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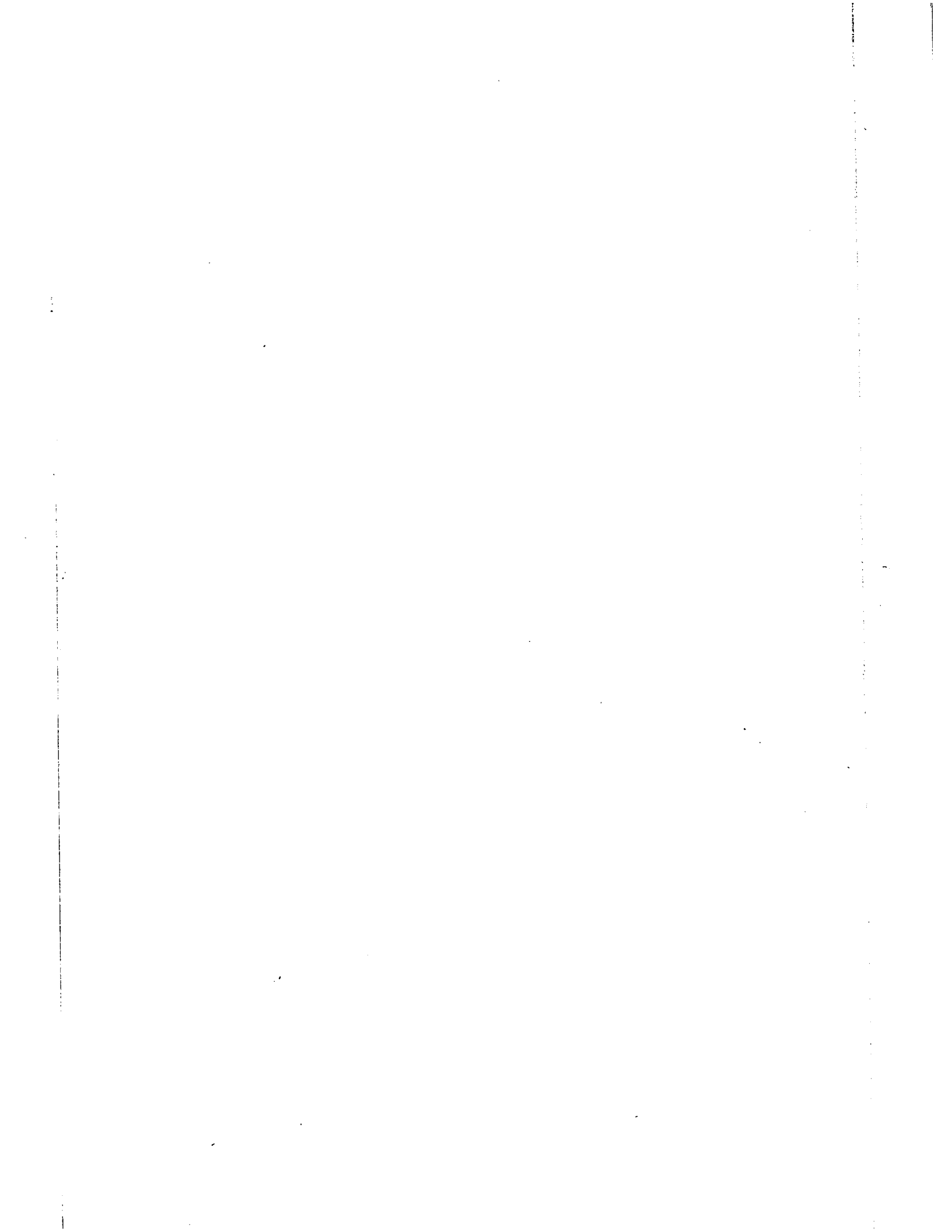
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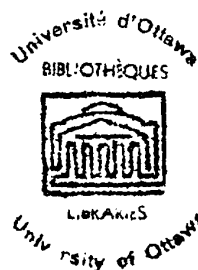


STUDIES RELATING TO MIDDLE AND INNER EAR DISEASE IN
THE ALBINO RAT

A THESIS

Submitted to the faculty of Comparative Pathology and
Immunology, University of Ottawa, in partial fulfilment
of the requirements for the degree of Master of Science.

by



Harold Cecil Grice, D. V. M. V. S.

1957

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I GENERAL INTRODUCTION

Disease of the middle and inner ear of the albino rat has been recognized as a problem for many years in laboratories where these animals are employed. The condition is a chronic infection to which the albino rat seems to have an inherent susceptibility. It is probably bacterial in origin with several varieties of micro-organisms playing a primary or secondary role.

The condition was present in a colony composed of approximately 5000 albino rats, the property of the laboratory of the Food and Drug Division of the Department of National Health and Welfare. This colony was used as an experimental unit in this study. Several aspects of the disease were considered. Detection of the condition in the asymptomatic animal was a problem as was the determination of a suitable method of eradicating the disease. The gross and microscopic pathology and the reasons for the peculiar symptoms required investigation; also the effect of antibiotics on invading agents. No attempt was made to determine why albino rats are unusually susceptible nor was any attempt made to rule out a virus as a possible etiologic factor. This study has been confined to the various factors mentioned above in relationship to this particular strain of rat.

II HISTORICAL REVIEW

In 1914 Casmajor (1) discussed an infectious ear disease of rodents in which the affected animals exhibited symptoms of disturbed vestibular function. He suggested that the symptoms occurred as the result of infection of the inner ear and the vestibular and cochlear nerves. He mentioned destruction of the ganglia of these nerves with degeneration of the nerve roots to their central terminations. The disease was described under natural or experimental conditions by a number of subsequent investigators (2-13).

The etiology of the condition has been extensively investigated. Daniels Armstrong and Hutton (3) called attention to the prevalence of middle ear disease in young rats fed a diet lacking in vitamin A.

In 1925 Sherman and Storms (6) considered vitamin A deficiency as an etiologic factor in middle ear disease when they found the middle ear infected in 75 per cent of a group of 134 rats dying of vitamin A deficiency. Bradford (7) studied a group of 31 rats dying from avitaminosis A in which the incidence of otitis was 100 per cent. The possibility of the infection being bacterial in nature was suggested by Bradford (7) when he isolated an encapsulated bacillus of the mucosus group from the middle ears of his rats.

Turner (8) studied the bacterial aspects of the disease more extensively. He produced a fatal septicemia in rabbits by injecting organisms isolated from the suppuration of the

upper respiratory tract and middle ear of albino rats suffering from a vitamin A deficiency. The organisms from these rats were principally Gram-negative cocci though Gram-positive organisms also were isolated. Gram-negative bacilli appeared in his experiments to have a higher percentage incidence in the control group.

Nelson (10) studied the bacterial flora of the infected middle ear in adult rats, isolating 9 different species of bacteria from adult rats and 6 different species from young rats. He identified a variety of organisms from cases of middle ear disease in rats over one year old and in rats three to four months old. In older rats, Bacillus actinoides occurred in 43.7 per cent, an unidentified streptococcus in 32.5 per cent, and diptheroids in 23.7 per cent. In the young rats, diptheroids were present in 26.4 per cent, streptococcus in 20.5 per cent, and Bacillus actinoides in 14.7 per cent of the cases. His findings did not implicate a specific causative organism. although he mentioned the fact that Bacillus actinoides is particularly well adapted for development in the middle ear of rats. Nelson (11) later reported on the infective capacity of three bacteria commonly encountered during a study of natural middle ear disease by direct intraural injection in young rats. One week after introduction of Bacillus actinoides, 75 per cent of the rats had a purulent exudate; with hemolytic and non-hemolytic streptococci, 75 per cent showed a serous or mucoid exudate; with diptheroids, 18 per cent gave a gross reaction.

Until 1932 it had been suggested that middle ear disease

was probably associated with a vitamin A deficiency. At this time, however, Freudenberg (12) published a paper on the incidence of middle ear disease in normal rats fed a balanced diet. He found the percentage incidence to be 60 per cent in a group of 99 one year old rats suggesting that infection was probably not related to a lack of vitamin A in his rats.

Other workers mentioned the incidence of the disease in their colonies. Donaldson (5) stated that only 1 per cent of the animals in his colony were affected.

In 1930 Nelson and Gowen (9) reported that the incidence of middle ear infection in adult and young albino rats and in wild Norway rats in their colony was as is shown in Table I.

TABLE I

Incidence of Middle Ear Infection in Albino and Norway Rats

Class	Number of Rats	Cases of Middle Ear Infection	% Incidence
Adult Albino	75	52	69.3
Young Albino	70	23	32.8
Wild Norway	58	1	1.7

Pathological changes were described by several workers. Fortuyn (2) described the pathology in three rats with symptoms of alteration in vestibular function. He utilized the degenerative changes produced by the otitis in the VIII nerve and its ganglia for the purpose of studying the tracts of the nerve. He noted in particular that the degeneration of the medullary

sheaths of the root-fibres of the VIII nerve in the brain bore no relationship to that in the medullary sheaths of the fibres of the VIII nerve in the auditory organs, the latter greatly overshadowing the former.

McCordock and Congdon (4) also described a type of inflammatory process observed microscopically in the tympanic cavity. They noted a dense leucocytic exudate in the lumen of the middle ear and on its mucosa, with marked destruction of the latter and replacement by granulation tissue. They mentioned also an osteitis of the bony ear with extensive bone absorption and excessive new bone formation. They noticed a diffuse inflammatory infiltration of both leucocytes and small cells in and about the vestibular and cochlear nerves.

The first method of control of middle ear disease was described by Nelson and Gowen. Their method was one of selective breeding with removal of infected animals. In 1939 King, in discussing labyrinthitis in the rat and a method for its control, suggested that in all probability middle ear disease was a non specific infection in which a number of different organisms were involved. King's (14) method of control was one of selective breeding and substitution of wild Norway rats as foster mothers. King, in the same paper, described the symptoms of animals with labyrinthitis associated with middle ear disease: "The clinical signs of labyrinthitis are definite and distinctive. The first indication of its presence is a slight tilting of the head to one side. Subsequently the rat holds its head continually close to the floor of the cage with the nose pointing away from the medial line. When sus-

pended by the tail the body invariably rotates rapidly".

The results of recently reported investigations from the Laboratory of the Food and Drug Division of the Department of National Health and Welfare (15) have supported the findings of these various groups of workers. Table II from this paper of Matheson, Grice and Connell (15) indicates that by 30 days of age over 90 per cent of the rats in their colony were found to be suffering from this ear condition.

TABLE II

Age of Onset of Infection of Middle Ear Disease in Albino Rats

Age in days	Number of Swabs	% Positive Bullae
1	30	0
7	30	0
14	30	3.3
18	30	16.6
25	30	36.6
30	30	93.3

Pasteurella multocida was the most prevalent organism followed by Diplococcus pneumoniae, Streptobacillus moniliformis, diptheroids, Microoccus aureus and Microoccus albus. The three most common organisms isolated alone and in combination were Pasteurella multocida, Diplococcus pneumonia and Streptobacillus moniliformis.

The present study, with which this thesis is concerned, represents a continuation and extension of this earlier investigation. It deals with other aspects of the problem, more particularly with the gross and microscopic pathology of the

disease and with the nature of the disturbed equilibrium in the affected rats.

During the course of the study a method of detecting the disease in the living animal was developed, which on application in the rat colony appeared successful in reducing the incidence of the disease very appreciably therein.

III MATERIALS AND METHODS

Standard materials and recognized methods were employed in this investigation as far as possible. Therefore, in discussing the materials and methods, a detailed description is given only in those instances where modifications or alterations of the standard and recognized procedures were made.

1. ANIMALS - The majority of animals used in these investigations were albino rats of the Wistar strain which have been bred in this colony for 16 years. In the studies on the diagnosis of middle ear disease, rats from two other sources were used as well as rats from this colony.
2. APPARATUS - The apparatus employed in these investigations is listed below.

General Electric Model D Type III X-ray machine

Welch Allyn otoscope

Cornwall syringe

Emesco No. 90 dental drill

Orthophot microphotographic camera (Silge and Kuhne)

Super Panchro - Press, Type B Film (Kodak)

DK 50 developer

Zeiss - Winkel microscope

Martin Sweets ionic bone decalcifier

Autotechnicon

Spencer Microtome

3. GENERAL LABORATORY METHODS

A. Bacteriological Methods

(i) Media

Standard bacteriological media (Difco) and equipment were available for use at this laboratory. Sheep's blood and horse serum was obtained from the Animal Disease Research Institute, Hull, P. Q.

(ii) Procedure

In all bacteriological studies the rats were killed with ether and the ramus of the mandibles were split with heavy scissors, the lower and upper jaws being separated past the level of the tympanic bulla. The bulla was then exposed by blunt dissection of the overlying muscles and was seared with a hot spatula. A sterile number "11" Bard Parker blade was inserted in the bulla and by applying leverage to the blade handle, the internal surface of the bulla was exposed. A small sterile swab dipped in sterile physiological saline was inserted into the internal portion of the bulla and then streaked on petri plates of veal infusion agar containing 10 per cent sterile horse serum. Slides were prepared from each swab, stained by Gram's method and examined microscopically.

After four days aerobic incubation of the petri plates at 37 degrees C. in a moist atmosphere the organisms were counted and grouped according to colony form and the results of microscopic investigation. Representative colonies were further identified by the use of

differential media.

During the early part of this investigation liver veal agar plates (incubated anaerobically) and MacConkey's agar plates (incubated aerobically) were also inoculated. Since no anaerobes or enteric organisms were found, these were discontinued in later studies. Furthermore, as several types of organisms appeared to be associated with infection, it became the practice to identify the organisms by macroscopic and stereoscopic examination of the colonies and by smears of the colonies stained by Gram's method, only reserving differential media for doubtful cases.

B. Histopathological technique

Tissues for histopathological study were prepared in the following manner:

- (a) Fixation - 10% formalin in normal saline
- (b) Decalcification
- (c) Imbedding - Autotechnicon

Schedule	50%	ethyl alcohol	$\frac{1}{2}$ hr.
	70	" "	$1\frac{1}{2}$ hr.
	80	" "	2 hrs.
	90	" "	2 hrs.
	95	" "	2 hrs.
	100	" "	$1\frac{1}{2}$ hrs.
	100	" "	4 hrs.
	xylol		2 hrs.
	xylol		2 hrs.

Paraffin 60 degrees C. 1 hr.

Paraffin 60 degrees C. 3½ hrs.

(d) Sectioning - 4 - 6 microns

(e) Staining - Heamatoxylin and Eosin (16)

Heidenhains (17)

Thionine (18)

(f) Microscopic examination

IV GENERAL INVESTIGATION

1 LESIONSGross and Microscopic Pathology of Middle and Inner
Ear DiseaseIntroduction

Gross pathological descriptions are derived from observations made at autopsy over a four-year period including animals on general projects as well as animals on middle and inner ear disease studies.

Microscopic examination was made on over 5000 sections of tissues. The microscopic descriptions are derived from the observations made on studying these tissues.

A Middle Ear(1) Gross Pathology

The anatomical relationships of the structures of the middle ear of the rat are such that inflammatory processes affecting one structure are coexistent and identical with inflammatory change in another structure. It was felt that under these circumstances it would be adequate as well as convenient to describe the inflammatory change in the bulla and to consider these changes as representative of inflammation in the middle ear in toto.

The normal dissected bulla viewed from below is seen to have a semitranslucent ventral portion and an opaque lateral portion with a definite line of demarcation between the two

portions at the internal attachment of the tympanic membrane. The remaining surfaces of the bulla form the points of attachment to the bones of the skull. If the dissected bulla is held to the light it may be seen to enclose an apparently hollow cavity. Another feature of notable interest is the fact that the dissected bulla floats in water. These features serve as diagnostic criteria inasmuch as they are peculiar to the non-inflamed bulla.

When infection first takes place the bulla loses its translucency, becomes somewhat dulled and the cavity contains milky purulent material. As the inflammatory process progresses the bony bulla becomes chalky in appearance, increasingly opaque and somewhat roughened. The bulla is eventually irregular and quite thickened, appearing to be a solid bony mass.

The contents of the inflamed bulla vary considerably depending generally on the type and stage of inflammation. One may observe varieties of inflammatory change from a light, liquid, purulent material to a dense parchment-like sac of fibrous tissue. Various types of inflammatory exudates between the two above described types are encountered and these again depend essentially on the type and stage of inflammation. Table III summarizes the types of inflammation that were seen in a group of 43 of our rats with middle ear disease (15).

TABLE III

Types and Incidence of Inflammation of Infected Bullae

Type of Inflammation	Number of Bullae	% Total Abnormal Bullae
Purulent.....	21	48.9
Mucopurulent.....	6	14.0
Serous.....	5	11.6
Serosanguineous.....	5	11.6
Sanguineous.....	3	6.9
Fibrinous.....	2	4.7
Fibrinopurulent.....	1	2.3
<hr/>		
TOTAL.....	43	100.0%

(ii) Microscopic Pathology

The gross pathology of the middle ear suggested that the histopathology might be expected to present much variety. Microscopic examination of sections of the diseased middle ear confirmed this expectation.

When infection first takes place in rats of about 30 days of age a number of polymorphonuclear leucocytes are to be noted in and on the mucosa of the middle ear more particularly on the tympanic membrane. Occasionally a serum-like material is present on the mucosa in which these leucocytes are imbedded. There usually appears to be a paucity of red cells in the inflammatory cellular assembly even though sanguineous exudates are encountered in the gross. There may be some oedematous separation of the mucosa from the underlying membranes of the bulla and in most instances the tympanic membrane is quite swollen and oedematous. Figures I to V illustrate these early changes.

As the infection progresses the bulla becomes filled with purulent material varying greatly in its inflammatory composition. This acute phase may either subside, as occasionally an inflammation of a subacute nature is observed, or it may pass on to a chronic stage in which there is much proliferation of fibrous tissue and in some instances the formation of a granulation type tissue within the bulla. When this stage is reached the bulla has already undergone considerable change, with bone destruction and new bone formation taking place, the latter process usually overshadowing the former. Fairly frequently one encounters a bulla with greatly thickened walls containing a dense fibrous sac which is adherent to the walls of the bulla and which may or may not contain purulent material. This latter picture represents the usual end result of a typical uncomplicated infection of the middle ear. It appears reasonable to suggest that structures contained in the middle ear such as the auditory ossicles would be subjected to the inflammatory processes that are described as occurring in the bulla (Figures VI and VII).

B Inner Ear

(i) Gross Pathology

A small percentage of animals with middle ear disease are also affected with disease of the inner ear. In our colony the percentage varies seasonally, usually ranging around 4 per cent of the rats affected with middle ear disease. Infection in the inner ear in all probability occurs by extension from the middle ear either through the round or oval

window or by erosion of the bones separating the two portions of the ear.

The bones containing the component structures of the inner ear present an irregular shape and are small being roughly 2 mm. in diameter. Because of its minute size it is difficult to appreciate the condition of the inner ear by gross examination, but generally the bones are denser than normal, usually with a few areas that appear as irregular, chalky, white spots. Aside from these two features, in order to detect inflammation, it is necessary to examine the ear microscopically.

(ii) Microscopic Pathology

In describing the gross pathology of the inner ear it was stated that inflammation varies, being approximately 4 per cent. This percentage indicates the number of living animals that exhibit clinical symptoms of a labyrinthitis. In reality the percentage incidence is higher, for it is not uncommon to observe signs of inflammation in the ampulla, utricle, saccule, semicircular canals or the cochlea of the labyrinth in a rat that shows no disturbed equilibration. Apparently in such animals the mechanisms producing symptoms of a labyrinthitis are not brought into play.

In animals described in the literature as "twisters" the deviation from the normal histology of the component inner ear structures is usually marked, so much so, that morphological identity of individual structures is often impossible. In the mild case, that is a rat exhibiting only a minor dis-

Figure I

Figure I Normal tympanic membrane
from a 16-day old rat,
200 x magnification Hema-
toxylin and eosin stain.

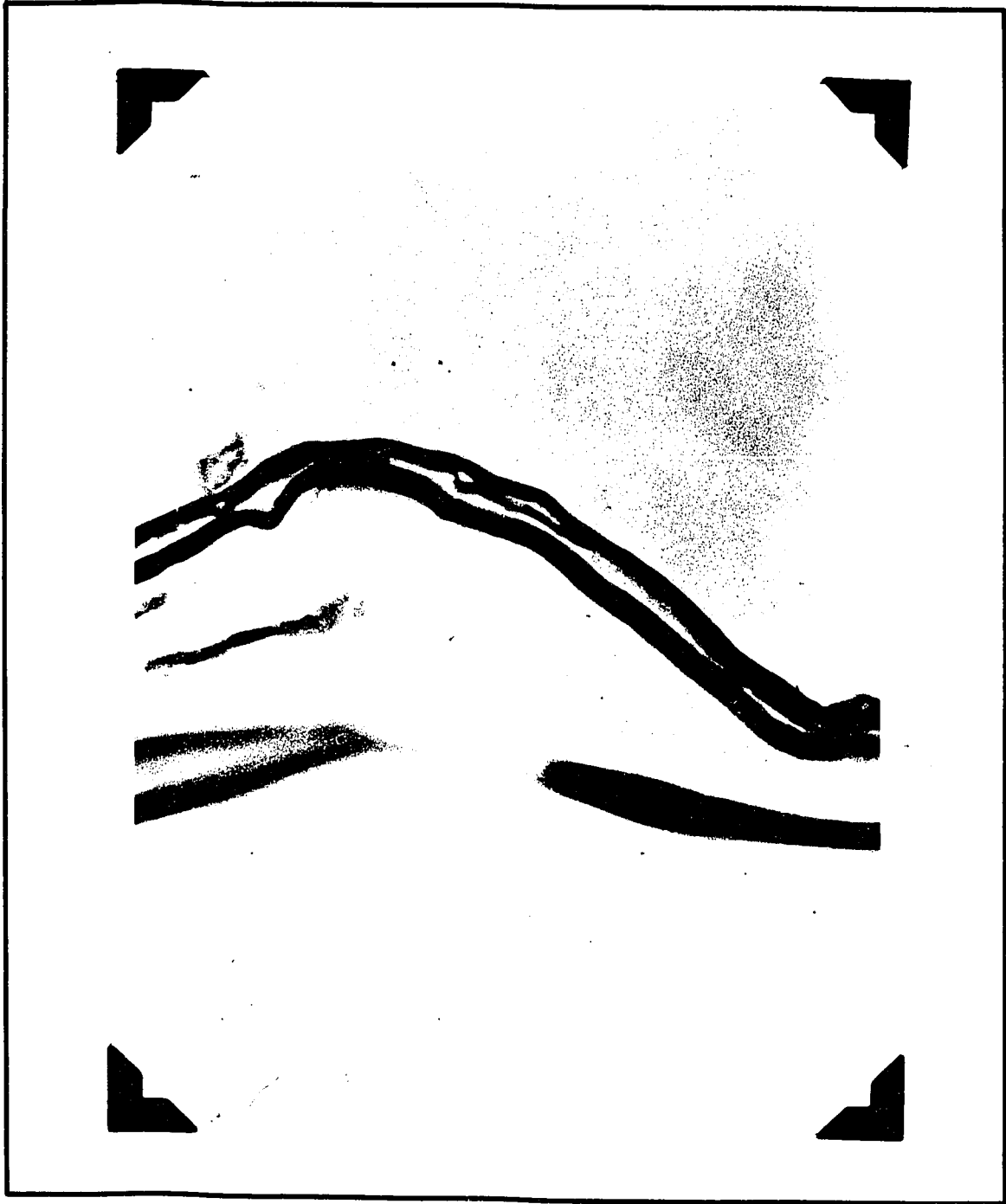


Figure II

Figure II Photomicrograph of a cross section of tympanic membrane from a 16 day old rat. Early inflammation with edematous separation of the membrane. A few leucocytes embeded in serum are seen on the inner surface of the membrane, 400 x magnification. Hematoxylin and eosin stain.



Figure III

Figure III Photomicrograph of a section of tympanic membrane from a rat 18 days of age. The Inflammatory process is slightly more advanced than in figure II. At this stage the gross tympanic membrane would be opaque, 400 x magnification. Hematoxylin and eosin stain.



Figure IV

Figure IV Tympanic membrane from a rat 30 days of age. Only a small portion of the membrane can be identified. This is seen at the lower right hand portion of the photomicrograph. The membrane is heavily invaded with white blood cells. A layer of serum containing white blood cells is present on the inner surface of the membrane, 400 x magnification. Hematoxylin and eosin stain.



Figure V

Figure V Photomicrograph of an infected tympanic bulla from a rat 30 days of age. The bulla contains considerable purulent material. The bony portion of the bulla is being invaded by white blood cells. The gross bulla at this stage would have lost its glossy semi-translucent appearance, 400 x magnification. Hematoxylin and eosin stain.

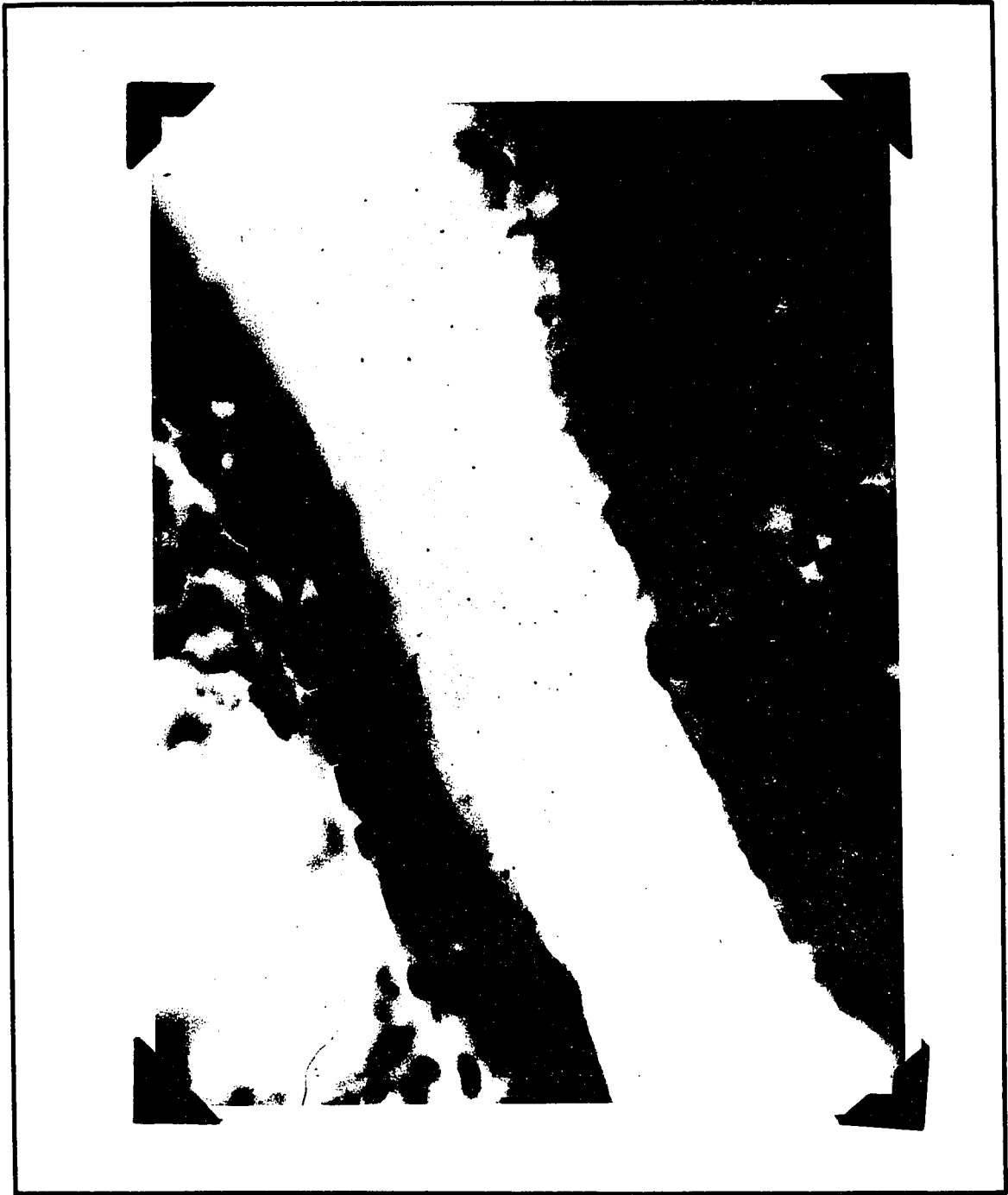


Figure VI

Figure VI Photomicrograph of a cross section of the tympanic bulla of a 60 day old rat infected with middle ear disease. Bone destruction, formation and replacement by fibrous tissue is taking place. There is little active inflammation in this section, 100 x magnification. Hematoxylin and eosin stain.



Figure VII

Figure VII Photomicrograph of a cross section of the tympanic bulla from a rat 200 days of age. This is typical of the usual uncomplicated end result of infection of the middle ear. The bone of the bulla is thickened the internal epithelium is obliterated. The internal portion of the bulla is occupied by a fibrous tissue sac. There is obliteration of the structures of the middle ear such as the malleus incus and stapes. A few neocapillaries are noted in the walls of the fibrous tissue sac at the left hand side of the photomicrograph. 100 x magnification. Hematoxylin and eosin stain.



Figure VIII

Figure VIII Photomicrograph of a cross section of the inner ear of a rat. The spiral ganglion is seen on the lower right. In the middle upper left hand portion a few white cells can be seen in the neuro-epithelial layer. 100 x magnification. Hematoxylin and eosin stain.



turbance of vestibular function, structural preservation of neuro-epithelial cells, the crista and macula, is commonly observed. Cross section may show area erosion of cells but more often there are only occasional cells missing with a few inflammatory cells adhering to the mucosa or epithelium of the vestibular structures, Figure VIII. Animals with advanced microscopic change usually exhibit symptoms of marked alteration from normal vestibular function. They revolve in a longitudinal axis, their heads are tilted laterally and if held by the tail they spin quite violently. These rats experience considerable difficulty in prehension and rapidly lose weight and die in a few weeks after symptoms of disturbed equilibrium are first noted.

C Nature of the Disturbed Equilibrium

Introduction

At the time studies were being carried out on the pathology of the inner ear, the question concerning the nature and cause of the disturbed equilibrium presented itself. It was felt advisable to inquire into the nature of this disturbed function because of the close association between altered function and observed pathology.

There was a possibility that the disturbed function was brought about by excessive stimulation of nerve endings which resulted in abnormal overactive vestibular function. The excessive stimulation could occur as the result of toxic or mechanical inflammatory irritation. A second and more likely possibility was that impulses from the affected side were greatly reduced because of the erosion of nerve endings.

Impulses from the opposite and normal side would then be proportionately in excess of those from the affected side, with resultant disequilibrium from comparative over stimulation. Either possibility could be proved or disproved experimentally by stopping impulses from one side of the animal and observing its behaviour.

Method

Anesthetized normal animals were utilized for experimental purposes. A number 90 Emesco dental drill with a No. 10 bit was used. The dental bit was introduced, at an angle predetermined by dissection, into the ear canal and through the tympanic membrane. The inner ear was then carefully drilled away, small portions being obliterated at a time, so that different degrees of disequilibrium might be observed.

When the animals revived, varying degrees of altered vestibular function, depending on the extent of obliteration of the inner ear structure, were noted. These ranged from animals that tilted their heads only slightly to animals that revolved horizontally so rapidly that when held by the tail, the skin of the tail would tear away from the underlying coccygeal vertebrae. The spinning tendency could be altered or abolished by drilling away portions of the opposite inner ear. In a like manner it was possible experimentally to increase the spinning effect in the rats with inner ear disease by drilling on the affected side and to decrease and reverse the effect in mild cases by drilling on the normal side.

Conclusions

The symptoms of altered vestibular function occurring as the result of disease process and developing over a period of weeks may thus be produced in a period of a few minutes by selective destruction of the inner ear of a normal rat. The complete train of symptoms elicited by a rat affected with inner ear disease, from the slight tilting of the head to the violent twisting in a horizontal plane, are observed in an experimental animal that has had a portion of its inner ear destroyed. The symptoms observed in both experimental and diseased rats may be altered or eliminated by selective destruction of the inner ear on the opposite side of the rat. The altered vestibular function in such rats results from an imbalance in the number of nerve impulses from the opposite side of the inner ear with a decrease in the number of impulses from the diseased or destroyed side. In the infected or experimental animals the impulses from the non-affected side are in excess of the impulses from the affected side and the degree of excess determines the extent of vestibular alteration.

D. Inner Ear Organs of Hearing

No comprehensive consideration was given to inflammatory change in the organs of hearing, primarily because this phase of the study was concerned essentially with the pathology of organs of the vestibular apparatus and the correlation of the lesions with the observed symptomatology. Furthermore in animals with middle ear disease the tympanic bulla along with

the malleus, incus and stapes, are subjected to such destructive inflammatory change it is very unlikely that sound waves would pass through the bulla to reach the inner ear and from this standpoint is quite probable that the majority of animals are deaf before the inner ear becomes infected.

The changes occurring in the cochlear portion of the labyrinth of the animals studied were of essentially the same nature as those described for the vestibular portion. Pus cells may be present in the scala vestibuli, and scala tympani, and the cochlear duct. Generally speaking, changes in the cochlea accompanied those in the other portion of the inner ear but only in the most advanced cases of disease of the inner ear was there obliteration of structures to the point where morphological identity was lost.

E VIII Nerve

Histopathology

No study was made of the changes occurring in the VIII nerve but these have been described in detail by Fortuyn (2).

F Central Nuclei and Tracts

Introduction

There is nothing in the symptomatology in these diseased rats that would lead one to believe that there would be alterations in the central portion of the vestibular apparatus. Nystagmus, a common symptom of inner ear disease in the rabbit, has never been observed in our rat colony. The lateral deviation of the heads of infected animals and the tendency to spin when held by the tail can be explained on the basis of

injury to the peripheral vestibular apparatus. Nevertheless for sake of completeness sections of the central divisions of the vestibular apparatus were examined.

(i) Vestibular Nuclei

Thionine stained serial sections which included the lateral, superior, medial and spinal vestibular nuclei did not reveal any degenerative or inflammatory change or any alteration from the normal histological picture in these nuclei (Figures IX to XII).

(ii) Lateral Vestibular Spinal Tract, Medial Longitudinal Bundle

Serial sections with Heidenhain's stain did not reveal any change or alteration from the normal histological picture in the fibres of the lateral vestibular spinal tract or the medial longitudinal bundle.

G Meninges

A rare and fatal complication of infection of the inner ear is an inflammation of the meninges. Rats with meningitis secondary to a labyrinthitis exhibit few obvious symptoms other than complete lethargy and anorexia. They die within a few days after infection of the meninges sets in or at least after the condition becomes apparent.

Grossly there is a purulent patch of liquefaction necrosis. Microscopically an acute purulent meningitis is observed with liquefaction necrosis of the brain and the production of purulent material.

Figure IX

Figure IX Photomicrograph of a section through the medial and lateral vestibular nuclei of a normal rat at about the level of the facial nerve, 100 x magnification. Thionine stain.



Figure X

Figure X Photomicrograph of a section through the medial and lateral vestibular nuclei of a rat with labyrinthitis taken at the level of the facial nerve. There is no cellular alteration from the normal histological picture. 100 x magnification. Thionine stain.

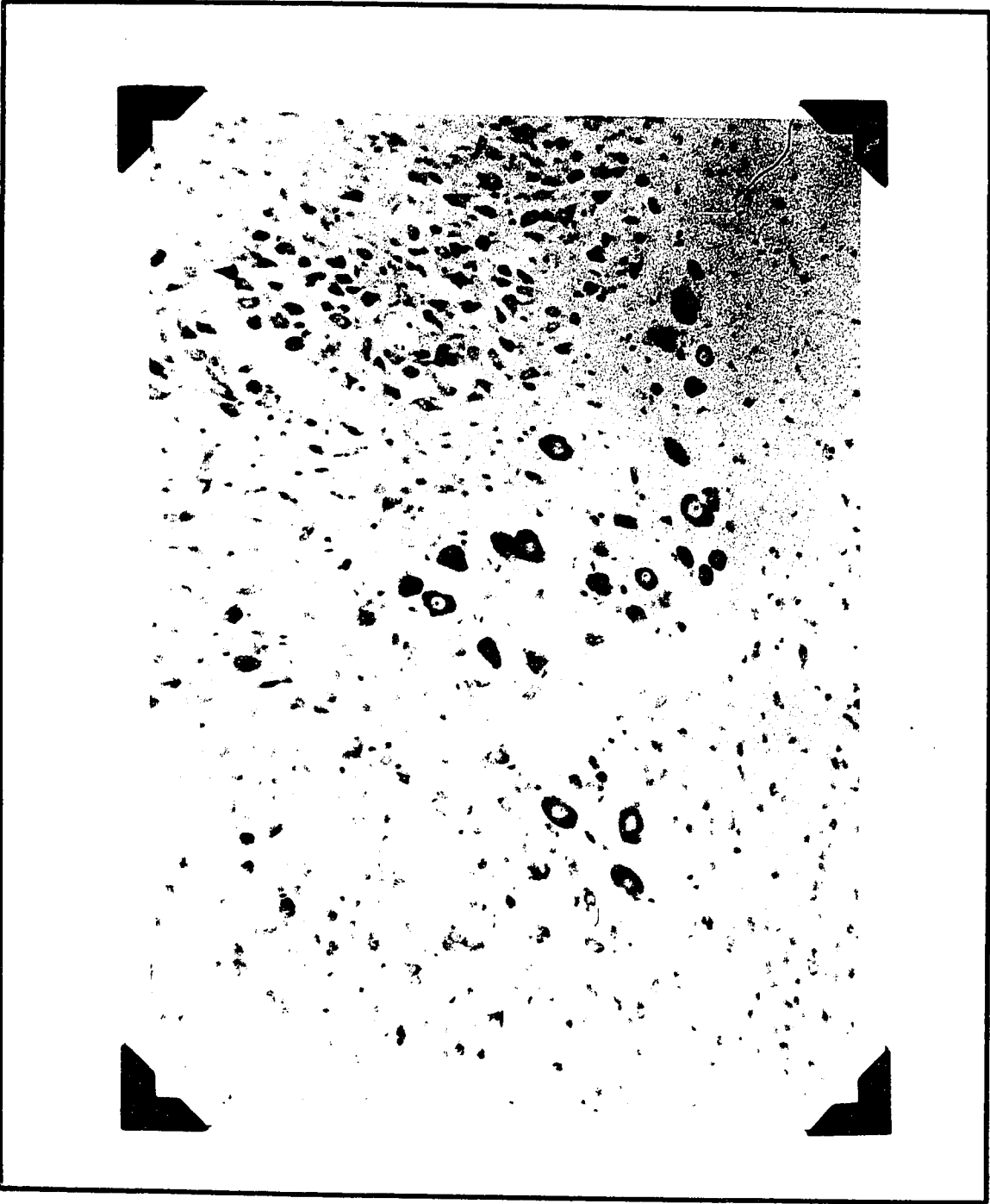


Figure XI

Figure XI Photomicrograph enlargement of vestibular nuclei of figure IX to illustrate cellular detail (normal). Thionine stain, enlargement approximately x 4.



Figure XII

Figure XII Photomicrograph enlargement of the vestibular nuclei shown in figure X to illustrate cellular detail. Thionine stain, enlargement approximately x 4.



Conclusions

The lesions of middle and inner ear disease follow the general pattern of inflammation in any tissue. Acute to chronic types were noted with the chronic type of response generally prevailing.

In the middle ear the inflammatory forces were of such a nature that the animals were undoubtedly rendered deaf as there was much destruction and obliteration of anatomical structures to the point that the bulla became a mere bony shell containing the products and results of inflammation.

In the inner ear erosion or destruction of the neuro-epithelial cells in the vestibular portion of the labyrinth leads to a progressive disequilibrium. Lesions were not observed in the vestibular nuclei or nerve tracts in the central nervous system. Lesions were found however in the central nervous system when there was an extension of infection along the VIII nerve to the brain with the production of a fatal purulent meningitis.

2 DIAGNOSIS

Detection of Middle and Inner Ear Disease in the

Asymptomatic Animal

Introduction

There are no clinical signs of middle ear disease, the condition being recognizable only when there is involvement of the inner ear with an associated disturbance of vestibular function. In the Food and Drug laboratory colony approximately four per cent of the animals exhibited symptoms of disturbed

equilibrium, the remainder being asymptomatic. It was decided to attempt to develop a method of detecting the disease in these asymptomatic animals.

Methods

A White Blood Cell Counts

Two series of white cell counts were made on a group of fifteen rats with middle ear disease at one week intervals to establish if a diagnostic difference existed between rats with middle ear disease and non-infected rats. Table IV gives the white cell counts of the rats.

TABLE IV

White Cell Counts of Rats with Middle Ear Disease

	<u>1st. Reading</u>	<u>1 Week Later</u>
1	14,150	18,400
2	13,750	13,350
3	9,280	11,450
4	11,850	22,900
5	12,000	12,250
6	9,320	10,200
7	11,900	7,300
8	13,750	13,350
9	10,750	11,950
10	9,320	10,200
11	10,200	11,900
12	10,700	11,900
13	11,800	12,300
14	8,280	9,800
15	9,900	12,150

Mean	11130	12456.6
t =	4.552	= 3.901
P =	1/5000	= 1/1250

The white blood cell count in the normal adult rat ranges from 6,000 to 18,000, with an average of 9,000 (19). Although the individual readings given in Table IV fall within this "normal" range, on comparing the means by Student's t test, a significant increase over the 9,000 average value can be shown in this group of rats. Such an observation may suggest the presence of a chronic infection in a group of animals, but it does not allow detection of middle ear disease in the individual animal.

B Otoscope Examination

It was noted on autopsy that when initial infection takes place the tympanic membrane becomes infected and somewhat opaque losing its pearly semi-translucent appearance. This finding suggested that detection of the condition in the living animal could probably be made by viewing the tympanic membrane and assessing the nature of any observed change.

Experimental

Attempts were made to devise an otoscopic speculum which would permit visual observation of the tympanic membrane. Several specula were designed with elongated tips and narrow lumens that were adaptable to the Welch Allyn otoscope. It was found, however, that it was necessary to use a speculum of such a small bore (less than 1.5 mm.) that insufficient light passed through the lumen from the source to permit a satis-

factory examination of the membrane. Consequently this approach to the problem was abandoned as impractical.

C X-ray Examination

In examining the tympanic bulla of normal and infected rats it was observed that the bulla of the normal rat is quite thin and has a pearly translucency. An infected bulla, however, loses this translucency, becomes thickened, somewhat roughened, opaque and whitish, while the cavity contains considerable pus. These observations suggested that the diseased bulla would filter sufficient x-rays to permit an x-ray plate differentiation between the infected and the normal bulla. Preliminary plates of rats' skulls were very encouraging in that infected and non-infected bulla were readily differentiated.

X-ray films were taken of the skull with the rat lying in various positions on the X-ray cassette. The rat was placed so that the rays passed either in a dorso-ventral, ventro dorsal, lateral, or tangential plane through the skull. The proper time, killivolt and milliampere settings, were determined by employing various combinations of the different factors. The time factor was varied from 1/8 of a second to 2 seconds. Killivolt settings were made from 30 to 50 killivolts and milliampere settings ranged from 10 to 20 milliamperes. The following technique was finally adopted in taking roentgenograms of rat skulls to detect infection of the middle ear.

Technique

The rat, under ether anesthesia, was placed in a horizontal position with the head slightly extended so that the

incident rays passed dorso-ventrally through the skull, the lip of the machine cone being $1/8$ " from the head of the rat. The equipment used was a General Electric Model D type 3 machine with a cone 13" long and 3" in diameter. The other factors were approximately 17 milliamperes, 65 killivolts with an exposure of $\frac{1}{4}$ second. The rat was properly placed in the true dorso-ventral position and held so that its head was immobilized. In this way, detail and excellent contrast were obtained as is shown in Figures XIII and XIV.

In the infected ear the thickened bone of the bulla together with the purulent material filtered out a considerable number of the rays as shown in Figures XIII and XIV. It will be seen that the bulla appeared irregular and hazy, while the normal bulla which filtered out very few rays appeared as a black spot on the X-ray plate.

This procedure has been employed successfully in detecting the disease in over 100 animals from this and other colonies. Table V shows the X-ray diagnosis and the autopsy findings in a group of 60 rats from three colonies. In the two doubtful cases the head was not held in the true dorso-ventral position which resulted in a super-imposition of the post tympanic hook and to a lesser extent the squamosal bones. In both cases the bullae appear irregular, hazy and resemble the infected bullae.

TABLE V

X-ray Detection of Middle Ear Disease and Autopsy Findings
in Tympanic Bullae of Rats

Colony	No. of Rats	X-ray Findings			Autopsy Findings	
		Positive	Negative	Doubtful	Positive	Negative
Food and Drug	30	28	1	1	29	1
Industrial Health	20	3	16	1	4	16
A.D.R.I.	10	9	1		9	1
Total -	60	40	18	2	42	18

Figure XIII

A - X-ray plate of a rat's skull. This animal was free of middle ear disease. The tympanic bulla appears as a dark spot. Bulla encircled.

B - X-ray plate of a rat's skull. This animal had middle ear disease. The tympanic bulla appears as a hazy spot. Bulla encircled.

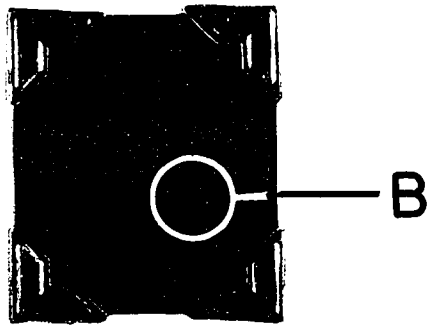
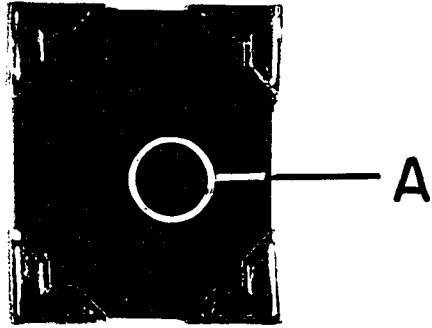


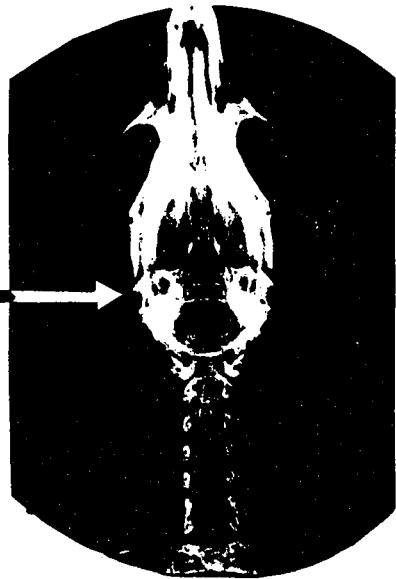
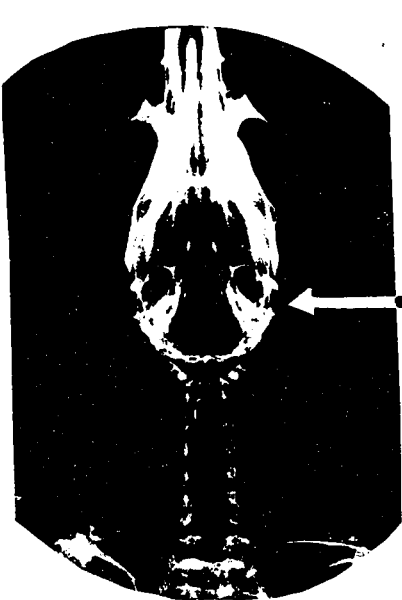
Figure XIV

Figure XIV

Photographs of X-rays of rat's skulls

The arrow points to the tympanic bullae.

The normal bulla (left) appears as a black spot. The infected bulla (right) is hazy and indefinite in outline.



Summary

Roentgenograms of rats' skulls serve as reliable diagnostic criteria of middle ear disease in that they reveal the condition of the middle ear in the living animal. It is possible to detect accurately the presence or absence of inflammatory products in the middle ear. There are selected cases of middle ear disease that appear to recover spontaneously and the reliability of roentgenograms might be questioned in such cases. It has been noted in these animals that although resorption of inflammatory debris is complete, restitution particularly of the bullae is never complete. The bulla never returns to its original, thin, semi-translucent state. The bone remains sufficiently dense to filter out rays to the extent that differentiation between a recovered animals and a non-infected animal is possible.

3. Control

The Application of the X-ray Method of Detecting Middle Ear Disease to the Control of the Disease

Introduction

Other investigators (13, 14) have met with difficulty in establishing an albino rat colony free from middle ear disease in that they have been unable to diagnose the condition in the living animal and consequently have had to rely on chance mating in their breeding programs. The primary objective of this study was to establish a colony free of middle ear disease in a shorter time period than prescribed methods and with less expenditure of breeding animals. The X-ray method of detecting

the condition in the living animal seemed to offer a practical solution to the problem.

Method

Breeders that were negative for middle ear disease on X-ray plates formed the nucleus of the breeding program. In the initial phases of the program the parents from any mating were sacrificed and the ears examined as a safety precaution. This practice was soon discontinued and the X-ray plates served as the sole diagnostic criteria. Roentgenograms were taken before matings and after parturition. The young from all matings were checked by X-ray at weaning and periodic X-ray checks were made until the breeding time.

All animals positive by X-ray were destroyed. Although conditions were unsuitable for strict isolation procedures and sanitary measures, the animals were kept in a room separate from the main colony. Two men were assigned duties pertaining to the care of the animals in the isolation room. Rubber boots and clean uniforms were available for use by the attendants at all times and no personnel other than the attendants were allowed entry to the isolation room. The room was sprayed with a 3 per cent Dettol aerosol twice a week and the animal cages were frequently steam sterilized.

Results

Ten months have elapsed since the initiation of this method of selective breeding for the control and elimination of middle ear disease. During this period, the number of affected animals has been reduced from over 90 per cent to less than 4 per cent.

Discussion

It is not possible to state whether or not the sanitary measures and isolation procedures employed in conjunction with the X-ray for selecting negative breeders were of benefit in reducing the incidence of the disease as there was no control group. Nevertheless the incidence of the disease in the animals kept in the main colony remained at over 90 per cent. These latter animals have now been replaced with non-infected animals.

It is interesting to note that in addition to the reduction in the incidence of middle ear disease in the colony there has been a decrease in cannibalism from 21.9 per cent to 4.5 per cent. Furthermore, there has been a reduction in death rate of nursing animals from 5.0 per cent to 1.8 per cent, and the incidence of pneumonia has dropped from 10 per cent to 1.4 per cent. Whether these associated conditions can be attributed to an improvement in the health of the animals as result of the elimination of the chronic infection, to the sanitary and precautionary measures employed, or to the fact that animals inherently resistant to infection were used for breeding, is undecided.

Conclusions

The method of selecting breeders through employment of the X-ray method for detection of the presence of disease, combined with sanitary measures and isolation procedures has reduced middle ear disease in this rat colony from over 90 per cent to less than 4 per cent. In addition to this there has been a reduction in cannibalism and pneumonia, and a lowering of the

death rate in nursing animals.

4 THERAPEUTICS

The Efficacy of Antibiotics in Middle Ear Disease in Albino Rats

Introduction

A review of the literature did not reveal any information concerning the use of antibiotics in middle ear disease in rats.

Several trial experiments were carried out to assess the efficacy of antibiotics for use in conjunction with the controlled breeding experiments. Bacterial sensitivity discs were not employed because of the mixed bacterial flora and because an extensive investigation of therapeutic agents was not intended. Antibiotics that are reported to be effective against the bacteria encountered in middle ear infection were used in this study.

A Streptomycin and Aureomycin

Twenty-four rats, twenty-one days old, were divided into three groups of eight each. One group received 400 mcg. of aureomycin at weekly intervals for a total of five weeks. A second group received 18 u. of streptomycin at weekly intervals for a total of five weeks. The third control group received no treatment.

Method

Anaesthetics were not used. The rats were held by one operator while injection was made by inserting a 22-gauge needle in the outer ear canal and through the tympanic membrane which gave a characteristic click when it was punctured by the needle.

The antibiotic in aqueous solution was then injected into the bulla. It was not possible to be sure that the injection always entered the bulla because of difficulties encountered in trying to inject the solution and restrain the animal at the same time. One week after treatment the animals were sacrificed and the bullae were examined bacteriologically.

Results

The results are given in Table VI

Discussion

The results indicate an absence of Staph. aureus and some decrease in other bacteria in the ears of the treated animals, suggesting that streptomycin and aureomycin might be employed as adjuncts to breeding method for the control of middle ear disease.

B Combined Antibiotics

Introduction

The results of the previous trial experiment seemed promising enough to justify a second trial experiment using higher dosage levels of the antibiotics and incorporating other antibiotics with streptomycin and aureomycin. It was noted in the previous experiment that it was not always possible to determine whether the antibiotic had been deposited in the bullae. The slightest movement of the animal's head disrupted the injection technique. It was also difficult to steady the head, make the injection and keep the needle in the bulla all at the same time. The technique was greatly simplified by modifying the Cornwall syringe for injection purposes in the manner indicated in Figure XV. These modifi-

TABLE VI

Bacteriological Results# of Antibiotic Treatment of Middle Ear Disease

Antibiotics Employed	Staph. aureus	Staph. albus	Streptobacillus moniliformis	Past multocida	Dip. oococcus
	Type of Growth *				
	H. M. L.	H. M. L.	VH. H. M. L.	H. M. L.	VH.
None	12.5 12.5	6.2 6.2	6.2 6.2 18.7	6.2 6.2 18.7	50.0 3.2
Streptomycin		6.2	2.5 18.7	18.7	68.7 3.0
Aureomycin		12.5	12.5	12.5	12.5 1.7

The numbers given in the table represent the percentage of swabs taken that gave positive bacteriological results.

* The symbols given indicate the type of growth:

VH - very heavy; H - heavy; M - moderate; L - light

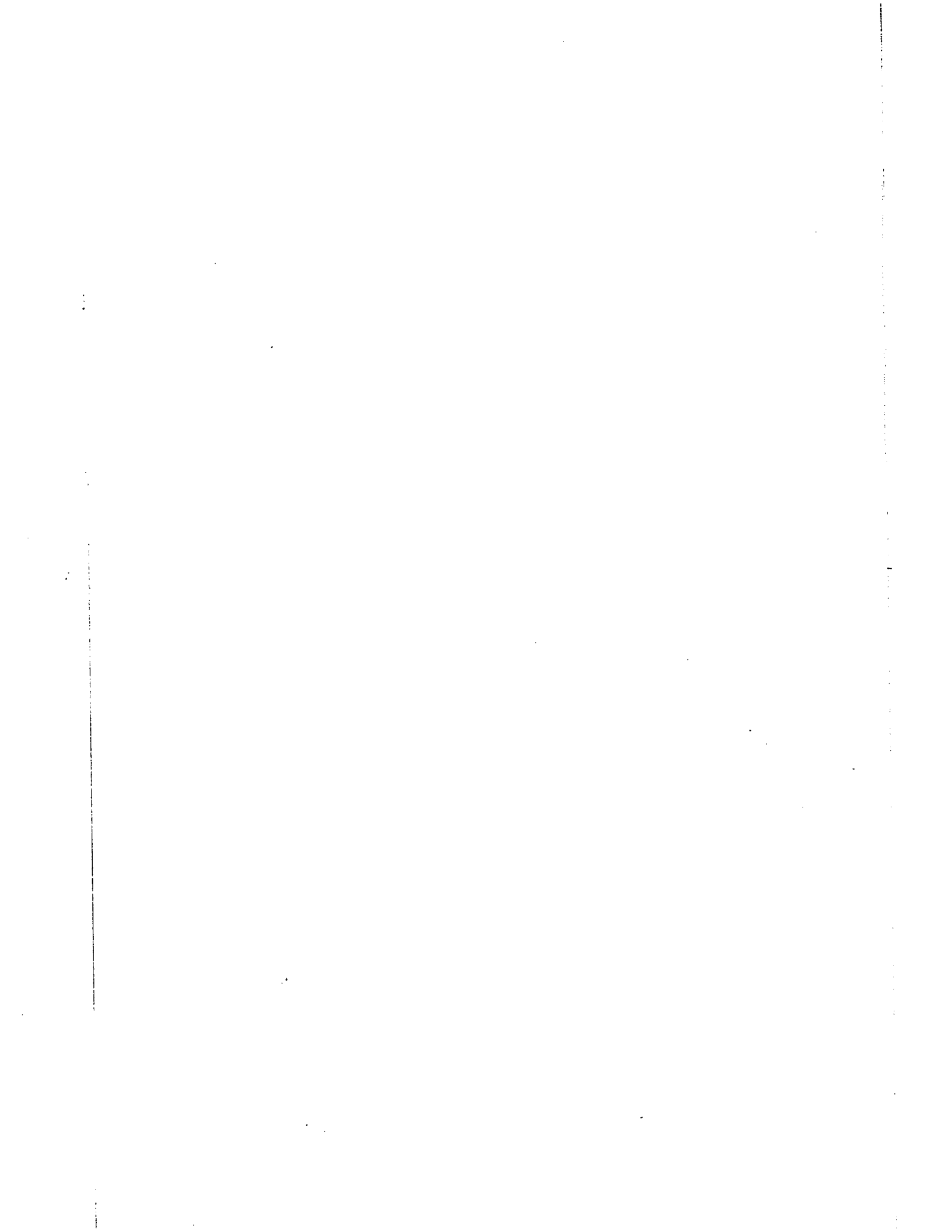


TABLE VI

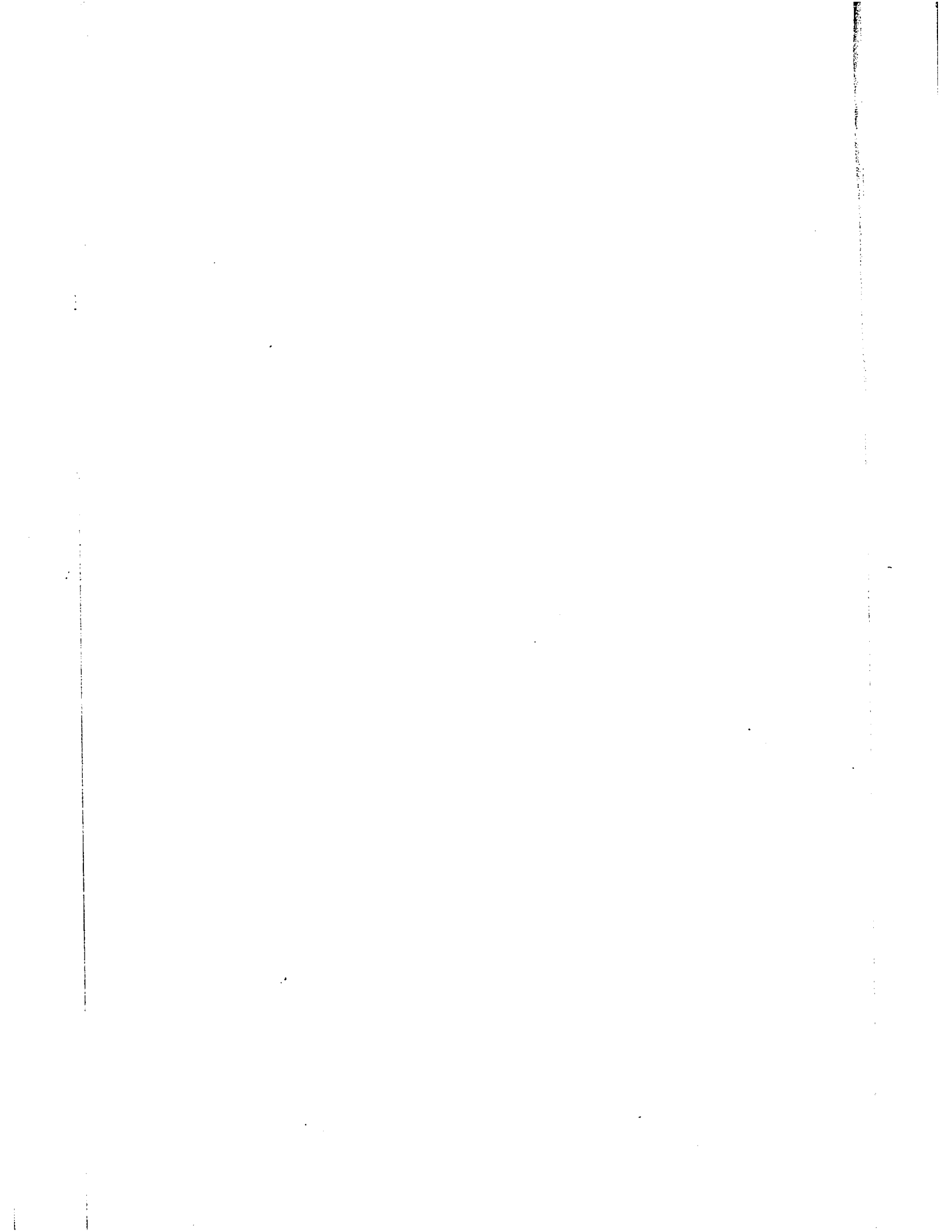
Antibiotic Treatment of Middle Ear Disease

Streptobacillus moniliformis		Past multocida		Diplococcus pneumoniae		Diphtheroids		Achromobacter	
Type of Growth *		H. M. L.		VH.		M. L.		H. M. L.	
VH.	H. M. L.	H. M. L.	H. M. L.	VH.	M. L.	H. M. L.	H. M. L.	H. M. L.	H. M. L.
6.2	6.2 18.7	6.2 6.2 18.7	50.0 3.2 12.5	6.2	18.7 6.2 12.5	6.2	6.2 6.2 18.7	6.2	6.2 12.5
	2.5 18.7	18.7	68.7 1.0 6.2		6.2		6.2 18.7 12.5		
	12.5	12.5	12.5 1.7 37.5						

* The percentage of swabs taken that gave positive

growth:

H - Heavy; L - light



ations made it possible for one operator to control the injection while a second operator introduced the needle, maintained its position in the ear and steadied any head movements on the part of the animal.

Experimental

Thirty mature animals were divided into two groups of 15 animals. A mixture of antibiotics in the following proportions: 1500 I.U. procaine penicillin, 0.25 gm. streptomycin, 2.5 mg. aureomycin, and 0.025 gm. of terramycin in distilled water, was injected through the tympanic membrane. The injection was continued until the antibiotic solution was observed at the nasal orifice indicating that the antibiotic had traversed the eustachian tube and was emerging from the nasal sinuses. Fifteen animals received no treatment.

The animals were sacrificed three weeks after the injection and bacteriological examinations of the tympanic bullae were made.

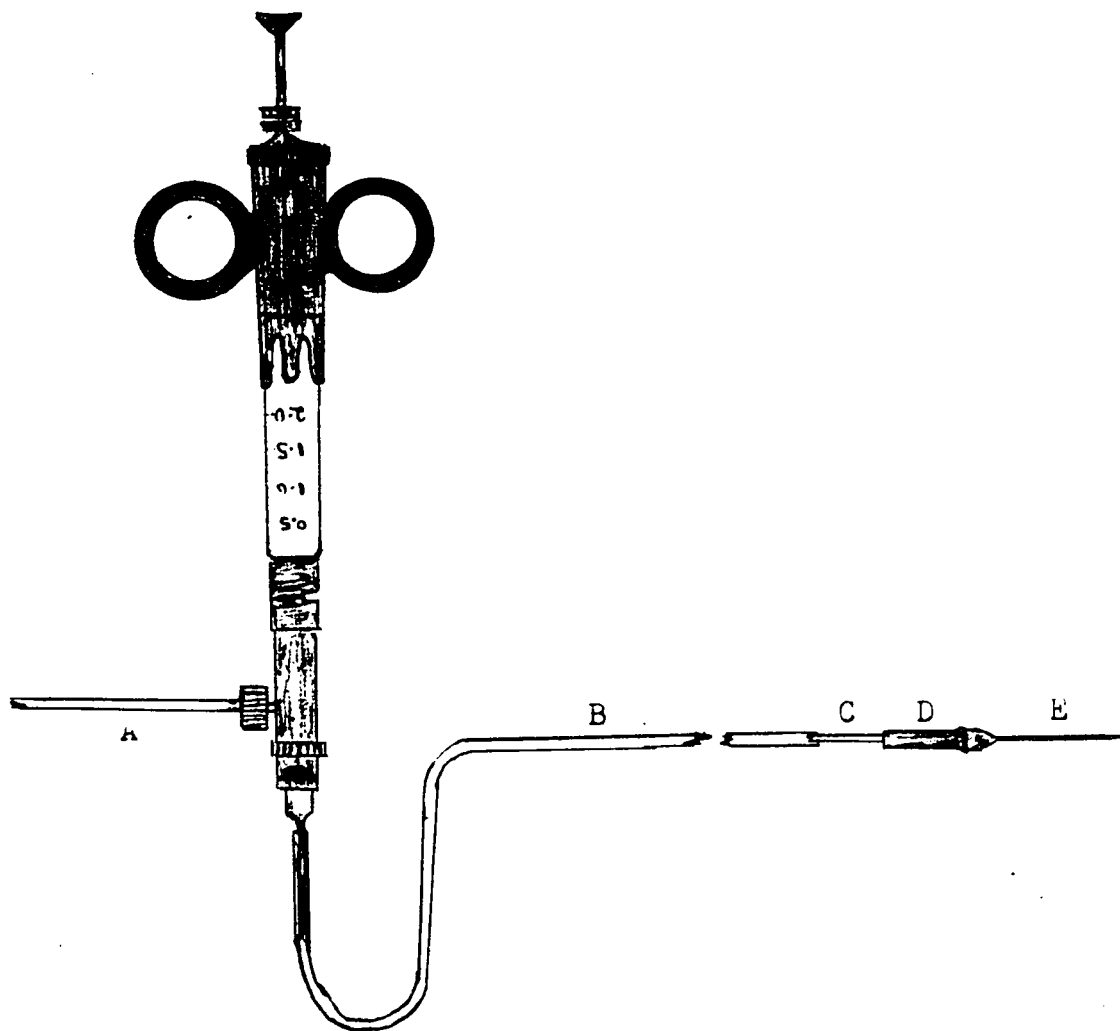
Results

The results are given in Table VII and VIII.

Figure XV

Figure XV illustrates the modifications of the Cornwall syringe which simplified the injection of antibiotics into the middle ear through the tympanic membrane.

- A - polyethylene tubing leading to solution for injection.
- B - polyethylene tubing from injection needle to glass rod.
- C - glass rod from polyethylene tubing to rubber hose.
- D.- rubber hose connecting tube C with injection needle E.
- E - injection needle. This needle is passed through the tympanic membrane by one operator, a second operator controls the injection.



Bacteriological Results# of Intra-aural Antibiotic Treatment of Middle Ear Disease

Staph. albus Staph. aureus Past multocida Streptobacillus moniliformis Pseud. aeruginosa

Group Type of Growth *

VH. H. M. L. VH. H. M. L. VH. H. M. L. VH. H. M. L. VH. H. M. L.

Treated 7.0 7.0 7.0 21.1 35.7 14.7 7.0 21.1

Control 23.0 7.6 15.2 7.6 7.6 30.4 38.0 7.6 15.2 22.8 38.0 7.6

Numbers in the table indicate the percentage of swabs taken that were positive.

* Type of Growth: VH - very heavy; H - heavy; M - moderate; L - light.

TABLE VIII

Pathological Results of Intra-aural Antibiotic Treatment of Middle Ear Disease

Group	No. Bullae examined	% Positive		% Negative	
		Both bullae	One bullae	Both bullae	One bullae
Treated	28	21.4	28.5	50	
Control	26	53.0	45.3	0	50

Discussion

The most significant finding in this group of animals is the 50 per cent reduction in the number of animals affected with middle ear disease. Considering the fact that only a single injection had been given and this three weeks previous to examination of the bullae, it is apparent that this method gave promise as an adjunct to control measures, particularly for treatment in enzootics. This method has, however, the disadvantage of requiring trained personnel for injection procedures.

The possibility of causing permanent ear damage to the rats by injecting the solution into the bulla has not been considered. There is an open passageway from the bulla through the Eustachian tube so that pressure damage is unlikely. No obvious sign or symptom of temporary or permanent damage was recorded in any of the rats that received an injection, and in any event it is not likely that the damage resulting from such an injection would affect the animals as adversely as would a bacterial assault on the middle ear.

Conclusions

The results of these trial experiments suggest that antibiotics, when properly employed, would prove effective as adjuncts to a controlled breeding program or for treatment of disease outbreaks in a colony free from middle ear disease.

V SUMMARY

1. Gross and microscopic examinations have been made of the lesions in some 1000 cases of middle and inner disease in rats. The lesions observed were typical of acute and chronic inflammation of non-specific bacterial origin. The lesions were principally confined to the middle ear with occasional involvement of the inner ear and VIII nerve.
2. The symptoms were restricted to animals with a complicating labyrinthitis and in these instances these symptoms were distinctive and diagnostic in that they were correlated with obviously disturbed vestibular function.
3. The disturbed equilibrium has been shown to result from a destruction of neuro-epithelial cells in the infected inner ear with a subsequent decrease in the number of impulses from the diseased side and a comparative over stimulation from the non-affected side. This imbalance in the number of impulses from the two sides bring about a disequilibrium on the part of the animal. The disequilibrium could be produced experimentally in the normal animal by erosion of the neuro-epithelial cells in the inner ear. The effect could be altered or abolished in the experimental or diseased animal by destroying the neuro-epithelial cells in the inner ear of the opposite side.
4. A method of detecting middle ear disease in asymptomatic animals using roentgenograms of the rat's skull has been developed.

5. The X-ray method of detecting middle ear disease has been applied to select animals for breeding purposes in a disease-control program.

6. Antibiotics were found to be effective to a degree suggesting their applicability as therapeutic agents for use in conjunction with disease control programs.

VI CLAIMS TO ORIGINAL RESEARCH

1. The detection of middle ear disease in rats by the appearance of the tympanic bulla on X-ray plates.
2. The gross and microscopic pathology of middle and inner ear disease as found in the Food and Drug Colony.
3. The nature of the disturbed equilibrium in the rat with disease of the inner ear.
4. A method of control for the elimination of middle ear disease.
5. The use of antibiotics as therapeutic agents in middle ear disease in rats.

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