BLOOD SUGAR STUDIES.

I. Rapid Alterations in the Blood Sugar Level of Rabbits as Result of Intravenous Injections of Killed Bacteria of Various Types.

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The effect produced by bacteria on the metabolism of the body into which they are introduced is an almost unexplored field of investigation. While many changes associated with infection, such as increased heat production, changes in functional activity of the circulatory and excretory systems, leucocytosis, and the development of immune bodies, have been elaborately investigated, little attention has been paid to the direct chemical changes resulting from bacterial invasion. The metabolic products of the bacteria, the enzymes they elaborate, the chemical composition of the bacterial body itself, aside from the specific toxins of the particular organism, must exert profound effects upon the chemistry of the body of the host. Recently Menten and Manning (1), in producing hyperglycemia in animals by the injection of killed organisms of the enteritidis-paratyphoid B group, have shown the possibilities of experimental work in this direction.

In order to investigate one phase of this problem, experiments were conducted to determine what effect on the blood sugar level of rabbits was produced by injecting intravenously killed organisms of various types. Any effect that would be produced would then be due to the chemical composition of the bacterial body, since killed organisms were used, thus eliminating the factor of the biological activity of the bacteria. It was found that certain organisms caused an immediate rise in blood sugar to a high level, while other organisms produced no effect.

Experimental Procedure.

18 hour cultures of various organisms on agar were suspended in physiological salt solution, killed by heat, and 1 cc. of the suspension, containing usually approximately 2 billion organisms, was injected intravenously into young adult male rabbits. Blood was withdrawn from the ear veins immediately before injection and at short intervals thereafter. Blood sugar determinations were made by the Folin-Wu method and leucocyte counts were made on each sample of blood. In cases where the blood pressure became extremely low, blood was obtained by heart puncture. In many cases the rectal temperature was taken immediately before each sample of blood was withdrawn.

In the following experiments a marked hyperglycemia was produced.

Experiment 1. B. proteus.—B. proteus, recently isolated from the urine of a patient with cystitis, was suspended in salt solution, killed, and injected intravenously. See curve of Fig. 1.

Time.		Blood sugar.
		per cent
Nov. 20, 1924		
a.m.		
10.45	Before injection.	0.104
11.00	*B. proteus* injected.	
11.15		0.137
11.30	Vasoconstriction and great weakness.	0.163
11.45		0.188
p.m.		
12.05	Animal prostrated.	0.253
12.20		0.275
12.50		0.264
1.50	" recovering.	0.269
5.00		0.146
6.00	" apparently well.	0.145

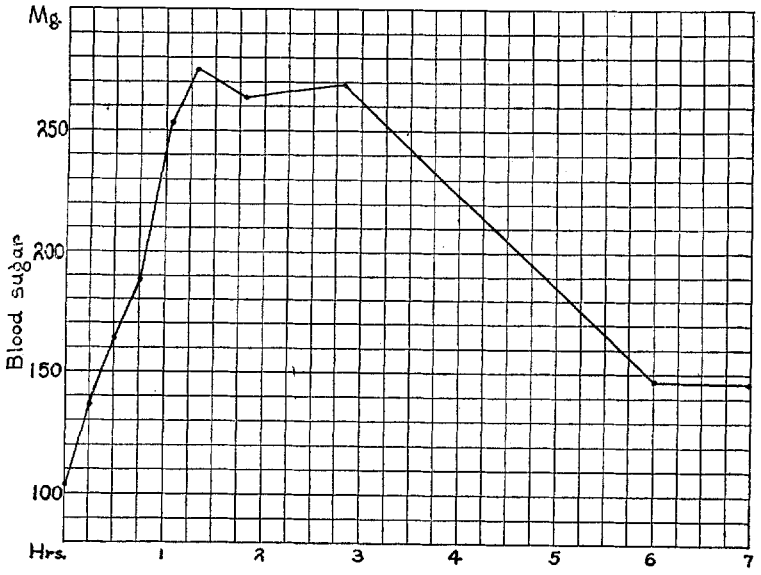

FIG. 1. *B. proteus.*

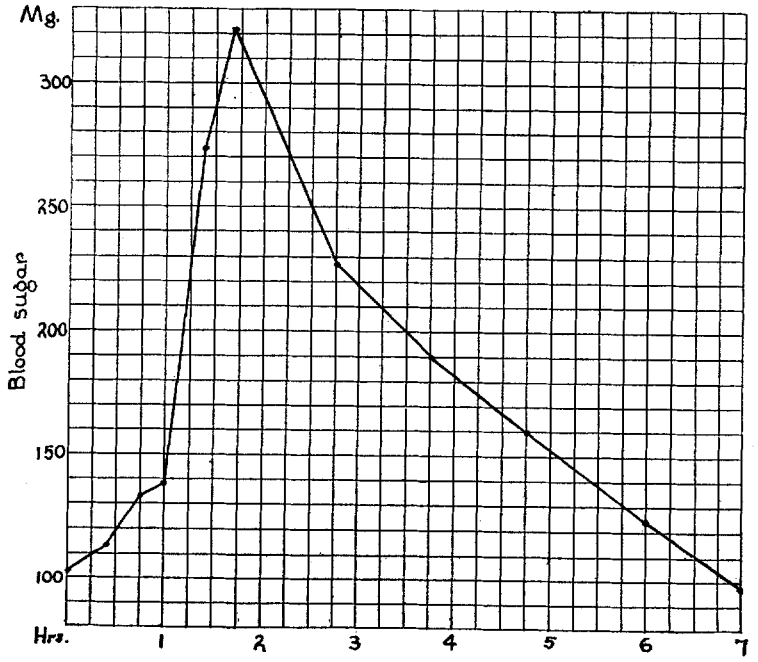

FIG. 2. *B. proteus.*

Experiment 2. B. proteus.—A large dose of killed *B. proteus* was given to the same rabbit used in Experiment 1, 1 week later. For curve, see Fig. 2.

Time.		Blood sugar.	W.B.C.
		per cent	
Nov. 28, 1924			
a.m.			
10.45	Before injection.	0.101	5,500
11.00	*B. proteus* injected.		
11.25		0.114	4,800
11.42	Vasoconstriction of ears.	0.134	2,200
m.			
12.00	Animal becoming weak.	0.138	1,900
p.m.			
12.25		0.274	1,200
12.40	Prostration.	0.321	1,250
1.45		0.227	600
2.45		0.190	700
3.45	Animal better.	0.155	1,600
5.00		0.124	3,800
6.00	" well.	0.097	4,700

Experiment 3. B. proteus vulgaris.—A stock culture of *B. proteus vulgaris*, which had been growing on artificial media for a long time, was used.

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°F.
Feb. 28, 1925				
a.m.				
8.45	Before injection.	0.095	8,600	101.2
9.00	*B. proteus vulgaris* injected.			
9.15		0.097	1,800	101.4
9.35	Vasoconstriction, copious diarrhea.	0.113	1,800	103.4
9.55		0.106	1,200	103.2
10.15		0.127	2,100	102.4
10.30		0.118	2,350	101.7
11.00	Animal prostrated.	0.147	1,600	101.0
m.				
12.00		0.118	1,650	100.4
p.m.				
1.00	" better.	1.108	2,900	101.3
2.00	" lively.	0.108	3,200	102.2

Experiment 4. B. coli.—B. coli communis, recently isolated from normal human feces, was suspended in salt solution, killed, and injected intravenously. See Fig. 3.

Time.		Blood sugar.	W.B.C.
		per cent	
Jan. 14, 1925			
a.m.			
10.00	Before injection.	0.117	5,600
10.20	*B. coli communis* injected.		
10.35		0.117	1,600
10.50		0.129	800
11.05		0.132	600
11.20		0.158	1,400
11.35		0.175	1,800
11.50	Animal prostrated.	0.244	1,400
p.m.			
12.30		0.182	1,400
1.30		0.132	1,600
2.30		0.120	1,200
3.30		0.116	2,400
4.30	" well.	0.116	4,600

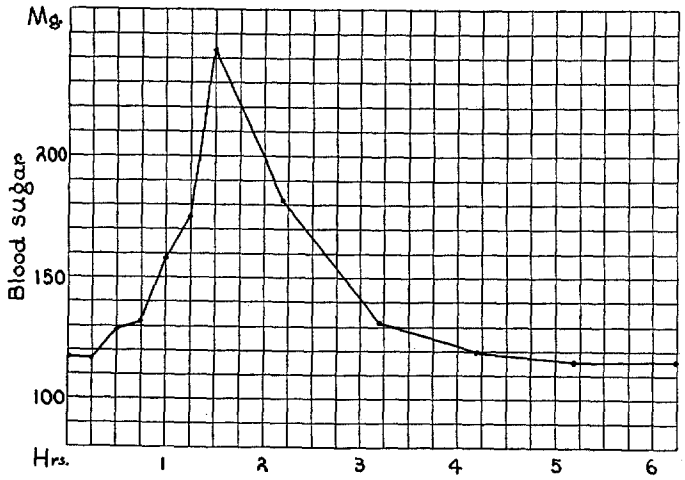

FIG. 3. *B. coli.*

Experiment 5. B. coli.—

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°F.
Mar. 27, 1925				
a.m.				
10.00	Before injection.	0.097	6,400	103.2
10.30	Injection of same organism as in Experiment 4, grown in media 2 mos.			
10.50		0.099	1,800	104.0
11.05		0.098	2,250	104.0
11.25		0.105	1,300	104.6
11.40		0.141	1,120	104.8
11.55		0.160	1,000	104.6
p.m.				
12.25		0.176	450	104.6
1.00		0.161	650	103.0
2.00		0.145	700	102.2
3.00		0.169	650	101.6
4.00		0.165	850	
6.00		0.112	3,650	

Experiment 6. B. paratyphosus B.—A stock culture of *B. paratyphosus* B was used. See Fig. 4.

Time.		Blood sugar.	W.B.C.
		per cent	
Jan. 27, 1925			
a.m.			
9.00	Before injection.	0.104	5,600
9.10	*B. paratyphosus* B injected.		
9.25		0.114	2,200
9.40		0.114	2,200
9.55	Animal weak.	0.104	1,700
10.10		0.130	1,300
10.25		0.160	1,600
10.45	" prostrated.	0.208	1,800
11.15		0.200	1,800
11.45		0.170	1,800
p.m.			
12.45		0.157	
1.45		0.113	3,000
2.45	" well.	0.090	4,800

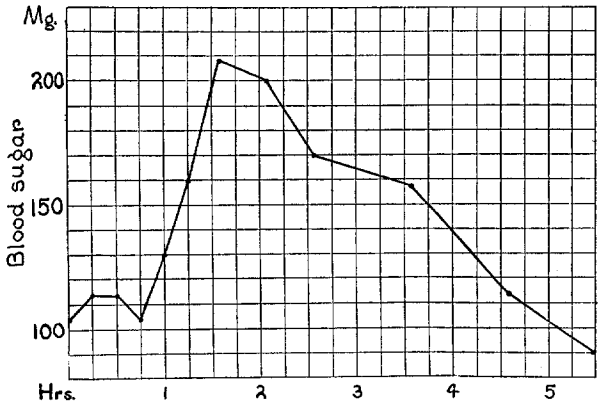

FIG. 4. *B. paratyphosus* B.

Experiment 7. B. paratyphosus B.—See Fig. 5.

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°F.
Mar. 10, 1925				
a.m.				
8.00	Before injection.	0.088	5,500	102.4
8.30	*B. paratyphosus* B injected.			
8.45		0.097	1,400	101.8
9.10	Diarrhea, weak.	0.114	700	101.6
10.00		0.187	850	101.4
10.30	Prostrated.	0.196	800	100.8
11.45		0.150	1,000	98.0
p.m.				
1.00		0.125	1,800	99.0
2.30		0.103	2,600	100.2
4.20	Recovery.	0.090	5,000	

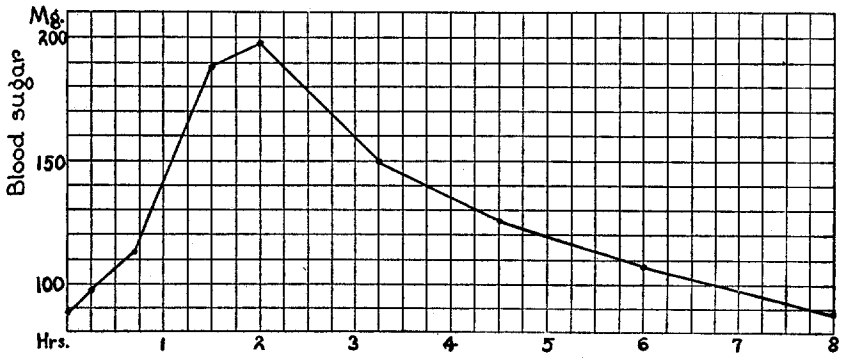

FIG. 5. *B. paratyphosus* B.

In the following experiments, a slight irregular rise in blood sugar occurred.

Experiment 8. B. paratyphosus A.—

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°F.
Feb. 17, 1925				
a.m.				
10.00	Before injection.	0.097	5,600	103.2
10.15	*B. paratyphosus* A injected.			
10.30		0.102	1,200	103.0
10.45		0.113	1,600	103.4
11.00	No symptoms.	0.098	1,200	103.8
11.15		0.098	1,000	103.6
11.30		0.120	1,200	103.6
m.				
12.00		0.110	1,600	103.4
p.m.				
2.30		0.099	3,600	103.4

Experiment 9. B. paratyphosus A.—

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°F.
Apr. 15, 1925				
m.				
12.00	Before injection.	0.096	4,400	103.2
p.m.				
1.15	*B. paratyphosus* A injected.			
1.30		0.096	2,400	103.0
1.45		0.101	1,600	104.0
2.00	Fall in blood pressure.	0.105	1,400	104.2
2.15	Slight weakness.	0.125	1,300	104.6
2.45		0.133	800	103.3
3.15		0.141	700	103.6
3.45		0.124	1,100	103.6
4.45		0.125	1,600	103.4

Experiment 10. B. enteritidis of Gaertner (Lethal Dose).—

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°F.
Feb. 17, 1925 *a.m.*				
8.30	Before iniection.	0.108	6,600	104.6
8.45	*B. enteritidis* of Gaertner injected.			
9.00		0.097	2,600	104.8
9.15	Weak; low blood pressure.	0.123	2,000	105.2
9.40	Copious diarrhea, prostration.	0.132	1,600	105.4
10.00		0.120	900	105.2
10.15			2,600	104.6
10.20	Shallow, rapid respirations.	0.132	2,800	104.0
10.22	Dead.	0.122		

Experiment 11. B. enteritidis of Gaertner.—

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°F.
Apr. 15, 1925 *a.m.*				
10.10	Before injection.	0.093	15,200	102.8
10.45	*B. enteritidis* of Gaertner injected.			
11.00		0.089	1,100	102.4
11.15		0.084	1,400	102.6
11.37	Marked prostration.	0.083	950	
11.50		0.082	1,300	103.2
p.m.				
12.15		0.088	700	102.4
12.35		0.091	2,200	101.4
1.00	Recovery.	0.095	2,000	100.8

The following organisms produced no notable fluctuations in the blood sugar level.

Experiment 12. B. fœcalis alkaligenes.—

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°*F.*
Feb. 25, 1925				
a.m.				
10.30	Before injection.	0.185	9,000	101.4
11.00	*B. fœcalis alkaligenes* injected.			
11.15		0.161	2,200	101.6
11.30		0.178	2,400	101.8
11.50	Weak.	0.196	1,800	101.1
p.m.				
12.15		0.188	1,600	100.0
12.45		0.187	1,700	99.3
1.30	Recovery.	0.185	3,600	100.1

Experiment 13. Streptococcus hæmolyticus.—

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°*F.*
Feb. 25, 1925				
a.m.				
9.30	Before injection.	0.097	4,100	103.2
9.45	*Streptococcus hæmolyticus* injected.			
10.15		0.101	4,300	103.3
10.45	No symptoms.	0.106	7,600	103.4
11.15		0.106	2,200	103.6
p.m.				
12.45		0.101	4,600	103.6
2.30		0.103	7,000	103.0

Experiment 14. Streptococcus viridans.—

Time.		Blood sugar.	W.B.C.
		per cent	
Jan. 19, 1925			
a.m.			
10.30	Before injection.	0.075	7,600
10.45	*Streptococcus viridans* injected.		
11.00		0.066	3,200
11.15			2,800
11.30		0.072	3,600
11.45	No symptoms.	0.072	2,400
m.			
12.00		0.073	3,000
p.m.			
12.15		0.072	2,600
12.30		0.072	2,600
1.00		0.072	3,800
2.15		0.072	6,600
3.15		0.066	5,800

Experiment 15. Streptococcus viridans.—

Time.		Blood sugar.	W.B.C.
		per cent	
Mar. 16, 1925			
a.m.			
10.00	Before injection.	0.091	4,400
10.05	*Streptococcus viridans* injected.		
10.10		0.114	3,000
10.20	No symptoms.	0.108	1,800
10.30		0.098	1,400
10.40		0.090	1,600

Experiment 16. Staphylococcus aureus.—

Time.		Blood sugar.
		per cent
Dec. 1, 1924		
a.m.		
9.00	Before injection.	0.109
9.15	*Staphylococcus aureus* injected.	
9.30		0.115
9.45		0.125
10.00	No symptoms.	0.109
10.15		0.108
10.30		0.126
10.45		0.113

Experiment 17. Staphylococcus aureus.—

Time.		Blood sugar.
		per cent
Feb. 3, 1925		
a.m.		
8.30	Before injection.	0.124
8.45	*Staphylococcus aureus* injected.	
9.00		0.125
9.15	No symptoms.	0.124
9.45		0.125

Experiment 18. B. pyocyaneus.—

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°F.
Mar. 23, 1925				
a.m.				
11.30	Before injection.	0.096	7,500	101.6
11.45	*B. pyocyaneus* injected.			
m.				
12.00		0.087	2,600	103.2
p.m.				
12.20		0.090	1,600	104.6
12.40	Weak.	0.083	1,500	
1.05		0.099	600	105.8
1.30		0.090	1,800	104.2
2.05	Recovery.		1,800	105.2

Experiment 19. B. typhosus.—

Time.		Blood sugar.	W.B.C.	Temperature.
a.m.		*per cent*		°F.
9.15	Before injection.	0.099	6,600	102.0
9.30	*B. typhosus* injected.			
9.45		0.099	3,900	102.0
10.00		0.099	2,400	102.8
10.15	No symptoms.	0.086	2,100	103.4
10.40		0.099	2,200	103.8
11.00			2,000	103.8
11.30			2,140	103.8

In instances giving no rise in blood sugar, systemic reactions were in some cases severe, while in other cases, as after staphylococci and streptococci, practically no symptoms were manifested. These negative results served as controls for the positive results, as the animals were exposed to the same stress of handling and bleeding. The slight fluctuations in blood sugar during the course of these negative experiments were negligible in comparison to the regular curves of marked increase in the positive cases.

Organisms producing hyperglycemia are less effective after prolonged growth on artificial media. The saline suspension must be used promptly, as, after standing, a change of some sort occurs which renders it ineffective in causing hyperglycemia. This was accidentally noted in using an old saline suspension of *Bacillus coli* and of *Bacillus paratyphosus* B which had no effect on the blood sugar, but which had previously, when freshly made, caused marked hyperglycemia.

The leucocyte counts were made merely to give an index of the depressed state of the animal and to show that the fall in leucocytes occurred simultaneously with the rise in blood sugar. The recovery of leucocytes lagged behind the return to normal of the blood sugar level. The fluctuations in temperature appeared to have no relation to the blood sugar curve or leucocyte count.

DISCUSSION.

The rapid rise in the blood sugar level produced by injections of killed organisms of the type of *Bacillus proteus*, *Bacillus coli*, and *Bacillus paratyphosus* B was followed by a return to normal in a few hours. A slight irregular rise occurred with *Bacillus enteritidis* of Gaertner and *Bacillus paratyphosus* A.

The results with *Bacillus enteritidis* and *Bacillus paratyphosus* B are in accord with the results of Menten and Manning (1) (1924), who found a rise in the blood sugar of rabbits spontaneously infected with organisms of the enteritidis-paratyphoid B group. After injecting intravenously killed organisms of these types, they found a rise in blood sugar which attained a maximum of 130 to 160 mg. and returned to normal in a few hours. Lethal doses produced a

rise to 235 to 250 mg. followed by a decrease until a low value of 40 to 50 mg. was reached at time of death.

In our experiments the hyperglycemia produced by various organisms was in general of higher degree than in the experiments of Menten and Manning. At no time have we obtained a hypoglycemia, as they did.

Evidence that the mechanism of the production of these changes in the blood sugar level is by increased glycogenolysis, probably due to sympathetic stimulation, will be presented in a subsequent paper.

SUMMARY.

A rapid rise in the blood sugar level of rabbits was produced by intravenous injections of killed *Bacillus proteus*, *Bacillus coli*, and *Bacillus paratyphosus* B, which returned to nearly the previous level in a few hours time.

A less pronounced rise in blood sugar was produced by killed *Bacillus paratyphosus* A and *Bacillus enteritidis*.

BIBLIOGRAPHY.

1. Menten, M. L., and Manning, H. M., Blood sugar studies on rabbits infected with organisms of the enteritidis-paratyphoid B group, *J. Med. Research*, 1924, xliv, 675.