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Host-Parasite Relations in Initiation of Infection II. Hyperglycemia and Stress in Experimental Infection with L. Monocytogenes

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ABSTRACT

The effects of applied stimuli on the initiation of experimental infection were studied on lemmings, rabbits, hamsters, and guinea pigs. Stress was simulated by injection of cortisone acetate, or by trauma, heat, or cold. *L. monocytogenes* was the organism used for experimental infection. The results showed that the course of the infection was influenced in favour of the invading microorganism by the stress agents used. Hyperglycemia was present in the animals that developed the infection to overt disease. This suggests that hyperglycemia may be the trigger mechanism.

INTRODUCTION

In the paper "Occurrence of Listeriosis in Arctic Mammals, with a note on its Possible Pathogenesis", an upset carbohydrate metabolism, whether initiated by stress (over-population, anxiety, fatigue, concurrent disease, gestation, etc.) or a dietary imbalance, was suggested as the trigger mechanism that makes conditions favourable for the proliferation of *L. monocygenes*, the infective agent.

This hypothesis is the foundation for the work reported in this and in the following paper dealing with certain phases of Host-Parasite relations. The investigation developed into a study of the effects of applied stimuli (or stress) on the cause of experimental infection, rather than a further study of Listeriosis per se. Because *L. monocytogenes* was involved in the initial observations and because of its tendency to latency, it was the obvious test organism.

In the work reported here, attempts have been made in the laboratory to reproduce conditions of stress as they might occur in nature, and to study their effect on the incidence and course of experimental infection with *L. monocytogenes*. Both normally susceptible and resistant animals were used.

Blood-sugar levels in both "stressed" and "non-stressed" animals have been determined, and the incidence of the simultaneous presence of infection and hyperglycemia in the two groups is compared.

It is not implied that every case of Listeriosis, whether in man or in domestic or wild animal species, depends on a hyperglycemic condition for its occurrence. Although hyperglycemia is among the earliest manifestations of a body's response to stress, it is only one of several effects that may influence the course of a superimposed infection. However, in the initiation of an acute listeric infection or in the activation of a latent listeric infection, a high blood-glucose or tissue-glucose level may be a deciding factor.

Once infection has been established in one or more highly susceptible (hyperglycemic) individuals, with the attendant rapid multiplication of the invading organisms and an increase in virulence through subsequent host passages, a hyperglycemia is probably no longer necessary for infection to become established in new individuals. The infection may then quickly reach epidemic proportions.

EXPERIMENTAL PROCEDURES

The experiments reported serially below were carried out on lemmings, rab-

bits, hamsters, and guinea pigs. Distribution of the animals into experimental and control groups, details of treatment, and results obtained, are reported in the following sections.

Stress was usually simulated by injection of cortisone acetate, but some guinea pigs were also exposed to trauma, heat, or cold, and in some groups gestation was an added stress.

Exposure to experimental infection with *L. monocytogenes* was made by injection intraperitoneally of an undiluted culture of the organism.

TABLE I

Activation of Latent Infections in the Lemming by Injection of Cortisone (simulated stress)

No.	Dosage per day	Body weight, gm.		Survival Time, Days	Bact'gy		Pathology
		Initial	Change		Brain	Liver	
1	CORTISONE 2.5 mg. (4 days) then 5.0 mg. until death	58	− 3	D 19	−	X	Liver: midzone necrosis ++ Lung: congestion +
2	"	75	− 9	D 16	−	X	Liver: cong. +++; central necrosis Lung: Edema ++
3	"	75	− 10	D 16	−	X	Liver: fatty degen. + Lung: Edema ++
4	2.5 mg.	44	− 11	D 4	+ Listeria	+	Liver: fatty degen. +; small abscess # Kidney: abscess ++ # Lung: Cong. ++
5	"	37	− 5	D 12	--	−	Liver focal necrosis with abscess #
6	SALINE 0.1 ml. (4 days) then 0.2 ml. until death	35	+ 10	D 21	−	X	Liver: necrosis (large)
7	"	38	+ 2	K 42	−	−	No findings
8	"	43	− 1	K 42	−	−	Kidney: tubular dilation
9	0.1 ml.	35	− 13	K 21	−	−	No findings
10	"	68	− 23	K 21	−	−	No findings

NOTE: K = killed on day indicated D = died on day indicated X = organisms other than L. *monocytogenes*
+ = L. *monocytogenes* isolated # = Listeria lesions

Culture

The strain of *L monocytogenes* used was obtained from Professor E. G. D. Murray, Department of Bacteriology, McGill University. It was identified as Strain 80: Type IV (Paterson's Strain 5214). It was held in lyophilized vials at refrigerator temperature.

When this strain was to be used to infect animals, 250 ml. of Bacto Tryptose Broth with thiamine hydrochloride was inoculated from a vial of Strain 80 and incubated for 24 hours. Bacterial counts were made direct from this culture, by means of the "Drop Plate" method of Reed and Reed (1).

Blood sugar was determined by the Folin-Wu method on samples of blood collected while the animals were under ether anaesthesia.

Body weights were recorded before experimental treatment, and then periodically until the animals died or were killed to end the experiment. Various tissues were collected at autopsy for histo-pathological examination and bacteriological culture. Identification of pathogenic organisms was made from macerated tissues that were held under refrigeration until plated on tryptose agar, as previously described (3). *L. monocytogenes* was easily identified under a dissecting microscope in oblique light, as smooth greenish colonies. The identity was confirmed by other tests. Other pathogenic organisms were sometimes isolated also, as noted in the tables of data.

Agglutination titres were determined in some experiments. The sera were collected for these tests both before and following infection. A flagellar antigen was used.

EXPERIMENT I

Activation of latent infection in the lemming by injection of cortisone.

Previously it was reported that *L. monocytogenes* was not isolated during autopsies of apparently normal lemmings at Fort Churchill, but was isolated from other animals from the same group, following shipment to Kingston (3). As the isolation had been reported on two previous occasions (4,5), the chance of infection en route can be ruled out. The hypothesis that some stress factor was activating a latent Listeria infection was tested on an experimental basis. Ten lemmings that had become acclimatized to conditions in the laboratory and had remained healthy for six months, were used. The lemmings were divided into 2 groups and treated as shown in Table I.

Results

Table I shows survival times expressed as days from beginning of treatment, and changes in body weights, as well as the bacteriological and pathological findings. *L. monocytogenes* was isolated from one lemming in the cortisone-treated group. The animals of this group died within 19 days with fatty degeneration, necrosis, or abscess formation of the liver. The other pathogenic bacteria involved included streptococci, staphlococci, and salmonella.

EXPERIMENT II

Influence of cortisone on experimental L. monocytogenes infection in hamsters.

Hamsters are normally resistant to Listeriosis. Nineteen animals were treated in experimental and control groups as shown in Table 2.

Results

All of the experimentally infected hamsters became ill in 24 to 48 hours. The cortisone-treated animals became progressively ill, while the control animals improved and went on to clinical recovery, with the exception of one that died on the 4th day. This was a gravid female that had one hind leg completely skinned from hip to toe on the 2nd day, presumably from fighting with a cage mate. The second gravid control aborted early in the experimental period, and survived.

TABLE II

The Production of Infection with L. monocytogenes in a High Resistant Host (Hamsters)

Animal		Dosage per day	Body weight, gm.		Survival Time, Days	Bact'gy		Remarks
			Initial	Change		Brain	Liver	
Gravid Females	1	CORTISONE 5 mg.	165	− 44	D 4	+	+	No abortion
	2	"	135	− 50	D 14	−	+	Unobserved abortion
	3	Nil	129	− 10	D 4	+	+	Partial abortion. Trauma
	4	Nil	135	− 58	K 24	−	−	Early abortion
Non-Gravid Females	5	5 mg.	135	− 47	D 19	−	+	
	6	"	125	− 53	K 24	−	+	
	7	"	130	− 37	D 19	−	+	
	8	Nil	115	− 27	K 24	−	−	
	9	Nil	125	− 43	K 24	−	−	
	10	Nil	125	− 50	D 27	−	−	10 mg. cortisone daily from 24th day
Males	11	5 mg.	100	− 23	D 22	−	+	
	12	"	102	− 8	D 9	+	+	
	13	"	112	− 48	K 24	−	+	
	14	Nil	110	− 20	K 24	−	−	
	15	Nil	108	− 28	K 24	−	−	
	16	Nil	106	− 26	K 27	−	−	10 mg. cortisone daily from 24th day
	17	5 mg.	93	− 8	D 18	−	−	No L. monocytogenes injected
	18	"	92	− 8	D 11	−	−	No L. monocytogenes injected
Female	19	"	105	− 18	K 24	−	−	No L. monocytogenes injected

NOTE: D = died on day indicated K = killed on day indicated

In the non-gravid female group, the three controls survived to the 24th day, when two were killed. The third (No. 10) was injected with 10 mg. cortisone acetate from the 24th day, and died on the 27th day.

L. monocytogenes was recovered from all cortisone-treated, infected animals. It was not recovered from any non-cortisone-treated control except the gravid female with trauma (No. 3). It was not recovered from the non-infected controls (Nos. 17 to 19).

EXPERIMENT III

Activation of induced latent infection in rabbits by cortisone.

Male rabbits of about the same age were divided into three groups of four and treated as shown in Table 3. All animals were injected with a culture of *L. monocytogenes*. Infection was evidenced by inactivity of the rabbits, lack of appetite and accelerated breathing. By the 5th day these signs were followed by apparent recovery. Agglutination titres on the 5th day also gave evidence of infection. The animals were then considered latently infected.

Treatment with cortisone acetate was started on the 5th day. Signs of illness reappeared in groups II and III almost immediately and lasted 5 to 7 days, again followed by recovery except in Nos. 5 and 11, which died. The remainder were killed on the 21st day after infection.

Blood-sugar levels remained reasonably constant in Group I (controls), while Groups II and III showed increases on the 5th and 13th days.

L. monocytogenes was not isolated from any animal in Group I, and all agglutination titres rose. The organism was isolated from three animals in each of Groups II and III, and titres were relatively stable.

Pathological lesions were found only in the rabbits that died, as noted in Table 3. Brain, lung, liver, spleen, kidney and adrenal tissues from the survivors showed no changes, except non-specific changes in Nos. 8 and 12.

EXPERIMENT IV

The effect of cortisone on the pathogenesis of Listeriosis in the Hamster.

Hamsters from the stock colony were divided into 4 groups of 15. Animals in Group I were injected with a culture of *L. monocytogenes*, followed by cortisone acetate in 5 mg. doses daily except on the 5th and 12th days; Group II were injected with *L. monocytogenes* only, Group III were injected with cortisone acetate only, same level as Group I. Group IV were untreated controls.

On each day of treatment except the 5th and 12th, one animal from each group was anaesthetized and a blood sample taken for glucose determination. The animal was then killed and autopsied. Brain and liver tissues were examined from all animals, other tissues alternately as shown in Table 4.

Results

L. monocytogenes was isolated from all but one animal in Group I, and from all animals of Group II that were killed in the first 6 days after injection of the organism. Other pathogens were isolated as shown in Table 4.

The range of individual blood-sugar levels was considerable, but group averages were higher in the cortisone-treated animals (Group I and III), as indicated in Table 4.

Histo-pathological findings in Group I showed that 7 hamsters had lesions due to *L. monocytogenes;* a further two had lesions that were suggestive, and these were considered positive because the organism was isolated from both animals.

In Group II, only one hamster showed histopathological evidence of such infection. In the non-infected Groups III and IV, only non-specific lesions were seen. That is, infection progressed to disease in Group I, but in no instance did it do so in Group II.

TABLE III

Activation of Latent Infection in the Rabbit with L. monocytogenes by Cortisone (stress)

Animal Number	Cortisone Dosage per Day	Body Weight, gm. (days after infection)				Blood Sugars, mg./100 ml. (days after infection)				Agglutination Titre* (days after infection)		Bact'gy		Pathology
		Init.	6	17	20	Before	2	5+	13	5	17	Brain	Liver	
Group I 1	Nil	2395	+ 99	+160	+195	102	104	105	103	1:160	1:640	–	–	Nil
2	"	2385	+ 50	+305	+540	107	123	105	116	1:320	1:1280	–	–	Nil
3	"	1965	−270	−585	−435	106	125	97	126	1:640	1:1280	–	–	Nil
4	"	2325	− 20	− 80	− 55	103	110	107	131	1:160	1:1280	–	–	Nil
Group II 5	12.5 mg.	2410	−104	−160	−685	127	102	176	112	1:320	1:320	+	+	Purulent meningitis and encephalities # Liver abscess ++ # Paralyzed 11th day. died on 15th day of cortisone inj.
6	"	2375	− 20	− 65	−227	106	108	156	129	1:640	1:640	–	+	Nil
7	"	1925	− 1	−160	+ 48	99	114	148	123	1:640	1:320	+	–	Nil
8	"	2000	−214	−110	−503	116	105	128	149	1:160	1:160	–	–	Kidney — focal tubular dilation
Group III 9	25 mg.	2125	+150	+ 55	−190	111	107	122	135	1:160	1:160	–	–	Nil
10	"	2675	+ 85	+ 25	− 45	108	115	138	146	1:640	1:320	–	+	Nil
11	"	1825	+116	+ 25	–	112	118	144	–	1:640	–	+	+	Liver abscesses + # Died on 9th day of cortisone inj.
12	"	2465	−353	−530	−559	107	98	139	135	1:160	1:1280	–	+	Kidney — focal tubular dilation

+ = Cortisone started on 5th day after infection. All survivors killed on 21st day after infection
* = All titres negative before infection
# = Listeria lesions

TABLE IV

Effect of Cortisone on the Pathogenesis of Listeriosis in the Hamster (Results of Cutlure for Listeria)

Survival time in days	Group I: Infection plus 5 mg. cortisone daily							Group II: Infection only							Group III: Cortisone only, 5 mg. daily							Group IV: Controls							Blood Sugar mg./100 ml.			
	Brain	Liver	Spleen	Adrenal	Lung	Kidney	Blood	Brain	Liver	Spleen	Adrena	Lung	Kidney	Blood	Brain	Liver	Spleen	Adrenal	Lung	Kidney	Blood	Brain	Liver	Spleen	Adrenal	Lung	Kidney	Blood	I	II	III	IV
1	+	+	0	0	+	+	+	−	+	0	0	+	+	−	−	−	0	0	−	−	0	0	0	0	0	0	0	0	0	0	0	0
2	+	++	0	0	++	++	+	+	++	0	0	++	++	+	−	−	0	0	−	−	X	−	−	0	0	−	−	X	176	104	132	110
3	−X	+	+	+	0	0	+	−	+	−	+	0	0	+	−	−	−	0	0	X	−	−	−	0	0	0	0	0	224	210	0	186
4	−X	++	0	0	+	+	−X	−	++	0	0	−	−	−	−	−	0	0	−	0	−	−	0	0	−	−	0	0	182	64	228	99
6	+	++	+	+	0	0	+X	−	+	−	+	0	0	−	−	−	−X	−	0	0	−X	−	−	−	−	0	0	−	228	190	136	140
7	−	+	0	0	+	+	+	−	−	0	0	−	−	−	−	−	0	0	−	−	−	0	0	0	0	0	0	−	156	130	137	148
8	−X	−	−	+	0	0	+X	−	−	−	−	0	0	−	−	−	−	0	0	−	−	0	−	0	0	0	0	−	380	72	266	176
9	+	+	0	0	+	+	−	−	−	0	0	−	−	−	−	−X	0	0	−X	−X	−	−	−	0	0	0	0	−	162	142	154	138
10	+	+	−	−	0	0	+X	−	−	−	−	0	0	−X	−	−	−	−	0	0	−	−	−	0	0	0	0	−	262	142	132	94
11	+	+X	0	0	+	+	+X	−	−	0	0	−	−	−	−	−	0	0	−	−	−	0	0	0	0	0	0	−	0	0	0	0
13	+	−	−	−	0	0	−X	−	−	−	−	0	0	−X	−	−	−	0	−	0	−	0	0	0	0	0	0	−	190	126	128	154
14	−	+	0	0	+	+	+	−	−	0	0	−	−	−	−	−X	0	0	−	0	−	−	−	−	0	0	0	0	−	0	0	0	0
15	−X	−X	−X	−	0	0	+	−	−	−	0	0	0	−	−	−X	−	−	0	0	−	−	−	−X	0	0	0	0	−	0	0	0	0
16	+	+	0	0	+	+	0	−	−	0	0	−	−	0	−	−	0	0	−	−	−	−	−	−X	0	0	−	−	−	0	0	0	0
17	−	0	+	0	+X	+	0	−	0	0	0	−X	−	0	−	−	−	−	0	0	−	−X	−	0	0	0	0	−	0	0	0	0

NOTE: 0 = tissue not examined X = organisms other than L. monocytogenes

EXPERIMENT V

The influence of cortisone, trauma, heat and cold on experimental L. monocytogenes infection in guinea pigs.

A total of 102 guinea pigs in groups of varying size were treated to produce actual or simulated stress, with and without simultaneous injection of *L. monocytogenes*. The distribution is indicated in Table 5.

Cortisone acetate was injected daily as follows, except that none was injected for 2 days at each week-end during the experiment: 6.5 mg. for 5 days, 12.5 mg. for 2 days, then 25 mg. until termination by death.

Trauma was inflicted under anaesthesia, by removal of the skin from hip to toe of one hind limb.

For heat exposure, the guinea pigs were kept in a constant temperature room at 37°C. The door was left open for 10 minutes 3 times a day for ventilation.

For cold exposure, the guinea pigs were kept in a room at 4°C, similarly ventilated, or in a refrigerated cage kept at -10°C.

Animals that did not die were killed on the days shown, expressed as days

TABLE V

Experimental Listeriosis in Guinea Pigs

Group	Treatment	No. of Animals			Remarks and Reference
		Total	Died	L. monocytogenes Isolated	
1	Exp. infection with L. monocytogenes	10	0	2	Table 6
2	Exp. infection plus cortisone	13	1	5	Table 7
3	" " plus trauma	11	1	9	Table 8. Long incubation, short illness
4	" " plus heat	16	7	13	Table 9. Short incubation and acute illness
5	" " plus cold 4°C.	12	1	5	Table 10. Short, mild incubation and illness
6	" " plus cold — 10°C.	4	2	4	Table 10. Short, violent illness
7	Normal	16	0	0	Table 11
8	Normal plus cortisone	8	0	0	Table 12
9	Normal plus trauma	3	1	0	Table 12, Died on 3rd day
10	Normal plus heat	4	1	0	Table 13. Died on 3rd day
11	Normal plus cold 4°C.	4	0	0	Table 13
12	Normal plus cold — 10°C.	1	1	0	Table 13. Died on 2nd day

TABLE VI

GROUP I — L. monocytogenes Only — (10)

Animal Number	Survival Days	Blood Sugar	Agglutination Titres	Bacteriology			Pathology
				Brain	Liver	Blood	
1	K 2	196	—	—	X	–	–
2	K 3	178	–	–	+	–	–
3	K 7	208	–	–	+	–	#
4	K 8	184	–	–	–	–	–
5	K 9	188	1:2560	–	–	–	–
6	K 10	129	1:640	–	–X	–	–
7	K 13	132	–	–	–	–	–
8	K 14	130	–	–	X	–	–
9	K 15	132	–	–	X	–X	–
10	K 16	176	–	–	X	–	#
4 gravid females D		0	0	0	+	0	#

NOTE: K = killed on day indicated D = died X = organisms other than L. monocytogenes
+ = L. monocytogenes isolated # = Listeria lesions

of survival, in Tables 6 to 13. A blood sample and tissues were collected and examined, and cultures made at autopsy as described above.

Results

Normal controls (that is, not experimentally infected) survived the different forms of stress, except as noted in Table 5.

In the experimentally infected series, isolation of *L. monocytogenes* was made from all animals that died. Lesions seldom appeared in the organs before the 3rd day after infection, and were usually observed between the 3rd and 8th day.

The course of the disease may be divided into 4 periods, on the evidence presented in Tables 6 to 13.

1. Incubation, in which most animals were ill but no deaths occurred. Few bacteriologically positive tissues and only peritoneal lesions were found in killed animals.
2. Acute Illness, in which most of the deaths occurred, and most tissues were bacteriologically and pathologically positive.
3. Recovery, in which clinical improvement took place and no deaths occurred, no lesions were found, but bacteriologically positive tissues were still frequent.
4. Latency, with apparently complete recovery, but positive cultures were obtained from a few tissues.

DISCUSSION

The bringing together of host and parasite does not always result in infection and disease. The internal en-

vironment of the host at the time must be such that it will meet the parasite's essential metabolic requirements. If these are not met, a lag period will result that allows the host's defensive mechanism to overcome and destroy the pathogen. Should they be met, microbial multiplication will occur and overwhelm the defence mechanism, with disease as the outcome.

In a discussion regarding the hypothesis that the occurrence of an elevated blood or tissue glucose level may be the trigger mechanism in the initiation of certain infections, other factors should be included. In the first series of experiments designed to investigate the effect of hyperglycemia acetate. Cortisone simulates stress by initiating reactions, both physiological and pathological, essentially similar to those caused by external stimuli to which the average host is subject.

For the experimental production of hyperglycemia, cortisone has the disadvantage of initiating a variety of other phenomena also associated with stress. These include increased potassium excretion by the kidneys, increased protein breakdown, and retarded growth. It also accelerates the destruction of white blood cells and depresses antibody formation (formation of gamma globulin). Long (7) suggests that the cortical hormone stimulates those processes of gluconeogenesis by which blood glucose is main-

TABLE VII

GROUP II — L. monocytogenes plus Cortisone — (13)

Animal Number	Survival Days	Blood Sugar	Agglutination Titres	Bacteriology			Pathology
				Brain	Liver	Blood	
11	K 1	144	–	–	–	–	
12	K 2	212	–	–	+	–	#
13	K 3	236	–	+	+	+	#
14	D 7	0	0	–	+	+	#
15	K 8	270	–	–	+	–	#
16	K 9	276	–	–	–	–	–
17	K 10	200	1:640	–	–	–	#
18	K 13	154	1:80	–	+X	–	–
19	K 14	126	1:320	–	–X	–	–
20	K 15	118	–	–	–	–	–
21	K 15	184	–	–	–X	–X	–
22	K 16	136	–	–	–	–	–
23	K 16	146	–	–	–X	–	–
3 gravid females	D	0	0	0	+	0	#

NOTE: K = killed on day indicated D = died X = organisms other than L. monocytogenes
+ = L. monocytogenes isolated # = Listeria lesions

TABLE VIII

GROUP III — L. monocytogenes plus Trauma — (11)

Animal Number	Survival Days	Blood Sugar	Agglutination Titres	Bacteriology			Pathology
				Brain	Liver	Blood	
24	K 1	186	–	–	+	–	#
25	K 2	204	0	–	+	–	–
26	K 3	246	–	–	+	–	#
27	D 5	0	0	–	+	–	#
28	K 7	176	–	–	+	–X	#
29	K 8	240	1:40960	–	+	–	–
30	K 9	162	–	–	–	–X	–
31	K 10	129	1:60	–	+	–	–
32	K 13	154	–	–	+	–	–
33	K 14	140	1:80	–	–	–	–
34	K 15	118	1:640	–	+	–	–
3 gravid females	D	0	0	0	+	0	#

NOTE: K = killed on day indicated D = died X = organisms other than L. monocytogenes
+ = L. monocytogenes isolated # = Listeria lesions

tained at the expense of tissue proteins. Selye (8) has shown that exposure to damaging agents always increases the blood sugar during the first hours following their impact on the body. It would seem justifiable to say that these phenomena in themselves would not adversely affect the course of a superimposed infection in its initial stage (12 to 24 hr.).

Bjorneboe (9) reports that "the administration of cortisone was found to result in a reduction in the concentration of anti-pneumococcal antibody in the circulation of rabbits. Marked atrophic changes in lymphoid tissue and a diminution in the number of various types of mononuclear cells followed upon the hormone administration". As stress, actual or simulated, is known to cause a depression in antibody formation coincident with atrophic changes in lymphoid tissue, this may be a significant factor in a lowered host resistance. At the same time, it is very unlikely that antibody formation and activity play a decisive role in the early stage of infection. Usually it is some days before an agglutinating titre appears in the serum of an infected animal. In our experiments, titres were not found before the 5th day after infection.

In Experiment I the isolation of *L. monocytogenes* from one lemming provides additional evidence (3,4,5) that this organism may be present in a latent form in the lemming, and that hormonal influences are capable of activating the organism and precipitating a full-blown infection. However, the fact that *L. monocytogenes* was isolated from I out of 5 cortisone-infected lemmings and

that the remaining 4 also died with bacterial infections, confirms the observations made by other workers that this phenomenon is true for a number of pathogenic bacterial species.

In Experiment 2 it was shown that cortisone administration was capable of producing infection with *L. monocytogenes* in the hamster, which in this laboratory has shown itself to be resistant to experimental infection with this organism. It is interesting to note that the only control animal which died was a gravid female. This animal, in addition to being subjected to the normal physiological stress of gestation (6), had been subjected to trauma by having a leg seriously injured, presumably from fighting. The second control gravid female aborted early in the experimental period and survived.

In three groups of Experiment 5, a few female animals in the early stage of gestation were inadvertently included. These animals were excluded from computation of the results, but have been recorded at the foot of the appropriate tables. Without exception they either died or aborted at the beginning of the experiment, further emphasizing

TABLE IX

GROUP IV — L. monocytogenes plus Heat (16)

Animal Number	Survival Days	Blood Sugar	Agglutination Titres	Bacteriology.			Pathology
				Brain	Liver	Blood	
35	K 1	160	–	–	+	–	#
36	K 2	178	–	–	+	–X	#
37	D 2	0	0	–	+	–	–
38	K 3	170	–	–	+	–	#
39	D 3	0	0	–	+	–	#
40	D 4	0	0	–	+	–	–
41	D 5	0	0	–	+	–	#
42	D 5	0	0	–	+	–	#
43	D 6	0	0	–	+X	–	#
44	D 6	0	0	–	+X	–	#
45	K 7	238	–	–	+	–X	#
46	K 8	276	–	–	+	–	–
47	K 9	162	–	–	–	–	–
48	K 10	134	1:60	–	–	–	–
49	K 13	144	–	–	+	–	–
50	K 14	144	–	–	–X	–	–

NOTE: K = killed on day indicated D = died X = organisms other than L. monocytogenes

\+ = L. monocytogenes isolated # = Listeria lesions

TABLE X

GROUP V. — L. monocytogenes plus Cold (16)

Animal Number	Survival Days	Blood Sugar	Agglutination Titres	Bacteriology			Pathology
				Brain	Liver	Blood	
4°C							
51	K 1	176	–	–	+	–	#
52	K 2	188	–	–	+	–	#
53	K 3	146	–	+	+	+	#
54	D 6	0	0	–	+	–	#
55	K 7	162	–	–	+	–	–
56	K 8	206	1:40960	–	+	–	#
57	K 9	0	1:1280	–	–	–	–
58	K 10	0	–	–	–X	–	–
59	K 13	128	–	–	–X	–	–
60	K 14	126	1:2560	–	–	–	–
61	K 15	154	1:640	–	–	–	–
62	K 16	132	–	–	–X	–	–
−10°C							
63	K 1	77	–	–	+	–	#
64	K 2	148	–	–	+	–	#
65	D 3	0	0	–	+	–	–
66	D 4	0	0	–	+	–	#
2 gravid females	D	0	0	0	+	0	#

NOTE: K = killed on day indicated D = died X = organisms other than L. monocytogenes
+ = L. monocytogenes isolated # = Listeria lesions

that the gravid female is more susceptible than the non-gravid female or the male. Gestation itself is stated to be diabetogenic (6) and both the maternal and fetal membranes are reported to be rich in glucose, as is the fetal liver.

In this Experiment it was found that, irrespective of the form of stress imposed on the animals injected with a culture of *L. monocytogenes,* those in which the infection became established (as evidenced by subsequent re-isolation of the organism) showed blood sugar values on the hyperglycemic level. But those that did not become infected showed blood sugar values normal for the species.

In Experiment 4 it was shown that if the normal hamster was injected with a culture of *L. monocytogenes,* to which it is highly resistant, infection occurred but did not progress to disease. The blood sugar level remained generally

unaltered, showing that infection alone in a non-susceptible host does not constitute sufficient stress to elevate this level. However, when cortisone was administered to either the normal or the infected animal, a hyperglycemic level was produced. In this environment the organism was evidently capable of multiplying to produce disease. As a result of establishment of the infection, this itself would become a stress agent and further activate the cortisone output, with further increase of the hyperglycemia. There was no increase in the antibody titre.

It has been believed for a long time from clinical evidence that diabetes predisposes to infection, notably staphylococcal and tubercular. Steinbach and Deskowitz (10) found that the dog was highly refractory to experimental infection with a particular human strain of tubercle bacillus (H37), but became definitely susceptible when rendered diabetic by pancreatectomy. While Dubos (11) believes that it is the ketosis resulting from diabetes and other conditions that is responsible for the enhanced susceptibility to various infections we have data from unreported experiments with rabbits show that ketosis did not accompany hyperglycemia in the early

TABLE XI

GROUP VI — Normals (16)

Animal Number	Survival Days	Blood Sugar	Agglutination Titres	Bacteriology			Pathology
				Brain	Liver	Blood	
67	K 1	128	–	–	–	–	–
68	K 2	164	–	–	–	–	–
69	K 3	170	–	–	–	–	–
70	K 7	180	–	–	–	–	–
71	K 8	150	–	–	–	–	–
72	K 8	176	–	–	–	–	–
73	K 8	184	–	–	–	–	–
74	K 9	206	–	–	–	–	–
75	K 9	172	–	–	–	–	–
76	K 9	158	–	–	–X	–	–
77	K 10	140	–	–	–	–	–
78	K 13	132	–	–	–	–	–
79	K 14	133	–	–	–	–	–
80	K 15	108	–	–	X	–	–
81	K 16	92	–	–	–	–	–
82	K 16	125	–	–	–	–	–

NOTE: K = killed on day indicated X = organisms other than L. monocytogenes

TABLE XII

GROUP VII — Cortisone Only (8)

Animal Number	Survival Days	Blood Sugar	Agglutination Titres	Bacteriology			Pathology
				Brain	Liver	Blood	
83	K 1	170	–	–	–	–	–
84	K 2	178	–	–			–
85	K 3	152	–	–	–	–	–
86	K 7	146	–	–	–	–	–
87	K 8	256	–	–	–	–	–
88	K 9	226	–	–	–	–	–
89	K 15	132	–	–	–	–	–
90	K 16	262	–	–	–	–	–

GROUP VIII – Trauma Only — (3)

91	K 3	205	–	–	–	–	–
92	D 4	0	0	–	–	0	–
93	K 15	200	–	–	–	–	–

NOTE: K = killed on day indicated D = died X = organisms other than L. monocytogenes
+ = L. monocytogenes isolated

stages of infection.

In a discussion of infection with *L. monocytogenes,* the conclusion cannot be avoided that emphasis must be put on the state of gestation. In the experiments reported the gravid females were always the more vulnerable. This observation led to the exclusion of all gravid females that died in Experiment 5, as their inclusion was thought to weigh too heavily in favour of the hypothesis. An interesting observation is that when abortion took place early in the experimental period, the animal usually survived. When abortion did not take place, a septicemia developed and the animal succumbed. This may be attributed to two factors: (a) The maternal and fetal membranes and the fetal liver, being high in glucose, offered the most suitable environment for rapid multiplication of the organism and for establishment of a focal infection. As a result of the infective process in the uterus, the fetus died and was expelled with the membranes. (b) When gestation was thus terminated, the blood sugar level returned to normal.

Potel (12) and Seeliger (13) both described Listeriosis in the human newborn. Potel called it granulomatosis infantisepticus, because of the histological features of the fetal liver. In the bovine species listeric infection is also often associated with abortion (14).

Since the first preparation of this

paper in 1957 as an internal report, several articles have appeared in the literature, which have a significant bearing on the subject of Host-Parasite relations. One (15) refers to the occurrence of a low metritis, caused by *L. monocytogenes*, with infection of the uterine contents leading to abortion or perinatal death in many mammalian species. One (16) deals not only with the "Revival of Microbial Slumbers", but also with their "Retreat into Latency".

One additional comment, although hypothetical, might be added in support of the influence of carbohydrate metabolism on infection with *L. monocytogenes*. The organs most consistently involved in this infection in the monogastric animal are the liver and the brain. Significantly this is also the case in the liver of the young ruminant, as long as it remains monogastric; liver lesions do not appear to any extent in the bovine after initiation of rumination, but brain lesions do. Listeriosis in the adult most often takes the form of an encephalitis. It has been shown that blood on passing through the brain loses glucose, the loss being about 10 mg. per 100 ml. of blood. This indicates that glucose is an important fuel of brain tissue.

It would not be illogical to correlate the change of the site of lesions with the change in the digestive processes. When the calf is several weeks of age, it commences to eat solid food and soon starts to ruminate. The function of the 4 stomachs possessed by the bovine species then comes into play. The glycemic levels of the newborn ruminant approximate those of the non-ruminant mammal. These decrease rather rapidly during the first 6 to 7 weeks of life, and then more slowly up to about the sixth month, when approximately adult values are reached (60 mg. per 100 ml.). The adult ruminant exhibits both qualitative and quantitative differences in its digestive processes. These in turn alter the intermediary metabolism of the animal (McCandless and Dye). The importance of the cellulose-fermenting bacteria to the nutrition of the adult ruminant hardly needs to be mentioned. According to Phillipson and McAnally the end products of cellulose digestion by these bacteria are not soluble carbohydrates, but rather volatile fatty acids. Not only cellulose but also starch, glucose, fructose, and sucrose are acted on by rumen bacteria with the production of similar end products. They have also shown that volatile fatty acids do not pass out of the rumen into the abomasum (the true stomach), but are absorbed into the portal circulation via the rumen epithelium. Fractionation of the fatty acids found in portal blood from the rumen indicates that the chief fatty acid so absorbed is acetic acid with lesser amounts of propionic and butyric acids (Barcroft et al). Of these, propionic acid, which represents 20 per cent of the fatty acids absorbed from the rumen, is known to be glycogenic. In other words, in the monogastric animals, irrespective of species, glucose is absorbed directly into the blood stream via the portal vein and the liver without intermediary steps being necessary, while in the adult ruminant carbohydrate metabolism depends on the conversion of intermediary volatile fatty acids such as propionic acid into glucose or glycogen by the liver (Dye et al) (15).

When considering that in the monogastric animals liver lesions are of almost constant occurrence in Listeriosis, while in the adult ruminant they are practically absent, one wonders if the reason may be, in addition to an altered carbohydrate metabolism, a sufficiently high residual content of propionic acid present in the liver of the latter animal to prevent the growth of the organism. We have found that the addition of .01M of propionic acid to our *L. monocytogenes* cultures completely inhibited the growth of the organism.

Further evidence in the attempt to incriminate hyperglycemia resulting from stress as the trigger mechanism

in acute infections and in activation of latent infections will be reported shortly. Employing genetically hyperglycemic mice and without invoking any stress syndrome, we have found that the survival time is significantly shorter in hyperglycemic than in normal mice, when both are experimentally infected.

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TABLE XIII

GROUP IX — Heat only (4)

Animal Number	Survival Days	Blood Sugar	Agglutination Titres	Bacteriology			Pathology
				Brain	Liver	Blood	
94	K 3	156	–	–	–	–	–
95	D 3	0	0	–	–	0	–
96	K 7	182	–	–	–	–	–
97	K 15	112	–	–	–	–	–

GROUP X — Cold only (– 4°C) – (4)

98	K 3	146	–	–	–	–	–
99	K 7	188	–	–	–	–	–
100	K 9	162	–	–	–	–	–
101	K 15	132	–	–	×	–	–

GROUP XI — Cold only (– 10°C) – (1)

102	D 2	0	0	–	–	0	–

NOTE: K = killed on day indicated D = died X = organisms other than L. monocytogenes
+ = L. monocytogenes isolated

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