STUDIES ON NATURAL IMMUNITY IN THE CAT

by

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A.B., University of Montana, 1934
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Submitted to the Department of Bacteriology and the Faculty of the Graduate School of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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February, 1940

ACKNOWLEDGMENT

The author appreciates the interest and encouragement of Dr. H. P. Sherwood and gratefully acknowledges his patient and helpful criticisms throughout the course of this work.

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STUDIES ON NATURAL IMMUNITY IN THE CAT

INTRODUCTION

The phenomenon of natural immunity is of peculiar interest to the experimental immunologist. The host specificity of disease among different species of animals still offers many unexplained problems. Natural immunity is not so important to the clinical immunologist because he deals only with diseases which a patient has or may acquire. He does not concern himself with viruses and bacteria which attack plants because, as far as is known, these infectious agents never attack man. However, research into the nature of a plant virus, for example, may lead to the discovery of basic principles which can be applied to the study of poliomyelitis, in which the clinical immunologist is very much interested. Similarly, determining the causes of natural immunity in the cat may lead to discoveries of value in treating diseases of man.

As will be shown, the cat enjoys a state of highly developed natural immunity to many organisms. There is no apparent practical application of knowledge concerning an infection which normally never occurs. Yet, from a didactic point of view the question of natural immunity in cats is an interesting one.

Physiologists have long realized the advantages of the cat for their experimentation, but immunologists have used this laboratory animal very little.

If a more concrete reason for this paper were needed, we might say that any knowledge as to why the cat fails to contract certain diseases, might shed light upon the paradox that other animals do contract them.

REVIEW OF LITERATURE:

Studies on susceptibility of cats to infectious agents are not numerous in the literature. There are isolated papers in which the cat has been used for various purposes. Many of these deal with anatomy, with physiology of muscle-nerve and nerve preparations, etc., and will not be quoted here. Brodie (1900) described the exaggerated sensitivity of cats to intravenous injections of foreign proteins. Many others have noticed this reaction in cats. Kabler (1938) and Kabler and Sherwood (1938) have reviewed the literature dealing with anaphylaxis in cats. From their own work and that of others they show that it is difficult or impossible to actively sensitize the cat. They were able to demonstrate anaphylactic shock in forty percent of cats passively sensitized with rabbit antiserum for crystalline egg albumen. Fall in blood pressure, kidney volume, smooth muscle reaction of excised uterine horns and intestinal strips were demonstrated. Seastone and Rosenblueth (1934) report that contraction of the nictitating membrane in the intact cat is a good criterion of anaphylactic shock. From the meager data at hand it would appear that cats fail to produce precipitins

that can be demonstrated by the ring technic or anaphylactic sensitizers that can be demonstrated by clinical shock or the Schultz-Dale reaction. There is little antibody response to injection of E. typhi (Kabler and Sherwood). We wondered if cats also fail to produce agglutinins for S. enteritidis, since cats are constantly exposed to infection through eating infected mice.

That the cat may passively transmit disease by contaminating his fur, teeth and claws with blood of infected animals is undoubtedly true. By dragging the carcass of an infected rodent into a barnyard the cat might be instrumental in bringing about infection of domestic animals without himself suffering from the disease. Rudesell (1937) reports a case of human tularemia contracted from the bite of a nursling kitten. The kitten had presumably been infected by a ground squirrel which the mother cat had brought from the fields. David and Schlessler (1939) have recently succeeded in infecting cats by parenteral and oral administration of middle European strains of Past. tularensis.

Veterinary journals such as Vet. Med., and J. A.V.M.A. contain occasional articles suggesting that ringworm and favus are common diseases of domestic cats. It is generally conceded by such authoritative

sources that man may contract ringworm from infected cats and conversely that cats readily become infected through contact with infected persons.

According to Pasteur's statistics on the incidence of rabies, of 12,000 persons bitten by rabid animals only 700 were bitten by cats while 11,000 were bitten by dogs.

Leasure (1934, 1937) has made extensive studies of feline enteritis. He finds that it is caused by a filterable virus, no bacteria being consistently present, and he concludes that bacteria play no role in the disease. The mortality in this disease is about ninty-two percent in young animals. The serum of convalescent cats confers protection and apparently is useful in treatment.

Hammon and Enders have recently (1939) described a malignant panleucopenia of cats, a highly fatal epizootic caused by a filterable virus. Eosinophilic infiltration and panleucopenia were the most striking pathological findings.

The cat has been suspected of transmitting various diseases to man (Frick (1937) but as pointed out by Savage (1920), many of these reports are based on circumstantial evidence.

Simmons (1920) has isolated virulent diphtheria

bacilli from two cats which were pets of a person who contracted a fatal diphtheritic pharyngitis. Savage re-examined the whole subject, and concluded that cats do not suffer from diphtheria. He failed to infect young kittens with cultures of the Klebs-Loeffler bacillus, and found that these organisms usually disappear within twenty-four hours when implanted into the nasal cavity and upon the throat. He concludes that there is no evidence that cats serve as carriers of diphtheria. Nonvirulent bacilli morphologically indistinguishable from Klebs-Loefflor bacillus were frequently isolated from healthy cats.

Hemmer in 1866 tried to determine the nature of pus by injecting it into cats and rabbits.

According to Huidepaper (1903), glanders is transmissible to cats. Doleman and Wilson (1938) review several years of experience with the Doleman test for staphylococcus enterotoxin. The prepared filtrate is injected intraperitoneally into the kittens. If enterotoxin is present the kitten shows symptoms of severe vomiting and diarrhea with prostration for several hours.

The cat is bothered by few bacterial infections but is commonly infected by several parasitic protozoa. Rees (1929) has studied the pathology of experi-

mental amebiasis in the kitten. Kessel (1928) was able to experimentally infect kittens with endameba from the pig, man and monkey. He also obtained amebae identical with Endameba histolytica from naturally infected kittens.

On the other hand, Heathman (1932) was able to infect only five out of forty-nine young kittens using four supposedly pathogenic strains of E. histolytica.

Helminth infestations are common. The cat being intermediate and final host to the tapeworm Echinococcus granulosus, acts as a reservoir of this harmful parasite of man.

The cat harbors also Dibothryocephalus latus, the fish tapeworm, and Dipylidium caninum, the dog tapeworm; infection with the latter is acquired by the ingestion of fleas and lice which harbor the intermediate stage of the parasite. (Frick, 1937).

Wenyon(1923) has reported extensive studies on coccidiosis in cats and dogs.

Lillie (1931) reports a case of mast myelocyte leukemia in the cat. He collected eleven cases from the literature both lymphatic and myelogenous.

STATEMENT OF PROBLEMS:

The purpose of this paper is to record experimental data accumulated over the past three years pertaining to natural immunity in cats. Rabbits and mice were also used. In some places the data represents intensive work while in others it is in the nature of a survey only. No attempt was made to do extended experimentation with a large number of infectious agents. However, several were employed and the results are recorded in later paragraphs. Many problems were revealed which have not yet been solved. The infectious agents most used were two members of the Salmonella group, viz., S. enteritidis, S. cholera suis. These organisms were chosen because they are a common cause of fatal epizootics in mice. Frequently whole colonies of white mice in captivity are destroyed by S. enteritidis. The disease is also common in wild mice and rats. With this in mind we wondered if cats eating infected mice might be infected.

We have proposed several questions to be answered by experimentation, namely:

1. To what extent are cats naturally immune to virulent infectious agents? Are cats immune to ingestion of Salmonella enteritidis?

2. Do cats produce antibodies when inoculated with Salmonella enteritidis?

3. What is the effect of Vitamin C on antibody production? Can the reports in the literature that Vitamin C stimulates antibody productions in rabbits be confirmed.

(a) Will "refractory" rabbits be stimulated to greater antibody production by administration of Vitamin C?

(b) What is the effect of Vitamin C on antibody production in unselected rabbits? Will d-iso ascorbic acid or other unrelated acids adjusted to equal PH value be capable of simulating the effect of Vitamin C?

(c) What is the effect of Vitamin C in cats?

4. What is the normal intestinal flora of cats? Of kittens?

(a) Can a new organism (Salmonella) be established in the intestinal flora of kittens? Of cats?

(b) What is the fate of Salmonella organisms fed orally to adult cats? To kittens?

5. What is the fate of Salmonella organisms injected into the blood stream of cats? How

long can they be isolated from the blood stream, various organs, intestinal tract, urine and bile?

6. To what extent do normal bacteriolysins play a role in the immunity of cats to Salmonella infection?

7. Are cats naturally immune to:

(a) Ingestion of encysted Trichinella spiralis larvae?

(b) Experimental subcutaneous injection of Pasteurella tularensis?

(c) Subcutaneous injection of Bacillus anthracis?

(d) Intracutaneous innoculation with Vaccinia?

(e) Intracutaneous injection of Pneumococcus Type III?

TECHNIC

Infectious organisms were always identified and checked by suitable means. Serological typing or agglutination, morphology and staining, fermentation and cultural reactions were used as necessary. Virulence of the culture was checked on some suitable laboratory animal other than the cat. This data is recorded with the results of experimentation. Virulence was maintained by animal passage and frequent transfer. Cultures were also preserved by desiccation in vacuo (Sherwood and Coriell, 1937). Agglutination tests were made with one half cubic centimeter of serum dilution added to one half cubic centimeter of antigen suspension. Incubation was in 56 C degree water bath for three to four hours. A second reading was made after storage at 6°-8° C degree. Readings were made with a concave mirror.

Precipitin tests were done by the ring technic with readings at one half hour, one hour, and two hours. Precipitin antibodies were also detected by the agglutination technic using antigen filmed collodion particles. In preparing the collodion particles the method of Cannon (1939) was employed. Briefly this is as follows:

PREPARATION OF COLLODION PARTICLES:

Collodion USP (non-flexible, Merck) is poured into a large volume of distilled water the while it is stirred with a glass stirring rod. A solid white stringy mass of collodion separates from the solution, freed from most of its alcohol-ether solvent mixture. The collodion is pressed between the hands to dry, and is then washed through three changes of distilled water. Tap water should not contact the collodion in this or in any subsequent stages of the preparation, as it leads to flocculation of the pellets, when formed. The mass is dried by pressure between the hands and finally on filter paper in a 40°C. incubator until completely dry. The dried product is then weighed approximately, and an 8-10 per cent solution by weight in acetone is prepared with stirring, at 40°C. Glass stirring rods etc., should be used throughout, avoiding metal. This collodion-in-acetone solution may be stored indefinitely, or used immediately in preparing the particles.

With a mechanical stirring device of moderate speed and power, (using a glass stirring arm) the collodion-acetone solution is stirred rapidly in an appropriate-sized beaker surrounded by a water jacket

at 40°C, while to it is added drop by drop (from a funnel emptying through a capillary tube) a mixture of 3 parts water to 1 of acetone. For all subsequent stages it is best to use triple distilled water where water is to be added. With about 100 cc. of collodion solution to start, approximately 35 cc. water acetone mixture will be required. The end point is reached when a heavy gelatinous phase separates from a water-clear supernatant, which may become cloudy on addition of excess of water-acetone mixture, (moderate excess does not harm in any way). The supernatant is decanted off and diluted while shaking with about 5 times its volume of triple distilled water, whereupon a profound cloudiness develops, due to separation of the particles. The gelatinous phase may be redissolved in acetone and the process may be repeated as often as desired, the yield of particles being pooled in a large flask, or it (the acetone solution) may be stored until needed again. Acetone is removed from the suspension of particles by attaching the flask to a water-aspirator pump or other source of vacuum for a time (2-5 hours). (If this is omitted, attempts to wash the particles will result in their sticking together and being lost.) The cloudy suspension is then centrifuged at a brisk speed (3000 RPM in

Angle Centrifuge) for 5-6 minutes, the supernatant decanted temporarily until the precipitated particles can be removed from the tube and resuspended in wash water by agitation. The supernatant is centrifuged again for a similar period and the above repeated until the supernatant appears clear, the resuspended particles precipitated out of it being pooled as they are recovered. In the same manner they are washed twice more to insure thorough washing and freedom from acetone in the solution. The finally resuspended particles are then placed in the centrifuge and whirled more slowly for about a minute, in order to eliminate the larger particles. The supernatant is then poured off and retained as the stock suspension of particles, most of which should be in the size range of staphylococci.

To sensitize the particles, a suspension heavy enough to have a milky appearance is added to an equal volume of a clear solution of antigen (5 percent for CEA or 20 percent horse serum) and the mixture is allowed to stand over night. Some settling out occurs, but shaking resuspends particles instantly. Very slow, gentle centrifuging at first is adequate to bring down many of the particles, and the first centrifuging should not exceed three minutes. The supernatant is transferred to other tubes to continue centri-

fuging as precipitation is not complete, and the precipitated particles resuspended by agitation in triple distilled water. The washing method is identical with that used in preparation of particles originally, with the exception that the particles are now more easily precipitated and lower speeds are necessary. If the particles are thrown down forcibly they cannot be well resuspended. Three complete washings are required, after which the particles are resuspended in triple distilled water adequate to give the desired turbidity or concentration to the suspension: add 1 part in 10 of a buffer solution pH 6.07 prepared with 12.5 cc. of 0.2 M.Na Ac and 0.3 cc. 0.2 M.H Ac diluted to 250 cc. We found the particles coated with horse serum to be more stable at pH 6.7. It is best to make stock in a concentrated suspension and dilute to desired turbidity for test as used. Such suspensions keep from 1 to 3 weeks. The particles are now coated with antigen and are suspended in an antigen-free aqueous medium. Dilution with saline does not break the colloid for 1-2 days.

Best results seem to occur with this method when the collodion pellet suspension is mixed with serial dilutions of anti-serum, shaken, and allowed to stand at room temperature for 10-15 minutes. The

tubes are then centrifuged and the resuspension technique employed for reading. If centrifuge agglutination is used in serum tests, minimum effective speeds and time periods should be used, to avoid agglutination of particles spontaneously from the centripetal force exerted upon the suspension.

Vitamin C determination in blood was done by the reduction of the red dye sodium 2:6 dichlorobenzene indophenol (Farmer and Abt, 1935). The results are reported in milligrams per hundred cubic centimeters of plasma. Determinations were made in triplicate.

In plating out intestinal contents for isolation of organisms, several media were employed. These include plain one and one-half percent nutrient agar, litmus lactose agar, eosin methylene blue agar (Billings, Clawson, Sherwood, 1938), Endo's medium, and brilliant green agar. Plates were scanned and picked after twenty-four hours and again after forty-eight hours incubation.

In securing fecal samples from cats at various intervals, an enema of sterile saline was the most convenient way of obtaining an uncontaminated sample. To inject and withdraw the saline we used medicine dropper pipettes with a 10 cc. rubber bulb attached.

To this procedure the cats objected most strenuously. However, the cats could be quite securely held by one man if rolled in a blanket or large sack.

Routinely the services of three men were required to hold a cat and pass a stomach tube. Soft rubber catheters, gauge 14, were used as stomach tubes.

When the cats were to be bled from the heart or skin tested, they were etherized. A special ether chamber was constructed for this purpose. This box was fitted with hinged lid, windows for viewing animal, and inlet for ether-air mixture. Ether was admitted to the closed box by bubbling compressed air through a pint jar of ether and then into the box. This insured a mixture of oxygen and ether. We have never lost an animal due to suffocation when this chamber was used. The animal was removed from the box for bleeding or injecting.

Titration of bacteriolytic properties of serum were made with the technic of Mackie and Finkelstein (1931). The serum or whole blood was used undiluted and added to logarithmic dilutions of bacterial suspension in saline. Incubation was at 37°C water bath with frequent shaking for three hours. Plates were then poured with plain agar and incubated at 37° C. Readings were made by plate count after twenty-four

hours incubation.

To determine the phagocytic activity of cat leucocytes we used the technic of Keller, Pharris and Gaub (1936) modified to better fit our own needs. Their test is called the opsonocytophagic reaction and is recommended as an aid in the diagnosis of undulant fever. We used heparin as an anticoagulant instead of sodium citrate. Blood was withdrawn from the heart, heparinized and one-tenth cubic centimeter placed in a small tube. An equal volume of a heavy suspension of washed living bacteria was added to the blood. The tubes were mixed and incubated for fifteen minutes at 37° C. Smears were made as for the differential count. Slides were fixed in methyl alcohol and stained with methylene blue. The total number of organisms in twenty-five neutrophiles was counted and the average number of bacteria per cell computed.

ARE CATS IMMUNE TO INGESTION OF SALMONELLA ENTERITIDIS?

EXPERIMENTAL:

The strain of organism used in these experiments was isolated from an epidemic in our colony of white mice. The organism was identified as Salmonella enteritidis by morphology, staining, fermentation, and cultural reactions and agglutination with known antisera. Adult white mice which had been kept separate from the infected strain were found susceptible to oral feeding of a twenty-four hour broth culture. Since the dosage of organisms was always indefinite by oral administration, we turned to intraperitoneal injeation of one-tenth cubic centimeter of a twenty-four hour broth culture as the infecting dose,(Grinnell 1932). With this technic four out of five mice developed fatal infection. The mice died in four to eight days after infection. Mice were autopsied soon after death and cultures made from heart blood and cut surface of the liver. The original culture was isolated from these sources and identified by cultural reactions and agglutination.

Two cats were fed infected mice. The mice were previously injected with S. enteritidis and showed symptoms of illness. Two other cats were fed broth

cultures of S. enteritidis. Five cubic centimeters of a twenty-four hour culture was added to fresh milk. The cats were observed to drink the mixture. This dose was repeated after four days and again after ten more days. Twenty-four hours after the last feeding of infected milk the two cats were sacrificed. Cultures were made from heart blood, intestinal tract and mesenteric lymph nodes. Agglutination tests were run on all these cats before the experiment started and at its conclusion.

RESULTS:

None of the four cats showed any symptoms of being ill during the course of the experiment. No normal agglutinins were found for S. enteritidis. No agglutinins were found up to fifteen days after experimental infection. Autopsy showed no gross pathology. Blood cultures were sterile. S. enteritidis was not isolated from feces or mesenteric lymph nodes.

DISCUSSION:

These four cats were apparently immune to our culture of S. enteritidis. Various aspects of this problem will be taken up separately in later paragraphs.

One of the most interesting results was the fail-

ure of the cats to produce agglutinating antibodies. We therefore ran a larger series of cats. Both living and formalized suspensions were used. The route of injection was also varied. This data and the results are shown in Table I.

TABLE I.

Cat. No.	Material	Date	Amount	Route	Highest Agglutinin titer
1	Formalized Enteritidis	3/13	0.5 cc.	i.v.	1-20
	" "	3/26	0.5 cc.	i.v.	
2	Formalized Enteritidis	3/13	0.2 cc.	Subcut.	
	" "	3/15	0.5 cc.	"	
	" "	3/17	0.5 cc.	"	
	" "	3/19	1.0 cc.	"	
	" "	3/24	1.0 cc.	"	1-40
3	Formalized Enteritidis	4/3	1.0 cc.	i.card.	
	" "	4/13	1.0 cc.	"	
	" "	4/25	1.0 cc.	"	0
4	Formalized Enteritidis	5/12	0.1 cc.	i.card.	
	" "	6/2	2.0 cc.	i. perit.	0
8	Living Culture Enteritidis	3/13	1.0 cc.	Subcut.	
	" "	3/16	1.0 cc.	"	
	" "	3/20	1.0 cc.	"	1-8
12	Living Culture Enteritidis	5/20	1.5 cc.	orally	0
11	Formalized Enteritidis	3/21	1.5 cc.	i. card.	
	" "	3/25	1.0 cc.	"	0

From the data in Table I it may be seem that both living and formalized suspensions of S. enteritidis were used. The routes of injection were intravenous, subcutaneous, intra-cardial, intra-peritoneal and oral. The sera of all the cats were negative for agglutinins before injection. Three cats out of the seven produced agglutinins in low dilution, i.e., 1-40, 1-20, and 1-8. The suspension of enteritidis was shown to be antigenic by giving a rabbit a series of injections. The rabbit's serum agglutinated S. enteritidis up to a dilution of 1-2400. The possibility was present that the orally administered organisms did not leave the intestinal tract and consequently had no contact with antibody producing tissues. For this reason we employed the parenteral routes of injection shown in Table I. Both cats injected subcutaneously produced agglutinins in low titer. The serum of one of the cats injected into the blood stream produced agglutination in dilution of 1-20.

The cat is able to produce agglutinins for this organism. However, the response is slight or absent after enteral or parenteral introduction of the whole organism. Either the cat's antibody producing mechanism is poorly developed, or S. enteritidis is not a suitable antigen for the cat. Kabler and Sherwood

(1938) in a series of twenty animals showed that cats do not produce demonstrable precipitins for crystalline egg albumin. Perhaps the cat is able to dispose of or eliminate foreign protein without producing antibodies. However, we have shown that the cat can produce antibodies in high titer for another antigen--F. tularensis.

WHAT IS THE EFFECT OF VITAMIN C ON ANTIBODY PRODUCTION?

In looking over the literature we noticed that Jusatz (1936) had reported the use of Vitamin C to stimulate antibody production in rabbits. He reported a five-fold increase in antibody production in rabbits when horse serum was mixed with Vitamin C before injection. Madison and Manwaring (1937) repeated this work and found a twelve to thirty fold increase in precipitin production for horse serum in rabbits when injected with Vitamin C. From their evidence it appears that Vitamin C may greatly stimulate antibody production in rabbits. Both workers found the greatest stimulating effect when the vitamin was mixed with antigen previous to injection. If antigen and vitamins were injected separately, the antibody stimulation was less pronounced. From the report of Madison and Manwaring it is not altogether clear whether they always got stimulation. One graph shows an average precipi-

tin titer of between 40-50,000 in the treated rabbits while another graph shows average titer of less than 4,000 for treated rabbits. In view of these contradictory reports we decided to re-investigate the validity of the claims for Vitamin C. The rabbit was chosen as the experimental animal for this purpose and we decided to use the same antigen used by Madison and Manwaring - namely, horse serum. Before doing this, however, we tried the effect of Vitamin C on rabbits which were refractory to production of sheep cell hemolysins. Ten rabbits were collected which had failed to produce anti-sheep hemolysins following repeated injection with sheep red blood cells.

EXPERIMENTAL:

Three injections of sheep red blood cells plus Vitamin C were given intraperitoneally over a period of eight days. The Vitamin C used was crystalline Cevitamic acid (Squibb). Each rabbit received three intraperitoneal injections of five cubic centimeters of five percent sheep red blood cells plus twenty-five milligrams of Vitamin C. Injections were spaced four days apart and a test bleeding was made on the eighth day following the last injection. Five of the rabbits received the sheep cells and vitamin mixed together.

Five others received the sheep cells and vitamin injected separately.

The results are recorded in Table III, showing that Vitamin C does not stimulate refractory rabbits to greater production of hemolysins.

TABLE III.

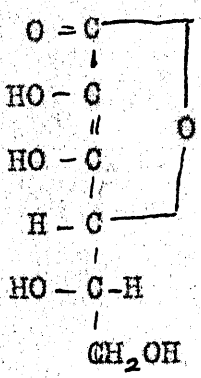
EFFECT OF VITAMIN C ON REFRACTORY RABBITS:

All rabbits had received 3 or 4 series of injections of sheep cells previously and failed to develop hemolysins in appreciable amount.

Rabbit No.	Highest titer reached previously	Injections of Vitamin C	Final titer
269	1-300		1-100
664	500		100
280	1000		500
288	100		100
306	100		100
*309	100		100
*321	100		100
*292	300		200
*336	200		200
*339	100		0

*Vitamin C and r.b.c. mixed previous to injection into rabbits.

Vitamin C has marked chemical properties in addition to its antiscorbutic action. It is strongly acid in concentrated solution (pH of 2.6) and rapidly reduces Fehling's solution in the cold. It was necessary, therefore, to control the effect of Vitamin C by using some other chemical which has similar reducing action and acid pH. These requirements were met by d-iso ascorbic acid. Reichstein (1933) has shown that this dextro isomer of Vitamin C is physiologically inert as an antiscorbutic. Iodine equivalents and alkali equivalents of the two compounds were identical within one-half of one percent. The structural formulae of the two isomers of ascorbic acid are as follows:



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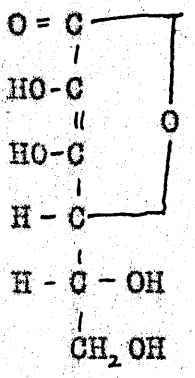

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EXPERIMENTAL:

Thirty-six white New Zealand rabbits weighing approximately 2,000 grams each were divided into three groups of twelve. In the first group, each rabbit was injected intravenously with one-half cubic centimeter of horse serum mixed with fifty milligrams of crystalline L. ascorbic acid (Eastman) or Vitamin C. The second group served as controls, each rabbit being injected intravenously with one-half cubic centimeter of horse serum. The third group served as additional controls. Each rabbit was injected intravenously with one-half cubic centimeter of horse serum mixed with fifty milligrams of crystalline d-iso ascorbic acid (Eastman). Test bleedings were made at intervals of 4, 6, 10, 14, 21, 31, 40, 70 and 80 days following the original injection. Precipitin titers were determined by the ring technic and by agglutination of collodion coated particles.

Vitamin C determinations were run on the blood of selected animals from all three groups forty days after the original injection. These rabbits were chosen in regard to their antibody production, i.e., some had given high precipitin titers while others had responded rather poorly. After the termination of the experiment skin tests were run on rabbits from

each of the three groups. Antigens used were: horse serum, horse serum plus l-ascorbic acid, horse serum plus d-ascorbic acid, l-ascorbic acid alone, d-ascorbic acid alone. Other rabbits from the three groups were injected with horse serum, l-ascorbic acid, or d-ascorbic acid to see if antibodies would reappear.

RESULTS:

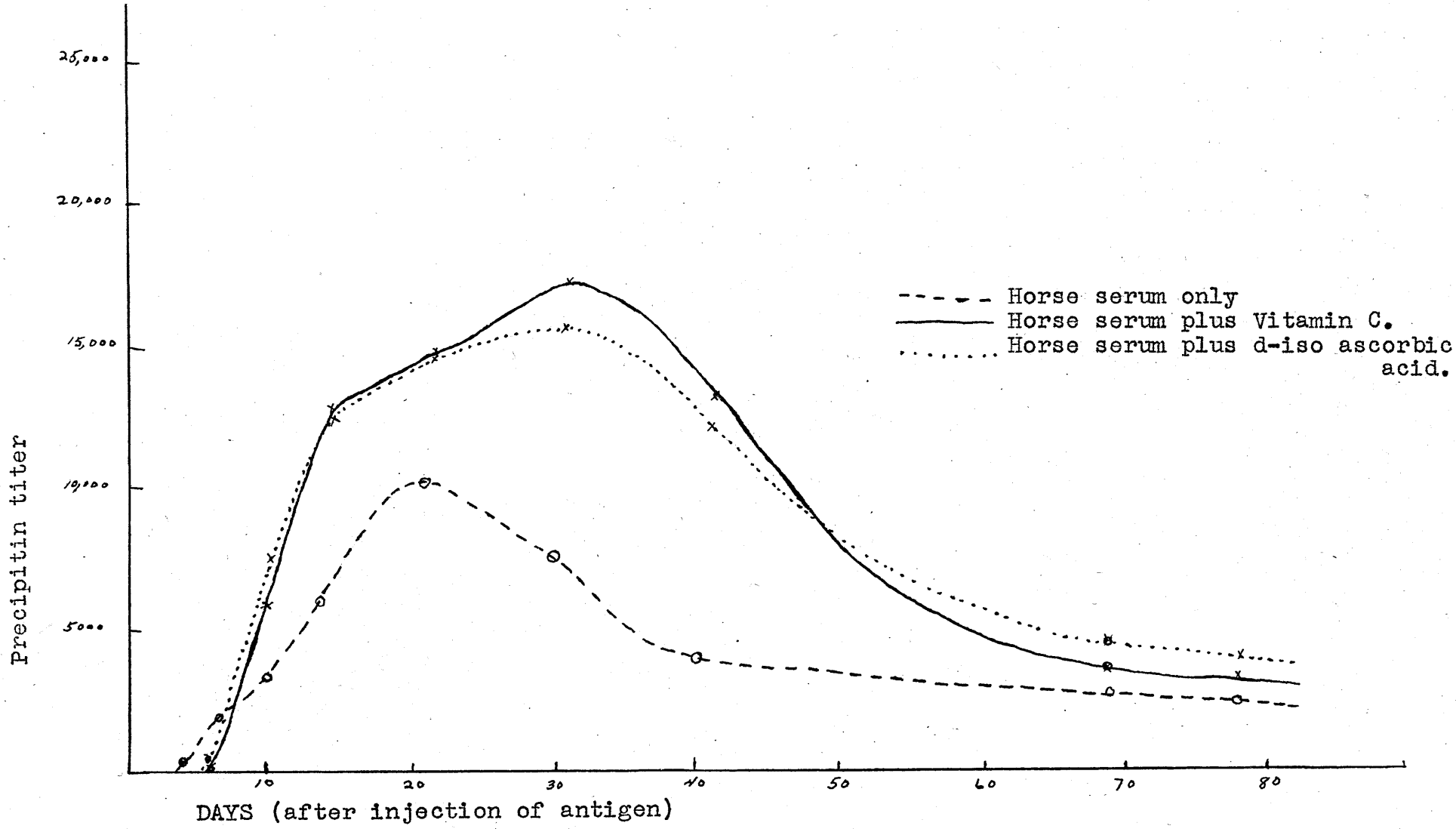
The antibody response in the three groups of rabbits is shown graphically in Table IV. The graph is plotted to show the average titer of all twelve rabbits. The following points may be seen on the table:

1. There was about a seventy percent increase of antibody production in the rabbits injected with treated horse serum as compared with those injected with horse serum only.

2. The antibody response to Vitamin C treated horse serum was not significantly different from the response to d-iso ascorbic acid treated horse serum. In fact there is a remarkable parallelism between the two curves.

3. Antibody production was delayed in the rabbits injected with treated antigens. Precipitins were first detected on the sixth day in the treated

TABLE IV.


25,000
20,000
15,000
10,000
5000
Precipitin titer
10
20
30
40
50
60
70
80
DAYS (after injection of antigen)
Horse serum only
Horse serum plus Vitamin C.
Horse serum plus d-iso ascorbic acid.

rabbits. Precipitins were evident on the fourth day in the rabbits injected with untreated horse serum.

4. The precipitin titer obtained by the ring technic was found to parallel that obtained by the agglutination of collodion particles technic except when the titer was very low. The agglutination technic would detect antibodies before they were apparent by the ring technic and would show a slightly higher titer with sera which gave a titer by the ring test of less than 1-1,000.

It was found that Vitamin C content of rabbit blood varied from 0.15 milligram percent to 0.20 milligram percent. An average value was about 0.18 milligram percent. There was no correlation between ability of a rabbit to produce antibodies in high titer and the Vitamin C content of his blood.

That the specificity of the horse serum used as antigen had not been changed by chemical treatment was shown by the following:

a. The precipitin titer of antisera from the three groups of animals was the same whether the antigen used was horse serum, horse serum treated with l-ascorbic acid, or horse serum treated with d-iso ascorbic acid.

b. The suppression phenomenon of Landsteiner

was not obtained when antisera from the three groups of animals were treated with small quantities of l-ascorbic and d-ascorbic acid previous to titration. Table V gives the results of three rabbit sera so treated. Serum number 782 is from a rabbit in group two which received only horse serum. Serum number 464 is from a rabbit in group one which received horse serum plus Vitamin C. Serum number 793 is from a rabbit in group three which received horse serum plus d-iso ascorbic acid.

c. Skin tests on rabbits from each of the three groups were made. No reactions were observed except when horse serum or treated horse serum was used. The l- and d-ascorbic acids alone gave no skin reactions in rabbits from any of the three groups.

d. Injection of horse serum, of l-ascorbic acid, and of d-ascorbic acid separately into animals of each group were made several months after the termination of the original studies. Antibodies did not reappear except in those animals reinjected with horse serum. In these animals a rapid rise to high titer occurred, the anamnestic or "hair-trigger" reaction.

TABLE V.

SUPPRESSION EFFECT OF VITAMIN C AND D-ISO

Antisera were absorbed by adding 0.1 mgm. of pure crystaline chemical to 0.5 cc. antiserum and allowed to stand fifteen minutes before titrating.

	Anti-serum absorbed with	Dilution of Horse Serum 2560	5120	10240	20480	40960	81920	Control
Control #782	none	+	+	+	+	-	-	-
	Vitamin C	+	+	+	+	-	-	-
	d-iso Vit.	+	+	+	+	-	-	-
Vitamin C #464 horse serum	none	+	+	+	+	+	-	-
	Vitamin C	+	+	+	+	+	-	-
	d-iso Vit.	+	+	+	+	+	-	-
d-iso Vitamin C. #793 horse serum	none	+	+	+	+	+	-	-
	Vitamin C.	+	+	+	+	+	-	-
	d-iso Vit.	+	+	+	+	+	-	-

DISCUSSION:

By none of these tests were we able to demon-

strate a union or combination of the horse serum with the ascorbic acid. If any combination does occur, it is not detectable by these immunological procedures. Lloyd and Shore state that "for a globulin hydrochloric acid system in dilute concentrations of acid (pH 2.0) the protein salts exist in an almost completely ionized condition". They also state that acid denaturation of egg albumin starts at pH of 3.9; so it is possible that some of the proteins of horse serum are slightly denatured when treated with ascorbic acid. The strong possibility remains that the effect is on the host tissues as well as on the antigen. Coriell (1936) has reviewed the literature on the effect of colloidal particles in stimulating antibody production. It was shown that crystalline egg albumin is a better antigen when absorbed on the surface of colloidal carbon particles (India ink).

Further studies were made using other acids both organic and inorganic in place of ascorbic acid. Acids used were hydrochloric, acetic, and propionic. They were adjusted to the same pH value as ascorbic acid (2.5 to 2.6), incubated with horse serum in the same way, and injected into small groups of rabbits. The antibody production in these rabbits was similar to the response when ascorbic acid was used, namely,

delayed initial response and later a sharp rise to high titer.

These results indicate that the stimulating effect of ascorbic acid is due to its acidity acting on the antigen and possibly on the host tissues (anamnestic reaction). Future work with acidified antigens may prove them superior to the usual antigens dissolved or suspended in neutral saline.

WHAT IS THE EFFECT OF VITAMIN C IN CATS?

In view of our work with rabbits it is probable that Vitamin C would not stimulate antibody production in cats. The acidity of Vitamin C might give the same effect as in rabbits.

EXPERIMENTAL:

Four adult cats were employed. A culture of S. suipestifer isolated from a mastoid infection in man by Sherwood (1936) was used. In each case where Vitamin C was used, it was mixed with the organism suspension just before injection. Vitamin C determinations were run on the blood of cats 56 and 57 before injections were started. Cats 10, 57, and 56 were normal cats. Cat 11 had been given a series of injections with S. enteritidis eight months previously and had

failed to develop demonstrable agglutinins for this organism (see Table I).

The data and results are seen in Table VI.

Cat No.	Antigen	Vitamin C.	Date	Amt.	Route	Highest Agglutinin titer
10	S. suipestifer living	25 mgm. Squibb	10/31	1.0 cc.	i.perit.	
	" "	"	11/4	"	"	
	" "	"	11/24	2.0 cc.	"	1-1280
11	" "	"	11/11	2.0 cc.	i. card.	
	" "	"	11/24	2.0 cc.	i. perit.	1-5120
57	S. suipestifer formalized	none	4/23	1.0 cc.	i. perit.	
	" "	"	5/3	1.0 cc.	Subcut.	
	" "	"	5/9	1.0 cc.	Subcut.	1-1000
56	3% Egg Albumen	none	4/23	1.0 cc.	Subcut.	
	" "	none	5/3	"	"	
	" "	none	5/9	"	"	
	" "	25 mgm.	5/16	"	"	
	" "	"	12/22	2.0 cc.	i. perit.	no precipitins

Cat number 11 is the same cat shown in Table I, which failed to produce agglutinating antibodies for S. enteritidis. Eight months after the last injection

of Cat 57 shown above its serum still agglutinated S. suipestifer in dilution of 1-40. On intraperineal injection of two cubic centimeters of living broth culture at this time caused the agglutinin titer of the cat serum to rise to 1-640 one week later.

DISCUSSION:

Vitamin C did not give any clear cut enhancement of antibody production in these four cats. The acidity may have enhanced antibody production in cats 10 and 11, but cat 56 injected with egg albumin did not produce demonstrable precipitins for this antigen. Precipitins could not be detected whether by the ring technic nor by the agglutination of sensitized collodion particles. Three cats injected with S. suipestifer developed agglutinins in high titer. Whether the culture was living or formalized seemed to make no difference. Two cats injected wtih organisms plus Vitamin C gave good antibody response. One cat injected with formalized organisms alone gave good antibody response. We may conclude that this culture of S. suipestifer is effective in stimulating antibody production in cats. The blood Vitamin C level of cat 56 was 0.27 milligrams; of cat 57 it was 0.23 milligrams per hundred cubic centimeters of blood.

SUMMARY OF WORK WITH VITAMIN C:

The conclusions of our work with Vitamin C may be stated as follows:

1. Refractory rabbits are not stimulated to greater hemolysin production by injection of Vitamin C.

2. In a larger series of unselected rabbits there is an increased precipitin response in those animals injected with Vitamin C treated horse serum.

3. An almost identical response occurred when the physiologically inert d-form of ascorbic acid was substituted for Vitamin C.

4. The appearance of precipitins in the blood stream occurs later in rabbits injected with treated horse serum than in those injected with untreated horse serum.

5. The antigenic specificity of the horse serum was not altered by treatment with the l- or d-forms of ascorbic acid.

6. Work with unrelated acids in place of ascorbic acid gives rise to an antibody response in rabbits which is comparable to that obtained when ascorbic acid is employed.

7. The blood level of Vitamin C in our rabbits

was about 0.13 milligrams percent.

8. Vitamin C did not stimulate cats to greater antibody production when using S. suipestifer as the antigen. This organism was found to stimulate the production of agglutinins without the presence of Vitamin C.

9. Vitamin C did not stimulate one cat to produce precipitins for egg albumin.

WHAT IS THE NORMAL INTESTINAL FLORA OF CATS?

Before doing further work on the feeding of Salmonella to cats, we decided to study the normal bacterial flora of the intestine. If normal cats ordinarily harbor bacteria of the Salmonella group, it might account for their natural immunity to infection with the organism. Furthermore, the normal presence of such organisms would make it difficult to know when we were dealing with our original culture and when with a new contaminant.

EXPERIMENTAL:

The intestinal contents of eight normal adult cats and of eight kittens was examined. The kittens were from the same litter, aged six weeks. Feces samples were withdrawn by enema and plated on suitable

media. All different types of colonies were picked and run through routine culture media for identification.

RESULTS:

The results of this work can be stated briefly. No organisms belonging to the Salmonella group were ever found in any of the sixteen animals examined. A typical coli-aerogenes flora was present. The most frequent organisms found were Es. coli, A. aerogenes, A. cloaceae, Proteus vulgaris, Alkaligenes fecalis, aerobic Gram positive spore formers, molds, and from one cat, Pseudomonas aeruginosa.

DISCUSSION:

The organisms present in cat feces were no different than those commonly encountered in other mammals. The fact that there were no members of the Salmonella group makes it possible to use the cats for feeding experiments. We have previously shown that adult cats could not be infected by oral feeding of S. enteritidis. This organism was never recovered from the feces or blood stream of the adult cats and agglutinins were not developed following the oral feeding of living organisms. It was thought that

young kittens might be more susceptible to infection. The eight young kittens used in the previous experiment were tested.

EXPERIMENTAL:

The eight kittens were divided into two groups of four each. One group was infected by feeding two cubic centimeters of a twenty-four hour broth culture of S. enteritidis in milk to each kitten. The remaining four kittens were not infected. The course of infection was followed by making blood cultures, feces examination, tests for presence of agglutinins, and bactericidal action of serum. In the bactericidal tests serum was used undiluted and diluted 1-50 with saline. One-half cubic centimeter of serum and of diluted serum were added to an equal amount of diluted living organisms. Suitable controls were included. Tubes were mixed, incubated at 37°C for one hour and plated in plain agar.

RESULTS:

None of the infected kittens were ill or off feed during the course of the experiment. Table VII gives the data.

TABLE VII.

Kitten Number	Infected 4/30	Blood Culture 5/1	Feces 5/1	Blood Culture 5/12	Feces 5/12	Agglutinins 5/12	Bactericidal 5/12
20	orally 2.0 cc. broth	Sterile	normal flora	sterile	normal flora	1-80	Sterilized
21	"	Sterile	normal	sterile	"	1-80	"
22	"	Enteritidis	normal	sterile	"	0	"
23	"	Enteritidis	Enteritidis	sterile	"	1-20	few colonies
24	none	sterile	normal		"	0	
25	none	sterile	normal		"	0	sterilized
26	none	sterile	normal		"	0	"
27	none	sterile	normal		"	0	"

DISCUSSION:

Two of the four infected kittens developed positive blood cultures twenty-four hours after feeding. One of these kittens had S. enteritidis in the contents of the colon. Twelve days after feeding all the kittens gave negative blood cultures and no enteritidis in feces. Three of the infected kittens developed agglutinins and one did not. The kittens were apparently susceptible to enteral infection with the organism. Infection was transient and not sufficiently severe to cause clinical symptoms. We base our conclusions that the kittens suffered transient infection on the presence of positive blood culture in two, positive feces culture in one, and production of agglutinins in three of the kittens. As shown in Table VI, the two infected kittens which did not show positive blood or feces culture developed agglutinins in dilution of 1-80. It will be recalled that adult cats fed orally did not develop agglutinins. Apparently the intestinal tract of kittens allows this organism to pass into the blood stream or by other means to gain access to tissues and stimulate antibody production.

On the other hand we found no difference in bactericidal power between the serum from the two

groups of kittens. The serum from all kittens gave complete sterilization of the culture with one exception. A few organisms survived in the 1-50 dilution of kitten serum No. 23. There was no correlation between agglutinin titer and bactericidal power of serum. The serum of normal six weeks old kittens have bactericidal properties equivalent to that of the innoculated and recovered kittens. Even in these kittens which are more susceptible than adult cats, we were able to recover our organism from the intestine in only one out of four. The next question to be answered was, what happens to Salmonella organisms fed orally to a cat? Are they destroyed in the stomach or intestine? How long do they survive in these locations?

A preliminary experiment was made by feeding a normal cat fifty cubic centimeters of autoclaved milk which was innoculated with a living culture of S. suipestifer. The cat's stomach was pumped after twenty minutes and again after eighty-five minutes. The bacterial count of the milk when fed was 47,000,000 per cubic centimeter. The count on stomach contents was: twenty minute sample, 33,000,000 per cubic centimeter; eighty-five minute sample, 1,450,000. Both samples were sterile after standing twenty-four hours

in the 37° incubator. The gastric juice was capable of killing all the organisms in the milk if given long enough to act in vitro. Would the organisms be exposed to the action of the gastric juice long enough to be all killed in vivo. This seemed unlikely. Physiologists tell us that the stomach begins to spill over its contents into the duodenum soon after food is eaten. If this is so, many of the injested organisms must be poured over into the small intestine. What becomes of them? Do they remain there or are they, too, soon destroyed?

EXPERIMENTAL:

Two normal adult cats were each fed 100 cubic centimeters of plain broth containing living S. suipestifer. The cats were fed by stomach tube. One hour later cat number 55 was bled from the heart and the cat sacrificed and autopsied. Blood cultures were made. Stomach contents were removed with a sterile syringe and analyzed. The gastrointestinal tract was removed and examined for S. suipestifer. Seven hours after feeding, cat 54 was sacrificed and autopsied as above.

RESULTS:

Cat 55 sacrificed and autopsied one hour after feeding:

Bacterial count of broth when fed to cat 12,000,000/cc.

Bacterial count of stomach contents at autopsy 10,000/cc.

Bacterial count of stomach contents after five hours at 37° C------200/cc.

Bacterial count of stomach contents after 30 hours at 37°C------sterile.

Gastric analysis of stomach contents at autopsy:

Free hydrochloric acid----------65

Bound acid----------53

Total acidity----------118

Blood culture sterile

S. suipestifer was found present in the duodenum, jejunum, and ileum; not in the colon.

Cat 54, stomach was pumped at the end of one hour and found to be empty. Cat was sacrificed and autopsied seven hours after feeding.

Blood culture sterile

Stomach empty and sterile

S. suipestifer was not found in the duodenum,

jejunum, ileum, or colon.

DISCUSSION:

These results corroborate our previous finding that the gastric juice is bactericidal. The strongly acid reaction no doubt accounts for much of this bactericidal action. Future work on fractionation of the gastric juice may clarify this mechanism. After entering the intestine the surviving organisms would escape the acidity of the stomach when neutralized by intestinal and pancreatic juices. However, the organisms which escaped into the intestine were also destroyed as shown by cat 54. We next decided to extract the intestinal mucosa of cats and try the bactericidal effect of the extracts. Perhaps there is a concentration of antibodies or "hormones" held to the tissues and not liberated into the blood stream.

EXPERIMENTAL:

Crude extracts of the intestinal mucosa were prepared. Extraction was patterned after the method used by Roberts, Macgraith, and Florey (1938) in preparing active lysozyme extracts. They found that a saline extract of acetone dried minced material contained the lysozyme.

Our cats were first bled to death and the blood preserved in the ice box. Bile was withdrawn from gall bladder under sterile conditions and saved. The entire small and large intestine were removed and flushed with water to remove fecal material. The mucosa was scraped off with a sharp scalpel and extracted in fifty cubic centimeters of physiological saline for fifteen hours at ice box temperature. The sticky mass was then filtered, first through cheesecloth and then through a Seitz filter. This was called the saline extract.

The acetone insoluble extract was made by adding 300 cubic centimeters of acetone to the mucosa pulp. This was shaken frequently and allowed to stand in the ice box for twelve hours. The white acetone insoluble precipitate was then dried, extracted with fifty cubic centimeters of saline, filtered, and tested for sterility. This was called the acetone insoluble extract. Agglutination tests were made using S. enteritidis, S. aertrycke, S. suipestifer asantigens. They were set up against blood serum, water extract, and acetone insoluble extract. Bactericidal properties for S. enteritidis were determined. Bile and urine were included in the bactericidal tests. Guinea pig complement was used in all the bactericidal tests.

Two cats were treated in this manner. Cat number 12 had been exposed to oral feeding of living S. enteritidis four months previously. Cat number 35 was a normal adult.

RESULTS:

No agglutination was observed with materials from cat number 12. The blood serum gave slight bactericidal activity, while the saline and acetone extracts were not active. The saline extract from cat number 35 was quite mucilaginous and tended to give nonspecific agglutination in low dilution. Serum was bactericidal in low dilution. Bile, urine and intestinal extracts gave no bactericidal action.

DISCUSSION:

Nothing was revealed by these experiments which would account for the disappearance of organisms from the intestine. The only clue we have is the gastric juice. Further work will be done with gastric juice in an effort to isolate its bactericidal elements.

Since S. enteritidis is so quickly eliminated from the intestinal tract, this may account for the failure of oral administration of organisms to provoke antibody production. Seven hours after feeding the

organism can no longer be found in the contents of the alimentary tract nor in the mucosa. It does not gain access to the blood stream following oral feeding in adult cats. The question naturally arose, What happens to the organisms when injected directly into the blood stream? Are they destroyed as quickly as in the alimentary canal? Previous experiments had indicated that the blood stream gives positive cultures as long as five hours after intravenous injection of living cultures into adult cats.

EXPERIMENTAL:

Six normal adult healthy cats were examined and no Salmonella organisms were found in the feces. Blood cultures were sterile. Each cat was then injected intracardially with one and one-half cubic centimeters of a twenty-four hour broth culture of S. enteritidis. At intervals thereafter the cats were sacrificed, blood cultures were made and all the organs examined for the presence of S. enteritidis. All levels of the intestine were examined by streaking on E.M.B. plates. Kidney, spleen, liver, lymph nodes, lung and other organs were removed aseptically. The organ was sliced with a sharp sterile knife and the freshly cut surface rubbed over an E.M.B. plate. A

piece of the organ was then dropped into a tube of sterile broth. If the direct smear plate proved to be negative, then the incubated broth was subsequently streaked out. Pericardial fluid, urine, and bile were tested by placing one cubic centimeter in lactose broth and streaking on E.M.B. agar. All plates and cultures were incubated at 37° C. Plates were scanned for presence of S. enteritidis and when found it was verified by cultural and agglutination reactions.

RESULTS:

Table VIII shows the results of this study. A plus sign indicates that S. enteritidis was recovered. A minus sign indicates that S. enteritidis was not recovered.

DISCUSSION:

The organism was not eliminated quickly when introduced into the blood stream. After ten hours the blood stream was still positive, so obviously the cut surface of all organs was positive. A few organisms were found in the spleen in one cat 120 hours after injecting. The presence of S. enteritidis in the intestine is interesting. We interpret this as meaning that leucocytes have ingested bacteria and

TABLE VII.

Cats autopsied - hrs. after injection -	Blood Culture	Colon	Jejunum	Ileum	Spleen	Ileocecal lymph n.	Liver	Kidney	Bile	Urine	Pericard. Fluid	Lung
10 hrs.	+	+	-	+	+	+	++	+	-	-	+	
24 hrs.	-	-	-		+ +	-	++	+	-	-		
36 hrs.	-	-	-	-	+ +		+	+	-	-		+
48 hrs.	-	-	-	-	+ +		+	+	-	-		+
72 hrs.	-	-	-	-	+		+	+	-	-		+
120 Hrs.	-	-	-	-	+ broth		-	-	-	-		-

migrated into the intestinal mucosa. After twenty-four hours the blood cultures became negative and organisms were no longer found in the intestinal tract. Further evidence of a phagocytic mechanism being active is the localization of the organisms in organs containing reticulo-endothelial tissue.

Bile and urine were found sterile in all cats. When the blood culture was positive, one would expect organisms to be eliminated through the kidney into the urine. Milligan (1917) found bacteria constantly eliminated in the urine in man. During periods of oral infection the causative organism was isolated from urine. This was not the case with cats. Our previous experiments showed that bile and urine of normal cats have no bactericidal effect on S. enteritidis. We conclude that these organisms are filtered from the blood stream by reticulo-endothelial organs and destroyed therein. Whether the bacteria are actually killed by phagocytes or find therein a haven of refuge from the bactericidal action of humoral elements remains to be investigated.

Opsono-phagocytic tests were run at this time on five normal cats, and one cat immunized with S. suipestifer. The organisms used were S. schottmulleri, S. suipestifer, and S. enteritidis.

We found the leucocytes of all normal cats quite actively phagocytic for the three organisms, there being no significant difference in reaction among the three. The average number of bacteria per leucocyte of normal cats was about seven, while the average in the case of the actively immunized cat was nine.

TO WHAT EXTENT DO NORMAL BACTERIOLYSINS PLAY A ROLE IN THE IMMUNITY OF CATS TO SALMONELLA INFECTION?

In view of the phagocytic activity of cat leucocytes, we wondered how effective these leucocytes are in killing bacteria. It was thought that the comparative bactericidal properties of heparinized whole blood and of plasma might give some information on this point. We have already shown that the serum of normal kittens is bactericidal for S. enteritidis. Some of the other questions which we hope to answer by experimentation are: Is the bactericidal property of normal cat serum destroyed at 55° C? Can an antibody-complement mechanism be demonstrated? Is the reaction specific for given organisms?

EXPERIMENTAL:

Presence of bacteriolysins was determined for

five organisms, S. enteritidis, S. suipestifer, S. schottmulleri, E. typhi, and E. dysentery flexner, by the method outlined in the section on technic. Fresh whole blood and plasma from the same animal were compared for bactericidal action on these organisms. Sera were heated for inactivation in a 55° C water bath for thirty minutes.

No normal cat serum could be found which did not have bactericidal properties, so in order to determine if a complement were active we had to absorb the normal cat serum at 0° C. Organisms were washed from French squares in sterile saline, packed in the centrifuge, and resuspended in fresh sterile saline. They were killed by heating to 60° C for one hour, tested for sterility, and centrifuged again to pack. Sufficient sterile saline was then added to make a thick mush and the clumps emulsified by drawing up and down in a sterile pipette. This suspension of organisms was added to the fresh serum at 0° C and incubated for two hours with frequent shaking. The organisms were then thrown down, and the supernatant reabsorbed with more organisms at 0° C. The resulting supernatant was called "absorbed" serum if inactive per se towards unsensitized bacteria. Living bacteria were sensitized by mixing with an

excess of fresh serum at 0°C for two hours, then washing in saline at 0°C. Likewise, living bacteria were sensitized in inactivated serum at a temperature several degrees above freezing, then washed in saline to remove the excess of serum. Sherwood had pointed out that some inhibitory substance might be present in the inactivated serum which would interfer with the later binding of complement. These bacteria were exposed to absorbed serum and to guinea pig complement. Guinea pig serum used as complement was titrated by the Kolmer technic and an amount equal to at least one full unit was always used. Many controls were included because it was found that guinea pig serum itself has some bactericidal effect when used undiluted.

Complement titrations were run on fresh cat sera, using the Kolmer technic.

RESULTS:

The blood and plasma of eighteen cats and kittens tested all had bactericidal properties for S. enteritidis. Whole blood was found to be more effective than plasma in killing S. suipestifer, but no more effective than plasma in killing the other four organisms. Plasma from none of the cats was very ef-

fective in killing S. suipestifer. This was in marked contrast to the effect of plasma on S. enteritidis, S. schottmulleri, E. typhi, and E. flexner. The whole blood and plasma of a cat actively immunized to S. suipestifer (Cat 57 of Table VI) was no more bactericidal than the blood of normal cats. This recalls to mind the similar experience with kittens (Table VII).

All bactericidal action of blood or plasma was destroyed by heating to 55° C. for one-half hour. It was impossible to reactivate this heated cat serum with guinea pig complement except in the case of S. enteritidis.

Heated cat serum always regained part of its bactericidal power for S. enteritidis when guinea pig complement was added, although it was not restored to its original potency. Neither was it possible to reactivate heated serum by adding absorbed cat serum.

When great precautions were taken to keep the serum at 0° C. we were unable to absorb the bactericidal property of fresh serum.* The bacterial growth from as much as eight French squares was used to absorb three cubic centimeters of serum without success. In one experiment when the serum was allowed to warm up a few degrees during absorption with S. enteritidis there was almost complete removal of antibodies

* After absorption period of 4 hours.

for this organism as well as for Para B and E. typhi but not for E. flexner. This absorbed serum would not reactivate heated cat serum, an altogether logical finding since any complement present would probably be bound at the higher temperature.

Living bacteria exposed to fresh serum at 0° C. for two hours then washed free of excess serum were not lysed by the addition of guinea pig complement. Likewise, living bacteria exposed to inactivated serum for two hours at a higher temperature and subsequently washed free of excess serum were not lysed by the addition of guinea pig serum. In neither case could we demonstrate that the bacteria were "sensitized". At 0° C. the bactericidal agent is inactive, nor is it bound to the bacteria*, while after inactivation it is so modified as to be unable to lyse or to sensitize at a higher temperature. Since we could not render fresh serum inactive per se toward unsensitized bacteria by absorption at 0°C. it was impossible to demonstrate a cat complement by adding "absorbed"serum to "sensitized" bacteria.

Fresh plasma of seven cats gave no trace of complement in a Kolmer titration. One cubic centimeter of a 1-10 dilution in saline was without detectable effect. Two of the cats gave partial hemolysis

* As a result of 4 hours of absorption.

of a Kolmer unit of red cells when one cubic centimeter of fresh plasma was added to the unit of red cells.

DISCUSSION:

It is not surprising that the blood of normal cats is bactericidal for these organisms. Immunologists have long known that fresh serum of many animals is bactericidal for E. typhi (Browning, 1927)(Von Fodor, 1886). Mackie and Finkelstein have recently (1931) studied the mechanism of natural bactericidal action of serum of ox, sheep, horse, rabbit, guinea pig, rat, and man for Dysenteriae shiga, E. typhi, Proteus vulgaris, and Vibrio cholerae. They did no work with the cat. In all these animals they demonstrated the presence of specific normal antibodies which were fixed within 4 hours at 0°C. stable at 55°C, but labile at 60-65°C. Complement from all species was interchangeable. Our work with the cat does not agree with their findings except perhaps on the question of specificity. Even on this point there seems to be a contradiction at first glance. In their work they used four organisms antigenically unrelated and found the antibodies specific, while we used four antigenically related organisms and one unrelated organism, E. flexner. It

was found that absorbing the serum with S. enteritidis, for example, removed the bactericidal properties for the group of related bacteria but not for E. flexner. We, therefore, conclude that the normal bacteriolytic principle is group specific.

We agree with Mackie and Finkelstein that the lytic effect of fresh serum is destroyed at 55°C, but beyond this point we could not get results in the cat similar to theirs with other animals and man. They say that the growth from twelve agar slants was sufficient to absorb the bactericidal properties from five cubic centimeters of serum in two hours at 0° C. This was two and one-half agar slants per cubic centimeter, while we were unable to absorb cat serum using the equivalent of fifty agar slants for four hours at 0°C. This would indicate a difference in amount of antibody to be absorbed or in avidity for antigen at this temperature, except for the fact that it failed to conform in other respects to the normal antibodies they described. First, guinea pig complement failed to lyse "sensitized" bacteria. Second, guinea pig complement failed to reactivate heated cat serum except for enteritidis. Lastly, we could not demonstrate complement in the fresh plasma of seven cats by the Kolmer technic. Apparently guinea pig comple-

ment will not activate most normal cat bacteriolysins and conversely, cat complement if such a distinct serum component exists in the cat, is not active in a rabbit-hemolysin sheep cell system. This statement must be qualified for S. enteritidis. In all the tests run it was found that guinea pig complement in amount which was inactive per se, was fairly active for this organism when mixed with inactivated cat serum. Dingle, Fothergill and Changler (1937) have found that guinea pig complement does not activate all immune bacteriolysins, although they did no work with normal bacteriolysins. The bactericidal function of anti H. influenzae horse serum was activated by human complement only, and not by rabbit, horse, or guinea pig complement; and anti-H. influenzae rabbit serum is activated by human and rabbit complement, but not by guinea pig complement. This is interesting in view of the fact that guinea pig serum will activate other rabbit amboceptors, such as sheep cell hemolysin. Complement is apparently more selective than was thought at one time. Muir and Browning (1908-1909) originally suggested that different moieties of complement are concerned in hemolysis and bacteriolysis respectively. Mackie and Finkelstein (1930) report that, unlike hemolytic complement, the complement concerned in bacteri-

cidal action is absorbed by charcoal. Our work with the cat indicates that guinea pig complement is not effective in activating normal cat bacteriolysins for Para B, suipestifer, E. typhi and flexner but is active when the antigen is enteritidis. Apparently the complements of these two species are not interchangeable.

CONCLUSIONS:

The blood of all normal cats and kittens tested was bactericidal for bacteria of the Salmonella group.

There was no correlation between presence of agglutinins and bactericidal properties. Normal agglutinins were never present while blood of all normal animals was bactericidal.

Whole blood was bactericidal for S. suipestifer whereas plasma was slightly active. No difference could be noted between whole blood and plasma for other organisms tested.

The bactericidal property of normal cat serum is destroyed by heating to 55° C for one-half hour. It can be partially reactivated for S. enteritidis by guinea pig complement, but not for the other bacteria used.

The bactericidal properties of fresh cat serum can not be absorbed within a period of 4 hours at 0° C by using an excess of bacteria, but can be absorbed at a few degrees above this temperature.

The completely "absorbed" serum does not reactivate cat serum which has been inactivated by heat.

The bactericidal property of cat blood is group specific for Salmonella and E. typhi. This is given a logical antigenic basis by the work of White (1929). He showed that the "RO" antigen was the same for all Salmonella and E. typhi.

Bacteria are not sensitized nor lysed in fresh cat serum at 0° C during a period of 4 hours, presumably because of failure of the toxic substance to be bound at this temperature. Neither were living bacteria, including S. enteritidis, sensitized in inactivated serum at a temperature several degrees above zero. This we interpret as further proof that the natural antibody in cat serum is destroyed or greatly modified by inactivation temperature.

The serum of seven cats gave no trace of complement when titrated in a standard Kolmer system.

If our absorption work is technically correct, it indicates further that the bactericidal action of normal cat serum does not depend on an antibody-com-

plement mechanism, but upon a thermolabile substance which is not fixed at 0°C and not capable of reactivation.

We call this bactericidal property of fresh cat serum "complement-like" because it is thermolabile and not fixed at 0° C. In reality it has more resemblance to a toxin; a substance which attaches itself to and causes death and degeneration of the bacterium. Van de Velde in 1894 first described a toxic substance present in staphylococcus broth cultures which is capable of destroying white blood cells. This so-called leucotoxin is heat-labile and fixed by the leucocytes; it is especially active on rabbit white cells, less so on those of other animal species (Gay, 1935). The hemotoxin or hemolysin of streptococci does not require complement for its action, is destroyed by oxidation and is readily destroyed by heating at 56° C. Even a toxin like diphtheria toxin cannot be reactivated when the toxophore group is once destroyed. We might justifiably call the bactericidal principle of cat serum a "bacterotoxin". It differs also from the "beta" lysins, "leukins" and "plakins" of the older literature in that they are all heat stable (75° for thirty minutes) and active as a rule only for gram positive bacteria. The so-

called "alpha" lysine are analogous to what Mackie and Pinkelstein (1931) have demonstrated i.e., a normal thermostable sensitizer which is dependent upon the presence of a thermolabile complement for its activation. Separation of serum proteins into albumen, globulin, complement, or ambocepter fractions is after all an artificial separation and they probably do not exist as separate entities in vivo.

Pending further experimental study we were unable to identify the bactericidal property of cat serum with anything that has been described heretofore. Since an antibody-complement mechanism could not be detected to account for the toxic effect of cat serum on bacteria we have proposed the name "bacterotoxin" to designate this substance. The immediate future work calls for further elucidation of the properties of this substance together with the development of new technics for demonstrating its mode of action.

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It was thought that a rapid survey of several other infectious agents might prove of interest and value in view of the meager data on experimental cat infections. Consequently, we tried to infect cats with several virulent organisms which were available in our laboratory.

ARE CATS IMMUNE TO TRICHINELLA SPIRALIS?

It would not be surprising to find the cat immune to infection with this swine nematode. The cat is constantly exposed to infection by eating trachina-infected mice and rats. If natural selection is at all active in immunity it might be expected that the cat would evolve some kind of protection against this common parasite of his stable food. The only data available is a report of Jensen (1937) that out of 451 cats examined in Copenhagen only 8 were trachinous. We proposed to determine if a young healthy cat would survive a massive infection.

EXPERIMENTAL:

An albino rat infected with trachina was obtained from Beahm (1939). The skeletal muscles of the rat were removed and ground in a meat grinder. The ground meat was thoroughly mixed and samples counted for worms by the pressed-preparation method. This strain of Trichinella spiralis regularly killed 250 gram albino rats when given by mouth in doses of 900-1000 larvae (Beahm and Downs, 1939).

A young healthy male cat weighing 1600 grams was selected. This cat had been raised in the labora-

tory from the time it was weaned. Infected rat meat containing 26,400 larvae was fed to this cat. Total white and differential blood counts were made daily before infection and at weekly intervals after infection.

RESULTS:

Twenty-four hours after infection the cat refused food, acted as if in pain, and emitted plaintive meows when handled. Loose watery stools were passed. The cat refused to eat meat for ten days and became thin and emaciated. Twenty-two days after infection the cat weighed 1150 grams. One month after infection the cat was eating normally and gaining weight rapidly. Three months after infection the cat weighed 2650 grams.

The average blood picture before infection was:

Total white cells-----average 23,500
Neutrophiles----------average 40.0 percent
Lymphocytes----------average 50.0 percent
Eosinophiles----------average 3.0 percent
Basophiles----------average 0.0 percent
Monocytes----------average 6.0 percent

At no time did the total white count go above 26,000. It fluctuated between 22,000 and 26,000. There was no

significant change in the differential count. The eosinophiles did not rise above four percent.

The cat had been made ill by the ingestion of the trachina infected rat meat, but had not succumbed to this massive infection. Now the question was, had the larvae encysted in the musculature of the cat? If so, were they viable? Were there any protective antibodies in the convalescent cat serum?

The cat was anesthetized, bled from the heart, and sacrificed. Examinations showed numerous encysted larvae in the skeletal muscles. The diaphragm contained an average of 192 larvae per gram. Intercostal muscles and triceps showed about half as many. Sufficient of this ground cat meat was fed to three rats to give them 900-1000 larvae each. Rat number one was given no protection. Rat number two was given 5.0 cc. of fresh convalescent cat serum intraperitoneally. Rat number three was given the same amount of cat serum and repeated next day, 10 cc. in all. All three rats died between 32-39 hours after infection.

DISCUSSION:

From our experience with this cat we may surmise that the small number of infected cats reported

by Jenson is not due to failure of the cat to become infected. The cat does become infected and the larvae encyst in the skeletal musculature. This cat withstood a per kilo dose five times as great as the fatal dose for albino rats. There was no accompanying leucocytosis nor eosinophilia. In this latter respect the cat differs from man and is similar to the rat. The encysted larvae in the cat are viable and infectious for the rat. No protective antibodies were demonstrated in the convalescent serum.

Leonard (1940) has worked with trachina infection in rats and rabbits. It is his impression that passive immunity is of no value. However, in rather extensive studies on infection of rabbits with the dog tapeworm he has been able to show a marked immunity to reinfection. He was unable to demonstrate precipitins in the serum of convalescent animals yet this serum was effective in passiv ely protecting rabbits. He believes the barrier to infection lies in the intestine since larvae injected into the portal circulation of passiv ely protected animals encyst normally in the liver. Anderson (1939) has demonstrated reinfection immunity to trachina in rats by an ingenious technic. Rats are infected with male or female adult worms only, then after four weeks the rats are fed and in-

fecting dose of larvae. At a later date the number of encysted larvae in the rats tissues are counted and compared to the number in normal rats fed the same infective dose of larvae. The rats previously exposed to male or female worms showed as much as ten fold reduction in the number of encysted larvae. Taliaferro (1929) finds the available literature indicats that convalescent serum does not produce immunity or parasitocidal action. It may neutralize the toxic effect of worms but the data is inconclusive. Our work suggests that convalescent cat serum is no more effective in conferring passive immunity.

ARE CATS IMMUNE TO SUBCUTANEOUS INJECTION OF PASTEURELLA TULARENSIS?

EXPERIMENTAL:

A culture of P. tularensis was obtained from Downs at the University of Kansas. This culture was designated at "Rasmussen". Downs had shown that the culture was extremely virulent for mice. One-tenth of a cubic centimeter of a 1-100,000 dilution was fatal for white mice when injected subcutaneously. A week old culture on dextrose-cystine blood agar was suspended in sterile saline and diluted to match the 500 p.p.m. silica turbidity standard. Dilutions were

made from this suspension.

Nine adult cats were tested for the presence of agglutinins for P. tularensis. Two of these were selected at random and given subcutaneous injections of living P. tularensis. Two injections were given eight days apart. The first injection was one-half cubic centimeter of standard suspension in saline. The second injection equalled two cubic centimeters of standard suspension. Fresh suspensions of organisms were made up each time. Viability of the culture was controlled by injecting white mice.

The cats were observed for local lesions, swollen lymph nodes, and for general state of health. Agglutination tests were made at intervals on the cats' blood serum.

Two other cats were given injections of another strain of P. tularensis isolated from a fatal human case of tularemia at the University of Kansas Hospitals. The culture used was a two month growth on dextrose-cystine blood agar made up to standard turbidity in saline. Simultaneous injections were made into guinea pigs to determine the virulence of the culture. The cats were observed as before. Total red and white blood counts were made at weekly intervals.

RESULTS:

The two cats injected with "Rasmussen" exhibited no lesions or symptoms of illness during a period of two months observation. All guinea pigs and mice injected with this strain died of generalized tularemia. Blood serum from both cats agglutinated a formalized suspension of the organism in dilution of 1-640.

The two cats injected with the second strain of P. tularensis showed no gross lesions nor symptoms of infection. Blood serum from these cats did not agglutinate P. tularensis at any time. None of the guinea pigs injected with this latter strain succumbed. Apparently the culture was non-~~viable~~ virulent when used. There was no change in total red or white blood count.

DISCUSSION:

As mentioned before, there have been instances in which cats have been suspected of transmitting tularemia to man through bites or scratches. If true, this would not indicate whether the cat was actually suffering from the disease or merely acting as a carrier. The latter is highly possible in view of the fact that the cat's teeth and claws are apt to be contaminated with the fresh blood of small rodents and rabbits.

Our experimental results indicate that the adult cat has considerable immunity. A massive dose of a highly virulent organism failed to cause death in two cats. Neither of the animals was visibly affected. However, they did respond by production of agglutinating antibodies. This may be evidence for some sort of a latent or transient infection especially since the two cats injected with a non-~~viable~~ virulent culture failed to produce agglutinating antibodies.

ARE CATS IMMUNE TO SUBCUTANEOUS INJECTION OF BACILLUS ANTHRACIS?

EXPERIMENTAL:

A culture of Bacillus anthracis was passed serially through guinea pigs to increase its virulence. One-tenth of a cubic centimeter of a twenty-four hour broth culture was injected subcutaneously into each guinea pig and proved uniformly fatal with septicemia in four days. A young cat weighing 1800 grams was injected subcutaneously with one-half cubic centimeter of this culture. Blood cultures were made from the cat's blood at two days and seven days after injection. Five cubic centimeters of blood were drawn aseptically, placed in 200 cc. sterile broth, and incubated at 37° C for one week.

RESULT:

The injected cat showed no outward symptom of being ill. It was never off feed at any time during the period of observation of two weeks. There was no loss of weight. Blood cultures proved sterile. There was no visible local lesion at the site of injection.

DISCUSSION:

Apparently this cat was immune to infection with B. anthracis. After running opsono-phagocytic tests on several normal cats, we found that the leucocytes of normal cats were actively phagocytic for B. anthracis. There were an average of sixteen to twenty phagocytized bacteria per cell.

ARE CATS IMMUNE TO INTRACUTANEOUS INNOCULATION WITH VACCINIA?

EXPERIMENTAL:

An Eli Lilly calf-lymph strain of vaccinia was used. Material for testing cats was obtained from Jay (1939) in this laboratory. The virus was carried through serial transfers in rabbit testicle by Jay. The suspension of virus for testing was made up by triturating rabbit testicle in fifty percent buffered

glycerine. The finished stock suspension represented about sixteen percent rabbit testicle in sterile fifty percent buffered glycerine. For skin testing in rabbits dilutions of the stock suspension were made in buffered saline. The sample of virus used in this experiment on the cat was found by Jay to be potent for the rabbit. Typical pustules were produced in the rabbit by twenty-five hundredths of a cubic centimeter of a 1-10,000 dilution. Occasionally a reaction was obtained with twenty-five hundredths of a cubic centimeter of a 1-100,000 dilution.

A normal young cat, weight 1600 grams, was depilated with barium sulfide. Decimal dilutions of the stock vaccine were made beginning with 1-10, 1-100, etc. up to 1-1,000,000. Twenty-five hundredths cubic centimeter of each dilution was injected into the skin of the cat. Observations were made over a period of two weeks.

RESULTS:

No inflammation developed, even in the 1-10 dilution of virus. Nothing which could be interpreted as a susceptible or immune reaction took place. Slight trauma was evident from injecting this large amount of fluid into the skin. It was the same in all dilutions.

DISCUSSION:

It would be unwise to conclude from this one experiment that all cats are immune to vaccinia. However, this particular young cat was immune, so we tried some protection experiments with cat serum. The cat in question was bled and the serum drawn off and frozen. Sera from six normal cats were also used. These cat sera were tested for the presence of neutralizing antibodies or virucidal activity by Mr. Jay. Each serum was mixed with serial dilutions of living vaccinia and the resulting mixtures injected into the skin of a susceptible rabbit along with suitable controls. Lesions developed in the rabbit when each of the seven cat serums plus vaccinia was injected, indicating that virucidal antibodies were not present in the cat serum, or present in quantities that were not detectable by the method employed. Jay has called attention to the vagaries of this method of testing for virucidal activity of serums.

ARE CATS IMMUNE TO INTRACUTANEOUS INJECTION OF PNEUMOCOCCUS TYPE III?

EXPERIMENTAL:

A virulent Type III pneumococcus (Goodner 1931) used in the production of fatal dermal pneumonia in

rabbits was employed. The organism was grown in one percent dextrose calcium carbonate blood broth with frequent transfer and animal passage to maintain virulence. It was preserved by desiccation in vacuo (Sherwood and Coriell) when not being used for producing dermal pneumonia in rabbits. Fatal dermal pneumonia was produced in rabbits by intracutaneous injection of two-tenths of a cubic centimeter of an eighteen hour broth culture. When injected into the skin of the groin of a rabbit there developed a spreading inflammation involving the whole abdomen. The rabbit became extremely toxic and died usually between the third and fifth days after innoculation. The belly of a normal adult cat was shaved and injected intradermally with two-tenths cubic centimeter of an eighteen hour broth culture of the virulent Type III Pneumococcus. Observations were made for evidence of local inflammation.

RESULTS:

Twenty-four hours after injection there was no visible hyperemia nor edema. There was a small lump about four millimeters in diameter where the injection had been made. It was not red nor warm to touch. Forty-eight hours later there was no visible lesion.

This particular cat was apparently highly immune to dermal infection with the pneumococcus. Future work may show that virulence of pneumococcus for cats can be increased by serial passage through cats and kittens.

GENERAL DISCUSSION

The term "natural immunity" is generally conceded to mean a state of resistance to infection which is independent of previous contact with the infectious agent, an innate quality of the host organism. It is usually contrasted with acquired immunity which is a state of heightened resistance due to having actually survived an infection or contact with the infectious agent or its products. Natural immunity may be further subdivided into species immunity in which a whole species exhibits a high level of resistance, and second, individual immunity as shown by a single member within an ordinarily susceptible species. Until the last few years the orthodox conception of individual natural immunity has been assumed to be the result of previous unrecognized specific or nonspecific infection. The review of Engalls (1937) lends credence to this theory. She reviews the evidence which shows that bacteria and other antigenic substances derived from widely diverse sources may show antigenically similar constituents. This is the theory subscribed to by Topley, Wilson and Greenwood and their associates. Experimental work bearing directly upon this question has been carried out in recent years

by Topley et al. in England and by Webster and his colleagues at Rockefeller Institute. The latter consider that inborn, non-specific resistance is the dominant factor in determining the progress of mortality during epidemics in a susceptible species. Greenwood, Topley, and their colleagues (1936) conclude that active immunization is probably more important than selection of innately resistant animals. Both groups admit the importance of environmental factors such as heat, overcrowding, diet, etc., and have attempted to keep these factors constant. Two rather different methods of experimental approach have been adopted although both methods have been used by the two groups. Webster (1933)(1924) working with mouse typhoid caused by S. enteritidis has been able to develop resistant and susceptible strains through selective breeding. From the parent strain of mice showing a mortality of 37.4 percent, he was able by line breeding and selection to develop a susceptible strain which gave 85 percent mortality, and a resistant strain which gave 15 percent mortality when infected under identical conditions. He further showed (1937) that this inherited resistance bred true and was specific for infective agents. Resistance to S. enteritidis and to St. Louis encephalitis virus were inherited independently of each

other; resistance was shown to be dominant and susceptibility recessive. Hill (1934) has criticized Webster's early work on the basis of his use of surviving individuals as resistant breeding stock since by this means he is introducing active immunization. This does not apply to the later work since Webster (1937) in recognition of this criticism has developed entirely new strains with no history of previous infection.

Topley and Wilson (1929, 1937) and Greenwood, Hill, Topley, and Wilson (1936) have maintained that use of selected animals does not represent the true state of affairs in a naturally occurring epidemic. They have shown that in a closed herd of mice they could reproduce the typical curve of an epidemic disease in man by introducing a few infected individuals into the herd. Eventually mortality and morbidity cease although latent infection and carriers remain. To keep the epidemic continually going it was only necessary to keep adding into the herd a few susceptibles from time to time. The death rate among animals which had passed through an epidemic was much lower than among fresh recruits. Largely on this basis Topley et al. maintain that active immunization due to previous sub lethal infection is the determining

factor as to whether or not an individual will survive exposure to infection. The latest work of Webster (1939) has seriously challenged this concept by producing epidemics in mice of known susceptibility. Under conditions in which mouse typhoid was allowed to spread naturally among herds of mice comprised of different proportions of individuals of innately high or low susceptibility eighty-five percent of the innately susceptible succumb to mouse typhoid in contrast to less than five percent of the innately resistant, regardless of whether either constitutes twenty-five, fifty, or seventy-five percent of the population respectively. The surviving population is, therefore, composed largely of individuals known at the outset to be innately resistant. Innately resistant recruits remained well unless subjected to some non-specific hazard, such as heat or over crowding. Finally, there was no tendency for known susceptibles to become immunized through exposure to epidemics or artificial innoculation. For the present, at least, it would appear that Webster has proved his hypothesis that inherited and general environmental components of resistance are of fundamental importance, whereas, immunological components associated with infection are of negligible importance in mouse typhoid infect-

ion. Lurie (1938, 1939) points out the differences between normal rabbits and those vaccinated with living human type tubercle bacillus. When subjected to infection with virulent bovine tuberculosis, there was only 13 percent difference in mortality between the two groups. Apparently natural resistance plays a very important role in immunity to tuberculosis.

From our work with the cat it appears that a similar mechanism is at work, i.e., an innate resistance of high order which is a characteristic of the species. Even young kittens were shown to have this innate resistance. As to the modus operandi of inherited resistance, little is to be found in the literature. We have shown that the blood of cats and kittens is strongly bactericidal and no doubt this is of importance. However, it is not the sole factor since immunity is not conferred to mice by passive transfer of cat serum. Cat leucocytes and macrophages have been shown to be active in removing bacteria from the blood stream, and organisms have been found to persist in the spleen. Webster (1939) noticed that innately resistant mice in an infected herd were likely to show positive stools and spleen cultures even though they remained healthy. One might speculate as to the role of phagocytes as a haven of refuge for bacteria.

Goodpasture (1937) and Goodpasture and Anderson (1937) have carried out some excellent work which lends support to this hypothesis. In early cases of typhoid fever small Gram negative intracellular bacilli, judged to be E. typhi, have been found at autopsy in the cytoplasm of young plasma cells, otherwise apparently unaltered, located in the lymphoid follicles of iliac and mesenteric lesions. Larger gram-negative bacilli were found in macrophages of the intestinal lesions in association with the remains of phagocytosed lymphocytes, or the nercrotic remnants of macrophages themselves. Goodpasture concludes that E. typhi grows and multiplies in both places because an almost identical picture is presented when E. typhi is cultured on embryonic chick membranes. He believes that the young plasma cell is an essential host for E. typhi in man and serves as a nourishing and protecting medium--not only during incubation but throughout the disease. Sherwood (1935) has called attention to evidence indicating that phagocytosis is not a one-sided affair--the bacterium may be killed or may multiply and destroy the phagocyte, or we might have a compromise between the two--the bacterium remaining dormant within the phagocyte. We may surmise from our studies of the bactericidal power of cat serum that as soon as a bact-

erum leaves the protection of the phagocyte, it will be destroyed in the serum. We believe that a complete study of the histology and physiology of the reticulo endothelial system of cats might prove of value in this connection. It is almost universally conceded at the present time that the reticulo-endothelial system is the site of antibody formation (Zinsser 1939). Lewis and Wells (1922), Zinsser (1939), Sherwood (1935), and many others have noted the close association of serum globulins with antibodies. The suggestion has been made and not without considerable evidence, that serum globulins are closely associated with, if not identical with antibodies. Sabin (1920) has shown that the original blood proteins come from liquefaction of the central mass of angioblasts in the blood inlands of the chick blast oderm. In her latest paper (1939) she presents evidence in support of the concept that throughout life the serum proteins, certainly as far as globulin is concerned, come from the sacrifice of a part of the cytoplasm of cells of the reticulo-endothelial system. On this basis her definition of an antigen is "a substance which can specifically modify the synthesis of the cytoplasm of the cells of the reticulo-endothelial system".

Our work with the cat indicates an actively phagocytic reticulo-endothelial system as well as active phagocytosis on the part of circulating leucocytes. Still, circulating immune antibodies were not uniformly produced. Are we to infer that the synthesis of cytoplasm of cat phagocytes cannot be readily modified? Another possible explanation is that S. enteritidis has a protein or haptene fraction antigenically related to some protein of cat tissues. If so one would not expect the cat to produce antibodies for S. enteritidis and thus for its own tissues. As yet we have no experimental proof but the question is being investigated.

At the beginning of this work we were under the impression that the cat does not produce immune antibodies to any great extent. Particular emphasis has been placed on the Salmonella infections because of the frequency with which cats must come into contact with this organism in nature. The available literature on antibody production in cats seems to bear out the fact that they are at best poor antibody producers. There has been an almost uniform failure to actively sensitize the cat to soluble proteins. The work of Kabler and Sherwood (1938) in which they failed to actively sensitize twenty cats to crystalline egg albumin is typical. Our meager work with egg albumin

mixed with Vitamin C has likewise been negative. Perhaps the failure of cats to develop allergy is in itself a protective mechanism. Allergy frequently leads to necrosis and secondary inflammatory reactions when the specific organism re-enters the tissues. Long, (1935), Rich and McCordock (1929), Myers and Harrington (1934) and others have pointed out the danger of necrosis and liquifaction accompanied by failure of organisms to be localized in animals and man allergic to tuberculosis.

However, we have been able to stimulate the cat to active antibody production when using certain other antigens, namely, Pasteurella tularensis and Salmonella suipestifer. Salmonella enteritidis caused slight antibody production in a small percentage of cats and kittens. Leasure (1935) reports that serum of cats convalescent from feline infectious enteritis has protective value. Obviously, then, the cat is capable of producing antibodies but does not do so for every foreign protein injected. We have shown that the lack of response to S. enteritidis is not due to failure of the organisms to reach all parts of the body. This was accomplished by injecting the organisms into the cat by various methods, i.e., intravenous, subcutaneous, intracardial, and intraperi-

toneal, as well as feeding in massive doses. For harmless foreign proteins and bacteria the cat apparently does not have to raise additional defensive mechanisms. We interpret this to be the ideal state of immunity mentioned by Theobald Smith (1933) in which the presence of foreign bacteria has no toxic effect on host tissues, no inflammation or antibody production is called upon, and the foreign object is simply removed by phagocytic action. It may be that we are a little over-optimistic anyway, when we expect to find good antibody production in every animal. Man himself must be classified as a poor producer of antibodies when compared with the rabbit. The rabbit is the animal par excellence for producing high titered antisera for almost any foreign protein introduced parenterally. Even in rabbits we occasionally encounter refractory individuals which do not respond. It may be that in cats this condition is the rule rather than the exception.

Another contrast between cats and rabbits is the amount of endotoxins of Salmonella which they will withstand. In this laboratory we have found rabbits very susceptible to intravenous injection of killed suspensions of Salmonella. One-tenth cubic centimeter of a standard suspension is the usual beginning

dose for a rabbit. More than this is likely to kill the animal and infrequently an animal will succumb to this first injection. We have never been able to kill a cat with intravenous injection of twenty or thirty times this amount. We wonder if there is some connection between the exaggerated sensitivity of vaso-motor mechanisms to injection of foreign protein (Brodie 1900) and ability to withstand injection of bacteria.

One could postulate the presence of a natural neutralizing antibody for endotoxins, or, perhaps a unique mechanism of lysis and destruction of the bacteria which does not involve the liberation of endotoxins.

Perhaps one of the most interesting outgrowths of our work on the cat was the demonstration of the nature of the natural bactericidal properties of cat serum. The properties of these normal bactericidal antibodies, if they be such, are different from normal bactericidal antibodies that have been described in other animal species by other workers. We have suggested the name "bacterotoxin" to designate the active agent in cat serum. Browning (1927) originally described a non-specific natural antibody-like principle

in the blood of guinea pigs which was capable of lysing E. typhi. It was fixed at 0°C by E. typhi as well as by the colon bacillus. Coal dust and powdered glass would also remove the principle from serum. Browning's work was later extended by Mackie and Finkelstein (1931) to include a study of normal bactericidal properties of other animal species. They conclude from their observations that immune antibodies have their precursors specifically differentiated in the serum of normal animals and that, in general, immune antibodies are not substances formed de novo. This concept is in line with Hirszfeld's (1924) view of natural antibodies as "biochemical organs" which vary in their specificity and distribution among individuals according to the genetic composition of the latter.

On the basis of the literature mentioned and on our own experimental findings we could propose as explanation of the immunity of cats the following hypothesis. Throughout a long period of exposure to virulent infectious agents the cat has by a process of natural selection and survival of resistant individuals developed a high degree of innate species resistance. We have spent most of our time investigating the mechanism of this resistance with particular emphasis on certain members of the Salmonella group

of bacteria. These studies have shown that the immunity of the cat to these organisms is not alone confined to any one tissue or system of cells but is in evidence whereever experimental procedure has been devised to shed light on one particular phase. The gastric juice is bactericidal, the intestinal wall is a barrier to penetration of the organism if not actually bactericidal, serum is strongly bactericidal, circulating leucocytes and reticulo-endothelial system are phagocytic and the general body tissues are quite resistant to endotoxins and bacterial growth products. We have speculated on the possible presence of anti-endotoxins. The failure to develop allergic inflammatory reactions is further evidence of an efficient tissue immunity.

Such observations as these we have just made may prove to be the pioneer work in elucidating the actual mechanism of innate resistance. Webster and his colleagues have demonstrated the importance of hereditary factors, but have not shown the physiological mechanisms involved. Sherwood (1935) has reviewed the literature and called attention to the possible importance of the suprarenal gland and resistance. Not all experimenters agree, but the conconsus of opinion seems to be that the presence of

some normal suprarenal tissue is necessary to maintain resistance to such agents as typhoid vaccine and diphtheria toxin. A deficiency of certain of the vitamins as well as other dietary factors have been shown to affect body nutrition and at the same time resistance to infection. In our hands Vitamin C did not stimulate precipitin production in cats nor increase precipitin production in rabbits any more than other acids adjusted to the same pH value.

Our work indicates that the cat has a similar high degree of innate resistance to such unrelated organisms as Trichinella spiralis, P. tularensis, B. anthracis, vaccinia, and the pneumococcus. Here we have a protozoan, three bacteria and a filterable virus. The hypothesis proposed above may apply to all these infections as well, but an alternate explanation to account for the cat's immunity to so many diseases may be that the cat is innately and fundamentally different in its physiological makeup from other laboratory animals such as the rabbit and the guinea pig.

To be sure the cat does become infected when fed a massive dose of trichina larvae, but the mere fact that the cat was not killed by the initial toxicity of the intestinal worms bespeaks great resistance. The cats tissues seem to be able to tolerate

the presence of foreign substances, which are toxic for other animals, without the usual secondary inflammatory reactions. We have indicated that the cats resistance to virulent pneumococcus and B. anthracis are probably due in part to active phagocytosis of the invading bacterium.

Antibody production against P. tularensis indicates a humoral mechanism may be active against this infection. It will be interesting to correlate the presence of agglutinins for P. tularensis with the bactericidal power of fresh cat blood throughout a period of active immunization.

Immunity to vaccinia appears to be a tissue reaction since the serum of six normal cats and one hyperimmunized cat gave no protection when mixed with vaccinia and injected into the skin of a rabbit.

CONCLUSIONS:

Our results and observations may be stated briefly as follows:

1. Adult cats as well as young kittens are immune to infection with Salmonella organisms of known virulence for white mice. The cats are immune whether the bacteria are fed orally or injected parenterally by various routes.

2. Circulating agglutinins in low concentration were produced for S. enteritidis by about fifty percent of the cats after prolonged exposure to living and killed suspensions.

3. When mixed with horse serum previous to injection into rabbits Vitamin C was shown to increase precipitin production about seventy percent. Similar results were obtained using d-iso ascorbic acid and other acids in place of Vitamin C. The enhancement of antibody production caused by Vitamin C is apparently due to its acid reaction. Vitamin C did not stimulate one cat to produce demonstrable precipitins for egg albumen.

4. By feeding heavy suspensions of S. enteritidis to adult cats and kittens we were unable to establish this organism in the intestinal tract. Organisms of the Salmonella group were never found normally in cats or kittens.

5. Gastric juice was shown to be sufficiently bactericidal to destroy S. enteritidis. When massive doses of living organisms were given by stomach tube they could not be isolated from the alimentary tract or blood stream seven hours later.

6. When living S. suipestifer was injected into the blood stream of cats, the bacteria were lo-

calized in the reticulo-endothelial organs, particularly the spleen, by the end of twenty-four hours. Spleen cultures showed the presence of viable organisms as long as five days after injection.

7. The opsono-phagocytic index of cat blood for Salmonella organisms was shown to be from five to twelve with an average of around seven.

8. Urine, bile, saline extracts and acetone insoluble extracts of intestinal mucosa were not bactericidal for S. enteritidis.

9. Blood of all cats and kittens was bactericidal for S. enteritidis. There was no correlation between bactericidal power and presence of agglutinins. Agglutinins were never found in normal animals.

10. The bactericidal property of cat serum is thermolabile, partially reactivated for S. enteritidis by guinea pig complement, but not reactivated for other bacteria. It is not fixed at 0°C, in 4 hours is fixed slowly at 4 to 5 degrees C, and not fixed at this temperature after inactivation. It shows group specificity for the Salmonella group and is not increased by active immunization. So far as is known a bactericidal substance having these properties has not been described heretofore. We have suggested that the term "bacterotoxin" be applied to this substance in cat serum.

11. Using small numbers of animals the cat has been shown to possess considerable immunity to infection with P. tularensis, trichinella spiralis, B. anthracis, vaccinia, and the pneumococcus.

12. It has been suggested that the immunity of the cat may be explained by,(1) innate inheritance factors; (2) survival of resistant individuals through process of natural selection; (3) fundamental inborn physiological makeup which differs from ~~other~~ susceptible animals.

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