MASTER

Aeromonas hydrophila as an Agent of Infection in Alligators
Phase I Final Report

PART I

PROGRESS REPORT FOR THE CONTRACT PERIOD SEPTEMBER 1, 1976 - SEPTEMBER 30, 1977

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ABSTRACT

An extensive, in-depth study of the interactions between *Aeromonas hydrophila* and *Americana mississippiensis* was initiated in September, 1976. Presently personnel from the following groups and institutions are actively involved in the research activities:

Wake Forest University (WFU)

Savannah River Laboratory (SRL)

Medical University of So. Carolina (MUSC)

So. Carolina Wildlife and Marine Resources Dept. (SCWMRD)

Savannah River Ecology Laboratory (SREL)

Northern Illinois University (NIU)

Rockefeller Refuge (RR) Louisiana Fish and Game Comm.

The valuable contributions of each group to the research effort is sincerely appreciated.

Basic goals of Phase I include (1) proof of Koch's postulates, (2) a study of possible routes of infection in the alligators, and (3) an understanding of the development and progression of the *A. hydrophila* infection. Many questions related to these goals have been answered. Additional information regarding immune responses to the infection has been generated due, in part, to the involvement of Dr. Glassman and coworkers from the Medical University of So. Carolina.

PHASE I

1. Koch's postulates have been satisfied.
2. Most, if not all, alligators have prior exposure to *A. hydrophila* as determined by
 - a) Presence of the bacteria externally
 - b) Presence of the bacteria in the habitat
 - c) Antibody titer levels of captured alligators
3. When captured, banded and transported the animals are highly likely to develop lesions on the jaws which progress to the abdomen, dorsal and caudal areas. These lesions are positive for *A. hydrophila*.
4. When exposed to increased levels of *A. hydrophila* in the H₂O or by injection the alligators produce antibody against the antigen.
5. Response to infection is demonstrated by changes in antibody titer and hematology parameters.
6. Temperature and stress may be involved in the alligators' immunological response.
7. Intraperitoneal injections of washed, live cells may result in a protective immune response.
8. IgA is apparently present in alligator serum. This has not previously been reported.
9. The immune responses of young alligators with exposure to infection, i.e., 6 month old growth chamber animals, are limited and reduced when compared to the responses of older, wild alligators.

Papers based on data from Phase I research will be presented at the Ga. Academy of Science meeting and at the national American Society for Microbiologists meetings in April and May, 1977.

INTRODUCTION

PHASE I Capture and initial observations

Experimental alligators were obtained Sept. 10, 1976. A total of 13 alligators (2.5'-4.5' in length) were captured in S. C. coastal ponds, transported to Flowing Streams Lab and placed in cleaned ponds. Within 36 hours each animal was inspected for external lesions and other evidence of *Aeromonas hydrophila* infection (all negative), tagged for identification and 1.5 ml of blood was withdrawn from the caudal vein for serology and hematology studies.

However, *Aeromonas hydrophila* was present in the ponds containing alligators by 9-13-76 and all 13 animals showed evidence of external lesions by 9-21-76. Lesions were first observed on the abdomen and jaw and then progressed to dorsal and caudal areas.

On 9-23-76 these alligators were measured for length, weighed, marked and randomly placed in three (3) tanks situated indoors. The tanks contained approximately 600 liters of water which was continuously pumped and cleansed through a biological filtering system. The water in each tank was thermostatically regulated at 30°C and the room temperature was maintained at 25°C. All alligators were exposed to similar types and degrees of handling and stress, i.e., animals were not fed during the experiment and were captured by hand over 45 times each.

Experiments in Phase I were designed to expose the experimental alligators to various concentrations of young, washed cells of *Aeromonas hydrophila* under controlled conditions. Responses of all alligators were monitored on the basis of:

1. observations of external lesions,
2. immunoglobulin production,

3. blood chemistry and hematology,
4. bacteriology, parasitology and pathology of internal organs, skeletal muscle and external lesions at necropsy.

Alligators in experimental tanks were exposed to *A. hydrophila* as follows:

Tank No.	No. of Animals	Type of Treatment
		<u>Concentration of <i>A. hydrophila</i></u>
1	4	Cells added to water in the tank
2	4	Control - none
4	4	Intraperitoneal injections

Tank #1. Alligators received *A. hydrophila* at two week intervals in increasing concentrations from 1×10^3 to 5×10^5 cells/ml mixed with the water in the tank during the experimental period. Bacterial cell levels were monitored daily.

Tank #2. Alligators were maintained as controls and had no further exposure to *A. hydrophila*. Bacterial cell counts ranged from 10-25 cells/ml of tank water.

Tank #4. Alligators were injected intraperitoneally with increasing concentrations of washed cells. From 1×10^3 to 5×10^5 cells/gm of body weight were administered during the experimental period.

Additional experiments were conducted on selected animals in Tank #2 and several animals in Tank #3.

Tank #2. Late in the experimental period, an additional experiment was conducted in which these infected alligators were given IP injections of 1×10^3 cells/gm of body weight. The two alligators with the most serious lesions were injected on 11-15-76, while the other two were given saline buffer injections.

Tank #3. Alligators in Tank 3 were captured on October 13, 1976, brought to SRL, weighed, measured and placed in Tank 3 water at 20°C. On November 15, 1976, 2 alligators from Tank 3 were injected IV caudal with 0.5×10^6 cells/gm of body

weight. Control alligators were injected with sterile saline solution only. Again, on 11-29-76, the same injection procedure was followed. On 12-6-76, the same procedure was used.

Prior to each series of injections or aquatic exposures to *A. hydrophila*, blood was taken from each alligator and antibody titers were determined. Furthermore, beginning with the 11-15-76 series, blood was withdrawn every two weeks for complete hematology studies. Whenever possible, large amounts (10-20 ml) of blood was obtained prior to necropsy.

At necropsy, tissue samples were obtained of heart, liver, lung, kidney, lesion and of skeletal muscle for bacteriology, parasitology and pathology studies (Table 1). Samples of tissue and blood were also sent to Dr. M. H. Smith, SREL, for electrophoreses studies.

SUMMARY OF NECROPSY DATA

Tank #1

Animals in Tank #1 received *A. hydrophila* in increasing concentrations ranging from 1×10^3 to 0.5×10^6 cells/ml of tank water. None of the internal tissues supported bacteria except #55 liver at 3.0×10^3 /gm. One animal had no lesions, one had no bacteria in the lesion cultured, and two of the four had approximately 10^4 cells/gm of lesion area.

Tank #2

Two animals which were most severely infected were given IP injections late in the experimental period. Alligator #70 died within 20 hours of the IP treatment and tissue loads ranged from 10^6 to 10^9 cells/gm. Alligator #70 died during the night and on necropsy showed internal loading ranging from 1.2×10^5 to 1.0×10^9 cells/gm weight of tissue. This *A. hydrophila* level suggests a generation time of

< 2 hr in the tissue, assuming no previous infection present. Alligator # 79 was again injected on 11-29-76 and also on 12-6-76 with 5×10^3 and 5×10^4 cells/gm of body weight, respectively. At autopsy on 12-10-76, # 79 showed little or no improvement in regard to external infection following the series of IP injections. Also, the *A. hydrophila* levels of internal organs were relatively low (see Table 1). Alligator # 79 received three weekly injections and supported from 10^3 to 10^5 cells/gm at termination. Two animals which had received sham injections of sterile buffer were terminated at the same time as # 79. Their tissue loadings were very low. The kidney of # 54 supported 1.4×10^3 cells/gm and all other tissue had no bacteria. Heart, liver and kidney of # 64 had bacteria loads of $10^2 - 10^3$ cells/gm, while the lung and skeletal muscle had no bacteria present.

Tank #4

All alligators in this tank received increasing IP injections ranging from 10^3 to 10^5 cells/gm body weight. Animals # 58 and # 69 were saved for Phase II studies. Liver, lung and skeletal muscle of # 60 had no bacteria while the kidney and heart had only 10^2 cells/gm. Alligator # 74 had from $10^1 - 10^3$ cells/gm of tissue.

Tank #3

Two animals were given sham buffer IV caudal injections. Alligator # 21 survived 3 injections of 5×10^6 cells/gm of body weight and has been retained as a source of serum for future studies of passive immunity and antibody memory. However, # 22 died shortly (40 hours) after receiving a massive injection of 1.0×10^9 cells. Tissues ranged from $10^5 - 10^9$ cells/gm. However, the heart tissue apparently supported no bacterial cells.

Two other animals which received sham injections, N-1 and N-13, were retained for Phase II studies.

These data suggest:

1. Internal infections are not likely to develop from external exposure to *A. hydrophila*.
2. IP or IV injections may cause massive buildup of *A. hydrophila* in susceptible animals --, possibly resulting in death.
3. Animals injected IP with increasing concentrations of the bacteria have very low internal loads of the infective agent, i.e., fewer than the number injected.

HEMATOLOGIC STUDIES

Data are now available for alligators in three age groups (0.5, 1.5 and 2.5 yrs) grown in growth chambers (Table 2). Peripheral blood cell sizes and chemical values (Table 3) are also available. These blood samples were taken from alligators hatched and raised in growth chambers at the Rockefeller Refuge, Grand Chenier, La. on February 4 and 5, 1977. Samples were obtained by heart puncture. Tissue samples from four animals were also obtained.

IMMUNOLOGY

Antibody titer measurements and, recently, total blood studies of the experimental alligators have been conducted. In a general manner, wild alligators from both South Carolina and Louisiana possess relatively low antibody titers. Following exposure to *A. hydrophila* which result in either (a) infection, or (b) apparent protection, the antibody titer increases by 2 to 3 doublings or from low to quite high titers; WBC (white blood cells) also increased dramatically under certain exposure conditions. Blood work will continue and analysis of these data is in progress.

Data on alligators (size), antibody titer, RBC, WBC and hematacrits are presented in Table 4. Note that the data on the latter three began on 11-15-77 when Dr. Glassman's group joined the study. Antibody data show that titer increases occurred even among control animals. Since all alligators have some exposure to *A. hydrophila* whether in nature or in growth chambers some level of antibody titer specific for this antigen is expected. Increases may be due to increased exposure to the antigen.

Additional data on these parameters will be available at the completion of Phase II (Note Appendix 1).

PARASITOLOGY AND PATHOLOGY

Haemogregarines, an intracellular parasite of alligator RBC has been found in South Carolina and Louisiana animals (Appendix 2).

Studies of the pathology of the tissue samples will be conducted during the summer. Presently tissues are being embedded, sectioned and slides prepared for microscopic observation at a later date.

Phase II experiments have been designed to meet these objectives:

1. To observe the effect of different temperatures on the alligators when they are exposed to *A. hydrophila* in the water.
2. To observe the effects of physical stress on the alligators and on their infection rate and immune response, and
3. To obtain information regarding the immune responses of these experimental animals to intraperitoneal injections of live or heat-killed cells following their exposure to *A. hydrophila* in the H₂O.

Additional experiments will be conducted which will involve the use of immune serum obtained from Phase I animals.

Briefly, the procedures will be as follows:

1. Animals will be distributed in 4 tanks in which the water temperature will be maintained at 20°, 25°, 30°, and 35°C.
2. All tanks will receive the same concentration of *A. hydrophila*; with increasing concentrations added to each tank and maintained for two-week intervals, i.e.,

1-10-77	1×10^3	cells/ml H ₂ O
1-24-77	5×10^3	
2- 7-77	5×10^4	
2-21-77	5×10^5	
3. All alligators will be physically stressed in a comparable manner.
4. All alligators will be fed once weekly.
5. All alligators will be bled biweekly or weekly.
6. A series of injections (intraperitoneal, live or heat-killed cells) is planned for late in the experiment, depending upon the condition of the alligators at that time.
7. Necropsy will be conducted at the time of death of experimental animals.

CONCLUSION

This report is a summary of data available in progress reports 1 and 2 plus some information recently turned in by Glassman and Bennett. It is an attempt to provide an overview of progress to date. For clarification please contact R. Gorden.

TABLE 1
PHASE I
NECROPSY DATA
CELLS/GM OF AEROMONAS IN TISSUE

ALLIGATOR	TANK #	TREATMENT	DATE OF DEATH	CAUSE OF DEATH	HEART	LIVER	LUNG	KIDNEY	SKELETAL MUSCLE	LESION
52	1		12-6-76	AC	0	0	0	0	0	0
55		in	12-5-76	NC	0	3.0×10^3	0	0	0	1.3×10^4
59		H ₂ O	12-7-76	AC	0	0	0	0	0	OG
71		only	12-7-76	AC	0	0	0	0	0	NO Lesion
66			11-12-76	NC						
54	2	Buffer	12-9-76	AC	0	0	0	1.4×10^3	0	0
64		Buffer	12-14-76	AC	8.8×10^3	5.6×10^2	0	1.30×10^3	0	7.9×10^4
IP 70		11-15 IP		NC	5.5×10^6	1.8×10^9	1.1×10^{10}	3.9×10^8	5.5×10^6	4.3×10^6
IP 79		11-15 IP		AC	1.78×10^5	6.4×10^5	1.4×10^5	2.1×10^3	0	0
IV 22	3	11-15 IV	12-8-77	NC	0?	2.25×10^9	1.28×10^7	7.6×10^5	2.2×10^7	1.7×10^7
21		11-15 IV	Saved for	future studies						
N1		Sham	Saved for	Phase II						
N13		Sham	Saved for	Phase II						
58	4	IP	Saved for	Phase II						
60		Series	12-14-76	AC	3.9×10^2	0	0	2.1×10^2	0	0
69		1×10^3 -	Saved for	Phase II						INT.
74		5×10^6 /gm	12-15-76	AC	1.6×10^3	5.9×10^2	5.8×10^1	6.6×10^2	7.7×10^2	2.8×10^3
1		outside	10-31-76	NC	7.0×10^2	1.2×10^5	3.4×10^4	4.1×10^4	0	0
5		in cold	12-9-76	NC	2.4×10^4	7.0×10^4	1.4×10^2	4.2×10^2	8.7×10^2	9.7×10^5
12		H ₂ O un-	11-19-76	AC	0	0	0	0	0	0
14		til death	12-13-76	NC	1.7×10^2	6.8×10^1	3.8×10^2	4.7×10^1	2.3×10^3	1.6×10^7
17			12-17-76	NC	0	0	0	0	0	1.3×10^5

TABLE 2
BASELINE HEMATOLOGICAL DATA - LOUISIANA NORMALS

AGE	2.5	1.5	0.5
	<u># 50(n=6)</u>	<u># 60(n=4)</u>	<u># 70(n=8)</u>
WBC	4.2 ± 1.1	4.5 ± 1.5	4.1 ± 1.2
RBC	0.46 ± 0.1	0.42 ± 0.1	0.36 ± 0.1
Hgb	7.3 ± 1.1	6.3 ± 1.4	4.9 ± 0.7
Hct	19.7 ± 3.6	18.6 ± 4.1	14.0 ± 0.9
MCV	430.2 ± 30.2	443.5 ± 31.3	394.6 ± 52.8
MCH	161.5 ± 30.2	150.5 ± 9.8	135.0 ± 8.6
MCHC	36.0 ± 6.4	34.0 ± 0.7	34.7 ± 4.0
Poly	59.5 ± 9.4	51.8 ± 17.8	67.9 ± 14.0
Lymph	14.5 ± 7.2	14.0 ± 5.5	16.4 ± 9.1
Mono	1.16 ± 2.0(0-3)	1.2 ± 1.8(0-3)	3.9 ± 5.3(0-9)
Eos	10.7 ± 4.1	12.5 ± 6.4	10.1 ± 4.8
Baso	14.2 ± 7.1	20.5 ± 15.8	4.7 ± 5.5(0-10)
Plt.	18,922 ± 9,553	27,643 ± 5,659	18,690 ± 7,159

TABLE 3

HEMATOLOGIC VALUES FOR ALLIGATOR MISSISSIPPIENSIS

	Per deciliter
Hemaglobin	7.2 ± 0.4 gm
Hematocrit	19.9 ± 2.0 %
Red blood cell #	$0.39 \pm 0.07 \times 10^6$
White blood cell #	$5.5 \pm 1.3 \times 10^3$
Thrombocytes	$23.0 \pm 7.6 \times 10^3$

White Blood Cell Differential %

WHITE BLOOD CELLS - MEAN $\pm$ 1 STD. DEV.

Neutrophilic macrophages	36.6 ± 9.2 %
Eosinophils	5.1 ± 3.4
Basophils	1.9 ± 2.6
Monocytes	4.0 ± 5.6
Lymphocytes	52.4 ± 10.7

THROMBOCYTES

Estimated #	$23.0 \pm 7.6 \times 10^3$	
Cell size-----	9.5 ± 1.6 (microns)	n=58
Platelet nucleus		
Long axis-----	6.6 ± 0.9	
Short axis-----	4.8 ± 1.2	n=20

RED BLOOD CELLS

MCV	$521.6 \pm 78.3 \mu^3$
MCH	$189.9 \pm 29.8 \mu\mu g$
MCHC	36.4 ± 2.2 %
Red blood cell size	

Long axis diameter

Short axis diameter

CHEMICAL VALUES

Iron	$6.5 \pm 4.2 \mu g$ %
* Iron Binding Capacity	$409.0 \pm 67.5 \mu g$ %
Sodium	139.4 ± 2.0 meq/L
Potassium	3.3 ± 0.3 meq/L

n=8
*n=3

TABLE 4
ALLIGATOR - AEROMONAS
PHASE 1
ALLIGATOR - AEROMONAS

TANK # & TREATMENT	ALLIGATOR #	LENGTH CM	WEIGHT KG	ANTIBODY TITER/LOG ²								RBC X 10 ⁵			WBC X 10 ⁵				HEMATACRIT		Death 12-
				9-11	10-3	10-14	10-26	11-22	12-9	Death or 12-16		11-15	11-29	12-6	11-15	11-29	12-6	Death 12-16	11-15	11-29	
1																					
<i>A. hydrophila</i>	52	70.48	.740	7.16	8.16	8.97	9.4	9.0	8.3	--		.47	.45		6.8	7.0			19.0	18.0	
H ₂ O	55	72.3	.840	7.60	8.64	5.68	8.0	9.3	--	--		.48	.57		12.3	16.4			21.5	19.5	
1 x 10 ³ /ml to	59	81.28	1.420	7.94	0	--	9.0	--	9.0	9.0		.62	.69		16.0	13.2			23.5	23.0	
0.5 x 10 ⁶ /ml	71	115.5	4.050	6.42		7.65	9.7	9.0	9.0	8.8		.45	.63		4.8	7.0			21.5	23.0	
2																					
No added	Buffer 54	82.5	1.280	7.03	--	--	--	9.2	9.2	8.7		.58	.61		6.3	17.3			22.0	20.0	
bacteria	Buffer 64	137.7	6.0	7.47	8.38	9.16	8.1	9.1	9.2	9.1		.69	.69		5.9	6.9		14.1	25.5	24.0	25.0
1 x 10 ³ /gm																					
to	IP 70	79.3	1.265	7.10	7.8	9.03		Dead	--	--									33.5		
1 x 10 ⁴ /gm	IP 79	111.7	3.480	7.35	--	9.11	9.5	8.9	8.7	9.0		.56	.78		3.9	7.5			27.0	31.0	
								8.7		9.1											
3																					
Sham control	N 1	91.4	1.80			9.08		8.9	--	--		.41	.49	.38	4.6	5.0		14.5	14.0	16.0	16.0
Sham control	N 13	128.27	6.0			7.47		--	8.7	--		.38	.60	.49	5.4	4.2		6.5	18.0	24.0	20.0
0.5 x 10 ⁶ /gm.	21	151.1	8.75			8.9		--	--	--		.17	.52	.32	1.6	23.1		10.0	6.0	20.0	20.0
IV	22	148.3	8.0					8.6	8.7			.55	.56		8.4	6.9		dead	24.5	21.0	
4																					
<i>A. hydrophila</i>	58	78.10	1.165	7.15	8.53			9.0	8.8	9.1		.47	.42	.28	9.3	5.8	13.1		20.0	11.0	12.5
by IP injection	60	81.9	1.535	7.93	8.26	5.71	9.4	8.9	9.1	8.9		ND	.55	.37	ND	11.1	7.2		ND	19.0	14.5
1.0 x 10 ³ /gm to	69	75.5	.930	7.10	8.21	9.06	9.06	8.9	8.9, 8.5	9.0		.39	.32	.31	12.8	5.2	6.1		16.5	11.0	14.0
0.5 x 10 ⁶ /gm	74	90.8	2.750	7.03	8.37	8.62	8.6	8.9	9.2	9.1		.49	.79	.37	5.7	4.6	4.9		19.5	23.0	21.0

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During the meeting of February 11, 1977 a fine spirit of cooperation was evident. All of the participants expressed a desire to foster continued cooperation. It was decided that Bob Gorden will serve as the coordinator in regard to authorship, sequence of authors and acknowledgments for papers and publications. In order to facilitate such action it was suggested that abstracts of papers and publications be sent to Gorden. He will review the content of the paper, the authors and the acknowledgments as included in the abstract, and will then contact other persons to get their reaction to these items. He will serve as referee in regard to suggested changes. We hope that this action will be acceptable to all concerned.

Several groups have made contributions to this research. Names and addresses are listed below, as are contract numbers of several groups. If there are corrections in address or in the spelling of names, places or groups, please contact Gorden.

It will not be necessary to acknowledge all of the groups listed below in each publication. However, please be aware of the contribution made by each group.

Please send names and addresses of those who are not now on our progress report mailing list and wish to receive these reports to B. Gorden, SRL.

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Alligator mississippiensis is being used as a host model for the epidemiologic studies related to the transmission of Aeromonas hydrophila in a natural environment. The measurements of host response to infection include white cell count and the percentages of various types of white cells in the peripheral smear. This study was performed to define normal ranges and morphology of alligator peripheral blood so that changes observed during the experiment could be interpreted. Peripheral blood smears were prepared from EDTA anti-coagulated blood and subsequently Wright stained for light microscopy. Buffy coat layers were prepared by centrifugation in micro-hematocrit tubes, fixed in glutaraldehyde, post fixed in osmium tetroxide, epon embedded, double stained with uraldehyde and lead acetate and viewed with a Zeiss emission electron microscope. The peripheral cells were identified and classified as follows: (A) Myelocytic series (neutrophilic macrophages, eosinophils and basophils); (B) Lymphocytes; (C) Monocytoid cells; (D) Thrombocytes; and (E) Nucleated red blood cells. The following mean and one standard deviation statistical values were obtained from a population of eight environmentally free, recently captured animals. Data obtained: hemaglobin 7.2 ± 0.4 grmas per deciliter, hematocrit 19.9 ± 2.0 , total white blood cell count $5.5 \pm 1.3 \times 10^3$. Differential counts in the peripheral smears revealed 36.6 ± 9.2 % neutrophilic macrophages, 5.1 ± 3.4 % eosinophils, 1.9 ± 2.6 % basophils, 4.0 ± 5.6 monocytes, 52.4 ± 10.7 lymphocytes and $23.0 \pm 7.6 \times 10^3$ thrombocytes. Electron microscopy revealed the cytoplasm of the neutrophilic macrophages to be filled with electron dense granules similar to ones seen in the myelocytic cell lines of humans. During infection, there is a significant shift in the percentages of the types of white cells in the peripheral smears. There is an increase in the overall white cell counts with the predominant increase being in the neutrophilic macrophages early in the infection with A. h. Peripheral blood morphology and cell make-up by light and electron microscopy in Alligator mississippiensis has been described for normal and infected animals.

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CONTINUATION OF THE Alligator mississippiensis RESEARCH

Additional chemical information has been obtained from frozen serum samples collected by Terry Hazen. The following data was obtained:

Sodium - 135.7 ± 15.9 meq/L (n=35), Potassium - 4.1 ± 1.4 meq/L (n=35)

Blood Glucose - 134.0 ± 59.4 (n=34), Iron - 59.2 ± 35.6 ug% (n=30), and

Iron Binding Capacity 275.7 ± 55.8 ug% (n=25). These values are from theoretically "normal" animals, although a very broad range of values were seen.

No distinctive trend is apparent at this time in Phase II of the project. It has been observed after three(3) samplings that the animals are apparently in much better physical condition (higher hemaglobin, hematocrit, and MCV) and that the animals exposed to the higher temperatures appear to generally have higher white blood cell counts.

We await normal whole blood and serum from the alligators on the Rockefeller Refuge in Louisiana in order to establish better baseline controls.

Serum protein electrophoresis has revealed a pattern of elevated α_2 concentrations in theoretical normals. In the presence of infection it appears as though there is an increase in the γ region, possibly reflecting increased antibody production of the IgG class. Some of the infected animals also have decreased albumin (a not unexpected finding particularly in chronic infection) and increased α_1 . Significant and more accurate interpretation with study of additional normal and infected animals, correlation with nephelometric values of IgG and IgM will be useful in proving the hypothesis of increased immunoglobulins being responsible for the increased γ region in the infected animals.

HAEMOGREGARINA SP. IN CHICK EMBRYOS

The chick embryo has been used as an experimental host for several parasites which, in nature, utilize only mammals as vertebrate hosts. The African trypanosomes of man, the leishmanias, and Trypanosoma cruzi, for example, develop readily in the bloodstream or visceral organs of the embryo while hatched birds are refractory to infection. Also, Entamoeba invadens, a parasite of the intestine and liver of snakes has been cultivated in chick embryos.

The availability of alligator blood infected with Haemogregarina suggested an attempt to determine whether that sporozoan parasite might find the chick embryo erythrocyte supportive for development. The Sporozoa are relatively host-specific, however, and attempts to infect chick embryos with malaria parasites of man or rodents have invariably failed. Nevertheless, should the haemogregarine successfully infect the embryo, that host might be valuable in studies of the biology of the parasite.

To date four (4) injections of infected alligator blood have been made into embryos via the intravenous route. Recipients ranged from ten (10) to fifteen (15) days incubation. While no embryo erythrocyte has been found to contain the parasite, it is apparent that alligator red cells survive intact for at least five (5) days in the embryo circulation. The haemogregarine also survives but has thus far only been seen in the alligator red cells.

The cycle of the haemogregarine includes an intraerythrocytic multiplication of asexual forms with subsequent invasion of other red cells. Whether this occurs in the peripheral circulation or is restricted to vessels of organs, e.g. bone marrow, is problematic. Thus, one can hardly expect to infect the embryo cells with non-multiplying parasites, i.e. gametocytes. Despite the possibility that only asexual forms may have been included in the inoculum in experiments performed thus far, several other inoculations will be done before the project is abandoned.

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