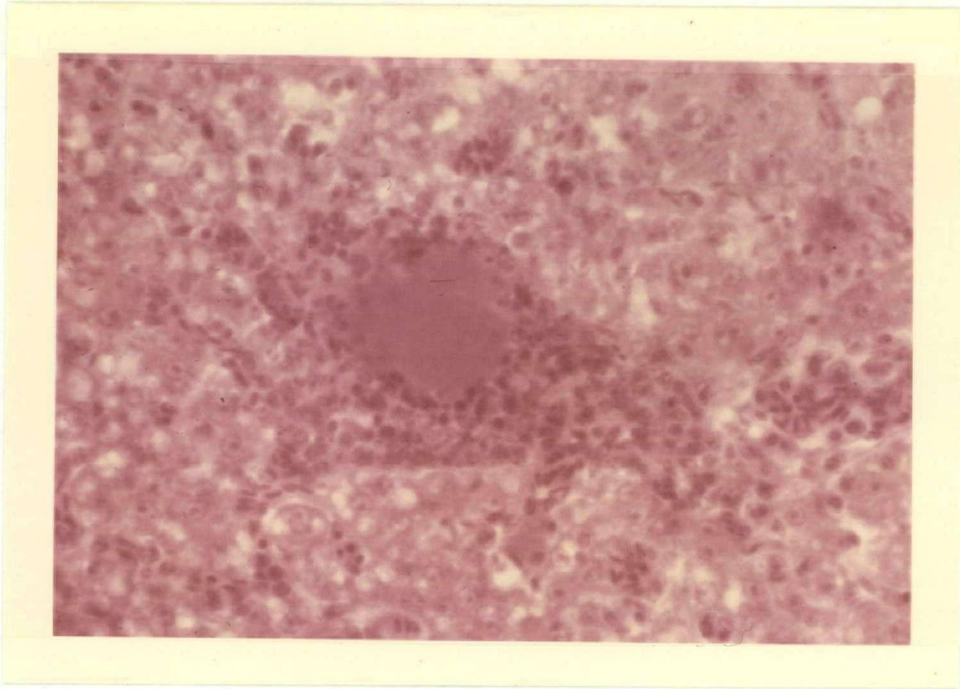
Figure 10
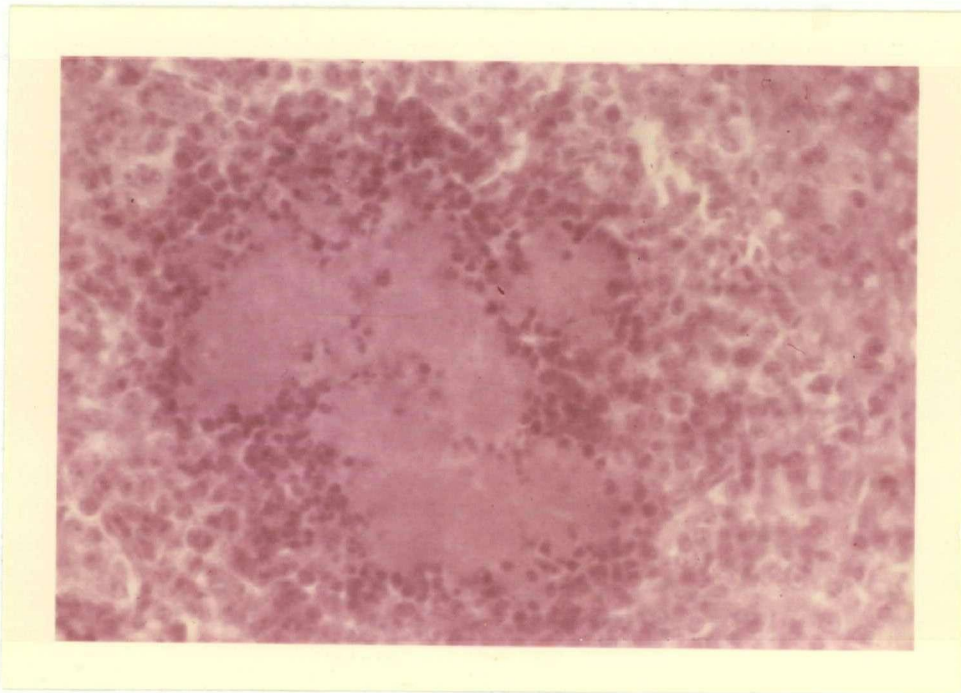
Figure 11

the spleen along lines of fracture due to structural weakening

of the organ.

EXPERIMENTAL STUDIES ON THE TRANSMISSION, FAECAL EXCRETION
RATE AND PATHOLOGICAL FINDINGS OF PSEUDOTUBERCULOSIS IN
CANARIES

When the epizootic commenced in Aviary No. 3, labora-
tory studies were carried out on canary pseudotuberculosis, to
increase the general knowledge of epizootiology and pathogenesis
of the condition. Two groups, totalling 35, of the affected
canaries were transferred to the laboratory and the progress of
the disease was studied. This work is reported as Experiment A
of this section.

As a result of the above study and of a concurrent
examination of the literature, a number of questions were raised
relating to both epizootiology and pathogenesis. It seemed
necessary to create experimental pseudotuberculosis by the
(assumed) natural, oral route in laboratory canaries to attempt
to answer these questions, along with others which were raised
by studying the epizootics in the three affected aviaries.
Attempts to arrange the transmission of pseudotuberculosis by
contact from experimentally infected to healthy birds were
carried out first (Experiment B). This work was followed by a
study of birds infected experimentally per orum (Experiment C).

Several of the questions are listed for which clarification was sought:

(a) Do most canaries become infected from each other during spontaneous epizootics or from a common source (such as rodent excreta)?

(b) In the event of recovery, do canaries remain as chronic carriers and excretors of P. pseudotuberculosis?

(c) What aspects of transmittance or of pathogenesis account for the extremely high mortality rate which is apparently common in canaries (close to 100%) and is almost without precedence among bacterial diseases?

(d) Do infected canaries pass significant numbers of viable P. pseudotuberculosis in their faeces, even though the gross pathological lesions are usually limited to spleen, liver and occasionally breast muscles?

(e) What is the significance, in terms of faecal excretion of the cecal abscess lesion which was noted on two occasions in spontaneously infected canaries (Nos. 1 & 3 Aviaries)?

Materials and Methods General

Housing Conditions for the Experimental Periods

Depending upon the purpose of the experiment, cages of widely varying design were employed and these are described in detail as each experiment is discussed. In the case of Experiment A, the primary objective was to give quarters with maximum comfort and space for the birds while the natural disease was being studied in the laboratory. In the case of Experiment B

involving contact infection attempts, two widely contrasting levels of sanitation were set up, with birds separated only by a common barrier of wire cloth. For Experiment C, with birds experimentally infected by the oral route, it was considered essential that the twenty-four birds should be housed close together, so as to be in a uniform environment yet completely in isolation one from the other from the point of view of direct spread of faecal or food materials. The faecal material had to be collected as an entire twenty-four hour sample, complete and without cross-contamination. For these reasons a battery cage was constructed with solid walls.

General Environmental Sanitation for Experiments

For Experiment A, every precaution was taken to control what was felt to be a hazard for human infection. The room air, faecal material and water containers were all sampled culturally, quarternary disinfectants were used on all surfaces and preliminary washing was carried out in the sink in the room with Roccal disinfectant before any equipment was moved out for sterilization. For later experiments, carried out for the purpose of faecal culturing, a complete avoidance was made of using disinfectants in the experimental room. All sterilising of materials that could not be discarded was carried out with an instrument steriliser or by placing materials in plastic pails and using chemical sterilization in an adjoining room. All

spilled seed was brushed up several times daily and the con-
crete floor flushed with copious amounts of cold water once
daily.

Feed, Water, and Supplements

For Experiment A, a mixture of three parts commercial
plain canary seed and one part mixed millet was used. This was
approximately the same formula as had been in use at the source
aviary (No. 3). This did not include the addition of paprika.
Towards the end of the experiment, the millet supply was dep-
leted and this fact probably had bearing on the results of the
experiment, and of Experiment B also, where again the plain
canary seed was in use. For Experiment C, a more complex mix-
ture of seeds (commonly recommended in this area) was in use,
while the birds were on hand. This was slightly modified when
the faecal culturing was actually in progress. (See Appendix).
Feed was offered in open dishes (cans) for Experiment A (Figures
12-13), and for the low-sanitation section of Experiment B.
For the high sanitation (top feeding) section of Experiment B,
and for Experiment C, clear polystyrene fountain-type dispen-
sers and white plastic hook-on cups were used (Figures 17-20).
Tap water from the Vancouver municipal supply was used. For
Experiment C, the water dispensers were emptied daily at the
time for faecal collection and thoroughly rinsed individually

under a jet of cold running water so that the chance of bacterial infection carryover from day to day was mechanically reduced to negligible proportions. Cuttle bone was supplied in all experiments. In Experiment C, a small piece of broken cuttle was kept in each feed dish. If the birds threw it out, it was replaced from stock daily. No. 10 grade granite grit was supplied in open containers except for the topfeeding section of Experiment B and for Experiment C. In the latter cases, the gravel was supplied in the polystyrene fountain-type dispensers.

No other supplements were used. Although the author considers that cage-bird nutrition is probably inadequate under these conditions, it was felt that no attempt should be made to favour the experimental groups with better nutritional care than was commonly practiced by canary aviarists in the area.

Identification of the birds

Some of the birds from all groups were already identified by aluminum leg bands which were installed by breeders at the nestling stage. In cases where such bands were not fitted, plastic numbered bands were placed on the birds in Experiment C. This was a precaution taken to permit the identification of possible escapees and for autopsy purposes, since the birds did not come in direct contact with each other for these experiments.

Source and preparation of the infecting inoculum

Two types of inoculum were used for the oral infection of canaries in Experiment B, and one type for a pilot experiment and Experiment C. The source in both cases was the pool of tissues from a canary in Group II which had died (Experiment A). The material was cultured and then stored at -20° C. The two inocula were:-

(a) A ten per cent suspension in saline of homogenized pooled tissue was prepared following thawing, and used for four (one-half) of the artificially infected birds in Experiment B.

(b) A re-constituted lyophilised culture, after being checked for motility well in advance, was held at room temperature in a tube of Robertson's Meat Mash. The day before the experiment the culture was grown at 37° C in a number of tubes of beef heart infusion broth, sedimented at 4000 rpm and re-suspended in a phosphate-buffered saline. The density was standardised to a turbidity approximating that of Brown's nephalometer tube No. 10. This type of inoculum was used for the other half of Experiment B, and for the pilot experiment. For Experiment C, the same procedure as above was followed, but with the organism being subjected to one further canary passage (in the pilot experiment) from which it was recovered in pure culture.

This strain was designated "Longworth F" on the basis of its original source in Aviary No. 3.

Air sampling

On several occasions an Anderson air sampler was used with blood and MacConkey Agar plates to sample the room air during Experiment B. Results were completely negative for Pasteurella pseudotuberculosis. Prior to obtaining this result, the wearing of face masks was made mandatory for persons entering the room.

Cultural procedures on artificial media

(a) From canary tissues in experimental infections

A tissue suspension in saline was prepared with a Teflon grinder. This was then plated out on five per cent bovine blood agar and onto MacConkey's agar. In addition, a few drops of the material were added to a 10.0 ml tube of Robertson's Meat Mash (RMM). Following incubation for twenty-four to forty-eight hours at 37° C, typical P. pseudotuberculosis colonies on plates were subcultured into both maltose and lactose broth fermentation tubes. If growth occurred only in the RMM then this culture was plated in the same way as the tissue.

(b)   From processed faeces

Following preparation of the plating inoculum (Table XV)
0.1 ml amounts were inoculated onto the surface of MacConkey
and blood agar plates: the inoculum being spread with a sterile
glass rod with the use of a hand turntable.   The plates were then
incubated at 37° C, checked twice daily, and colonies were picked
off for identification as above.   In the case of sugar broths
which showed fermentation of maltose with no gas, the maltose
broth was plated onto a segment of a fresh blood agar plate and
stored in the refrigerator to await biochemical tests to check
the identification.

Identification of Pasteurella pseudotuberculosis

For details of the appearance of P. pseudotuberculosis,
growth in primary isolation and identification media, and for
biochemical reactions used, see the foregoing section titled,
'Laboratory Diagnosis'.

Calculations for faecal counts of P. pseudotuberculosis
and faecal dry matter output

(a)   For faecal counts

For those plate counts which applied to colony types
later confirmed as P. pseudotuberculosis, a formula was used
with two variables and three constants (Table X).   In the
table, the constants are underlined.

FACTOR	PLATE COUNT (COLONY)	X	(10 X TOTAL VOL MLS OF DILUENT)	(10)	X	6/5=TOTAL FAECAL COUNT OF VIABLE ORGANISMS IN 24 - HOUR SAMPLE
E X P L A N A T I O N	Total count on plate (average of five small squares)		Extra dilution caused by use of 0.1 ml for plating (i.e. 1/10 of dilution unit)	This figure in mls represents the degree to which 1/10 total sludge was diluted to 1:50 (W/V) to arrive at a 1:500 dilution (W/V) of faecal sludge ————— These figures give the product of dilution on to plate of 1/10 of the total sludge	Removal of 1/10 aliquot of sludge by process of standardising to 10 ml vol and removing 1.0 ml		Correctional factor to bring 5/6 of faeces (on which count made) to total faeces collected for 24 hours
EXAMPLE	40	X	(10 X 12.5)**	(10)	X	6/5 = 60,000

** Faecal sludge weight being 0.25 grams

TABLE X   STANDARD CONVERSION FORMULA EMPLOYED FOR COMPUTING TOTAL EQUIVALENT VIABLE FAECAL COUNT OF PASTEURELLA PSEUDOTUBERCULOSIS PER TOTAL TWENTY-FOUR-HOUR FAECAL SAMPLE OF EXPERIMENTALLY INFECTED CANARIES

Several steps, illustrated by an example, were taken with colony counts to obtain the total equivalent faecal counts. These are shown in the table along with explanations.

In the example given here, an original weight of faecal sludge[1] of 0.25 gm was diluted to ten ml in broth. Thereupon, one-tenth of the broth suspension (containing 0.025 gm of sludge) was further diluted to a total volume of 12.5 ml, thereby constituting a 1:500 dilution (W/V) level of the faecal sludge. Plating was carried out with 0.1 ml of suspension (containing 0.002 gm faecal sludge) which is one-tenth of the unit of dilution. This procedure made a plate from any one bird truly comparable with another, since the same final dilution (thus the same amount - 0.002 gm) of faeces was used regardless of actual amount voided by the bird. The three constants give a product factor of 120. In practice, a set of simple tables was prepared which showed the saline volumes required for diluting all possible weights of faecal sludge corrected to two decimal places. Since the dilution factor was a product of five, the saline volumes came to every whole or half millilitre (actually between 8.0 and 22.5 ml). These values in turn were incorporated into a table with a multiplication factor (MF) which was the

---

1 That portion of 5/6 of original total faecal sample material capable of being sedimented by 10,000 rpm for 15 minutes in an angle centrifuge.

product of each saline volume and the constant factor of 120. In the example given, the MF was 1500. It remained then only to apply the actual (or computed) colony plate count to the MF to obtain the final figure required. In the example given, 40 x 1500 - 60,000. In many cases, the figures were large. The highest output recorded on any day (bird No. 16) was close to 152 million organisms (1.52×10^8) which was delivered in a volume of faecal material with a dry matter equivalent of only 0.66 gm. In this particular case the faecal sludge weight was exactly half of the calculated faecal DM for that bird on that day. At times the faecal sludge (which contained little water after 10,000 rpm centrifugation) weighed less than one-half of the faecal output in terms of dry matter. It was not known whether most of the weight was lost in heavy sediment which was left on decanting, or whether more of it was lost in soluble salts and urates along with micro-particles in the discarded supernatant following centrifugation. Certainly, this supernatant contained very few micro-organisms when test-cultured.

(b) Calculation of the dry matter (DM) equivalent weight of the total daily faecal output

This has already been discussed to some degree. The aliquot of one-sixth of the twenty-four hour sample of faeces was obtained by volumetric proportion, brought to air dryness at 37° C over a period of a week or so (humidity of walk-in incubator is approximately thirty-six per cent) and then weighed.

The resultant net weight figure is expanded by 6/5 to obtain
that figure which is plotted on the graph chart for each of the
inoculated birds.

Execution of Experiments

Experiment A:

Clinico-Pathological and Cultural Observations on Birds Trans-
ferred from the Premises of No. 3 Aviary to the Laboratory
During an Epizootic

Objectives

Detailed observations of the course of a canary
epizootic of pseudotuberculosis were not available to the author.
Such observations appeared to be a fundamental necessity for the
evaluation of the data already collected from the No. 1 aviary.
In particular, it was felt that the transfer of birds to the
laboratory might modify the progress of the disease or yield
information to explain the high mortality which had been recorded
previously and was already expected for this aviary (No. 3).
In retrospect this experiment was of considerable value for
comparative purposes in studying the artificially induced
infections in Experiment B and Experiment C.

Materials and Methods

(a) Source and description of birds

Two groups of birds from aviary No. 3 were used in
this experiment, (Fig. 7 and Key).

Group I: This group consisted of twenty canaries
and red-factor canary crosses. They varied in plumage colour
from pale yellow to orange in hue, with some possessing dark or
white wing feathers. Twelve of these canaries had been picked
from Flight "O" and various cages where heavy losses or loss of
mate had occurred. The remaining eight birds were from single
cages (four) or were birds which flew around loose in the
aviary.

In addition, four male budgerigars were included from
the semi-covered flight cages at the east end of the building.

Six canaries in good health were obtained from the
local zoo for use as contact and isolated controls. The latter
source flock had an excellent health history. Two of these birds
were maintained in other premises on the same feed as that used
in the laboratory.

Group II: This group was comprised of fifteen birds
mostly fledglings, also obtained from the No. 3 aviary, but
collected fourteen days later when the epizootic had been going
for six weeks and most of the birds were dead. Two of the bud-
gerigars received with Group I had been housed separately and

these were now added to Group II. One of these was then
inoculated orally with a culture of P. pseudotuberculosis.

(b) Housing conditions for experimental period
(Figs. 12,13)

The two groups of birds brought were housed in two
flights manufactured of slotted angle-steel and welded wire
cloth with apertures of one inch by one-half inch. The
flight for Group I measured six feet by four feet by four feet,
and that for Group II was slightly smaller. The flights had no
bottoms, but were set on a trestle table across which was fed a
roll of brown double-waxed paper for use as a litter carrier.
Perches were trimmed tree branches which gave a great variation
in diameter so that the individual birds could find their most
comfortable perch diameter.

Experimental Technique

General clinical observations were recorded, prelimi-
nary studies were made to determine the level of excretion of
the pathogen into the environment, and those birds which died
were studied both culturally and for gross pathological lesions.
Four contact control canaries were placed with the first of two
groups of birds after they had been nine days in the laboratory.

Figure 12   Experiment A. View of corner of
                 flight cage housing Group I
                 canaries (fromAviary No.3) in
                 the laboratory. Casual observat-
                 ion did not suggest that the
                 birds were sick; however, a dead
                 bird can be noted on the floor
                 and losses were heavy.

Figure 13   Experiment A. Close up view of
                 flight shown in Figure 12.
                 Note the tendency of sick birds
                 to huddle in open-type feeders
                 thereby increasing the faecal
                 soilage of seeds.
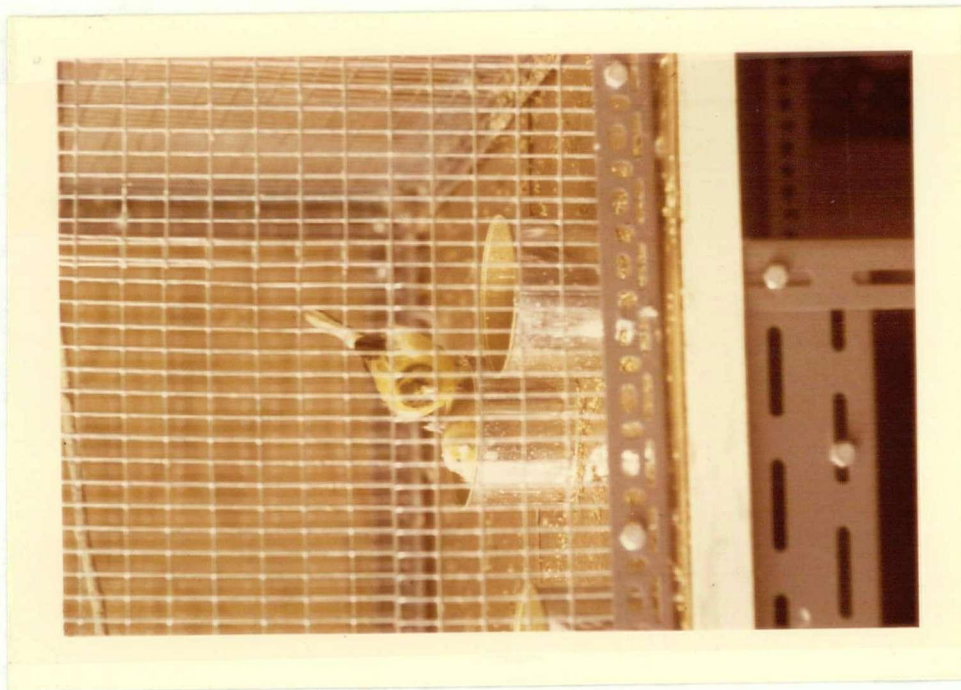
Figure 13.

Figure 12.

Results and Discussion

Clinically the birds were depressed inasmuch as there was no singing and many of them sat in a ruffled attitude on the perches. Practically every bird was active at times however, and they appeared to eat and drink as much as normal birds. When moving or showing interest, the birds uttered hoarse chirps. For a day or two prior to death, birds were usually unable to fly onto the perches and they would huddle in a corner on the floor.

As can be noted from the data (Tables XI & XII) 75% of the canaries of Group I, and 55% of those from Group II were dead by the 77th day following the commencement of the epizootic. This point corresponds to the reported date of death of the last bird on the premises of aviary No. 3 from whence the birds had been removed.

In addition to the frequently occurring yellowish nodular tubercles in spleen and liver, two birds showed the presence of what appeared to be abscesses in the caeca, which are vestigial organs, often termed caecal "buds" in the canary. One bird which showed the most advanced spleen and liver lesions recorded, also showed the presence of pseudotuberculosis in the breast muscles. (Figures 14 and 15).

Up to about the 60th day following commencement of the epizootic, isolations of P. pseudotuberculosis could still be obtained from birds which died with pseudotubercle lesions.

EXP GROUP DESIG.	BIRD NO.	SOURCE IN AVIARY	EXP DAY OF DEATH SINCE TRANSFER	SINCE 1ST LOSSES	TOTAL DEATHS CUM %	SPLEEN enlarged	SPLEEN tubercles	LIVER enlarged	LIVER tubercles	OTHERS description	ISOLATION OF PPT ORGANS CULTURED	ISOLATE SECURED	REMARKS
GROUP I													
See	1	flight 'O'	1	31	5%	++	++++	-	+	-	spleen	+	sick on arrival
Table	2	flying free	1	31	10%	+	-	-	+++small	-	liver	+	
Section 5C	3	flying free	1	31	15%	+	++	+	++	-	spleen	+	
	4	flight 'O'	2	32	20%	+++	++++	+++	-	-	spleen	+	
	5	intact pair	6	37	25%	++	+++	+	++at spleen	-	liver	+	
20 canaries	6	flying free	6	37	30%	++	++	+	+++minute	-	spleen	+	
removed											bowel	+	
from No.3	7	flight 'O'	8	38	35%	++	+++	+	++	-	spleen	+	
aviary											bowel	+	
30 days	8	intact pair	8	38	40%	++	+++	+	++	-	spleen	+	
after	9	single cage	9	39	45%	-	-	-	-	-	spleen	-	NB no gr.lesions
beginning											liver	-	
of	10	flying free	12	42	50%	++++	++++	+	++small	-	spleen	+	
epizootic											bowel	+	
	11	flight 'O'	14	44	55%	++++	++	-	-	-	spleen	+	Biochem atypical
	12	intact pair	19	49	60%	++++	++	+	++small	catarrhal enteritis	spleen	+	Stored in freezer 9 days
	13	intact pair	20	50	65%	++++	+++	+++	++large	emaciated	spleen	+	
											liver	+	
	14	intact pair	34	64	70%	++	++	++	++	thymus area	spleen	-	NB lesions. No
											liver	-	isolate
	15	flying free	36	66	75%	++	++	++	++	-	spleen	+	Thymus area
											thymus	+	independent
	16	single cage	53	83	80%	++	-	-	-	-	spleen	-	
	17	single cage	57	87	85%	-	-	-	-	-	liver & spleen	-	Tissue pooled
	18	single cage	70	100	90%	-	-	-	-	-	liver & spleen	-	Tissue pooled
	19	single cage	70	100	95%	-	-	-	-	-	liver & spleen	-	Tissue pooled

1 The surviving canary from Group I was later included in experiment. B. This bird, along with the survivors of Group II ultimately died from general debility.

2 Two of four male budgerigars were received from the aviary on the same date as the canaries. Two of these birds were placed with Group I as contact controls. They remained healthy for the 61-day observation period. When autopsied later, no pathological lesions were seen except for hypertrophy of the thyroids.

TABLE XI□ RECORD OF MORTALITIES, PATHOLOGICAL LESIONS, AND ISOLATIONS
OF PASTEURELLA PSEUDOTUBERCULOSIS FROM CANARIES TRANSFERRED TO LABORATORY FROM AN
AVIARY DURING THE COURSE OF AN EPIZOOTIC (GROUP I TRANSFERRED 30 DAYS AFTER LOSSES COMMENCED)

EXP GROUP DESIG.	BIRD NO.	SOURCE IN AVIARY	EXP DAY OF DEATH SINCE TRANSFER	SINCE 1ST LOSSES	TOTAL DEATHS CUM %	SPLEEN enlarged	tubercles	LIVER enlarged	tubercles	POST MORTEM OTHER description	ISOLATION OF PPT ORGANS CULTURED	ISOLATE SECURED	REMARKS
GROUP II													
See Table	1	Exact	1	45	6.6%	++	++	-	-		spleen	+	
Section 5C	2	sources not	1	45	13.3%	++	++	-	-	caecal absc. peritonitis	spleen bowel	+	
	3	known	3	47	19.8%	++++	++++	-	-	caecal absc. very large	spleen bowel	+	
15 canaries removed	4	Mostly	5	49	26.4%	++++	++++	++	++large		spleen liver	+	
from No.3 aviary	5	fledglings	8	52	33.0%	++++	++++	+	++small		spleen	+	
14 days following	6	from all over	16	60	39.6%	++++	++++	++++	++++	large breast musc.tuberc.	pool	+	
removal of Group I	7		16	60	46.2%	-	-	-	-	very poor flesh	pool	-	for inoc
Table	8		33	77	54.8%	+++	-	-	++small	emaciated	pool	-	
CONTROLS													
Four of six	1	N/A	21	N/A	25%	+	-	-	-	emaciated	pool	-	
contact	2	N/A	22K	N/A	50%	-	-	-	-	emaciated	pool	-	
control	3	N/A	50	N/A	75%	+++	-	-	-	emaciated	pool	-	
canaries placed in flight 9 days after Group I	4	N/A	61	N/A	100%	-	-	-	-	emaciated	pool	-	

1 The seven surviving canaries from Group II were later included in experiment B as potentially infected. As noted
all of these birds eventually succumbed from general debility.

2 The remaining two of four male budgerigars (footnote 2, Table XII) were placed with Group II canaries, and one bird, the
'infected control' was dosed orally with 0.5 ml of a broth culture of Pasteurella pseudotuberculosis. This bird died with classical
lesions of Pseudotuberculosis twelve days post inoculation. The other bird remained healthy during the obervation period.

3 The two remaining control canaries were utilized as isolation controls.

TABLE **XII** RECORD OF MORTALITIES, PATHOLOGICAL LESIONS, AND ISOLATIONS
OF PASTEURELLA PSEUDOTUBERCULOSIS FROM CANARIES TRANSFERRED TO LABORATORY FROM AN AVIARY
DURING THE COURSE OF AN EPIZOOTIC (GROUP II TRANSFERRED 14 DAYS AFTER GROUP I, I.E. 44 DAYS AFTER LOSSES HAD COMMENCED)

Figure 14   Experiment A. Canary from Group I.
             Spleen and liver lesions at autopsy
             (spontaneous infection). The spleen
             shows as a dark cylindrical organ.
             The liver lies either side of the
             spleen and below the gizzard in
             this picture.The kidneys are in the
             background to the right. Pseudo-
             tubercles were not seen in renal
             tissues.

Figure 15   Experiment A. Canary from Group II.
             Breast muscle and liver lesions at
             autopsy of a spontaneous infection
             case. The latter may be seen through
             the thin, unopened abdominal wall.
             (caudal to sternum)

             The affected tissues from this bird
             were stored frozen and later used
             to infect birds experimentally
             (Experiment B), by oral inoculation.

Figure 14

Figure 15

Following this point, six more birds died by the 100th day.
Of these, one only showed discrete pseudotubercles in the liver,
and this bird plus one other showed splenic enlargement. No
isolation of P. pseudotuberculosis could be obtained from
these six birds or any of the remaining eight which died without
typical cross lesions in a succeeding experiment at points
ranging between the 102nd and 241st day from the start of the
epizootic. (Experiment B). The four contact control birds sick-
ened and died, one with an enlarged spleen. No isolations of
P. pseudotuberculosis were obtained. Two additional isolation
control birds remained healthy on the same feed at the author's
residence.

Conclusions

Deaths continued at a high rate in the groups under
observation with 50% of Group I being dead by twelve days follow-
ing the transfer, and 46% of Group II being dead in sixteen days
following the move. It appears from this and from the compar-
ison already drawn between the events at the aviary and those
in the laboratory, that the disease was not significantly modi-
fied in its general course by the transfer.

Clinically, there did not appear to be any feature of
diagnostic significance which was observed in the affected
canaries under close observation. From the pathological and
cultural data, one could deduce that this infection tends to

burn itself out eventually, before all the birds are dead; but that the deaths continue due to general debilitation even after the bird has eliminated the viable pathogens from its system. Two possible factors arise here which could contribute to this effect. One is the production of bacterial endotoxin during the resolution of pseudotubercles, and the other is the increased difficulty which weakened canaries have in cracking their seeds so as to maintain the intake of digestible nutrients. It seems likely that the second factor contributed to the ultimate death of birds which survived beyond one hundred days, since they were emaciated at death. Undoubtedly the more comfortable environment of the laboratory extended the life of such debilitated birds beyond that period expected in this particular aviary.

Probably the most significant finding was that of two birds with abscesses in the caecal buds. Since both these birds were held in the freezer awaiting autopsy and culture, there was no histological study made of these particular lesions. This finding with its possible implication of heavy faecal dissemination suggests itself as a probable explanation for the apparently vigorous role of the canary in spreading infection to other members of the aviary.

In all cases with typical gross lesions where bowel culturing was carried out, an isolate of P. pseudotuberculosis was obtained. The four contact control birds sickened about

nineteen days after entry to the flight, and died between
the 21st and 61st day of the experiment. Of these, three
died and one was killed. It is felt that no conclusions of
any kind can be drawn from these deaths of control birds in
the absence of pseudotubercle lesions. Three budgerigars
remained healthy. One was inoculated orally with broth cul-
tures and died twelve days post-inoculation with typical lesions
and cultural recovery of P. pseudotuberculosis.

Experiment B:

Contact Infection Experiment with Healthy and Artificially-
Infected Birds

Objectives

A tentative first attempt to demonstrate contact
infection between naturally infected and healthy canaries had
been carried out in Experiment A. The four healthy birds
sickened and one was killed and three died. There was a debili-
tating process which was absent in their two mates maintained
on the same feed at other premises. P. pseudotuberculosis
could not be isolated when the birds were examined at autopsy.
However, this did not rule out the possibility of infection
having taken place during the first two weeks or so. In
light of this and the fact that the healthy canaries had

not been introduced to the infected group until so late in the course of the epizootic, it was decided that an experiment should be set up specifically designed for this purpose. In both No. 1 and No. 3 aviaries, the budgerigars had remained clinically free of pseudotuberculosis.

Since in the natural state, the budgerigar (a psittacine) is a top feeder among seed-bearing plants, whereas the canary (a passerine) is a ground feeder, the question arose as to whether the canary would be less likely to become infected if it was not allowed access to the caecally contaminated floor environment of the cage. For the purpose of testing this theory, a double cage was designed incorporating two extreme levels of sanitation.

Materials and Methods

(a) experimental birds

Sixteen healthy canaries were obtained from a healthy flock which had been closed for several years, and free of epizootics during that period.

(b) housing

For this experiment a cage of slotted angle steel and wire cloth was made with overall dimensions of two feet

by four feet by two feet. This was divided in half by a partition of the same welded wire cloth, and one one side there was a false floor, built up four inches from the cage bottom, constructed also of wire cloth, one inch by one-half inch.

Experimental Technique

Two groups of thirteen canaries were assembled, each sub-divided into two groups of four and one group of five. These sub-groups consisted of five healthy canaries obtained from an aviary in British Columbia (the remaining birds in this aviary served as 'off experiment' controls); three more birds from this source; one of the isolation control canaries from Experiment B (freshly brought on premises) which were to be introduced and infected; and four of the potentially infected, or perhaps immune, survivors of the No. 3 aviary epizootic.

The group held in the left hand side of the double cage were allowed access to the floor and were given floor feed and water receptacles which soon became heavily contaminated with faeces. This flight was designated 'standard' cage. The right hand flight contained a wire false bottom and the feed, water, and grit containers, as well as the cuttle bone were all suspended on the side wire as close as possible to the top of the cage. In addition to this, the wire floor and walls of the right hand flight were cleaned of all faecal

material once daily by use of a cellulose sponge and Roccal 500 PPM in warm water. The water fountains were flushed out and re-filled daily. This flight was designated 'test cage'.

The sub-groups of five healthy and four potentially infected (PI) birds were placed in the cage so as to total nine birds in each side. After eighteen days, eight more birds were introduced to the experimental room, infected with 0.1 ml of a reconstituted broth culture of the Longworth F strain of P. pseudotuberculosis by means of an 0.2 ml pipetter, and placed in the cages.

Results

The results will be presented in summary only since it is felt that the experiment was not successful, due in part to improper design.

The eight birds which were inoculated orally became infected and seven died. With one or two exceptions (Fig.16) they showed classical lesions of pseudotuberculosis. The potentially infected birds continued to die as described in Experiment B, and eventually all succumbed to a debilitating disease not recognizable as pseudotuberculosis. The healthy birds all became sick and nine of the ten eventually died. These also suffered from a debilitating disease not recognizable as pseudotuberculosis.

Figure 16   Experiment B.   Gross view at autopsy
              of thymus lesion in a canary which died
              34 days following oral inoculation
              with Pasteurella pseudotuberculosis.

              Previously, isolations were obtained
              on two occasions from smaller lesions
              in the thymus area in birds spontan-
              eously infected (Aviary No. 3).

              This bird also showed extensive
              lesions in lungs and abdominal
              viscera.

Figure 16.

Conclusions

Useful data were secured on the ability to artificially infect canaries. One isolation of P. pseudotuberculosis was obtained from the faecal droppings from the standard cage by means of inoculation of sediment into a guinea pig. Thus it can be stated that the healthy canaries were exposed to an infected environment. The healthy canaries evidently suffered from a debilitating disease which might have been transmissable; however, it was felt that their deaths were more directly due to nutritional deficiency. In following experiments, a better mixture of seeds was employed to enable sick birds to pick those seeds with hulls more easily cracked than those of the plain canary seed.

Experiment C:

Experiment with Quantitative Determination of Faecal Dissemination

Objectives

(a) To reproduce pseudotuberculosis by oral inoculation of canaries with a resultant disease course and pathological lesions similar to those recorded during the course of epizootics of the spontaneous disease in British Columbia.

(b) To measure, quantitatively if possible, the faecal dissemination level of P. pseudotuberculosis achieved by the inoculated birds from the day following inoculation to the day of death; or, in the event of recovery, the day of sacrifice.

(c) To shed further light on the probable role of the canary in the epizootiology of spontaneous disease in its own species, and to allow an estimate to be made of its possible role in zoonotic pseudotuberculosis.

Materials and Methods

(a) experimental birds

Thirty birds were received from a dealer in Vancouver. These were ordered from a closed-flock aviary in British Columbia possessing an excellent health record. The distribution of sex was six males and twenty-four females. Weights ranged from fifteen to thirty grams. Their breeding did not involve any hybrids, however the colour and wing markings of the canaries varied considerably. It should be noted that at no time have any observations made by this author on spontaneous or experimental pseudotuberculosis suggested that there is any sex differential in susceptibility of canaries to P. pseudotuberculosis infection. The birds used for this experiment were maintained at the laboratory in good health for a period of one month before the experiment commenced.

Figure 17   Experiment C. View of the canary
             battery unit which was used for
             the quantitative fecal dissemin-
             ation experiments.

             The operator removing hook-on
             seed and cuttle containers from
             the wire fronts of the cubicles.
             This was in preparation for the
             evening collection of twenty-
             four hour faecal samples.

Figure 18   Experiment C. Another view of
             the canary battery.

             Cleaned and sterilized metal floor
             trays with wire grids are replaced
             following faecal collection.

Figure 17

Figure 18

Figure 19   Experiment C. Further view of
canary battery.

This picture shows fountain-type
grit and water containers, hook-
on feeders,and spring clip wooden
perches.

The perches were replaced with sterile
substitutes daily,following faecal
sample collections. All wooden and
metal surfaces received a thin coat
of molten parawax.

Figure 20   Experiment C. Canary battery
showing close up of individual
cubicle.
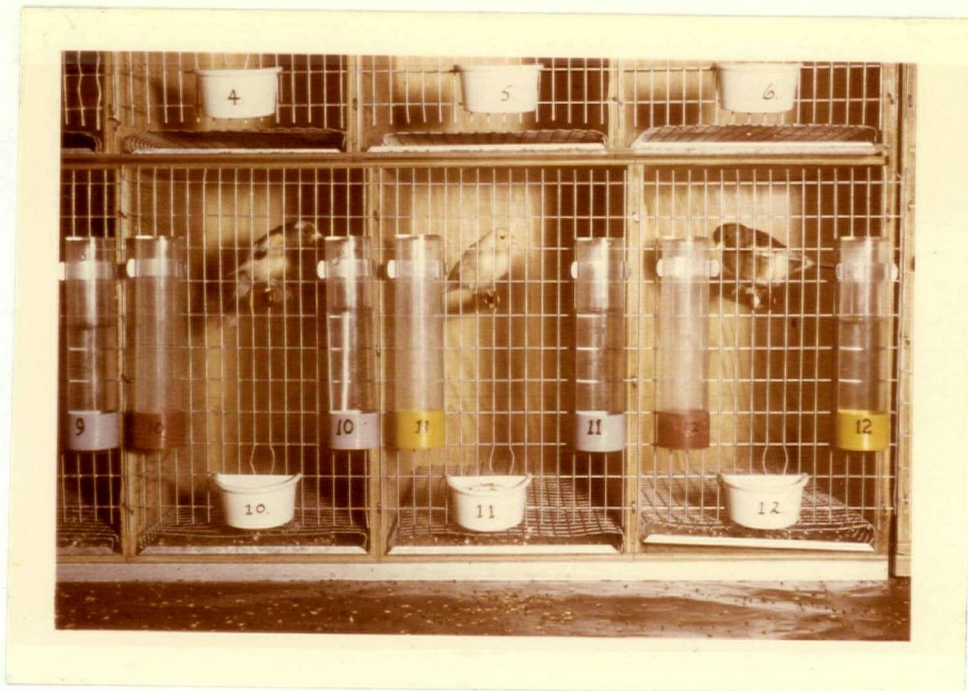
Figure 19.

Figure 20.

BIRD NO.	CLINICAL APPEARANCE	DEATH TIME DAYS PI	AUTOPSY LESIONS	ATTEMPTED CULTURAL RECOVERY		FAECAL CULTURES
				Tissue	Result	
1	Perky to the end	30	Enlarged spleen air sacs, yellow	liver kidney spleen air sac bowel	+ + +	not attempted initially
2	Non clinical recovered	70 sacrificed	None. Excellent condition	Kidney liver spleen bowel	- - -	not attempted initially
3	Ruffled after 3 days Very sick after 6 days	12	Typical nodules spleen and liver of p. pseudo	Kidney spleen liver bowel	+ + +	not attempted initially
4	Normal and perky Death sudden	57	Right lung caseous mass left air sacs caseous material	spleen kidney liver right lung cloaca	+ + +	not attempted initially
5	Rough on 7th day	8	Spleen enlarged 2 X. No other	spleen lung liver, rt kid bowel	+ + + +	4th day pos. (few cols) 5th & 6th days negative
6	Rough and depressed on 5th day	10	Both lungs and air sacs caseous material spleen enlarged with one tubercle	spleen liver lung air sacs bowel	+ + + + +	day death sample pos. 5th 6th, 7th days negative

TABLE **XIII**   EFFECT OF INOCULATION OF BROTH CULTURE ORALLY IN SIX CANARIES - STRAIN "LONGWORTH F". INOCULUM CONSISTED OF 0.2 ML BROWN #10 EQUIVALENT RESUSPENSION OF TWENTY-FOUR-HOUR GROWTH AT 37° C

(b) Housing

For these experiments, a battery of twenty-four cubicles was constructed of wood and wire (Figs. 17 - 20). The dimensions of the individual cubicles were eight inches by twelve inches by eight inches. (A full description of the construction will be found in the Appendix.)

Experimental Approach

Following the completion of Experiment B, a series of preliminary experiments were carried out to obtain guide lines in materials and methods for Experiment C. The preliminary work included a pilot experiment which has been previously referred to, wherein six birds housed in the specially built cubicle battery were infected orally. The results in terms of pathology and recovery of the organisms from tissues and faeces are to be found in Table XIII.

A number of conclusions were made from the preliminary work which may be listed under the following headings.

(a) Effectiveness of the inoculum

There was every reason to feel that the strain and dosage selected was satisfactory to infect the birds, and to produce classical lesions of pseudotuberculosis.

A total of ten birds had been inoculated with broth

cultures of the Longworth F.strain in Experiment B, and the preliminary pilot experiment. Of ten birds inoculated, one only recovered and seven died within seventeen days of inoculation. Two died at extended times later.

A total volume of 0.2 ml was used in the pilot preliminary experiment. This was felt to be too great for a single dose into the esophagus since birds regurgitated and suffered a brief collapse from anoxia, and the resultant lesions in the lung suggested that culture material had been inspired into the bronchial system. A similar volume (0.225 ml) was then used in Experiment C, but was split into three daily doses of 0.075 ml each.

In administering the inoculum, a serology pipette of 0.2 ml capacity was discontinued in favour of a stainless steel teat cannula.

(b) Collection and storage of the faeces

Collection methods were changed in several respects. The use of waxed paper drop sheets was discontinued in favour of coating the metal of the drop tray with a thin layer of molten wax. For the pilot experiment the faeces were stored at 5° C for days or weeks prior to culturing. Recoveries of P. pseudotuberculosis from the faeces of birds No. 5 and No. 6 of the pilot group (the first two to die) were quite inconsistent. Since storage at 5° C introduced an unknown

factor[1], it was decided to cut this delay to less than twenty-four hours, except for two days during the experiment when it was necessary to sharp freeze the material and process it later.

(c) Culture of faecal material

It was found that 0.1 ml of a 1:500 dilution of faecal sludge would yield a bacterial colony concentration suitable for counting with a Quebec Colony Counter on a MacConkey agar plate. For qualitative work, blood agar plates were also used, containing five per cent bovine red cells. (See Table XV).

In selecting the secondary isolation media, it was noted that the best growth with uniform turbidity occurred with beef heart infusion broth; however, maltose peptone water tubes (with gas tube) were quite satisfactory for supporting growth of P. pseudotuberculosis. Furthermore, other members of the canary bowel flora, possessing similar colony characteristics did not apparently have the ability to ferment maltose without gas production.

Experimental Technique

The complex series of steps involved in this exper-

---

1 It has recently been shown that P. pseudotuberculosis will multiply in certain media at this temperature refrigeration (Paterson and Cook, 1962).

iment is presented as a flow diagram in Tables XIV and XV.

The experimental work was made up of a number of phases which can be listed as follows:

(a) Acclimatisation phase

The birds were placed in the cubicles about one week before the experiment started, following thorough preparation of the surfaces.

(b) Inoculation phase

After collection of one pre-inoculation set of twenty-four hour faecal samples, the birds were inoculated daily over a three day period with 0.075 ml of a turbid suspension of P. pseudotuberculosis strain "Longworth F".

(c) Faecal collection phase

Each evening a complete set of twenty-four hour faecal samples was collected aseptically from the twenty inoculated and four control birds. The technique appears in Table XIV.

(d) Faecal processing and culture phase

The steps outlined on the accompanying flow

TABLE XIV ASEPTIC TECHNIQUE FOR COLLECTION OF 24-HOUR FAECAL
SAMPLES FROM CANARIES

1. Prepared 24 sterile, S/C, pre-labelled glass vials in wooden block

2. Removed all feed and water containers from wire fronts of cubicles to prevent possible cross contamination during collection procedures

3. Faecal drop trays removed from six cubicle cages at a time and a plastic baffle was hung in place to prevent escape of canaries through apertures

4. Faecal material adhering to grid over drop tray was teased loose with sterile tongue depressor, this fell onto drop tray ready for collection

5. Continued action with the tongue depressor loosened the faecal material from the drop tray, moved it to one corner and guided it into the collection vial corresponding to the cubicle number for the canary

6. While action No. 5 continued, the cleaned-off drop trays and grids were boiled for three minutes in an instrument steriliser which had a thin layer of molten wax on the surface of the boiling water

7. Upon removal from the steriliser, each grid and tray was wiped with a sterile paper towel. This left a thin layer of wax upon the sterile metal surfaces

8. While the trays and grids were out of the cubicles, the wire fronts were wiped with sterile paper towels dampened in cold tap water

9. Immediately following action No. 8, the clip perches were removed from the cages and placed in an instrument steriliser. A spare set of sterilised, waxed, and dried perches was replaced daily

10. The sterile trays and grids were replaced in the cubicles in sets of six. The thin wax layer prevented close adhesion of faecal material to the metal surface thereby making the above operation fast and efficient

11. All water fountains were emptied and rinsed thoroughly with cold running water. The feeder dishes were tipped out completely and half-filled with fresh seed. The cuttle was replaced in the same cup, or discarded if it had been thrown out by the bird

12. The plastic baffle which projected from between the decks (to protect the lower feed cups from contamination) was removed and washed thoroughly under running water

13. Freshly collected sets of 24-hour faecal samples removed to 5° C storage overnight

TABLE NO. XV   PROCESSING AND CULTURAL PROCEDURES USED IN
               OBTAINING FAECAL COUNTS AND FAECAL DRY
               MATTER OUTPUT ON A DAILY BASIS

INDIVIDUAL CANARY

battery with cubicles was
completely serviced at 20:00
hours daily

QUANTITATIVE CULTURING   QUALITATIVE CULTURING

Complete 24 hour faecal sample   discontinued after first
removed from drop tray to screw   ten days as found to be
cap (S/C) vial   unnecessary

       overnight at 5° C   One fresh canary dropping
                                          collected on waxed paper
10 ml SPSS added to soften   inserted when drop tray
faecal material in S/C using   removed for cleaning
Cornwall Syringe

       stand 30 mins at   With use 4 mm platinum
       R/T   loop plate out on

Vial shaken vigorously for 30
secs to homogenize faecal   Blood   MacConkey Agar
material in S/C vial   Agar   Plate (MacCAP)
                                          Plate
                                          (BAP)
       groups of four
       samples   incubate 24 hrs
                                                at 37° C

Tipped through stainless steel   Check plates for colonies
sieve to beaker. Vial rinsed   suggestive of being Pas-
and sieve flushed with total of   teurella pseudotubercl.
18.0 ml sterile physiological
saline solution (SPSS)

Beaker contents returned to
S/C. Vials and topped up to
grad mark of 30 ml with SPSS

Pick such colonies off
plate to maltose broth
tubes with gas tube

Shake again and allow
to stand for 10 minu-
tes to allow settling
heavy sediment

One 5.0
ml ali-
quot

Incubate to 72 hours at
37° C and check for acid
no gas

Decant to stainless steel
centrifuge tube of known
weight

Maltose fermenters
(no gas) and non-
fermenters

Gas
produ-
cers

Centrifuge 10,000 RPM/15
mins in angle centrifuge

Discard

Immediately machine stops
invert to discard super-
natant

Streak on segment
of blood agar
plate

drain for
30 seconds

Incubate 37° C for 24 hours

Store at 5° C

Weigh tubes on analy-
tical balance and subtract
tube weight to give net
weight damp sludge

Biochemical identification
check

Added nutrient broth to
give 10 ml W/V total
100 3.0 mm glass beads

agitated for
uniform re-
suspension

Removed 1.0 ml (1/10 of resuspension sludge) to saline blank so as to give 1:50 W/V dilution of suspension

Removed 6.0 ml to 12 x 85 mm plastic vial (cork stoppered)

labelled

shake thoroughly

-20° C deep freeze for use in emergency

Pipetted 0.1 ml onto surface of MacCAP and spread with sterile glass rod using turntable

At time of plating a broth culture of P. pseudo TB

incubate 37° C

streaked

Examined 24 hours, at 48 hours (most useful & 66-70 hours

Control MacConkey agar plate examined for colony size. If negative for suspicious colony

Suspicious colonies described and counted on Quebec Colony Counter

Discard

Picked off representative numbers to maltose broth tubes

37° C incubation to 72 hours

Acid formation No gas

Growth with no acid form

Acid + Gas

Plate on BAP

Further incubation at 37° C

Discard

Storage 5° C

DETERMINATION OF FAECAL DRY MATTER

Plastic Petri dish

dishes grouped
on trays

37° C incubator
Covered to keep out
dust and allowed to
dry for 7 days

Weighed on analytical balance

Calculation x 6 to give
total faecal dry matter
output for 24 hour period
for individual bird

chart (Table XV) took up much of the experimental time daily. However, in retrospect it was felt that the results justified this concentration of effort.

(e) Examination of cultures

In the early morning and late afternoon, all primary isolation plates were checked. Colony types were examined, selected, described, counted, and picked off into maltose broth tubes (see flow chart Table XV). Other tubes of maltose broth, already incubating, were examined daily for acid production with no gas. Tubes showing such characteristics, (or growth with no fermentation), were streaked out with a loop into blood agar plates for incubation and storage.

(f) Pathological examination

As birds died, they were chilled, autopsied, and cultured. Gross pathological examinations were carried out, and tissues were fixed. Surviving birds, all of which had ceased to shed viable P. pseudotuberculosis were autopsied and cultured on the 21st day after the conclusion of the course of inoculations, i.e. the 24th experimental day.

(g) Biochemical identification of isolations

Representative numbers of faecal isolates of

presumed P. pseudotuberculosis were checked biochemically for each bird following termination of the experiment. Checks were made of all first counts, peak counts, and final counts for individual shedder birds.

Results and Discussion

(a) Clinical appearance of the birds

Within about three days of completion of the series of oral inoculations, i.e. around the sixth experimental day, the birds appeared subdued. There was little or no singing from the inoculated birds. Two of those birds which subsequently died (No. 3 and No. 17) were noticed to be ruffled from this point onward. On some days they appeared to be improved. The activity of the birds was, of course, limited by the cubicle size and they did not have the stimulus of being able to see each other. One bird, No. 17, seemed to be particularly agitated as though constantly hungry and it emptied the feed dish daily. Most of this feed went on the floor of the room. It was interesting that this bird never showed a positive faecal count until the fifteenth experimental day which was eight days before death. Another bird, No. 12, was also ruffled towards the end of the experiment. It laid two eggs on the 11th and 14th experimental days, but according to the faecal count and autopsy findings, it reached the recovery point on the 20th experimental day. Bird No. 1 sat a great deal on the

wire toward the end of the experiment. A number of the birds suffered from wet stools transiently. Three of these were non-inoculated control birds. A lactose-fermenting mucoid organism appeared on the plates. This condition was noticed to be unrelated to the clinical signs of depression or of mortality due to the test organism. As the experiment progressed, the condition cleared up. The causative organism was assumed to be a low grade pathogen, the spread of which commenced before the birds were separated and was checked by the isolation of the birds from each other. As far as the influence on the faecal count of Pasteurella was concerned, it was noticed that thirteen of the inoculated birds never showed soft stools during the whole course of the experiment. Of this group, two died, seven additional birds gave a positive count, and four of the birds never showed a positive count. Doubtless it would not have been noticed if the faecal samples had not been collected. One bird, No. 13, was seen to be wheezing on the 18th experimental day. This was the same day that the bird reached the point of recovery. The distress may have been asthmatic, or due to endotoxin formation as the bacterial cells were broken down in the reticulo-endothelial system.

(b) Mortality

As stated elsewhere the mortality rate among inoculated birds was twenty per cent. This was lower than expected,

but in view of the large number of birds which showed positive faecal counts (80 per cent) the lower mortality may have resulted in the data being of greater significance. All the birds which died showed lesions and yielded a culture of P. pseudo-tuberculosis.

(c) Variance in faecal excretion and weight of birds

There were several reasons for this measurement being taken and recorded on the graph charts for each bird. First, it allowed direct correlation to be made between faecal colony count and faecal volume in terms of dry matter. This was desirable where marked fluctuations of high peaks are noted on the graph. Secondly, the relative food intake of the bird could be measured in no other way. As it turned out, the birds did not cease to eat, although some varied their intake sharply. Any relationship between peaks of faecal count, and appetite or excretory activity of the bowel could be noted on the graph chart. The total food intake could be roughly calculated for each bird during the course of the experiment, and correlated with weight variation which occurred during the twenty-five experimental days or until death.

Considering weight variations in retrospect there was an unfortunate omission in that the weight of the non-inoculated controls was not taken on the first experimental day. Actually the main function planned for the control group

was to provide bacteriological evidence particularly of
whether the live organism contained in the inoculum was capable
of spreading from one canary to another, and more important,
from one slide tray to another. This purpose was achieved
well enough. In addition there were four inoculated birds
which never passed the organism in the faeces. This group is
of greater comparative value in some respects than the non-
inoculated controls could have been. Of this group two gained
slightly in weight, one lost slightly, and one heavily. Of
the group which showed faecal counts all showed a weight loss
varying from about 5.7% up to 40.6%. These observations are
recorded in Table XVII which summarizes much of the data.
As uncontrolled clinical observations, the loss in weight of
all the shedders, but of only two of four non-shedders, would
appear to be definitely associated with the experimental dis-
ease. The very magnitude of the weight losses suggests that
the environmental and nutritional factors could not have been
solely responsible. Since most authorities describe varying
degrees of loss of condition for naturally occurring pseudo-
tuberculosis, the figures are not surprising.

    (d)   Presence and amplitude of faecal counts for
            viable <u>Pasteurella</u> <u>pseudotuberculosis</u>

      The data collected from testing of faecal material,
and pathological examination accompanied by bacteriological

culturing, allowed a fair assessment to be made of the course and pathology of experimental pseudotuberculosis in canaries. The main postulate for this experiment was based on the expected significant level of shedding of viable P. pseudotuberculosis organisms in the canary faeces following their inoculation per orum.

The experimental group, totalling 24 birds, falls into three groups based on the presence or otherwise of viable shed organisms. The first of these, group A, consisting of 16 birds, all shedders, can be further divided into three subgroups designated A-1 to A-3, based on variations in the pattern of output of viable organisms in the daily faecal excretions. (Table XVI). A more complete summary of data collected on individual birds appears in Table XVII, covering all birds which received the infecting inoculum.

From the above it can be noted that the postulate involving faecal shedding was fulfilled, since sixteen (80 per cent) of the twenty inoculated birds were shedders, many of them on a tremendous scale (Figures 21-40), even some which later recovered (as judged by cessation of shedding and negative culture upon autopsy). See also Figure 41 for totals.

Two graph charts have been prepared to aid in assessing the characteristics of experimental pseudotuberculosis in the group of twenty inoculated canaries. Figure

MAIN GROUPING	CRITERIA FOR GROUPING	SUB GROUPING	CRITERIA FOR SUBGROUPING	BIRD NO.	RECOVERED	DIED
A 16 birds	Shedders. All inoculated birds. All shed detectable numbers of <u>P. pseudo</u> on each of at least three days between 1st post inoculation day and the death of the bird, or end point (exp day No. 25)	A-1 4 birds	All birds died. High faecal counts until death. Culture recovered at postmortem. Monophasic.	3 16 17 21		day 14 day 23 day 23 day 23
		A-2 8 birds	No deaths. Faecal counts stayed high until 'recovery' point. No exacerbations. Monophasic. No recovery of culture on autopsy.	1 4 5 8 12 14 18 23	day 13 day 15 day 21 day 18 day 20 day 17 day 12 day 14	
		A-3 4 birds	No deaths. Faecal counts showed tendency to remissions and exacerbations Diphasic (7 & 13) or Triphasic(2 & 15). No recovery on autopsy.	2 7 13 15	day 21 day 15 day 18 day 19	
B 4 birds	Non-shedders. All inoculated. <u>None</u> shed detectable <u>P. pseudo</u> on any of 20 days of faecal testing		→	6 11 19 22		
C 4 birds	Non inoculated Non shedders		→	10 9 20 24		

TABLE **XVI**   GROUPING OF TWENTY-FOUR EXPERIMENTAL BIRDS ACCORDING TO FAECAL DISSEMINATION DATA

	COMPUTED FAECAL EXCRETION DETERMINATIONS					CHANGES IN CONDITION			GROSS PATHOLOGY			
Exp Bird No.	Daily Faecal Counts	Total Positive Counts	Day of Peak P. Counts	Peak Amplitude (Log)	Counts Over Log 6.0	Weight Loss %	Weight Gain %	Day of Death or Sacrifice	Spleen	Other Organs	Degree of Involvement	Total Cumulative Count Expont'l Per Bird
A-1												
3 F	10	9	12	6.25	1	16.3		14	++++	Liver + / Crop +++		2.38×10^6
16 M	19	.19	10	8.1	15	21.8		23	+++++	Heart + / Larynx + / Thymus + / Caecum ++++		5.02×10^8
17 M	19	6	21	6.75	1	27.6		23	++++	Liver ++ / Peritoneum++ / Breast muscle tumor		6.73×10^6
21 M	19	17	19	7.3	4	40.6		23	+++	Mandible ?		3.66×10^7
A-2												
1 F	20	8	11	5.0	0	21.9		S-25	++	nil		2.03×10^5
4 F	20	9	10	6.3	1	17.6		S-25	+++	nil		2.37×10^6
5 F	20	10	10	6.7	2	7.6		S-25	+.	nil		8.19×10^6
8 F	20	12	11	6.4	4	11.6		S-25	+	nil		1.26×10^7
12 F	20	12	15	7.0	3	22.1		S-25	+++	nil		1.38×10^7
14 F	20	10	8	6.75	1	11.6		S-25	+	nil		6.49×10^6
18M	20	7	6	5.5	0	11.2		S-25	+	nil		7.9×10^5
23M	20	8	9	7.6	6	7.0		S-25	-	nil		9.02×10^7
A-3												
2 F	20	11	6	6.4	1	6.1		S-25	+++	nil		3.29×10^6
7 F	20	5	10	5.1	0	11.8		S-25	-	nil		1.74×10^5
13F	20	3	10	5.1	0	5.7		S-25	++++	nil		1.39×10^5
15F	20	4	6	4.3	0	11.7		S-25	±	nil		2.2×10^5
B												
6 F	20	0	-	0	0	28.6		S-25	-	nil		nil
11F	20	0	-	0	0	6.6		S-25	-	nil		nil
19M	20	0	-	0	0		3.4	S-25	-	nil		nil
22F	20	0	-	0	0		4.5	S-25	-	nil		nil

TABLE XVI DATA SUMMARY SHEET FOR TWENTY
INOCULATED BIRDS (EXPERIMENT C)

Figure 21. Canary No. 3   Record of Faecal Shedding
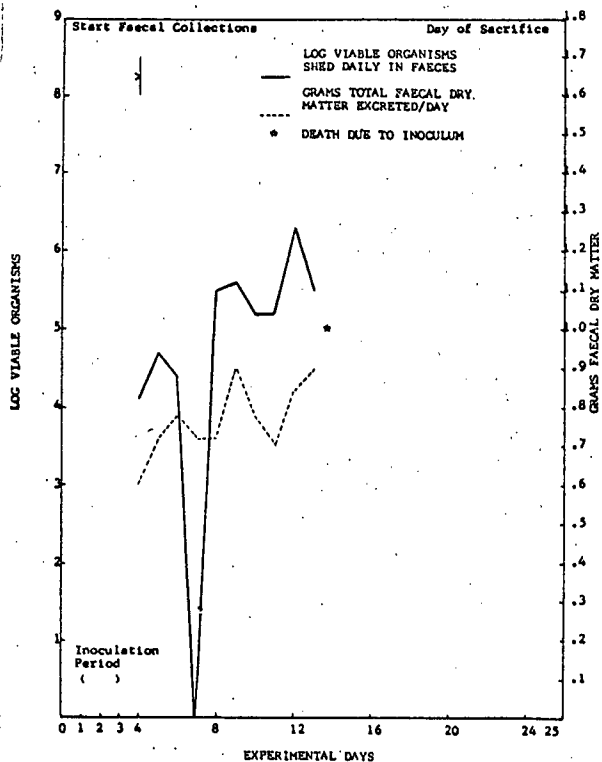
Figure 22   Canary No. 16   Record of Faecal Shedding
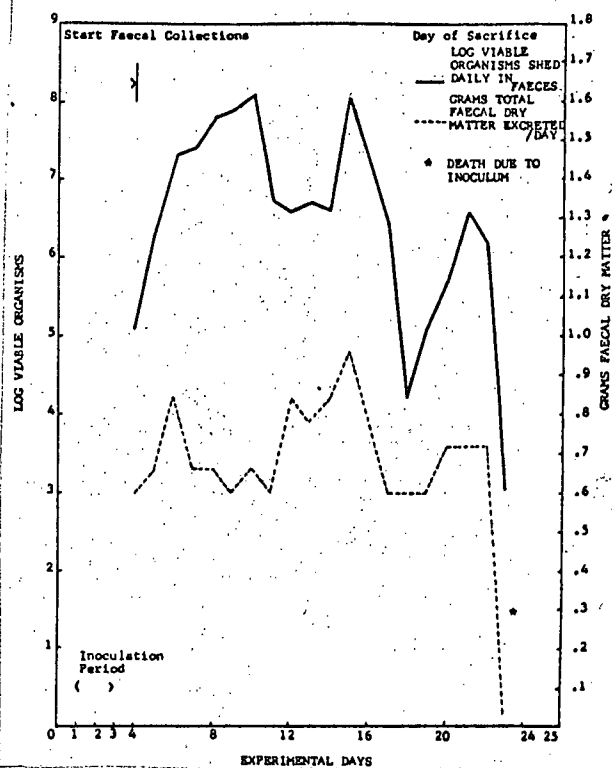
Figure 23   Canary No. 17   Record of Faecal Shedding
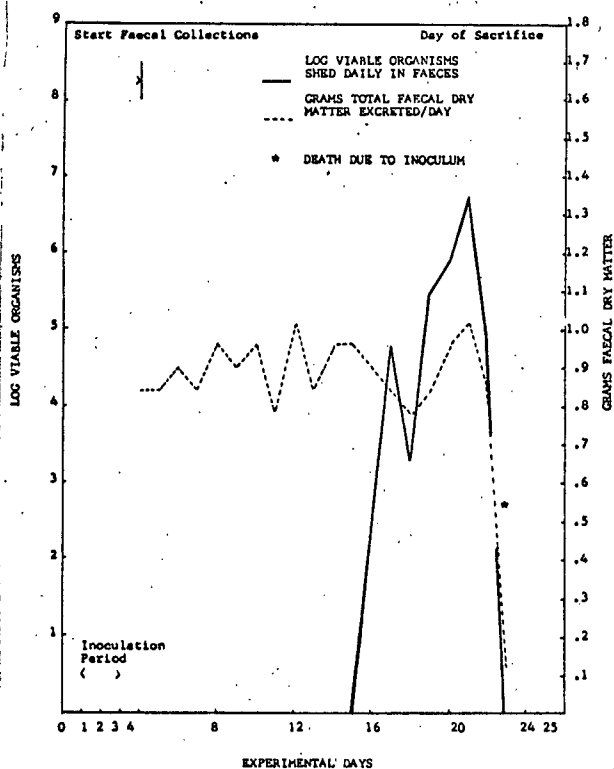
Figure 24   Canary No. 21   Record of Faecal Shedding
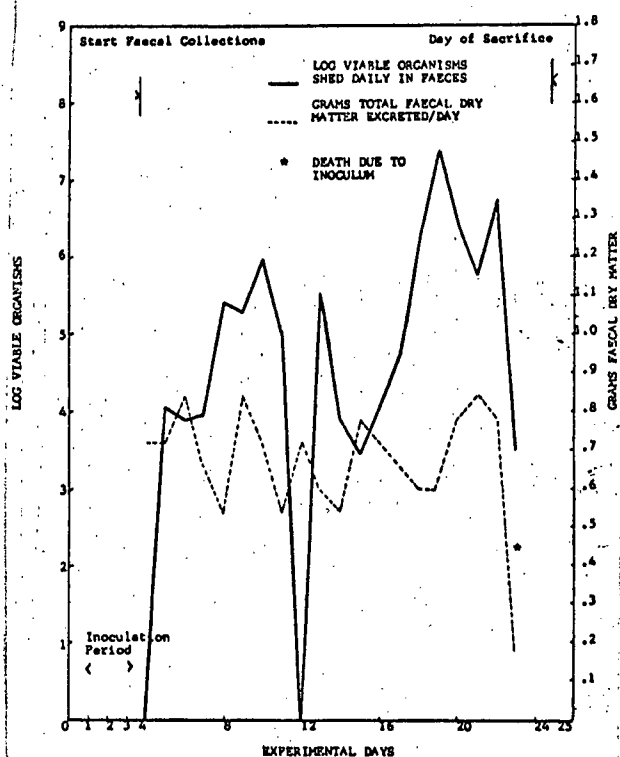
Figures   21-24.   Grpup A-1.   Four deaths due to experimental pseudotuberculosis. All shedders.
Graph charts of data collected daily on faecal counts of viable P. pseudotuberculosis, and faecal output in terms of dry matter.
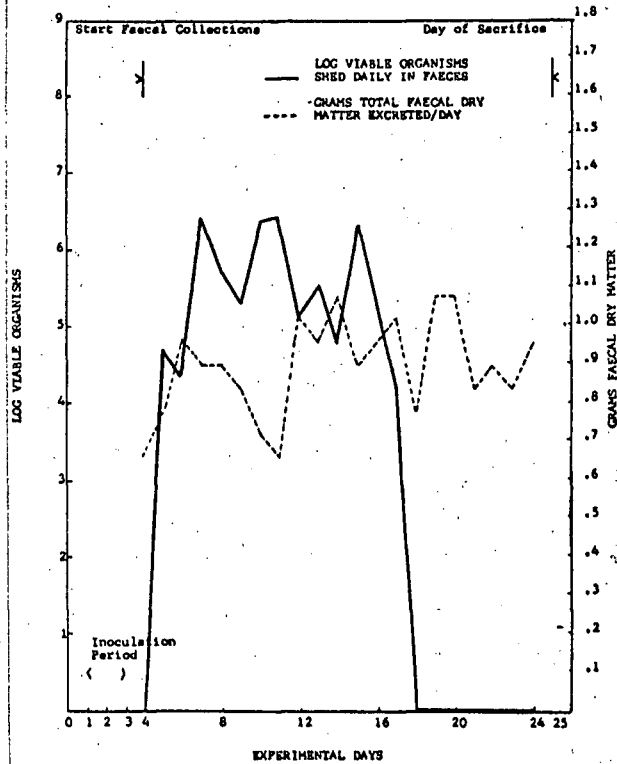
Figures 25-28   Group A-2.   Four of eight monophasic shedders
which recovered.
Graph charts of data collected daily on faecal
counts of viable P. pseudotuberculosis, and
faecal output in terms of dry matter.
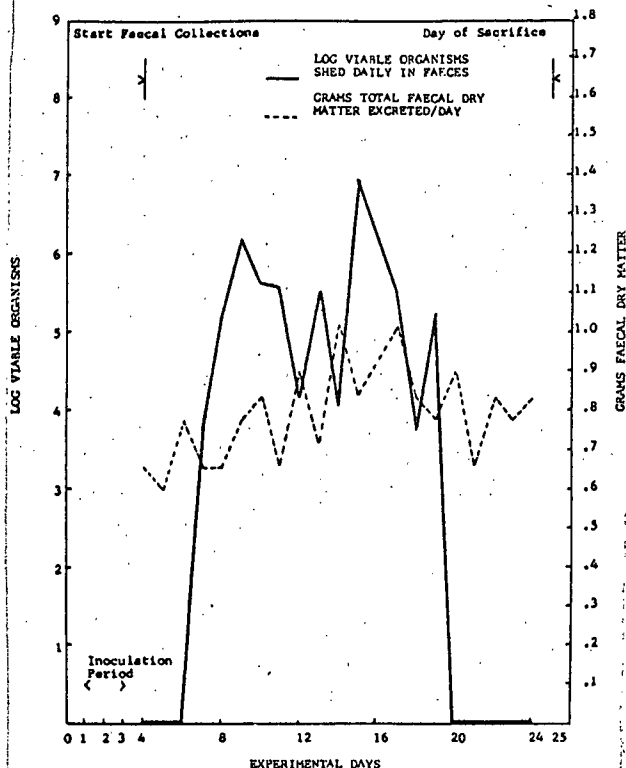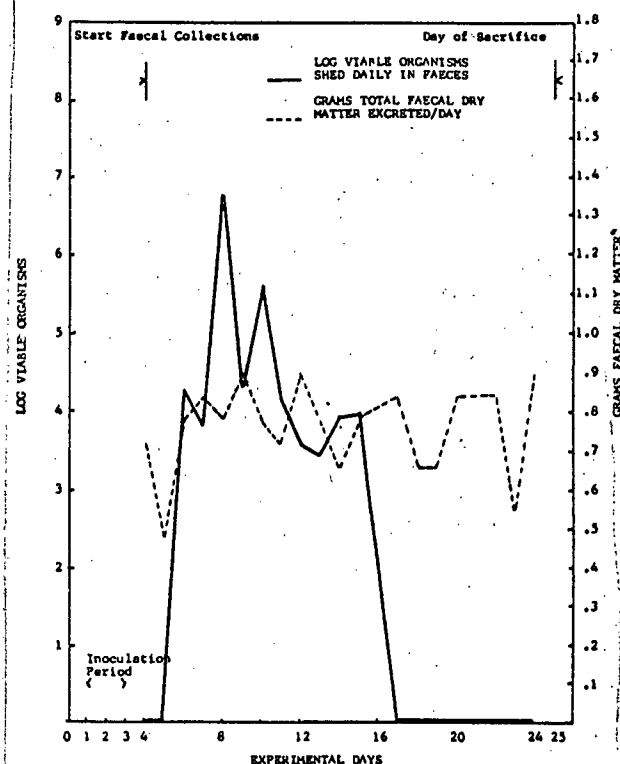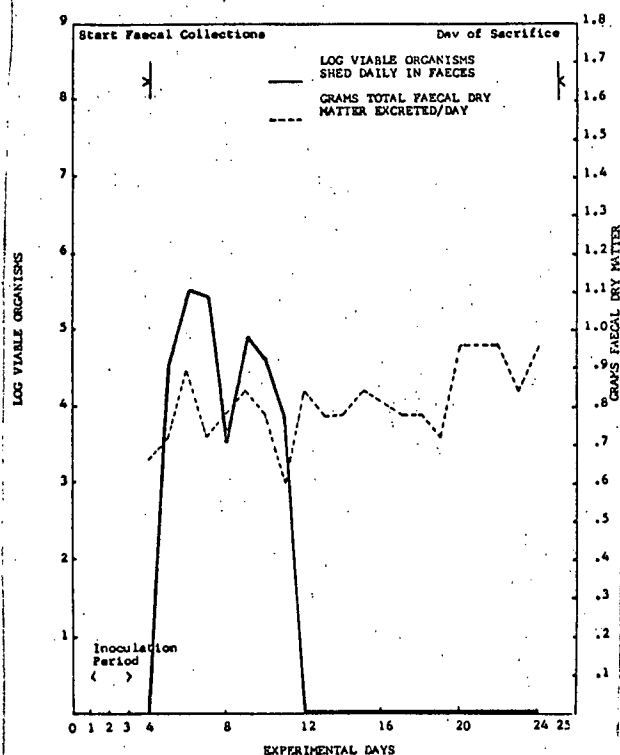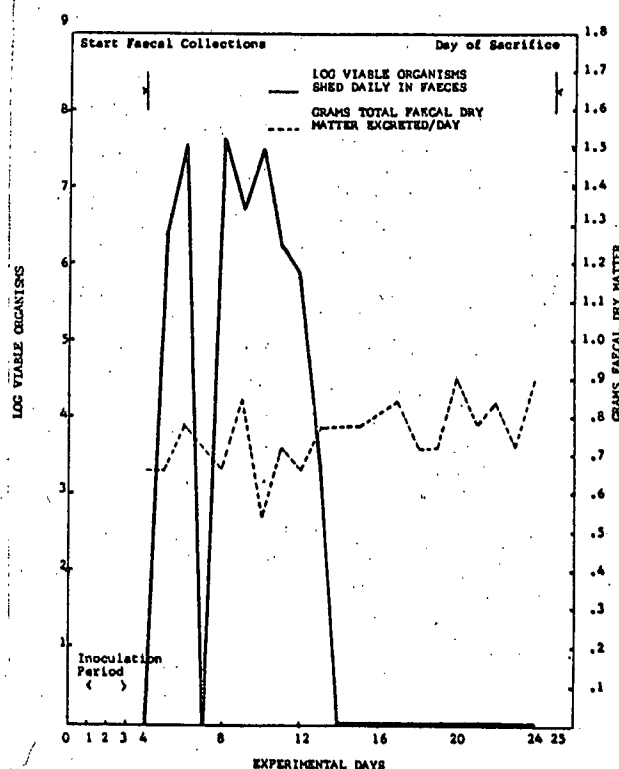
Figures 29-32   Group A-2 (contd). Four of eight monophasic
shedders which recovered.
Graph charts of data collected daily on faecal
counts of viable P. pseudotuberculosis, and
faecal output in terms of dry matter.
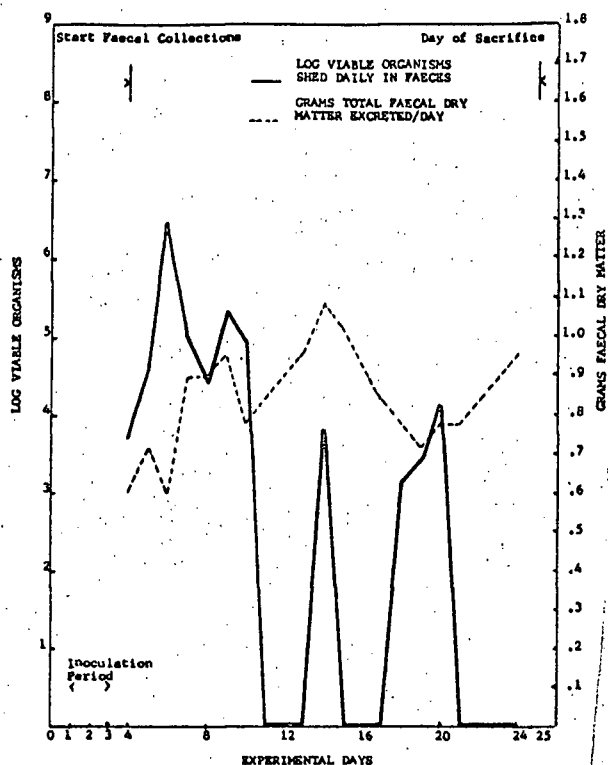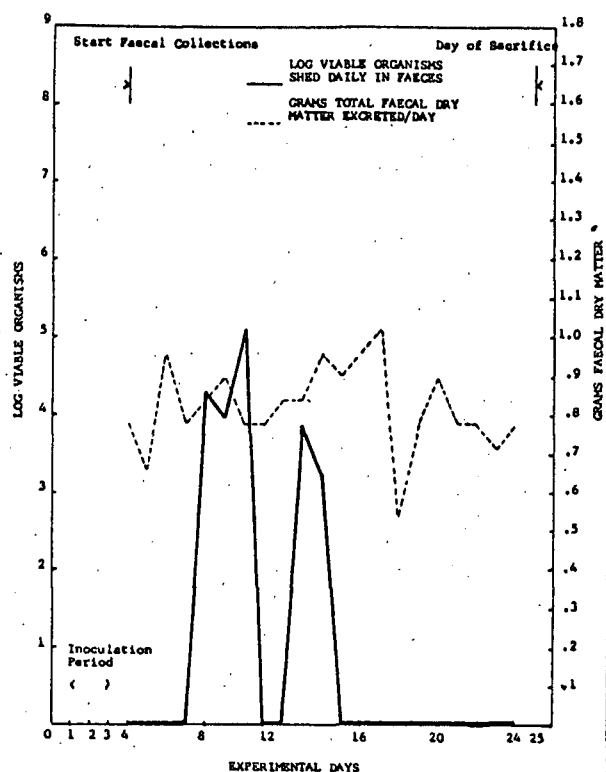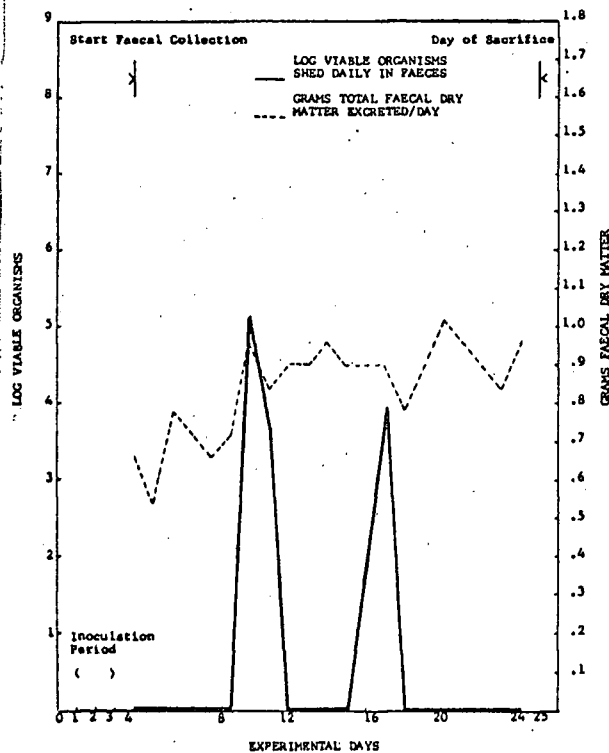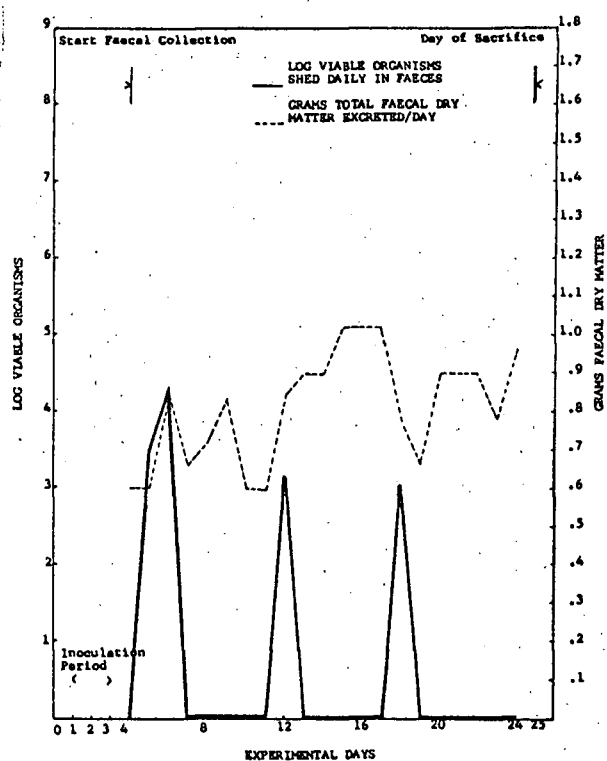
Figures 33-36   Group A-3.   Four di- and triphasic shedders
                which recovered.
                Graph charts of data collected daily on faecal
                counts of viable P. pseudotuberculosis, and
                faecal output in terms of dry matter.

Figure 37 Canary No. 6 Record of Faecal Shedding

LOG VIABLE ORGANISMS

Start Faecal Collections

Inoculation
Period
< >

LOG VIABLE ORGANISMS
SHED DAILY IN FAECES

GRAMS TOTAL FAECAL DRY
MATTER EXCRETED/DAY

Day of Sacrifice

EXPERIMENTAL DAYS

GRAMS FAECAL DRY MATTER
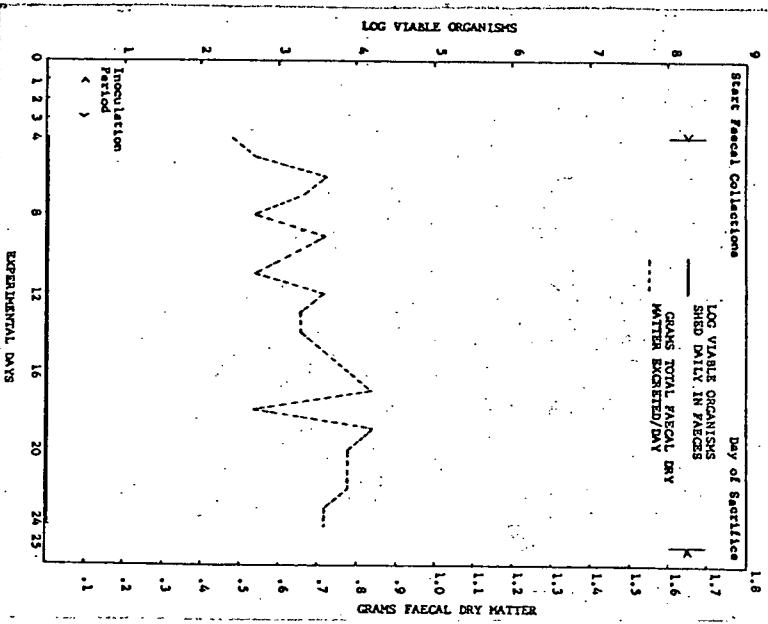
Figure 38 Canary No. 11 Record of Faecal Shedding

LOG VIABLE ORGANISMS

Start Faecal Collections

Inoculation
Period
< >

LOG VIABLE ORGANISMS
SHED DAILY IN FAECES

GRAMS TOTAL FAECAL DRY
MATTER EXCRETED/DAY

Day of Sacrifice

EXPERIMENTAL DAYS

GRAMS FAECAL DRY MATTER
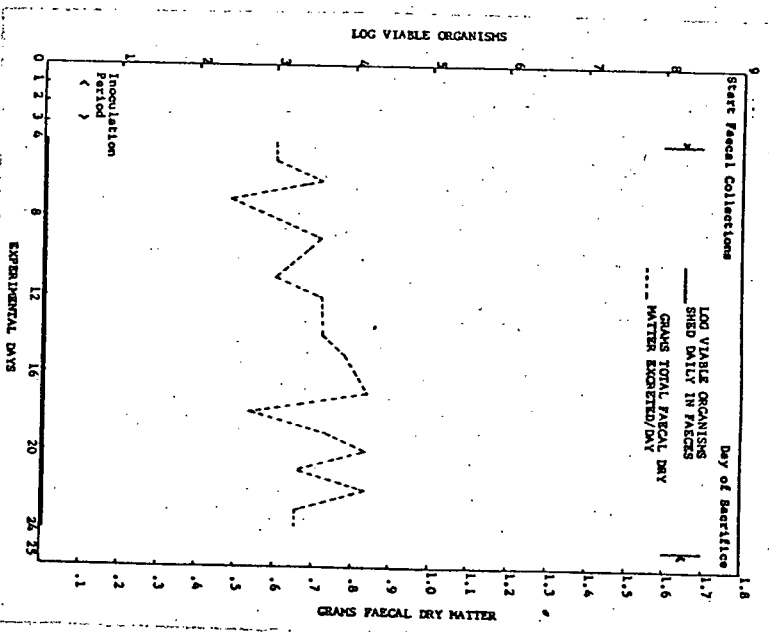
Figure 39 Canary No. 19 Record of Faecal Shedding

LOG VIABLE ORGANISMS

Start Faecal Collections

Inoculation
Period
< >

LOG VIABLE ORGANISMS
SHED DAILY IN FAECES

GRAMS TOTAL FAECAL DRY
MATTER EXCRETED/DAY

Day of Sacrifice

EXPERIMENTAL DAYS

GRAMS FAECAL DRY MATTER
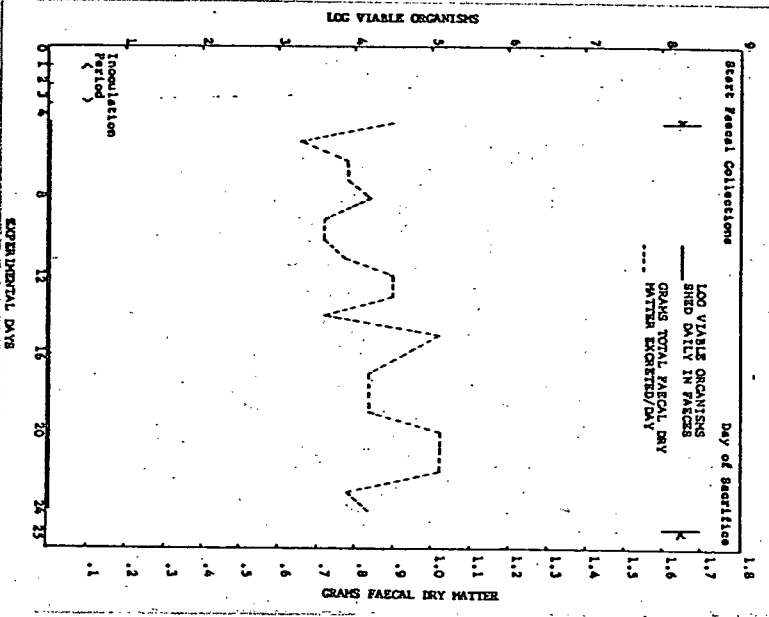
Figure 40 Canary No. 22 Record of Faecal Shedding

LOG VIABLE ORGANISMS

Start Faecal Collections

Inoculation
Period
< >

LOG VIABLE ORGANISMS
SHED DAILY IN FAECES

GRAMS TOTAL FAECAL DRY
MATTER EXCRETED PER DAY

Day of Sacrifice

EXPERIMENTAL DAYS

GRAMS FAECAL DRY MATTER
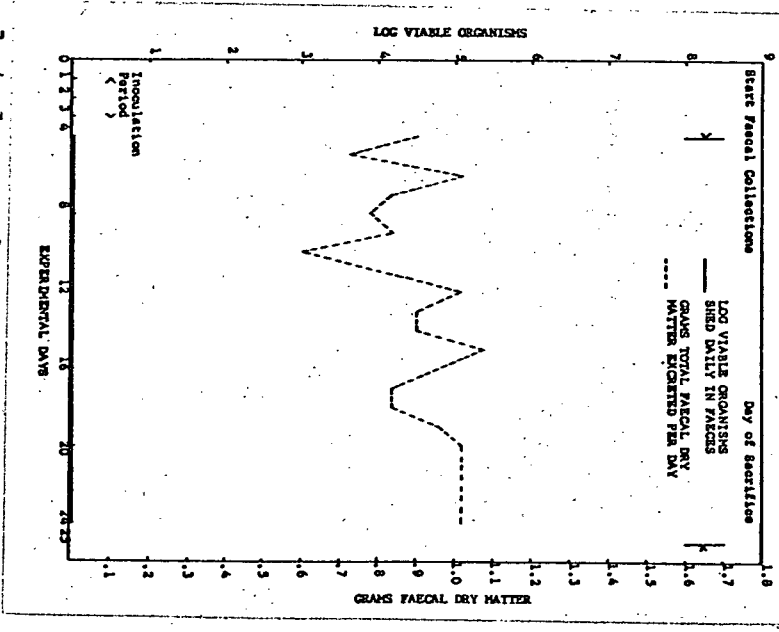
Figures 37-40 Group B. Four inoculated non-shedders.
Graph charts of data collected daily on faecal
counts of viable P. pseudotuberculosis and,
faecal output in terms of dry matter.
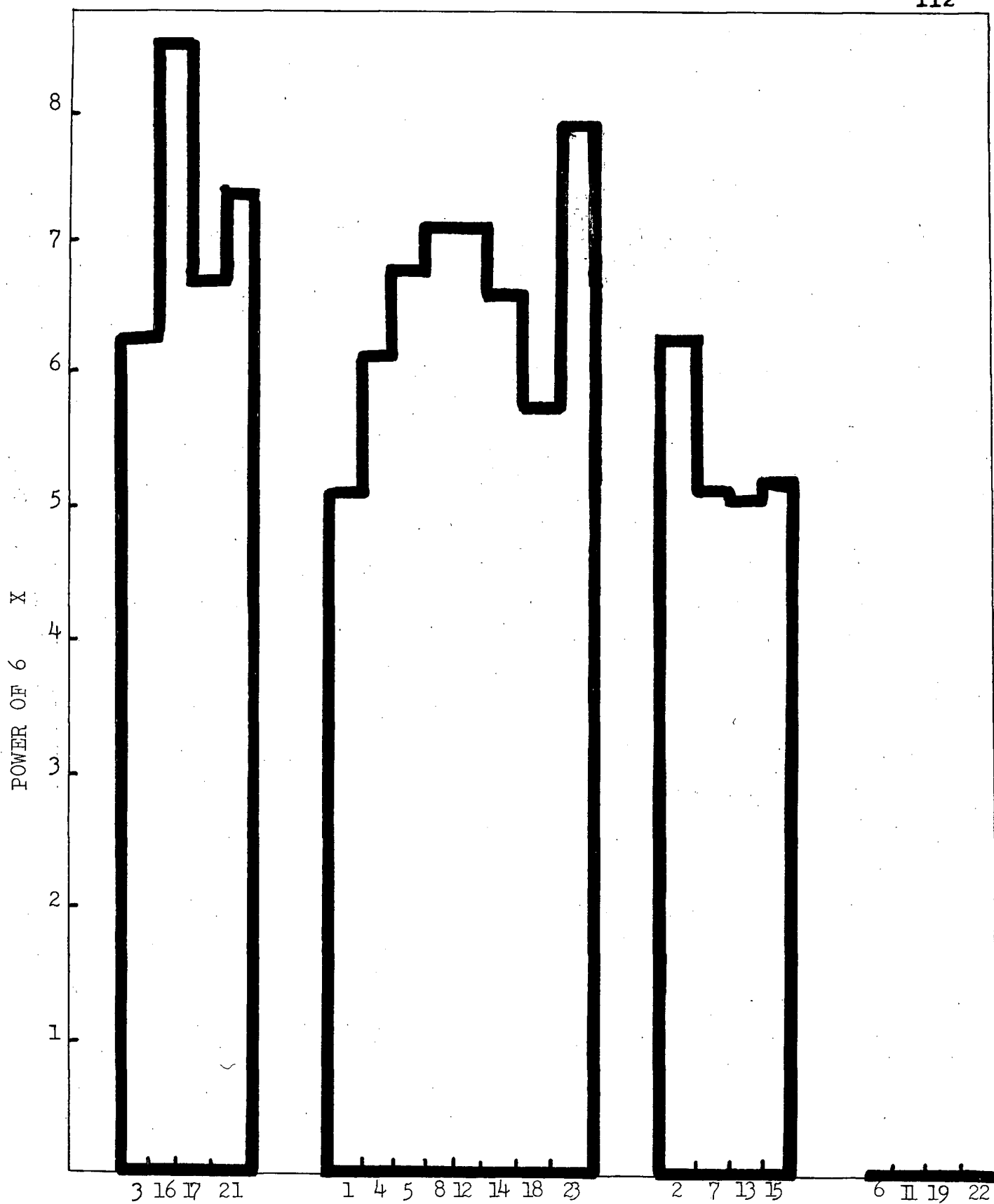
<u>Figure</u> 41   Excretion of viable <u>Pasteurella pseudotuberculosis</u>
by inoculated birds Totals for twenty experimental
days (exponential values)
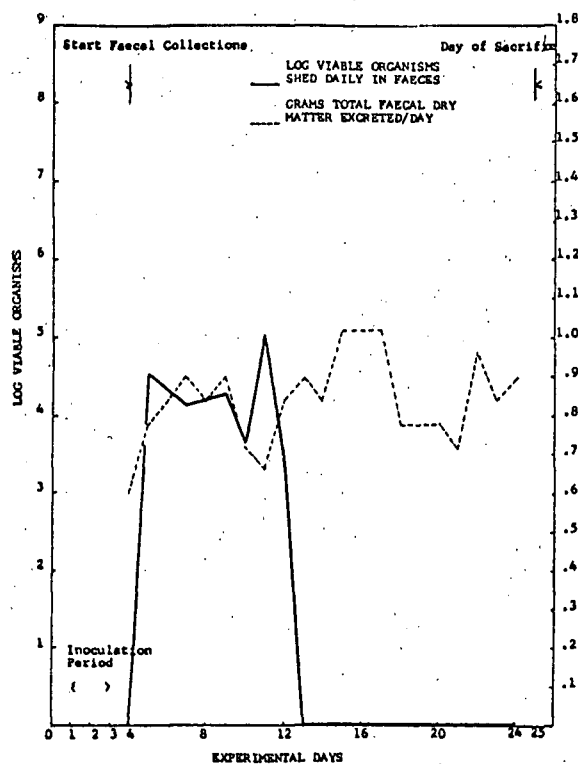
42 illustrates the daily totals of shedder birds (solid line)
(Group A Table XVI) out of the total survivors of the group
of sixteen birds which shed the test organisms at some stage
during the experiment (interrupted line). It will be noted
that the peak of excretion of the organisms in terms of num-
bers of birds shedding was reached on the tenth day. Since
the mean point of peak excretion in terms of numbers of
organisms for the sixteen shedder birds is 10.8 days into
the experiment (the median and mode being 13.5 and 10.0 res-
pectively) it seems likely that this figure (10 days) bears
relationship to the maximum bacterial invasion of the birds
as a group and hence of the greatest bacteremia. This in
turn suggests that the average incubation period of the exp-
erimental disease by the oral route could best be stated as
10 days. For this purpose incubation period is defined as the
time interval between first inoculation and the appearance of
maximum faecal excretion of the test organism.

In Figure 43 the total daily number of shedder birds
is again plotted (solid black line). This line descends fin-
ally to zero at the 24th experimental day as a result of four
deaths (dotted line) and twelve "recoveries" (i.e. birds
which have ceased to shed). The solid red line plots the
daily increase in numbers of recoveries (up to a total of
twelve). These two solid lines cross on the 18th experimental
day which it is suggested might be considered as the point of
maximum effective immunity in the group of affected (fecal
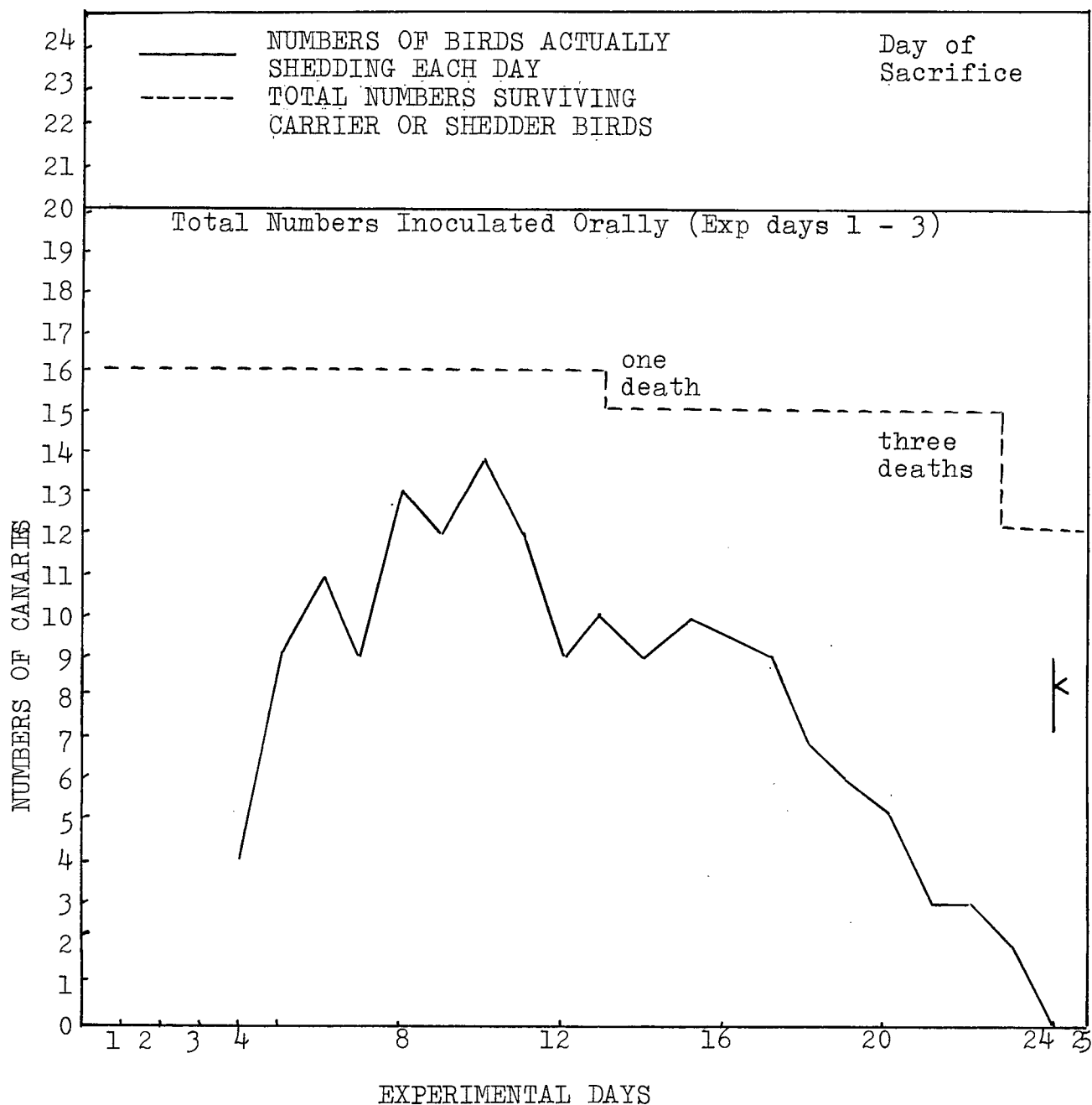
NUMBERS OF BIRDS ACTUALLY SHEDDING EACH DAY

TOTAL NUMBERS SURVIVING CARRIER OR SHEDDER BIRDS

Day of Sacrifice

Total Numbers Inoculated Orally (Exp days 1 - 3)

one death

three deaths

NUMBERS OF CANARIES

EXPERIMENTAL DAYS

1 Four of the total of twenty-four birds were non-inoculated controls

2 Four of the total of twenty-four birds were inoculated non-shedders

Figure 42 Daily record showing numbers of birds shedding (Group A-Table XVI) illustrated by a graph chart
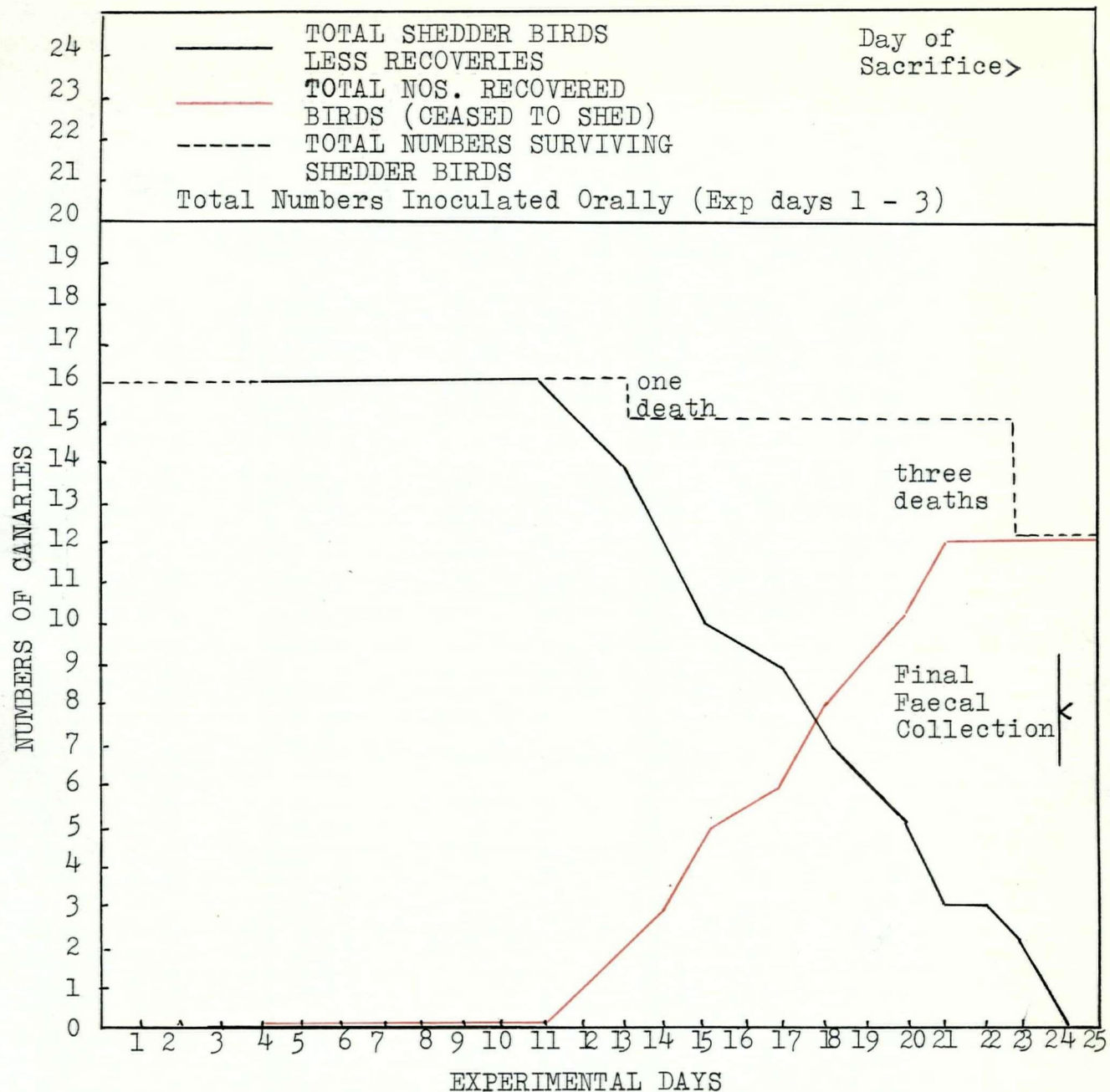
Legend:
- TOTAL SHEDDER BIRDS LESS RECOVERIES
- TOTAL NOS. RECOVERED BIRDS (CEASED TO SHED)
- TOTAL NUMBERS SURVIVING SHEDDER BIRDS

Total Numbers Inoculated Orally (Exp days 1 - 3)

Day of Sacrifice>

one death

three deaths

Final Faecal Collection

NUMBERS OF CANARIES

EXPERIMENTAL DAYS

1 Four of the total of twenty-four birds were non-inoculated controls

2 Four of the total of twenty-four birds were inoculated non-shedders

Figure 43 Daily record showing numbers of birds reaching the point of "recovery" in terms of ceasing to shed viable P. pseudotuberculosis (Group A - Table XVI)

shedder) birds. The median point between when the first and
the last birds reached the recovery point was sixteen days.
It can readily be seen that if canaries contact P. pseudo-
tuberculosis, become infected, shed viable organisms, and
do not recover on the average until sixteen to eighteen days
later, the chances of the infection spreading to other birds
is potentially high.

(e) Gross pathology and cultural recovery (Figures 44 - 48)

The four birds in Group A-1, all of which died,
were found to show typical pseudotuberculosis nodules in
various locations, and a pure culture of P. pseudotuberculosis was
checked biochemically and found still capable of fermenting
maltose. Of the remaining sixteen inoculated birds, twelve
were shedders, and five of these showed definite gross splenic
lesions, while several more exhibited a slight degree of splenic
enlargement and/or congestion. None of the four non-shedders
showed any such changes on autopsy. These findings are recor-
ded in Table XVII. Particular attention is directed to Bird
No. 16 which showed lesions in the caecal lumena (Figure 47).

(f) Histopathology

Pseudotubercles were studied in the caecum of one
bird and in the spleens of all which died. The left caecal
lumen of canary No. 16 (Figure 49) was noted to be filled with

Figure 44   Experiment C. Bird No. 16.
             Autopsy showing trmendous size
             of affected spleen.

Figure 45   Experiment C. Bird No. 16.
             Close up of spleen which is
             made up of a solid mass of
             expanding pseudotubercles.

Figure 44.

Figure 45.

Figure 46   Experiment C.   Bird No. 16.
             Lesion in thymus area of neck.

Figure 47   Experiment C.   Bird No. 16.
             Close up view of swollen and
             abscessed cecal buds.

             The left cecum can be seen to have
             a distinct yellow nodular abscess
             at the blind extremity. This shows
             through the serosa.

             This bird excreted daily counts of
             Pasteurella pseudotuberculosis of
             up to Log 8.1 for nineteen consecutive
             days until its death.

Figure 46.

Figure 47.

Figure 48   Experiment C. Bird No. 17. The
             autopsy disclosed lesions in
             the spleen and breast muscles.
             This type of spleen with yellow
             to white pseudotubercles, is
             pathognomonic for pseudotuber-
             culosis in the canary.

             In spite of these lesions, this
bird did not commence faecal shedding of the
study organism until twelve days following
conclusion of the course of three peroral
inoculations. It then came to a daily peak
of Log. 6.75 P. pseudotuberculosis organisms
shed 48 hours before death. (see Figure 23).

Experimental Pseudotuberculosis
Canary
Test Run No. 17   March 1960
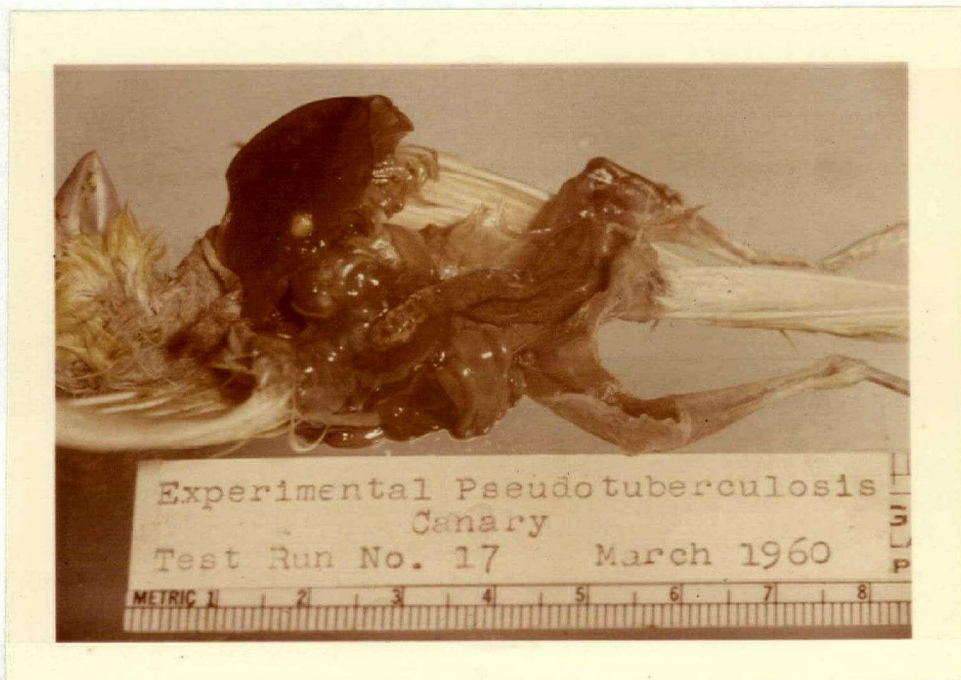
Figure 48

a mass of macrophages which had infiltrated and replaced the mucosal tissues and extended back into the muscularis mucosa (Figure 50). Centrally located was a necrotic centre which stained pink with H. and E. (Figure 50). At the periphery was a ring of large macrophages showing marked granularity indicative of bacteriophagia (Figure 51). Many giant cells of the Langhans type (Figure 52) were noted in the median area.

The spleen showing greatest enlargement was from the same bird (Figure 45). The microscopic picture was typical of other spleens. The changed organ was seen to be made up of a mass of expanding granulomas with caseating centres (Figure 53). An intense mass of macrophages with lessening degrees of degeneration extended out in concentric rings from the necrotic centre. At the periphery a ring of such cells contained ingested bacteria (Figure 54). The same tissues when stained with Twort's method using fast green for a counter stain, showed the central necrotic zone staining blue and the peripheral giant cells with their bacterial load showing as an intense red ring on low power (Figure 55). High magnification allowed illustration of the mass of red-staining bacteria showing the typical pattern of bipolarity (Figure 56).

H & E   40X

Figure 49   Experiment C.   Canary No. 16.   Exp-
erimental pseudotuberculosis.   The
two photomicrographs on this page
illustrate the histopathology of
the caecal abscess.

Low power cross section of colon
and caecal lumena shows the pink
staining necrotic centre of the
tubercle abscess in the right
caecum.

H & E   150X

Figure 50   Experiment C.   Canary No. 16.
A close up photomicrograph of
the abscess illustrated in
Figure 49.   The necrotic centre
is at the right, a mass of mac-
rophages (including giant cells)
in mid zone, and part of the ring
of giant cells with phagocytosed
bacteria (note blue smudging) at
left.
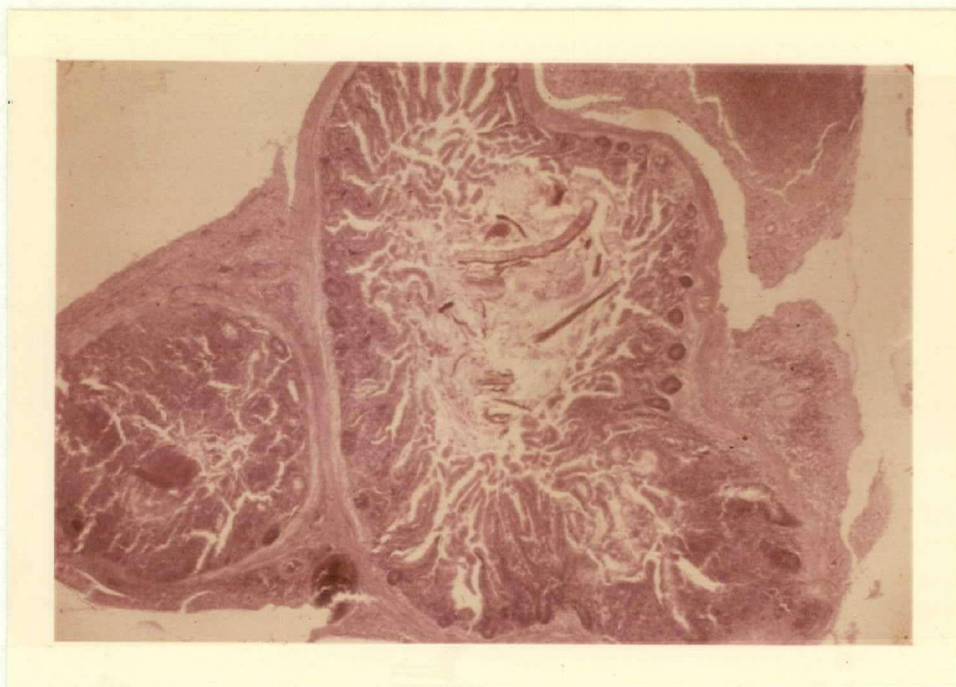
Figure 49.
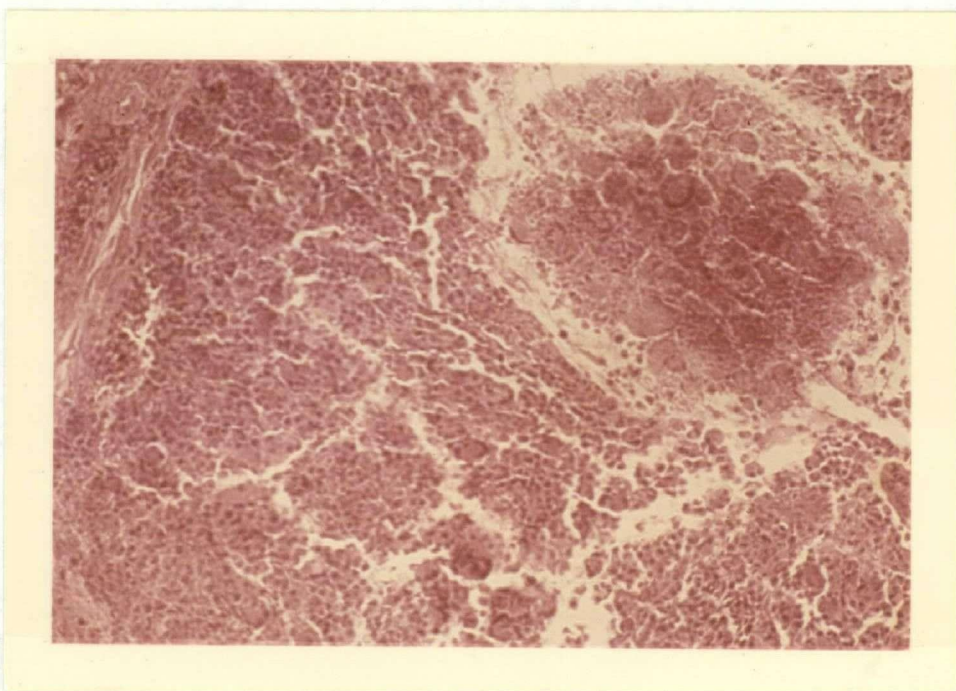
Figure 50.

H & E X 1500

Figure 51   Experiment C. Experimental
             pseudotuberculosis canary No.16.

             High power photomicrograph of
             the caecal abscess shows a part
             of the giant cell ring which apears
             in Figures 49 and 50. Numerous
             blue bi-polar-staining vacilli
             can be noted engulfed by the
             giant cells.

H & E X 1500

Figure 52   Experiment C. Experimental
             pseudotuberculosis canary No.16.

             High power photomicrograph of mass
             of giant cells in the mid-zone of
             the cecal abscess illustrated in
             Figure 50.
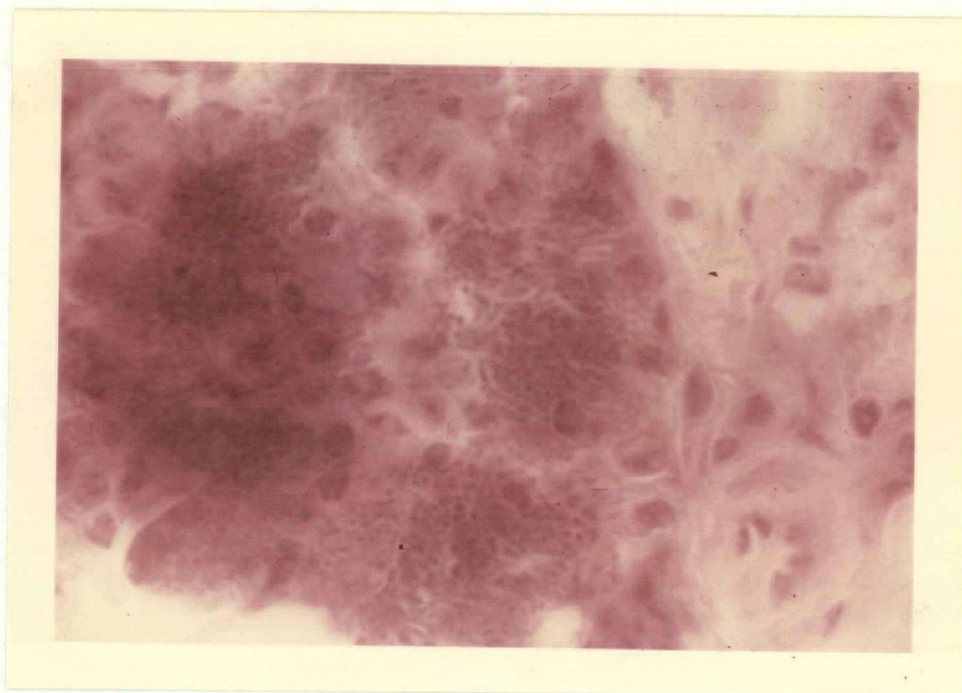
Figure 51.
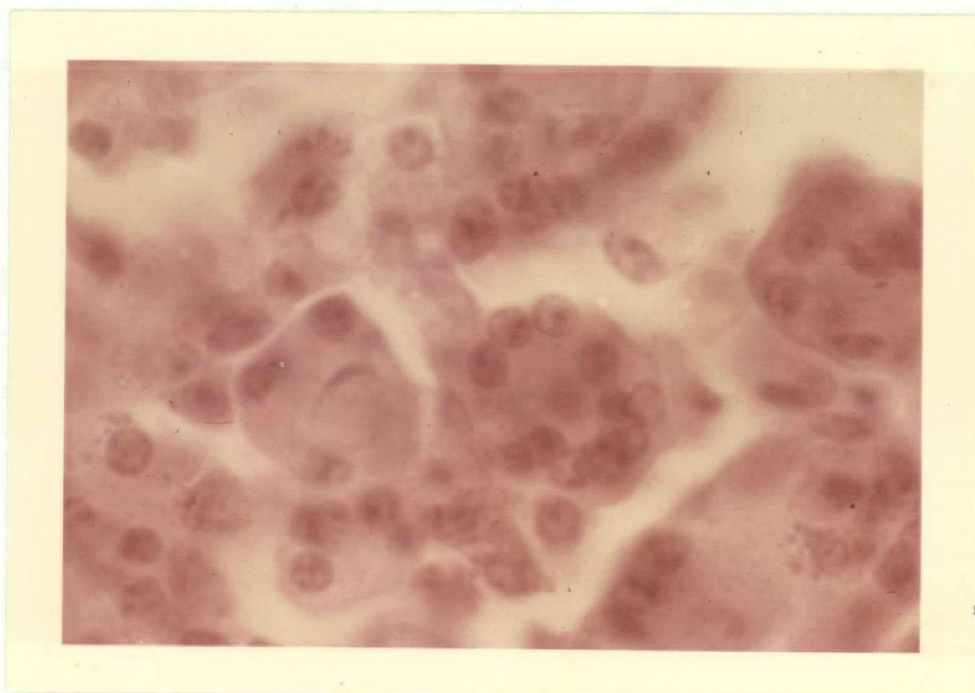
Figure 52

H & E X 30

Figure 53   Experiment C. Experimental
            pseudotuberculosis canary No.16.
            Low power photomicrograph of section
            of spleen which was illustrated
            in gross in Figure 44.

            The concentric rings indicate the
            expanding development of the
            pseudotubercles with their necrotic
            centers and peripheral rings of
            giant cell macrophages containing
            large numbers of bacilli.

H & E X 150

Figure 54   Experiment C. Experimental
            pseudotuberculosis canary No. 16.
            This high power photomicrograph
            shows the various zones of the
            pseudotubercles which are illust-
            rated in Figure 53. The necrotic
            centre is at the left and the
            giant cell ring at right.

Figure 53
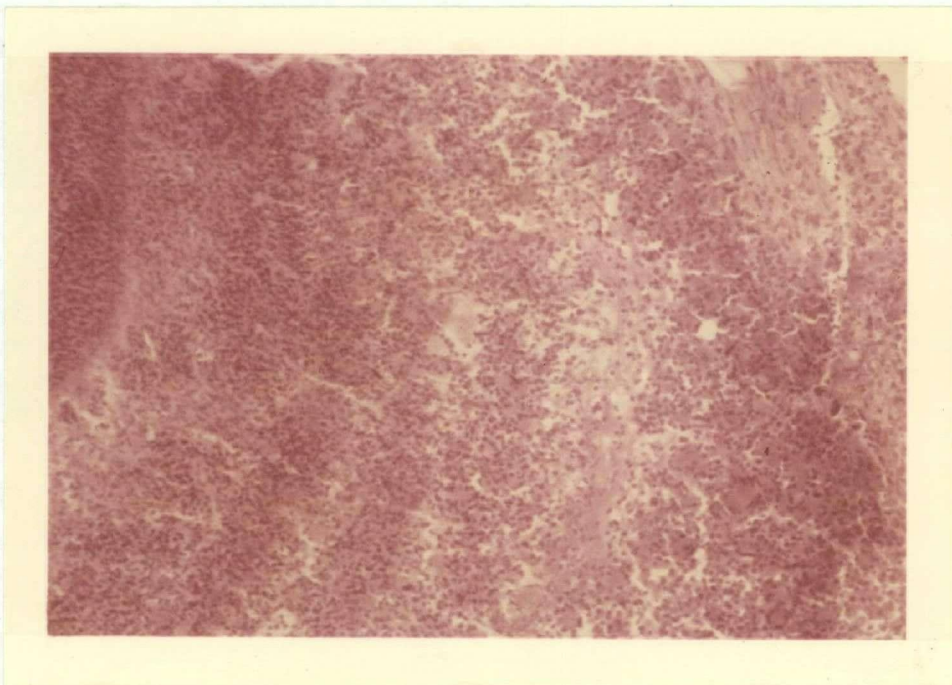
Figure 54

Twort's   X 50

Figure 55   Experiment C. Photomicrographs
             of the spleen illustrated in
             Figures 53 and 54.

             The stain used for this section is
             Ollett's modification of Twort's
             method. The necrotic centre retains
             the crystal violet, the gram negative
             phagocytosed bacteria are stained
             red, and the cytoplasmic areas take
             the Fast green counter stain.

Twort's   X 1200

Figure 56   Experiment C. Photomicrograph
             showing high pwer view of a
             section of the spleen in Figure
             55 (above). The neutral red
             staining bacteria are packed into
             the macrophages. The staining
             bi-polarity is clearly visible
             in this picture.

             This canary (No.16) was the heaviest
             faecal shedder of Pasteurella pseudo-
             tuberculosis.

Figure 55
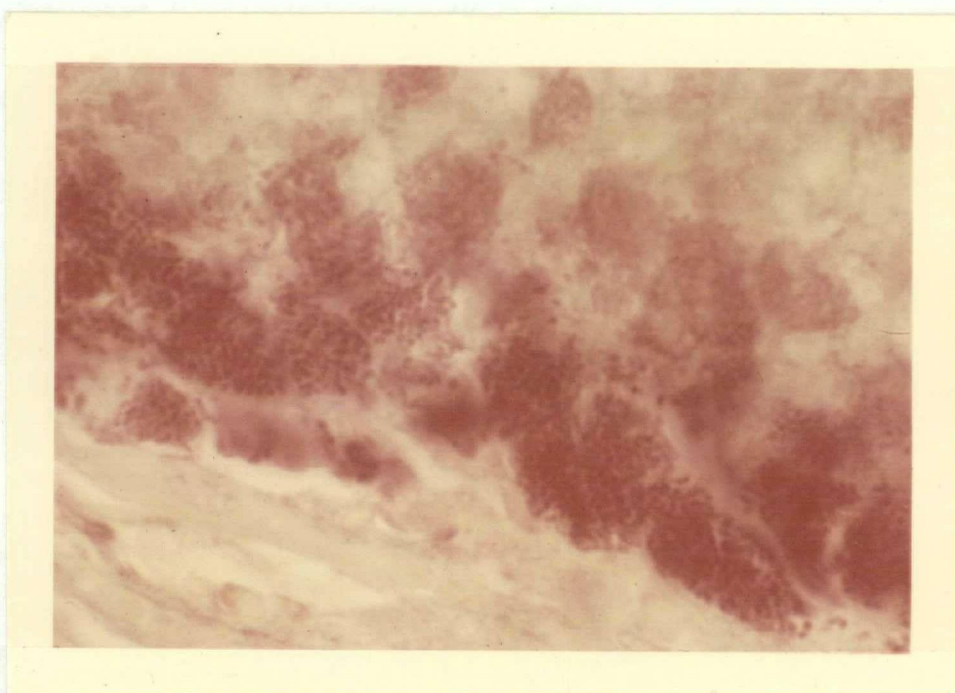
Figure 56

Summary and Conclusions

(a)   On the clinical appearance during the experiment

It was concluded that the superficial appearance of the birds was not a reliable indication of the outcome of the experiment. Many birds which showed high faecal counts for the inoculated organism and one of the birds which died (No. 16), showed no tangible clinical signs. Both inoculated and control birds seemed to have their bad days. The spread of a naturally occurring condition of soft stools among eleven of the twenty-four birds, including three of the control birds, is mentioned although it appears not to have influenced the course of the experimental disease.

(b)   On the course of the disease as measured by faecal data and weight

In summary it is felt that the experimental disease can be studied reasonably well in a small species (which cannot be handled without altering the disease course) by taking daily twenty-four-hour faecal samples. The results of faecal colony counts for viable P. pseudotuberculosis organisms suggest the following conclusions:

— The incubation period is probably about ten days, in an experimental infection of this severity (20 per cent

mortality, with 50 per cent of the survivors reaching recovery point by 2½ weeks).

The height of effective immune activity is possibly reached in about 2½ weeks from the first day of inoculation of live culture by the oral route.

The experimentally infected canary disseminates large numbers of viable P. pseudotuberculosis bacteria for extended periods even if it later recovers from the infection.

There was no evidence in this experiment that the canary becomes a long-term infected carrier. However, the course of the disease to death in birds which are heavy faecal disseminators can extend to over three weeks. This may be of great epizootiological import in the canary species, and could have zoonotic importance, as recently suggested by the findings of Paterson and Cook (1962) which are referred to in Appendix Section on pseudotuberculosis in man.

Tubercles or abscesses containing proliferating P. pseudotuberculosis were found in the mucous membrane of the bowel (specifically the caecum, a vestigial organ). This condition was seen in three birds with the naturally occurring disease from aviaries No. 1 and No. 3. The caecal abscess was postulated at that time to be indicative of ability to excrete extremely high counts of the causative organism, in

the faeces. This lesion was produced in the experimental disease in one bird, and logarithmic faecal counts of over 8.0 were recorded in as little as 0.66 gm (DM equivalent) of faeces excreted over a twenty-four hour period.

The experimentally infected canary continues to eat during the course of the disease, which in turn supports its ability to disseminate significant numbers of viable organisms from the bowel.

The experimental infection of canaries by the oral route using a homologous Type I strain (isolated in British Columbia) apparently requires a heavy dosage to achieve a uniform effect. The dose levels here used, with extension over a three-day period, but with no introduction of any gastro-intestinal irritant, caused 80 per cent of twenty birds to become infected. It is suggested that in the natural disease with its frequently high mortality (as occurred in British Columbia in 1958) the pathogenesis is based in part on predisposing stress factors.

(c) On the pathology of experimental pseudotuberculosis in canaries

In this experiment, pathological lesions were noted in four birds that died and in five others which were sacrificed. There appears to be no significant difference between

the experimental disease and the natural disease, in that the most concentrated focus of change is the spleen. Of probable great importance was the finding of abscess tubercles in the caecum of one of the birds (which was the heaviest faecal disseminator of the causative organism). This type of pathology has also been noted in three canaries from different avaries suffering natural epizootics (See Tables VII and XVII).

DISCUSSION AND SUMMARY OF STUDIES ON EPIZOOTIOLOGY AND EXPERIMENTAL INFECTIONS OF PASTEURELLA PSEUDO-TUBERCULOSIS

1. Pseudotuberculosis is now the universally recognized term for infections with Pasteurella pseudotuberculosis. This term should be reserved strictly for such infections (Meyer 1959).

2. The spectrum of hosts for this pathogen is perhaps the widest encountered for any bacterium (Burrows 1963) and the known hosts include man, in particular children, in whom appears a non-fatal mesenteric adenitis which can clinically resemble acute appendicitis.

3. For the last eighty years, since its discovery, P. pseudotuberculosis has been associated primarily with the continent of Europe. Of relative interest also is the apparent absence of detectable pseudotuberculosis in the Far East, as well as in South Africa, Madagascar, and Central Africa (Devignat 1953).

4. Three canary epizootics of pseudotuberculosis occurred in the Vancouver and Vancouver Island areas in 1958. There was every appearance that the foci of the infections were endemic to this area. The presence of field mice in the aviary (not actually identified but in one case sick or

or dying) appears to have been closely associated in two cases, and could have been a factor in the third case (Aviary No. 2). Although data relating to a possible concurrent field mouse epizootic is not available for any of the immediate areas concerned, there is one recorded observation for the Agassiz area of the Fraser Valley, a point about 80 miles from Vancouver. A trained agricultural observer (Clarke 1961) supplied evidence to suggest that the population of short tailed field mice in that area reached a peak in the fall and winter of 1957-58. Swampy pasture ploughed that fall was found to have the turf riddled with mouse nests. Several sleek almost tame coyotes followed the plough picking up mice from the furrow. Farmers complained of mouse damage to new stands of grass and clover. In the fall of 1958 no mice were seen. The coyotes disappeared and have not been seen down in the valley since.

5. From this one might conjecture that a major epizootic of transmissible disease (pseudotuberculosis?) probably occurred in one or more species of rodents in the lower mainland area. This probably involved field mice in the vicinity of the epizootics prior to or at the time of the first losses in canaries. This was assumed to be the case at the time of the first epizootic but in spite of requests no field mice were procurable for examination and time did

not allow execution of the survey that was obviously des-
irable.

6. Examination of the literature made it appear that
pseudotuberculosis infections were rare in birds, and in
mammals in North America, other than in guinea pigs and to
a lesser extent chinchilla colonies. The recent associa-
tion of guinea pig infections with the feeding of field
greens soiled by pigeon faeces (Paterson and Cook 1962)
in England, and the recent grackle epizootic in Maryland
(Clark and Locke 1962) when combined with the British
Columbia data, could suggest that avian and rodent epizootics
may be occurring more frequently, or have been overlooked
in North America. In Table XVIII which accompanies this
section, I have assembled the names of some of the known
hosts for P. pseudotuberculosis. These hosts are arranged
so as to fall into one or more of four different categories
thus:-

> I.   Suspected reservoir (carrier) hosts
>
> II.   Epizootic species in rural (feral) and
>   urban areas
>
> III. Epizootic domesticated or farm species
>
> IV.   Sporadic infection species (probably often
>   unnoticed)

The principal hosts are underlined and the groups are inter-
connected with directional arrows to illustrate what I feel

I SUSPECTED RESERVOIR (CARRIER) HOSTS

WILD RODENTS   WILD BIRDS   OTHERS

FIELD MICE   PIGEONS (England)   HARES (Europe)
Rats?   Others   Others?
Others?   Owls (Sweden)
                          Crows

II EPIZOOTICS IN RURAL AND URBAN AREAS

                          Grackles (New England 1962)
FIELD MICE   PIGEONS & DOVES   HARES (Europe)
Beaver   GALLIFORMES   Rabbits?
Muskrat?   Pheasant   Others?
Others   Partridge
                          PASSERINES
                          Others?

III EPIZOOTICS IN DOMESTICATED (OR FARM) SPECIES

GUINEA PIGS (worldwide)   CANARIES   MINK (Denmark)
CHINCHILLAS (N.America)   OTHER CAPTIVE HARDBILLS
Microtus agrestis   TURKEYS
    (Oxford, Eng)   CHICKENS
    Chitty 1962   OTHERS

IV SPORADIC INFECTIONS (OFTEN UNNOTICED)

CATTLE
SHEEP
CATS
Dogs   ?
PRIMATES   MAN (Germany, France
                              England, U.S.A.
                              Canada, others)

TABLE XVIII   CONJECTURAL PATHWAYS OF TRANSMISSION OF
              PASTEURELLA PSEUDOTUBERCULOSIS ASSOCIATED WITH
              EPIZOOTIC AND SPORADIC INFECTION

to be the principal pathways of transmission of pseudo-tuberculosis infection. One solid diagonal line illustrates the probable spread of infection from field mice to canaries in outside or accessible aviaries which could have taken place in British Columbia. An interrupted line shows the conjectural relationship between canaries and man (because of close spatial contact) which in part led to the decision to carry out experiments with artificially induced infection in canaries.

7. The canary appears to be highly susceptible to pseudo-tuberculosis judging by the early European literature and the experiences in Vancouver. Some predisposing stress factors appear to be necessary for an epizootic occurrence; but mortalities are extremely high although the disease pursues an insidious course of up to several months within the aviary.

8. A preliminary study of the three epizootics led into experimental work with canaries transferred from epizootic premises to the laboratory for closer study. From the point of view of lateral spread of infection among canaries, and from the considerations of human contact a phase was entered where the studies were based on experimental infections.

9. Indications were that the spread of infection to susceptible hosts (when suitably stressed) must take place through ingestion of faecal material. (Meyer 1958)(Manninger and Mocsy 1956). The gross pathological lesions did not appear to fit a pattern of high faecal shedding until it was noticed that some birds (about 5 per cent of those examined) showed the presence of caecal abscesses, lesions with an interesting parallel to alimentary and associated adenitic lesions in the human. In a carefully monitored experiment, the rate and pattern of faecal shedding of viable P. pseudotuberculosis was recorded over a three week period, following a three day oral inoculation course in twenty birds. There were four uninoculated control birds in the tested group.

10. Gross pathological and histopathological changes were assessed and evaluated in terms of the characteristics of faecal shedding and subsequent death or recovery (birds which ceased to shed and harboured no viable organisms in their tissues at the 25th experimental day).

11. The striking similarity of gross lesions between spontaneous and experimental pseudotuberculosis gave the impression that the oral route is indeed the natural one in canaries. This was further supported by the scale of faecal shedding of P. pseudotuberculosis from the four fatally affected birds (20 per cent) and the twelve transiently affected birds (60 per cent) which totalled sixteen (80 per cent) of those

inoculated.

The incubation period appeared to be about ten days with the mean recovery point for non-fatal cases being about one week later. Faecal shedding started early and in most cases stayed at high levels until death or recovery occurred. (Figures 21 - 32). Four of the recovered birds showed a tendency to regressions and exacerbations of faecal shedding however which might have been relatable to the clinical course of the disease. (Figures 33 - 36).

12. The development of characteristic caecal lesions in one of the experimentally infected birds (No. 16) was accompanied by the highest rate of faecal shedding for an individual bird (Figures 22 and 41). It is felt that probably other grossly undetected lesions occurred in the caecal tonsils or bowel mucosa of additional birds which shed the test organism. Generally speaking the fatal cases shed most organisms followed by the steady shedding, then the di- or triphasic shedders. This trend shows clearly in Figure 41.

13. In a spontaneous epizootic an incubation period of ten days or more accompanied by high levels of faecal excretion of P. pseudotuberculosis, could result in the more resistant birds being exposed by the oral route for long periods until their resistance broke down. In such a situation the high mortality rates would be expected since in all probability birds do not

begin to develop specific immune mechanisms until the first focus of infection has been established in the alimentary tract.[1] This long term exposure was lacking under the conditions of the experimental infection (Experiment C) which could account for the mortality being only 20 per cent.

14. Dispersal of birds from an infected aviary could have great potential danger to other aviary stocks and humans, in light of the prodigious ability of the canary to shed viable P. pseudotuberculosis organisms in the faecal material. This applies especially in the presence of caecal abscesses, which were shown to occur in both spontaneous and experimental canary infections.

15. Devignat through his studies of the development and spread of the varieties of the plague bacillus, Pasteurella (Yersinia) pestis, particularly in the European area; and Burrows and Bacon (1960) through comprehensive comparative studies which included that of V and W antigens in the plague bacillus and in P. pseudotuberculosis, have expounded and supported the theory that the latter may be a transmutant form of P. pestis to which it retains an ability to revert. If this is so, then two major mysteries may be explained, namely,

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1 In Experiment C, it was noted that towards the end of the culture runs faecal isolates were in a rough form on primary isolation. This could have been due to the effect of specific immune mechanisms. These rough forms were not actually tested for their expected loss of virulence.

the 'sudden disappearance of mediaeval plague from Europe' (Devignat); and the puzzling alternation of plague epidemic and quiescence throughout history (Burrows and Bacon). The former occurrence could have arisen from an extension of the _Pasteurella_ (Yersinia) _pseudotuberculosis_ infection in rodents in Europe (aided by avian and domestic animal epizootics), giving cross-immunity and rendering the terrain unfavourable for the maintenance of plague.

These theories appear to be supported by the claims of Russian authors to have achieved _in vitro_ the mutation of _P. pestis_ to the organism of pseudotuberculosis. One worker in the Belgian Congo when attempting to infect guinea pigs by the bites from rat fleas containing _P. pseudotuberculosis_, achieved only the isolation of a strain of _P. pestis_ from one of the guinea pigs. This organism had not been previously encountered in the guinea pig stocks. (Blanc 1944).

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APPENDIX

Material   Referred from Page

Dissolve 0.2 gm. fuchsin and 0.75 gm. methylene
blue in 20.0 ml. of absolute alcohol. Add this
dye solution to 200.0 ml. of 5% aqueous phenol
solution. Filter.

Stain smears for a few seconds; wash; blot dry.

Fixation is usually carried out with methyl hydrate.

Bipolar staining is enhanced in Pasteurella group
organisms.

A thin smear made by crushing a small amount of
pus between two slides and drawing them apart, or
an impression smear, is fixed by gentle heat. The
slide is flooded with a freshly prepared 1 in 10
dilution in tap water of 1 per cent carbol fuchsin.
This is allowed to stain without heat for 30
minutes, after which the slide is well washed with
tap water. Decolorization is then effected by
flushing the slide with 0.5 per cent acetic acid
for 30 seconds, although a longer period is necessary
for smears of more than one-cell thickness. The acid
is removed by washing and the film is counterstained
for 10-15 seconds with Loeffler's methylene blue.

Examination of the film reveals that the cytoplasm of
the cells and the amorphous debris of the pus are
stained blue or purplish blue. The cell nuclei and
Past. pseudotuberculosis are red, the characteristic
bipolar staining of the organisms being well marked.
In tissue smears stained by this method from the
liver and spleen of mice inoculated with a strain of
Past. pestis isolated from a human source in N. Africa,
it was of interest that the organisms clearly ex-
hibited a similar degree of acid-fastness.

    Plain canary   70%
    Rape   10%
    Niger   10%
    Flax   10%

Construction – Two double decked plywood batteries, each with twelve cubicles of 8" x 12" x 12" dimensions. Hinged doors are in the back, the fronts are of welded 1" x ½" galvanized wire cloth with expanded apertures for access to feed. Water and grit are supplied in gravity feeders and seed and cuttle in plastic cups, all mounted on the outside of the wire. Droppings collect on galvanized steel slides, which are protected by removable grids of ½" x ½" wire cloth; these slide in under the wire in the front.

Environment – The batteries were mounted on a wide shelf attached to the end wall of a 9 x 16' room in a concrete block and pumice walled building. Room temperature is controlled by a White Rodgers thermostat attached to two 750 watt Berke glass radiant heaters ceiling mounted to hang 8 feet from the floor and giving a diurnal variation of 4 - 8°F. Ventilation is achieved by a small discharge fan mounted in the glass brick window above the batteries and a louvred cold vent low down on one side of the room.

Pseudotuberculosis in Wild Mammals (including fur bearers chiefly bred in captivity)

(a) Fur Bearing Animals

An incomplete compilation has been made of some reports which are available on pseudotuberculosis in this group of animals. The number of reports involving Chinchillas, along with observations on pathology and the tendency to recurrence on a farm, all suggest that this species is one of the most susceptible of animals. (TABLE XIX).

Gorham (1955) obtained from Thal (15th Int. Vet. Congress) Group IV hare isolate, a strain of Pasteurella pseudo-tuberculosis (No. 32) which the latter had used for active (live) immunization of guinea pigs. He was able to confirm the inability of this strain of the organism to cause pregressive infection in a group of twelve guinea pigs. The same strain of organism, administered in the same way as a live vaccine to Chinchillas, killed nineteen of twenty test animals in four weeks. The guinea pigs were not challenged with virulent strains, but Gorham observes that the Chinchilla and not the guinea pigs should be the test animal for any vaccine trials.

SPECIES AFFECTED	AUTHOR	YEAR REPORTED	LOCATION OBSERVED	DETAILS OF OUTBREAK	PATHOLOGY	EPIZOOTIOLOGY CONCLUSIONS
Silver Fox	Rislakkiv	1942	Finland			Infection tends to died out on a farm
Dog Marten	Claussens	1934	Germany?			
Mole	Golovin	1930	Germany?			
Unspecified Fur Bearers	Marthedal	1954	Sweden			
Mink	Knox-?	1954 1961	Denmark	75 mink dead of unstated no	Yellowish caseous nodules liver spleen kidneys & intestinal wall	Due to feeding mink infected viscera of wild hares
Mink	Crews & Gorham	1961	Washington western state	Only 2 animals involved Organism isolated	Detail not stated but typical nodules seen	Ranch overrun with mice & animals ran up on pens; eaten by mink at times
Chinchilla	Chapman	1948	USA	Organism isolated by direct culture and guinea pig inoculation		
Chinchilla		1950	Corvallis Oregon	1 isolation from chinchilla No details	Not stated exactly, but nodules seen	
Chinchilla	Leader & Baker	1954	Washington state USA	Died 2 days after anorexia and depression Organism isolated often from nodules in affected livers	All livers with small yellowish white foci Histologically necrotic foci with lymphocyte & macrophage zone around V. Little encapsulation & giant cells	Premises were contaminated by wild rodents & exchange of animals between premises

TABLE **XIX** PSEUDOTUBERCULOSIS IN CAPTIVE FUR BEARING ANIMALS
SOME REPORTS BY VARIOUS AUTHORS

SPECIES AFFECTED	AUTHOR	YEAR REPORTED	LOCATION OBSERVED	DETAILS OF OUTBREAK	PATHOLOGY	EPIZOOTIOLOGY CONCLUSIONS
Chinchilla	Benito & Borrel	1957	France		Nodules liver & spleen Haem enteritis with multi necrotic foci in colon. Enlarged associated lymph nodes.	
Chinchilla	Bankier & Langford	1959 to 1962	British Columbia	Mod to severe losses in 8 premises. Some repeats.	Similar to rpt of Leader & Baker	Usually exchange of infected animals between premises
Nutria	Pallaske	1933	Leipzig	2 natural infections with isolations		
Nutria	Rislakki	1942	Finland	At least one outbreak on a farm		
Nutria	Wozniak & Chwalibog	1958	Varsova			
Nutria	Piletc et al.	1958	France	1 outbreak 5 dead of 500 Emaciation Anorexia Depression	Nodules in lungs chiefly. Also spleen, kidneys, liver	One outbreak controlled by isolation of affected animals and treatment with parenteral Oxytetracycline
				2nd outbreak: Diarrhoea symptoms	Lobar pneumonia Acute Enteritis	

TABLE XIX   PSEUDOTUBERCULOSIS IN CAPTIVE FUR BEARING ANIMALS
page 2   SOME REPORTS BY VARIOUS AUTHORS

151

(b) Hares and Wild Rabbits

Judging by the number of reports, the finding of
Pasteurella pseudotuberculosis infection in hares and perhaps
wild rabbits, is common in Continental Europe and possibly
Scandinavia. Apparently Lerche in Leipzig (1927) was the first
to mention hares when comparing rodent infections to those of
turkeys. Pallaske (1932) studied histopathology in the hare
using Lerche's material. In 1938 Olt reported an 'epidemic' in
hares in Germany. From Italy came a report by Avanzi (1948)
of Pasteurella pseudotuberculosis being isolated from a hare
in Tuscany, and later, from Slovenia, Rigler (1954) and Stefanovic
(1957) reported similar findings. In France Boquet (1937),
Vautrin (1949), and Goyon (1956) contributed to the literature
in this regard. Goyon studied the bacteriology and serology
of ten strains so isolated. From Sweden Thal (1954) reported
on the immunologic characteristics of 186 strains of Pasteurella
pseudotuberculosis many of them of Swedish origin. It is note-
worthy that no less than ninety-three of these strains were
from hares and rabbits. This was more than four times the
number attributed to the beaver, the second on the list of
mammalian contributors; and almost five times the number
isolated from turkeys, the highest avian contributor.[1] Soltys

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1 At a later date, Thal (1961) stated that between 1948
and 1960 some 154 (6.2 per cent) of 2500 hares examined
in Sweden were found to be infected with Pasteurella pseudo-
tuberculosis.

(1947) found infection with <u>Pasteurella</u> <u>pseudotuberculosis</u> in
a high percentage of hares in Scotland while conducting a survey
for Tularaemia. Other reports have been published by Weiden-
muller (1959) in Germany and Knox-Seith (1961) in Denmark. The
report by the latter on infection of mink due to feeding hare
viscera has already been mentioned.

(c) Wild Mammals other than the Hare

In view of the broad range of host susceptibility,
the few reports in Table XX seem sparse indeed. It is well
accepted that <u>Pasteurella</u> <u>pseudotuberculosis</u> is capable of
causing sweeping epizootics among wild rodents and birds. A
glance into the literature on plague gives ample justification
for the assumption that many such epizootics of sylvatic plague
are completely unobserved and are likely often undiscovered.
Doubtless as time goes on and more work is done in population
dynamics, a more accurate picture may be pieced together con-
cerning epizootics of pseudotuberculosis.

Table XXX includes two previously unreported in-
volvements of beaver in Canada with the possibility in one case
that the epizootic of pseudotuberculosis (which is assumed) may
have also involved muskrats in the same area, (Queen Charlotte
Islands).

AUTHOR	YEAR	SPECIES	LOCATION	DETAILS
Keymer	1959	Wild rodents in general	England	The disease is common among wild rodents which may spread it to captive hardbilled birds by contamination of the seed
Weidenmuller	1959	Field mice and house mice	Germany	The organism was found in faeces of ten of thirty mice (field mice and house mice). Mice were regarded as the chief carriers of infection
Haas	1938	Rat	USA	A strain was studied which was recovered from a rat
Rislakki	1942	Beaver	Finland	Not apparently in epizootic proportions
Bankier and Humphries	1952	Beaver Muskrat?	Queen Charlotte Islands Canada	An isolated dead beaver found in an area where both beaver and muskrats had died off. Presence of numerous discrete caseo-necrotic nodules in liver, spleen, liver and kidney. Pasteurella pseudotuberculosis isolated and identified
Langford	1954	Beaver	Jasper Park Canada	Epizootic of suspected tularaemia. Two beaver received. Pasteurella pseudo-tuberculosis isolated in both instances. Rabbits were killed with ease by IP injection of strains isolated
Claussen	1934	Muskrat	Germany	No details obtained

TABLE XX   SEVERAL REPORTS OF PASTEURELLA PSEUDOTUBERCULOSIS
INFECTION OCCURRING IN WILD MAMMALS

Pseudotuberculosis in Domestic Animals

(a) Farm Animals

Table XXI lists a number of references to pseudo-tuberculosis infection in sheep, cows, horses, goats and the pig.

Since all these animals graze, with the possible exception of the pig, one would expect them to be unavoidably in contact with the excretions or degenerated carcasses of birds and rodents which have been involved in epizootic or sporadic infections.

One can expect the level of reported incidence of pseudotuberculosis to parallel the degree of use of laboratory diagnostic facilities, since even provisional diagnoses of this condition are seldom if ever made in the field. In light of this, one must assume that the figures are probably not representative of the degree of involvement of farm animals.

It is interesting that Knapp (1959) raises the possibility of human infection from one source of contact with cows. When this author (Stovell 1953) obtained an isolation from a bovine mesenteric gland, no other references to cattle could be found on a preliminary literature search. It was felt, and still is, that a survey of diseased mesenteric glands from this species should be carried out before this finding deserves a report _per_ _se_.

AUTHOR	YEAR	LOCATION	SPECIES	DETAILS
Murray	1932	Australia	Sheep	Isolation of B. pseudotuberculosis (rodentium) from sheep
Truche	1938	Australia	Sheep	Identified a strain of P. pseudotuberculosis isolated by Gilruth (quoted by Jamieson Soltys, 1947)
Jamison & Soltys	1947	Scotland	Sheep (rams)	Epididymo-orchitis of rams associated with P. pseudo type 1B. 3 lambs with infection limited to testicle and epididymus. Tick activity was marked (July & August) infected hares in area
Placidi et al	1951	Morocco	Sheep	Details not obtained
Watson	1962	England	Sheep	P. pseudotuberculosis has been isolated from ovine foeti on six occasions since 1960
Boquet	1937	France	Cow	Mentions infection in this species (After Nocard 1889)
Mazzini	1897	Italy?	Cow	After Poppe (1928) cited by Knapp (1959)
Stovell	1953	BC, Canada	Cow	Organism isolated from mesenteric lymph node with caseo necrotic lesions suspected of being tuberculosis strain provisionally identified but culture lost
Knapp (Graber & Knapp)	1959	Germany	Cow Mastitis?	Human (milker) with appendicitis form. One cow with recently resolved purulent mastitis had ascending serum titre to 1:320. Other cows in area less than 1:50.
Pfeiffer	1890	Leipzig	Horse	Isolated from a horse suspected of having Glanders. Autopsy showed nasal and lung nodules with spleen, liver, and lymph nodes also involved
Stephan	1942		Horse	Isolated three strains from horses
Schlaffke	1921	German?	Horse	Isolated from a glanders-like case in a horse
Bauman	1927	Germany	Goat	Pseudotuberculosis in a young goat
Rajagopalan	1944	India	Goat	A case of Pseudotuberculosis in a goat
Wramby & Frederison	1941	Scandinavia	Pig	Described P. pseudotuberculosis infection in a pig

TABLE XXI)   PARTIAL DATA RELATING TO REPORTS OF PASTEURELLA
PSEUDOTUBERCULOSIS IN FARM ANIMALS

156

(b)   Cats and Dogs

Table XXII lists some of the literature references
to infections in these domestic animals.

It seems that several authorities rate the cat
highly as a potential source of human infections. The relatively
high reported incidence in this species is not surprising in
view of its dietary habits in connection with rodents, and the
relative frequency of laboratory diagnosis being carried out
in the case of deaths in pet cats.

AUTHOR	YEAR	LOCATION	SPECIES	DETAILS
Collet	1955	France	Dog	Liver infection of dog with P. pseudotuberculosis
Leblois	1920	France	Cat	Zoogleique pseudotuberculosis in the cat
Cocu	1931	France	Cat	A case of Pseudotuberculosis in the cat
Pallaske	1932	Leipzig	Cat	Used twelve spontaneous isolates from cats in Germany
Verge	1937	France	Cat	A case of Pseudotuberculosis in the cat
Tottire-Hippoliti	1942	Italy	Cat	Worked with two old strains from a cat
Communal	1945	France	Cat	Gives general description of infection in cats including personal observations on spontaneous cases
Goret et al.	1957	France	Cat	Describe laboratory diagnosis and pathogenesis in cats
Winkle	1955	Germany	Cat (in human disease)	States "cases in humans are very often due to infected cats'
Meyer (in Dubos)	1959?	General	Cats	Comments that human infection has been attributed to cats

TABLE XXII SOME REPORTS OF SPONTANEOUS PASTEURELLA PSEUDOTUBERCULOSIS INFECTION IN CATS AND A DOG

Pseudotuberculosis in Man

Until the publications of Masshoff and Dölle (1953)
human infections were proven in but sixteen cases. Of these,
fifteen cases ran a severe septicaemic typhoidal course with
eleven ending fatally.

Following the observations of the above authors, a
number of publications have appeared (mostly in Europe) con-
firming the occurrence of mesenteric lymphadenitis in humans
with a clinical onset resembling that of acute or subacute
appendicitis. The condition usually affects children and young
adults, particularly males between the ages of three and twenty-
three. A benign course with uneventful recovery is usual.

The mesenteric adenitis is usually described as
abscess-forming and reticulocytic in nature, (German literature
reports). In North America the infection has been diagnosed
in the San Francisco area (Goldman 1957) and more recently in
Alberta (Hnatko and Roden 1962).

Isolation of Pasteurella pseudotuberculosis from
the affected lymph nodes is often difficult; however, since
1954 Knapp (1959) has either through isolations of the bacterium
(thirteen lymph nodes; two blood) or through serologic tests
(ninety-four cases) and histologic examinations, fully proved
the nature of the infection. Serological titers of 1:80 to

1:12,800 have been recorded.[1]

The epidemiology is considered by Meyer (1959) to be obscure, although the vast animal reservoir with its carriers and shedders is naturally suspect. Undoubtedly the route of entry is per oral as judged by the history of cases and confirmed by the location of the focus of infection. The wide avenue of possible contamination of human food is well illustrated by the findings of Paterson and Cook (1962). These authors incriminated field greens which were contaminated by pigeon droppings as the cause of recurrent pseudotuberculosis in guinea pig stock colonies.

As mentioned earlier, in 1961 Daniels described the isolation of <u>Pasteurella</u> <u>pseudotuberculosis</u> from the faeces of a patient suffering from pseudotuberculosis mesenteric adenitis. In addition the organism was also isolated from the faeces of a canary belonging to the patient.

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[1] At the moment, it is necessary, due to serological aberrations, to test patients' serum by use of the Widal test with live smooth antigen.

CARBOHYDRATE	AUTHOR[***]								
	14	15	16	17	18	19	20	21	22
A USUALLY REPORTED POSITIVE									
Arabinose	+	-	+	+	+		+d	-	
Galactose	-	+	-	+d			+	+	
Glucose	+	+	+	+	+		+	+	+
Glycerol	+d	+	-		+			+	
Levulose	+	+	+	+	+		+		
Maltose	+	+	+	+d	+	-	+	+rt	
Mannitol	+	+	+	+	+	-	+	+	
Mannose			+	+				+	
Melibiose									
Rhamnose	+	+	+		+		+	+	
Salicin		+	+	±v	+		+	+	
Trehelose			+		+		+	+	
Xylose		+	-	+	+		+d		
B CONTROVERSIAL OR VARIABLE REACTIONS									
Adonitol									
Dextrin	-	-	-	-		-	±s	-	
Inulin		-	-		-		-	-	
Sorbitol			-		-			-	
Sucrose	-	±	-	-	+*	+r	-	+	
C USUALLY REPORTED NEGATIVE									
Aesculin			-					+	
Amygdalin							-		
Dulcitol	-	-	-	-	-		-	-	
Erythritol							-		
Inositol			-		-		-	-	
Lactose	-	-	-	-	-		-	-	
Melezitose							-		
Raffinose			-	-			-	-	
Starch							-	-	

*	on first isolation only		d	delayed reaction
***	refer to Key on page		rt	room temperature
			s	slight
			v	variable
			r	reverts (alkaline)

TABLE XXIII   CARBOHYDRATE FERMENTATIONS: REPORTED FERMENTATION
CONT'D   WITH ACID NO GAS

CARBOHYDRATE	AUTHOR[***]												
	1	2	3	4	5	6	7	8	9	10	11	12	13
A USUALLY REPORTED POSITIVE													
Arabinose	+	+	+	+	+	±	+	+	+10	+	+	+	+
Galactose	+			+		+		+	11	+		+	+
Glucose	+	+	+	+	+	+	+			+	+	+	+
Glycerol	+	+	+	+	+	+							
Levulose	+			+				+		+		+	+
Maltose	+	+	+	+	+	+	+		+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+		+	+	+	+
Mannose						+				+			
Melibiose	+			+		+	+						
Rhamnose	+	+	+	+	+	±	+	+	+10		+		
Salicin	+	+	+	**	+	+	+a		d11	+			
Trehalose	+			+		+	+						
Xylose	+	+	+	+	+	+	+	+		+			
B CONTROVERSIAL OR VARIABLE REACTIONS													
Adonitol	+					-				-			
Dextrin	±	-		**		+			±	+		+	
Inulin	-			-		+	-a		±	-			
Sorbitol	±			**		-	±		±		-	-	-
Sucrose	-	±	±	-	-	-	±		±	-	-	-	-
C USUALLY REPORTED NEGATIVE													
Aesculin													
Amygdalin	±			-		-							
Dulcitol	-			-	-	-a			-9	-	-	-	-
Erythritol	-			-		-			11	-			
Inositol	-			-		-							
Lactose	-			-	-				-	-	-	-	-
Melezitose						+							
Raffinose	±			-	-	-			-10				
Starch									11				

** varies between 22 and 37°C incubation
*** refer to Key on page

TABLE XXIII   CARBOHYDRATE FERMENTATIONS: REPORTED FERMENTATION
                   WITH ACID NO GAS

BIOCHEMICAL REACTION	AUTHOR**										
	1	2	3	4	5	6	7	8	9	10	11
Litmus Milk Reaction		sa	sa	a	nc			sa	a		nc
Indol Production	-	-	-	-	-*	-	-	-	-		-
MR Test	+	+	+	+	+			+	+		
VP Test	-	-	-		-			-	+		
Production of H_2S	±	+	+	+s	+	+	-		+	-	
Catalase Production		+	+	+	+			+	+		
Urea Decomposition	+	+		+a		+st	+	+s	+		
Nitrites Production	+	+		+	+	+		+	+		
Production of NH_3 from Peptone	+	+	+		+	+			+		
Methylene Blue Reduction Test	+	+	+	+				+s	+		
Gelatin Liquefaction								-	-		
Citrate Tests											
In MacConkey Media		+	+	+	+			+	+		
Bile Tolerance 1% 10% 40%											
37°C 7 day (final) pH in glucose peptone water											

*Claimed to be a differentiating feature from P. pestis (in error?)
**See Key.

sa slight alkalinity
a   alkaline formed
nc no coagulation
s   slow
st strong
w   weak

TABLE XXIV   BIOCHEMICAL REACTIONS AS REPORTED BY VARIOUS AUTHORS

KEY TO AUTHORS FOR TABLES XXIII AND XXIV

1   Knapp (1959)
2   Wilson and Miles (1955)
3   Bergey (1957)
4   Meyer (1959)
5   Zinsser (1957)
6   Devignat (1954)
7   Winkle (1955)
8   Zwick (1908)
9   Stovell (1963)
10 Hadley (1918)
11 Beck and Huck (1925)
12 Lerche (1927)
13 Truche and Bauche (1929)
14 Lesbouyries(1934)
15 Urbain and Nouvel (1957)
16 Kilian et al. (1962)
17 Rosenwald and Dickinson (1944)
18 Mathey and Siddle (1954)
19 Moss and Battle (1941)
20 Beaudette (1940)
21 Jamieson and Soltys (1947)
22 Wasielewski and Hoffman (1903)

BIOCHEMICAL REACTION	AUTHOR**									
	12	13	14	15	16	17	18	19	20	21
Litmus Milk Reaction					a		sa^3			sa^7
Indol Production					−	−	−			−
MR Test						+	only on + orig. is.			+
VP Test						−	−			−
H_2S Production	−					+w	$+s^3$			−
Catalase Production							+w			+
Urea Decomposition						+				
Nitrates Reduction & Nitrates Production						+	+			+
NH_3 Production from Peptone							+			+
Methylene Blue Reduction Test							+1			+
Gelatin Liquefaction						−	−			
Citrate Tests						−				
Bile Tolerance 1%										+
10%										±
40%										−

In MacConkey Media

$37^{\circ}C$ 7 day (final)
pH in glucose peptone
water

*Claimed to be a differentiating   sa slight alkalinity
 feature from P. pestis   a   alkaline formed
**See Key.   nc no coagulation
     s   slow
     st strong
     w   weak

TABLE XXIV CONT'D   BIOCHEMICAL REACTIONS AS REPORTED
                   BY VARIOUS AUTHORS