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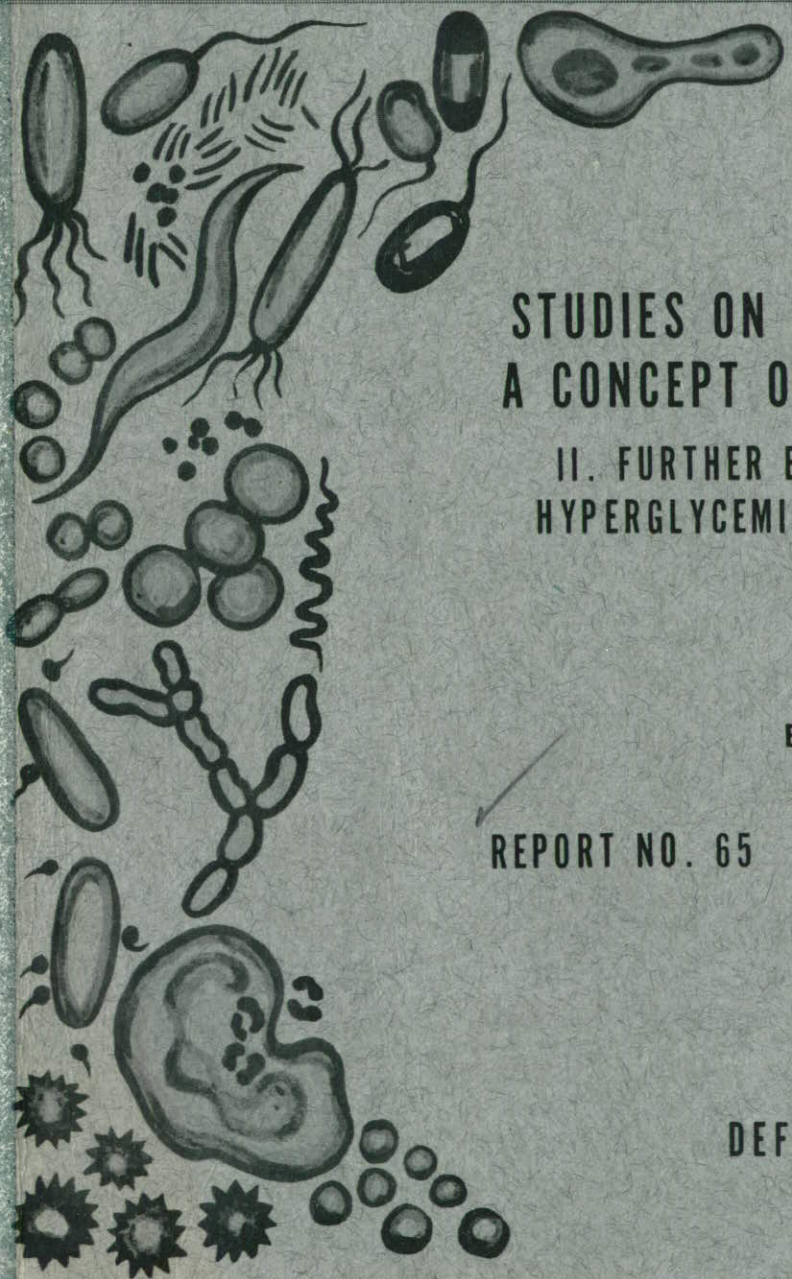

STUDIES ON HOST-PARASITE RELATIONS: A CONCEPT OF INITIATION OF INFECTION

II. FURTHER EVIDENCE ON THE INFLUENCE OF HYPERGLYCEMIA ON EXPERIMENTAL INFECTION

O.S. NORDLAND
and
BEVERLEY SANDERSON

REPORT NO. 65 APRIL, 1959

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STUDIES ON HOST-PARASITE RELATIONS – A CONCEPT OF INITIATION OF INFECTION

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INTRODUCTION

In the foregoing report on "Hyperglycemia and Experimental Infection with L. monocytogenes" (1), it was shown that the course of such an experimental infection can be influenced in favour of the invading micro-organism by various stress agents, whether simulated (cortisone) or naturally occurring (trauma, cold, heat). Throughout the experiments, one denominator common to the animals in which the infection progressed to overt disease was the presence of hyperglycemia.

In the search for a method to produce hyperglycemia in animals without invoking other physiological responses, as invariably occur when either naturally occurring or simulated stress agents are employed, and which might conceivably have a bearing on the outcome of an experimental infection, we first considered somatotrophic hormone (STH). This choice was prompted by a report by Selye (2) that a spontaneous infection in rats brought about by the injection of cortisone in toxic doses over a period of 12 days could be prevented by the simultaneous injection of somatotrophic hormone (STH). Kass, Lundgren, and Findland (3) found that STH did not increase resistance to pneumococcal and influenzal virus infections, and it failed to overcome the effect of cortisone in depressing resistance to these infections.

We were curious to see whether a combination of these two hormones might have any effect on an experimental infection of laboratory animals with *L. monocytogenes.* In addition, we could determine whether a correlation existed between the results obtained and the blood sugar levels of the infected animals.

At the same time, our attention was directed to the obese mice maintained at the Roscoe B. Jackson Memorial Laboratory, Bar Harbour, Maine (4). The obese hyperglycemic syndrome in these mice is due to the mutation of a single homozygous recessive gene. The hyperglycemia appears to be the fundamental disturbance. The levels of liver glycogen per gram of liver are similar in the obese hyperglycemic mice and their non-obese, non-hyperglycemic controls, but because of their larger livers the former have more than twice as much total liver glycogen. Significantly, however, the turnover of liver glycogen as measured by the incorporation of uniformly labelled glucose C^{14} is approximately six times as great per total liver glycogen, and three times as great per gram of liver and per milligram of glycogen, in the obese hyperglycemic mice as in their controls (5).

EXPERIMENTAL

The following experiments on hamsters and obese mice were based on the considerations outlined above.

Experiment 1: The effect of cortisone and STH singly, and in combination, on experimental infection with *L. monocytogenes,* and on the presence or absence of a concurrent hyperglycemia.

Method:

The choice of the hamster as experimental animal was prompted by its low susceptibility to experimental infection with *L. monocytogenes* (1). Forty hamsters, weighing 115 ±8 g., were divided into eight groups. Four groups were injected with the infecting organism, the remainder were used as hormone-treated or untreated (Group VIII) controls, as indicated below.

Group	Hormone		*L. monocytogenes* Injection
	Injection	Dose (mg.)	(ml. of whole culture)
I	STH	10	0.5
II	STH	10	NIL
III	Cortisone	5	0.5
IV	Cortisone	5	NIL
V	STH	10	0.5
	Cortisone	5	
VI	STH	10	NIL
	Cortisone	5	
VII	NIL	NIL	0.5
VIII	NIL	NIL	NIL

Six hours after administration of 0.5 ml. of whole culture, the first injection of hormones was given in doses indicated above. These hormone injections were repeated on the fwo following days.

Except when death interfered, one animal from each group was killed on the 2nd, 4th, 7th, 9th, and 11th day following infection; on the second day, the animal was killed before the final injection of hormones.

The animals were killed by ether administration. During anesthesia, a blood sample for blood sugar determination was obtained by incising the axillary region and severing the axillary artery. The blood was collected with a Pasteur pipette from the pocket thus produced between the front leg and the chest wall.

At autopsy, the same routine was followed as in earlier experiments (8). A piece of liver tissue was macerated in a grinder with a few millilitres of Tryptose broth. The grinder was emptied into an Erlenmeyer flask containing glass beads and approximately 15 ml. of Tryptose broth. The flask was shaken for 20 min., after which it was refrigerated for one week. Two drops of the suspension were then plated on

Tryptose agar (2 per cent). The plates were incubated for 24 hr. at 37°C and examined by the aid of a dissecting microscope, with transmitted light (6). The typical green colonies of *L. monocytogenes* were easily identified by this method.

In addition, blocks of tissue from the brain, lung, heart, liver, spleen, kidney and adrenal gland were placed in 10 per cent formalin for histopathological study. From these, paraffin sections were made later and stained with hematoxylin and eosin, and when required, with Lillie's Gramstain.

Results

The results are given in Table 1, and discussed below.

Clinical Observations

On the day following injection of the bacterial culture, the STH-plus-cortisone-injected animals (Group V) appeared quite ill. The cortisone-injected animals (Group III) were not as severely affected and the STH-injected animals (Group I) were influenced even less. The infected controls (Group VII) were only slightly affected. At 48 hr. this picture was accentuated, the Group VII animals being somewhat more affected. From this point the animals in Groups I and VII improved to complete clinical recovery, while the animals in Groups III and V deteriorated rapidly. One animal in Group III died on the 5th day, a second on the 7th day, and the two remaining were ill. It is doubtful that they would have survived had they not been killed. In Group V the animals were all practically moribund by the 4th day. This accounts for the well-below-normal blood sugar levels in the animals killed on the 4th and 7th day. In this group two animals were found dead on the morning of the 5th day. The animals in the 3 non-infected hormone control groups (II, IV, and VI) showed no clinical effects from the hormone injections.

Blood Sugar Determinations

The initial blood sugar levels in Group V (animals extremely ill almost from beginning, with two deaths by 5th day) were at the hyperglycemic level, 241 mg. per 100 ml. Furthermore, in the hormone-control animals (Group VI) 2nd and 4th day blood sugar levels were 234 and 210 mg. per 100 ml. This correlation was not present to the same extent in the cortisone groups (III and IV), but is still evident when these two groups together are compared with the two remaining group combinations (Groups I and II, and Groups VII and VIII).

Bacteriology

The infecting organism, *L. monocytogenes,* was isolated from all the animals in Groups III and V; from three animals in Group I; and from the two animals killed on the 2nd and 4th day in Group VII.

Histopathology

The results are given in Table 1.

The administration of STH did not offset the effects of cortisone when the two hormones were given together in this experiment in the doses employed. However, a correlation existed between severity of infection, based on clinical, bacteriological and pathological findings, and the presence of hyperglycemia in the initial stage of infection.

Experiment 2: A comparison of infection with a glucose-fermenting pathogen (*L. monocytogenes*) in hereditary hyperglycemic and non-hyperglycemic mice.

In our previous experiments, blood sugar determinations were made at the time the experimental animals were killed. The readings obtained from (a) infected animals (with *L. monocytogenes*) and (b) non-infected animals, both subjected to identical forms of stress, were compared with those from (c) non-infected, non-stressed animals. For this experiment the determinations were made before the experiment was started because we wished to obtain information regarding the comparative survival time in the hyperglycemic and normal groups of animals, rather than rely entirely on the morbidity figures, represented by the number of re-isolations of the organism from the animal tissues at the time of serial killing.

It was of interest to find what percentage of the total body weight of the hyperglycemic mice consisted of removable fat, and to investigate if the extra body weight influenced the course of infection. The dissectable fat amounted to approximately 30 per cent of body weight; therefore 70 per cent of total body weight was taken to represent 'fat-free' body weight.

Two groups of 10 hyperglycemic mice, ranging in size from 40 to 71 g., were injected with an 18-hr. culture of *L. monocytogenes*. Group I was injected with .02 ml. per g. of *total* body weight, while Group II was injected with .02 ml. per g. of *fat-free* body weight. This is equivalent to .014 ml. per g. of total body weight. One group of 9 non-hyperglycemic mice from the same stock as the hyperglycemic mice, ranging in weight from 25 to 36 g., was injected with .02 ml. per g. of total body weight (Group III).

Results (Table 2)

The average blood glucose for 100 ml. of blood was 297 mg. for Group I, 272 mg. for Group II, and 173 mg. for Group III.

The average survival time of the animals in Group I was 52, in Group II 72, and in Group III 95 hr.

Comparing the average blood sugar levels with the average survival time of the three groups, it is apparent that there is a definite correlation (inverse) between these two: the higher the blood glucose level the shorter the survival time.

Fig. 1 and Table 2 show that the animals in Group I died from 39 to 64 hr. after being infected, during a 24-hr. period. The animals in Group II died in a 9-hr. period between the 63rd and 72nd hr., with one exception. The period of incubation in Group II, with the smaller infecting dose, was longer than in Group I. Group III animals died from 54 to 162 hr. after infection, a period of 108 hr. This experiment indicates that the infection was more explosive in its onset and course in the hyperglycemic groups than in their controls.

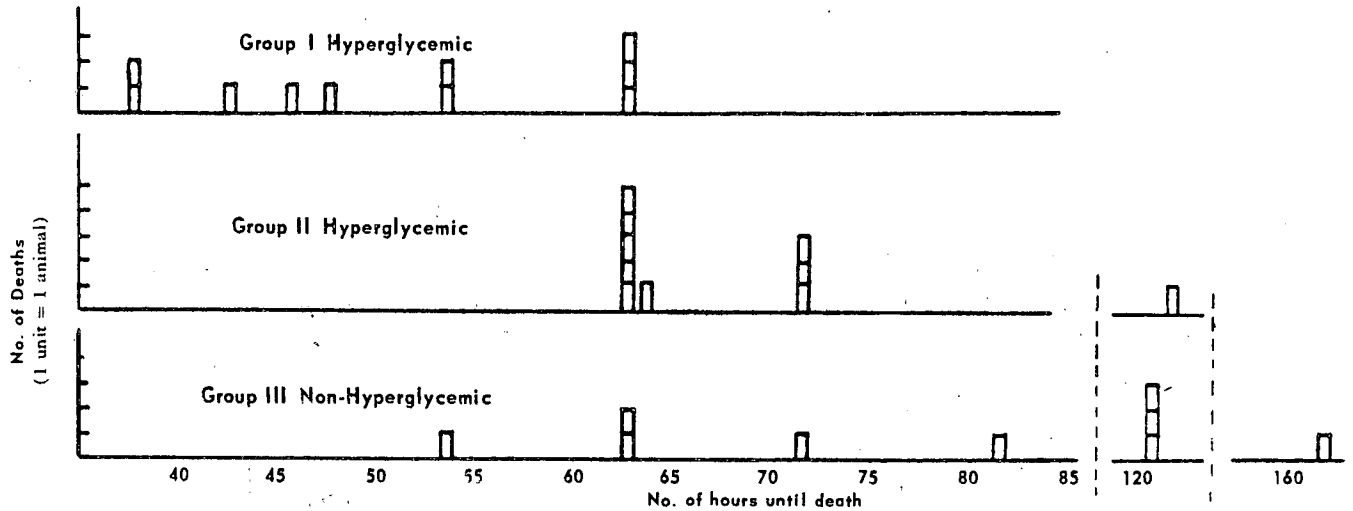

Fig. 1. Comparison of survival times of the three groups (data from Table 2)

Bacteriology and Pathology

Bacteriological and histopathological examinations were made on a few animals chosen at random. *L. monocytogenes* was always re-isolated from the tissues, and typical lesions of Listeriosis were observed (Table 2).

Experiment 3: A comparison of infection with a non-glucose fermenting pathogen (*Brucella bronchiseptica*) in hyperglycemic and non-hyperglycemic mice.

It has been indicated that the presence of hyperglycemia influences the course of an experimental infection with *L. monocytogenes*, a glucose fermenter, in favour of the infecting micro-organism. It was thought interesting to find what influence an existing hyperglycemia might have on an experimental infection with a non-glucose fermenter. Other things being equal, one would expect that the presence of hyperglycemia should not appreciably affect the course of the infection.

We obtained from Dr. D.A. Barnum, the Ontario Veterinary College, a culture of *Brucella bronchiseptica* (V36), a non-glucose fermenter. This organism differs further from *L. monocytogenes* in that, while the latter ferments a considerable number of carbohydrates as well as glucose, *B. bronchiseptica* does not ferment any carbohydrates whatsoever. It was therefore admirably suited to our purpose.

Twelve hyperglycemic and twelve control non-hyperglycemic mice were injected with 1 ml. each of a 1:500 dilution of a 48-hr. culture of *B. bronchiseptica.*

Results

Of the hyperglycemic animals, 10 died on the third day and 2 on the fourth day following infection.

Of the controls, one died on the second day, 7 on the third, 3 on the fourth, and one on the ninth day.

B. bronchiseptica was recovered from the tissues of every animal.

DISCUSSION AND CONCLUSIONS

Under the conditions of Experiment 1 and in the doses given, the cortisone and STH, when injected singly, did not influence the course of the infection as strongly as when given together. Cortisone, however, produced a more severe reaction than did STH. This may in part be explained by the observation by de Bodo and Siskoff that "both STH and (ACTH via) the adrenocortical hormones have a sparing action on carbohydrate utilization, including the deposition of glycogen; however, the former induces its effect primarily by inhibiting the entry of glucose into the cells, while the function of the latter appears to be concerned with the mobilization of amino acids from the body protein and increased gluconeogenesis by the liver." (7)

The correlation between severity and outcome of infection and level of blood glucose in the *initial* stage is of interest. The higher levels of blood glucose were found in the most severely affected groups of animals. This is consistent with earlier results (1).

In Experiment 2, in which we used hyperglycemic mice infected with a glucose fermenter, *L. monocytogenes,* we found the survival time in hyperglycemic animals to be shorter than in similarly infected non-hyperglycemic mice. Also, the onset and course of the infection was more rapid than in the control animals.

In Experiment 3, no appreciable difference was found in the survival time of hyperglycemic mice infected with a non-glucose fermenter, *Brucella bronchiseptica,* and in that of non-hyperglycemic mice infected with the same organism.

The results of these three experiments add evidence to support the validity of the opinion previously stated (8, 1), that an upset carbohydrate metabolism, initiated by stress (over-population, anxiety, fatigue, gestation, temperature changes or trauma) is the trigger mechanism that makes conditions favourable for the proliferation of *L. monocytogenes* and probably of other glucose-fermenting pathogenic agents.

In DRKL Report No. 64 (1) the influence of hyperglycemia on infection is discussed in relation to some of the additional effects of stress on the body. Two facts stand out in regard to the occurrence of hyperglycemia in stress; it occurs in the forms of stress investigated, and it occurs as an early manifestation of such stress. This early period is critical in host-parasite relations.

SUMMARY

(including references to associated Reports Nos. 47 and 64)

Reference was made in DRKL Report No. 47 to the isolation of *L. monocytogenes* from two groups of lemmings shipped from Fort Churchill to southern Ontario with subsequent development of fatal Listeriosis. In another study, on the deaths of lemmings maintained in the DRNL animal colony, the observed symptoms were diagnosed as diabetes mellitus; no bacteriological identification had been attempted in the latter study.

Serious illness of doubtful etiology in personnel at Fort Churchill, including an animal caretaker, led to an investigation by the author of possible Listeriosis in the DRNL lemming colony. Bacteriological, serological, and pathological examinations did not reveal the presence of *L. monocytogenes* in some 50 lemmings, although low antibody titres were found in a number of them. Low titres were found in 3 of 4 Eskimos and 1 of 4 whites examined, also. *L. monocytogenes* was isolated from the brain of a fox, however, after a quarantine period had shown rabies was not involved.

It appeared that Listeriosis might be indigenous to the area, sometimes in a latent state. This belief was supported by the development of a fatal infection in lemmings from the same colony after they had been transported to DRKL; *L. monocytogenes* was isolated from the brains of two lemmings.

It was improbable that infection had been incurred by three different groups during the brief shipment by air. Rather, external factors responsible for activation of an organism in a latent state seemed more likely. This hypothesis was further strengthened by the experimental production of Listeriosis by the injection of cortisone into apparently healthy lemmings, 6 months after their shipment from Churchill to Kingston. Details appear in DRKL Report No. 64.

The above observations led to further investigation of certain phases of Host-Parasite relations, which developed into a study of the effect of experimentally applied stimuli (or stress) on the course of experimental infection, rather than into further study of Listeriosis. *L. monocytogenes* was the obvious test organism, because it was involved in the initial observations and because its tendency to latency suited it for the purpose.

From the investigations reported in DRKL Reports 47, 64 and 65, it is submitted that the mere bringing together of host and parasite is not certain to result in disease. However, should the internal environment of the host be suitable to the invading organism, disease may be the outcome. Under conditions of stress as outlined, an invading pathogenic agent may produce a more acute illness than it ordinarily would. Similarly, a micro-organism present in the tissues of the host, but in a latent state, may be activated to produce overt disease.

A good deal of evidence is presented in these reports to indicate that, in certain instances, hyperglycemia may be the *trigger* mechanism that directly or indirectly initiates infection, when host and parasite are brought together.

ACKNOWLEDGMENTS

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TABLE 1

Group No. and Treatment	Animal No.	Blood sugar in mg./100 ml. Days after infection					Bacteriology	Pathology
		2nd	4th	7th	9th	11th		
Group I (*L. monocytogenes* plus STH)	1	114					+	focal necrosis of liver, perirenal inflammation †
	2		116				+	–
	3			131			–	–
	4				107		+	–
	5					102	–	perirenal inflammation †
Group II (STH only)	1	102					–	–
	2		172				–	–
	3			116			×	–
	4				117		–	–
	5					92	×	–
Group III (*L. monocytogenes* plus cortisone)	1	120					+	–
	2		154				+	focal necrosis of liver
	3			Died on 5th day			+×	focal necrosis of liver
	4			Died on 7th day			+	focal necrosis of liver
	5			110			+	nephritis †
Group IV (Cortisone only)	1	180					–	–
	2		111				–	–
	3			93			×	–
	4				170		–	–
	5					157	–	–
Group V (*L. monocytogenes* STH and cortisone)	1	241					+	focal necrosis of liver
	2		98 *				+×	focal necrosis of liver
	3			Died on 5th day			+×)	focal necrosis of liver, nephritis †, pneumonitis †, myocarditis †, adrenal abscesses †
	4			Died on 5th day			+×)	
	5			71 *			+	nephritis †
Group VI (STH and cortisone only)	1	234					–	–
	2		210				–	–
	3			110			×	–
	4				120		–	–
	5					138	–	–
Group VII (*L. monocytogenes* only)	1	61					+	–
	2		143				+	–
	3			121			–×	–
	4				128		–	–
	5					108	–	–
Group VIII (Normal controls)	1	128					–	–
	2		130				–	–
	3			118			–×	–
	4				128		–	–
	5					114	–	–

NOTE: + = Isolation of *L. monocytogenes*
– = No isolation of *L. monocytogenes*
× = Organisms other than *L. monocytogenes*
* = Moribund
† = Gram-positive rods *in situ*

TABLE 2

Results of Experiment 2

Type of mice and amount of *L. monocytogenes* culture injected per g. body wt.	Animal No.	Body Weight, g.	Actual Dose *L. mono.*, ml.	Blood Sugar, mg./100 ml.	Survival Time, hours	Bacteriology	Pathology
						Liver	Liver and Spleen
Group I 0.02 ml. Obese hyperglycemic	1	52	1.04	432	63		
	2	52	1.04	256	54	+×	#
	3	65	1.30	252	39	+	
	4	66	1.32	230	39	+	
	5	56	1.12	260	46	+	
	6	50	1.00	214	63		
	7	53	1.06	378	43	+	
	8	56	1.12	366	54		
	9	45	.90	322	63	+	
	10	71	1.42	256	47½	+	#
	Average	57		297	52		
Group II 0.014 ml. Obese hyperglycemic	1	57	.80	172	63		
	2	50	.70	374	72		
	3	40	.56	234	72	+	#
	4	56	.78	222	63		
	5	45	.63	270	63		
	6	48	.67	198	63		
	7	46	.64	406	63		
	8	45	.63	304	72		
	9	50	.70	186	66	+	#
	10	47	.66	356	121		
	Average	48		272	72		
Group III 0.02 ml. Non-hyperglycemic	1	36	.72	194	121		
	2	29	.58	182	54		
	3	33	.66	194	82		
	4	30	.60	142	63		
	5	36	.72	154	162		
	6	25	.50	182	121		
	7	27	.54	132	72	+	#
	8	29	.58	182	121		
	9	29	.58	196	63	+	#
	Average	30		173	95.4		

NOTE: + = isolation of *L. monocytogenes*
× = organisms other than *L. monocytogenes*
= Listeria lesions

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