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Research Article

IMMUNITY IN DIABETES. III. RELATION OF TISSUE GLYCOGEN AND BLOOD CHEMISTRY TO BACTERIAL DISSEMINATION, ANTIBODY FORMATION AND SURVIVAL AFTER INFECTION IN DIABETES

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In former publications from this laboratory (1, 2) it was shown that diabetic patients were unable to form agglutinin to as high a titer as normal controls following inoculation with a B. typhosus vaccine given according to the standard technique. It was further demonstrated that the bactericidal power of the blood from diabetic patients was definitely less than that of normal controls when tested against six pathogenic organisms. This failure was found to be due to the amboceptor, as the blood from the diabetics contained complement in an amount equal to that of non-diabetic controls. At the same time it was shown that the smallest amount of agglutinin was formed by those diabetics who were in the most unsatisfactory metabolic balance. Rabbits which had been kept on a low food intake for several weeks before the inoculation with vaccine always showed a similarly diminished ability to form agglutinin with some alteration in metabolic balance as evidenced by a marked loss in body weight. From these investigations it appeared that at least a part of the commonly recognized susceptibility of diabetics to infection might be concerned with their decreased power to form immune bodies as compared with normal individuals.

A large part of the previous work on the resistance of the body to disease has dealt only with the immunological aspect. In addition to the variations in antibacterial activity, there are, of course, many chemical and physical alterations in the tissues and fluids of the host which may have an influence on the ability of the body cells to react to such bacterial products as are not overcome by the antibacterial forces of the body. The bacteria themselves have a variable ability to attack the host with their toxins and to protect themselves by such means as their capsules. Thus infection with its accompanying inflammation would appear to consist of an interaction of the offensive and defensive forces of both the host and the infecting agent.

In this report are presented the results of some further observations and experiments on the formation of antibodies and the resistance to infection in diabetic patients and experimental animals. In the latter an attempt has been made to reproduce certain of the factors which are commonly present in the diabetic with a view to determining their influence on the ability of the body to resist infection. Among these factors are the amount of sugar of the blood, the total protein, albumin, globulin, and cholesterol of the serum and glycogen of the tissues. These factors have been studied in connection with (*1*) the ability to form antibodies in both diabetic patients and experimental animals, (*2*) the survival time after intravenous inoculation of animals with bacteria, (*3*) the spread of bacteria throughout the body from an experimentally inoculated focus, and (4) the ability of the blood to destroy the bacteria *in vivo*.

In experiments on the formation of antibody B. typhosus vaccine was used according to the standard technique. In all other experiments, suspensions in normal saline of an 18-hour broth culture of staphylococcus aureus were used. This strain of staphylococcus aureus, which has been used throughout the entire investigation, was isolated from the blood of a fatal case of bacteremia, and has been maintained for the past 3 years by semi-weekly transplants on plain agar plates. Only smooth colonies were fished for transplant so that few rough colonies appear in the culture.

The blood sugar was determined by the Benedict method, serum cholesterol by Bloor's method, and serum protein and serum albumin by Pregl's modification of the Kjeldahl. Serum globulin was taken as the difference between the total protein and the albumin. Glycogen of the tissue was determined by a modification of Pflüger's method.

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The agglutinative titer was measured by Dreyer's macroscopic method, using a formalinized antigen, readings being made after 2 hours in the water bath at 55° C. followed by 18 hours' refrigeration at 6° C.

Quantitative determination of bacteria in blood was made by plating one half cubic centimeter of blood in plain agar by standard bacteriological technique. Tissues were ground with sterile sand and plated in plain agar plates after proper dilutions with broth according to standard technique. Control cultures of bacteria-free blood and tissues were made to check the sterility of these procedures.

In an attempt to determine the influence of the amount of sugar in the blood and the amount of glycogen in the tissues on the survival time of experimental animals following an intravenous inoculation with bacteria, the following investigations were undertaken. Normal, adult rabbits weighing about 3 kgm. were used. Each rabbit was given intravenously from 1 to 7 cc. of an 18-hour broth culture of staphylococcus aureus. Different amounts of bacterial suspension were given in different experiments, but all the animals in each experiment received the same amount. At intervals after the inoculation blood was withdrawn from an ear vein for culture and sugar determination. For the sake of clarity the experiments will be described in three groups.

In Group I the rabbits were given glucose intravenously during the course of the bacteremia, in Group II they were given epinephrine subcutaneously during the course of the bacteremia, and in Group III they were given glucose intravenously previous to the inoculation with bacteria.

In Group I and II blood was withdrawn from an ear vein for culture and determination of sugar 3 or 4 times at intervals of 4 hours after the inoculation with bacteria. The animals were used in the same order for the inoculation and each successive bleeding, so that the elapsed time between successive determinations was as nearly equal as it could be made, and in each rabbit did not vary more than 10 minutes from the average for the whole group. The animals seldom showed any clinical evidence of the septicemia for from 6 to 8 hours after the inoculation. After this, however, some began to show symptoms of the infection and 11 or more hours after inoculation death occurred.

Group I. In 8 experiments 22 rabbits were given glucose intravenously and 13 controls were given an equal amount of physiological saline solution. The results of the blood cultures on these animals are shown in Figure 1. They are given as the ratio between the number of bacteria in 0.5 cc. of blood from the rabbits which had received sugar, and the number in the blood from the control rabbits used in the same experiment, and are plotted logarithmically. The comparisons were made only between the cultures taken an equal number of hours after the injection of the bacteria. If only one control rabbit was used with several rabbits receiving sugar, the blood cultures of the latter were each compared with the single control. If more than one control rabbit was used, an average of the number of bacteria in the blood cultures of all the control rabbits was used for comparison with the number of bacteria in the blood of each rabbit receiving sugar. These ratios are grouped according to the sugar in the blood of the glucose-treated rabbits taken at the same time as the blood culture. In the first sub-group are shown those in which the blood sugar was below 140 mgm., in the second sub-group those with blood sugar between 140 and 200 mgm., and in the third sub-group those with a blood sugar over 200 mgm. per 100 ml. of blood at the time the culture was taken.

It is evident that there is no significant difference in the number of bacteria in the blood of those animals given intravenous glucose during bacteremia, whether their blood glucose was normal or high, and the controls with a normal blood sugar.

Group II. In 3 experiments 7 rabbits were given epinephrine and 5 were used as controls. The bacteria were given intravenously 1 hour after the first injection of epinephrine while the blood sugar was above 200 mgm. per 100 ml. of blood. Blood for culture and sugar determination was withdrawn as in the experiments of Group I.

The results are also shown in Figure 1. It is apparent that there is no significant difference between the number of bacteria in the blood of the control rabbits and that of the rabbits with hyperglycemia produced by injections of epinephrine.

Group III. In 4 experiments in which 16 rabbits were given glucose and 9 were used as controls, 7 intravenous injections of from 2.5 to 5

grams of glucose were given at hourly intervals, followed, after the seventh injection, by the inoculation with bacteria as previously described. No glucose was given after the inoculation. Blood for culture and sugar determination was withdrawn 15 hours later.

The results of these determinations are also shown in Figure 1. It is evident that the injection of glucose before the bacteria were given had no significant influence on the number of bacteria in the blood.

In order to determine the effect of the bac-

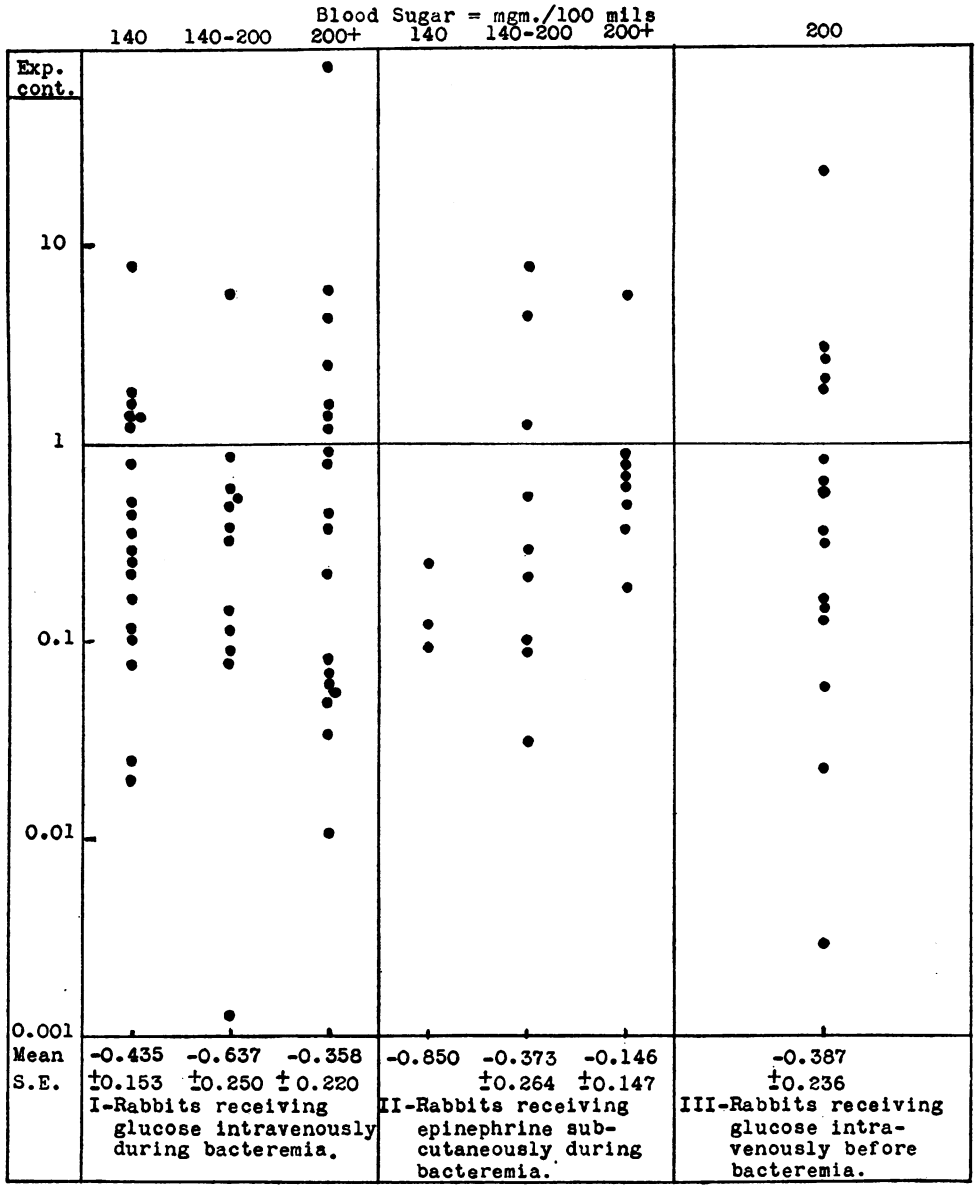

FIG. 1. RATIO OF BACTERIAL COUNT IN EXPERIMENTAL ANIMALS WITH RAISED BLOOD SUGAR TO BACTERIAL COUNT IN CONTROL ANIMALS BLED AT SAME INTERVAL AFTER INTRAVENOUS INJECTION OF BACTERIA

Blood sugar indicates the values found in experimental animals at moment when bled for blood culture. Mean $= \frac{\text{log. exp.} - \text{log. cont.}}{n}$ where log exp. and log. cont. are the logarithms (base 10) of the bacterial counts. S.E. = standard error of mean.

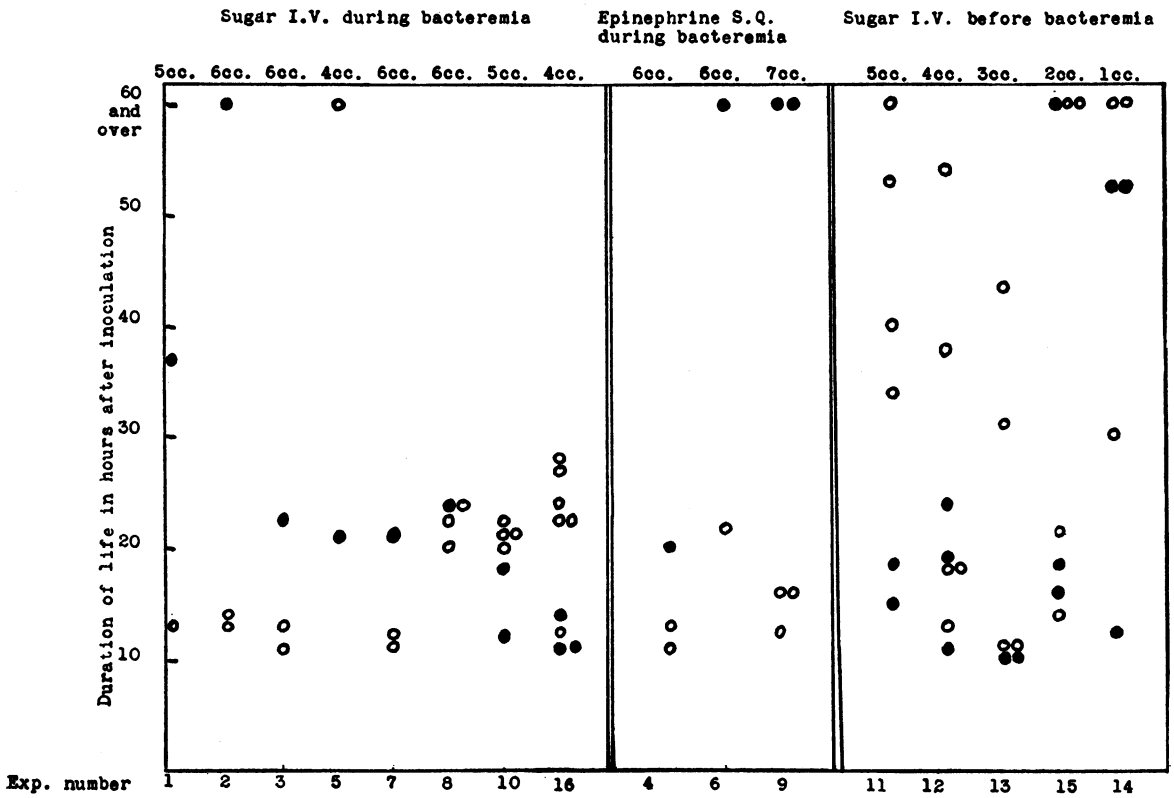

FIG. 2. DURATION OF LIFE IN HOURS AFTER INOCULATION WITH BACTERIA IN EXPERIMENTS NUMBERS 1 TO 16
○ = rabbits receiving glucose or epinephrine. ● = control rabbits.

teremia on the rabbits, their survival time after inoculation was studied. The results are shown in Figure 2. The difference in number of bacteria injected makes a comparison between animals in different experiments difficult. For this reason numbers 1, 5, 10, 16, 11 and 12, in which 4 or 5 cc. of the broth culture were used, were selected for analysis. In these experiments 12 rabbits had been given glucose during the bacteremia, 9 had been given glucose before the bacteremia, and 12 were controls. Table I shows the average duration of life in hours of the animals in these three groups.

Glucose appears to have no significant influence on the duration of life when given during the bacteremia, but when given for several hours previous to injection of the bacteria there is a greater increase in survival time, although the significance of this is not clear. As approximately the same number of bacteria had been injected into all these animals, and as there was no significant variation in the number recovered by culture, it would appear that the longer duration of life may have been due to resistance to the action of the bacteria provided by the glucose given before the bacteremia.

TABLE I

Survival time of rabbits without glucose and with glucose during or before the bacteremia produced by intravenous injection of staphylococcus aureus

	Hours of survival
Control rabbits (12)	17±1.8
Rabbits given I.V. glucose during bacteremia (12)	21±4.7
Rabbits given I.V. glucose before bacteremia (9)	34±6.2

In view of these experiments, which suggest that glucose, given intravenously, may increase the resistance of the body to the bacteria, the glycogen content of the liver was determined as evidence of the glycogen stores of the body. The averages of these determinations are shown in Table II. The tissue was taken under nembutal

anesthesia when it was evident that the animal would die soon. The greatly increased glycogen in the liver of the rabbits receiving glucose intravenously as shown in this table is correlated with the longer duration of life in the rabbits in Group III shown in Table I.

In view of these results it was decided to continue this work with depancreatized cats. It was thought that in this way an experimental diabetes could provide a more prolonged hyperglycemia than was obtained in the rabbits and certain other alterations in the chemistry of the body commonly found in diabetic patients.

TABLE II

Per cent of liver glycogen in rabbits which had received injections of bacteria with glucose intravenously or epinephrine subcutaneously

	Per cent of liver glycogen
Control rabbits	0.63±0.244
Rabbits receiving epinephrine subcutaneously	0.56±0.237
Rabbits receiving glucose intravenously	6.38±1.27

Normal cats about 2.5 kgm. in weight were completely depancreatized and maintained for from 3 to 60 days on a diet of fish, beef liver and pancreas. Insulin was given subcutaneously twice daily in different amounts, depending on the diet. No attempt was made to maintain normal blood sugar. In order to vary the nutritive condition of the animals the diets given varied from a normal diet to one slightly above basal requirements. Some cats refused food for several days before the experiments in which they were used.

Thirteen experiments were done, in each of which 1 normal and 2 depancreatized cats were generally used. At the beginning of each experiment, under nembutal anesthesia, blood was taken from the inferior vena cava for determination of sugar, serum cholesterol and serum protein, and tissue from liver and muscle for glycogen. One cc. of staphylococcus aureus suspension per kilo of body weight was then injected into the inferior vena cava. For each experiment an 18-hour broth culture was centrifuged and the bacteria resuspended in physiological saline solution so that each cubic centimeter contained 2,000 million bacteria. At 24 and 48 hours after the inoculation such animals as were still living were again anesthetized and blood and tissue removed as already described. Blood was removed at these times for culture also. One-half cc. of blood was plated with agar in Petri dishes according to standard blood culture technique. After 48 hours' incubation at 37° C. the colonies in each plate were counted.

In order to determine the effect of the operative procedures on the animals, 3 normal and 3 depancreatized cats were operated on from 2 to 4 times without inoculation with bacteria. One of these cats became infected and died. The other 5 lived. It would appear, therefore, that as both the depancreatized and the normal control cats were operated on in the same manner, the operative procedures were not a significant factor as between the two groups.

For purposes of comparison, the depancreatized and the normal control cats were divided into four groups according to the survival time after inoculation with bacteria. In the first group 6 depancreatized animals lived less than 18 hours after the inoculation. No normal cats died within this time. In the second group 5 depancreatized cats lived longer than 18 hours and less than 30 hours. One control animal died within these limits. In the third group 13 depancreatized cats survived to more than 30 hours and less than 80 hours. Eleven normal cats died within these limits after receiving the bacteria. The fourth group comprised those animals which recovered entirely from the infection. In this group there were no depancreatized cats, and 4 control cats.

TABLE III

Duration of life in hours after inoculation with bacteria, and glycogen of liver in depancreatized and normal cats

	Less than 18 hours	18 to 30 hours	30 to 80 hours	Survived injection
	per cent	*per cent*	*per cent*	*per cent*
Depancreatized cats	(6) 0.42±0.14	(5) 1.61±0.31	(13) 2.74±0.26	None
Normal cats	None	(1) 1.11	(11) 3.28±0.59	(4) 3.05±0.93

Number of animals in each group is shown in parentheses, liver glycogen in per cent.

The survival time and the glycogen of the liver of these cats at the time of inoculation with bacteria are shown in Table III. It would appear from these experiments that, as shown in this table, those depancreatized animals which had more glycogen in the liver at the time of inoculation survived a longer time after the inoculation with bacteria.

The amount of glycogen in the muscles of these animals at the time of inoculation is shown in Table IV. It is apparent that there is no significant correlation between the survival of the animals and the glycogen of the muscle at the time of

TABLE IV

Duration of life in hours after inoculation with bacteria, and glycogen of muscle in depancreatized and normal cats

	Less than 18 hours	18 to 30 hours	30 to 80 hours	Survived injection
	per cent	*per cent*	*per cent*	*per cent*
Depancreatised cats.....	(6) 0.90±0.18	(5) 0.99±0.15	(13) 0.99±0.08	None
Normal cats.....	None	(1) 0.97	(11) 0.98±0.08	(4) 0.99±0.17

inoculation in either the depancreatized cats or the controls.

The loss in the amount of glycogen in the liver during the 24 hours after inoculation of the cats which died is shown in Table V. The cats in Group I did not, of course, survive long enough to have a second operation.

TABLE V

Percentage loss of liver glycogen of depancreatized and normal cats during 24 hours following their inoculation with bacteria

	Group II 18 to 30 hours	Group III 30 to 80 hours	Group IV survived infection
	per cent	*per cent*	*per cent*
Depancreatized cats.	52±21 (3 cats)	46±9.7	(No cats)
Normal cats.....	61 (1 cat)	46±9.9	41±11.2

It is evident that there is not a significant difference in the percentile decrease of glycogen in the liver in depancreatized cats as compared with normal cats. In Group II, in which the animals lived for from 18 to 30 hours after the inoculation, only 3 depancreatized cats lived over 24 hours and were available for a second determination of glycogen.

The change in the glycogen of the muscle was found to be highly variable and very different from that of the liver during the 24 hours after inoculation. Of the depancreatized cats in Group III, which lived from 30 to 80 hours after the inoculation, 4 had an increase of from 13 to 28 per cent, while 7 cats lost from 7 to 74 per cent of the amount of glycogen present 24 hours earlier. In the normal cats 5 showed an increase in muscle glycogen of from 35 to 87 per cent, while 6 showed a decrease of from 8 to 79 per cent. There was thus no constant reaction of the muscle glycogen to the infection nor was there any correlation between it and the duration of life after inoculation. In those animals in which there was an increase in percentile muscle glycogen there was usually a greater decrease in the percentile glycogen in the liver. With such changes in distribution of the glycogen in the body no inferences can be drawn as to total body glycogen.

The number of bacteria in the blood 24 hours after inoculation varied greatly in both the depancreatized and in the normal animals. There was, however, no significant difference in this respect between the two groups. Table VI shows the results of these determinations.

TABLE VI

Blood sugar and number of bacteria per 0.5 cc. of blood, cultured 24 hours after inoculation with bacteria, in depancreatized and normal cats which died 18 to 80 hours after inoculation

	Blood sugar	Number of bacteria per 0.5 cc. of blood
	mgm.	
Depancreatized cats.....	Over 200	2226±1034
	Under 200	1009± 980
Normal cats.....	Over 140	363± 300
	Under 140	1279±1100

All cultures of the blood at the end of the 24-, 48- and 72-hour periods contained bacteria. In those animals which died there was no correlation between the number of bacteria in the blood at these times and the duration of life after inoculation with bacteria. In the 4 normal cats which survived the infection there appeared to be a definitely smaller number of bacteria per 0.5 cc. of blood at these times. Cultures on these animals made at the end of 24 hours contained 96 ± 43 bacteria per 0.5 cc. of blood. A study of the sugar and of the number of bacteria in the blood of the depancreatized animals shows no significant correlation between them.

The cholesterol of the serum in the depancreatized cats varied from 65 to 277 mgm. per 100 ml. of blood and showed no correlation with the hours of survival after inoculation with bacteria. In the normal cats the cholesterol of the serum was in general slightly lower than in the depancreatized cats, varying from 87 to 185 mgm. per 100 ml. of blood. No correlation appeared, however, between the survival time and the cholesterol of

the blood in these animals, either in the individuals or in the averages of the groups.

The total protein in the serum of the depancreatized cats varied between 6.07 and 8.65 per cent and of the normal cats from 6.54 to 8.15 per cent. No significant correlation was found in these animals between the protein of the serum and either the duration of life after inoculation with bacteria or the number of bacteria in the blood.

In the foregoing experiments in which the bacteria were given intravenously, it is recognized that this method of inoculation is not above criticism, in that it does not reproduce infection in the experimental animals in the manner in which it occurs in the patient. Seldom, if ever, does the blood stream receive bacteria in the large numbers given in those experiments. For that reason it was decided to continue the investigation by giving a much smaller number of bacteria intradermally, and after a certain number of hours to examine the blood and various tissues in order to determine whether the bacteria in the skin were destroyed, and whether there might be found in the normal and depancreatized animals any significant difference in the frequency with which certain other organs of the body had become infected from this focus. In this investigation cats of the same weight as those used in the earlier experiments were depancreatized and maintained in the same manner.

In from 3 to 65 days after pancreatectomy, 0.1 cc. of a suspension of staphylococcus aureus made from an 18-hour broth culture was injected into the skin of the abdomen of 2 depancreatized cats and 1 normal cat. The suspension was made so that 0.1 cc. contained 8 million bacteria. After 24 hours the animals were anesthetized with nembutal and the inoculated area of skin, as well as the blood, liver and spleen, was cultured by standard bacteriological technique. The cultures were made quantitatively so that the results showed the number of bacteria in 0.5 cc. of blood, 1 gram of liver, half of the spleen, and the entire inoculated area of the skin. In order to control the sterility of the method, a number of cultures of normal skin were made. No bacteria were recovered in these cultures. An adjacent piece of liver, spleen, a piece of skin from the side of the body opposite the area of inoculation and symmetrically located, and a piece of muscle were taken for determination of the glycogen content. Blood was also taken for sugar and for total protein, albumin, and cholesterol of the serum. The serum globulin was taken as the difference between the total protein and the albumin. Thirty-eight normal and 58 depancreatized cats were studied in this manner.

TABLE VII

Percentage of depancreatized and control cats in the organs of which bacteria were found 24 hours after in intradermal inoculation of staphylococcus aureus

	Normal	Depancreatized
Number of animals inoculated.....	38	58
Per cent with bacteria in liver.....	35	77
Per cent with bacteria in spleen.....	29	81
Per cent with bacteria in blood.....	16	19
Per cent with bacteria in skin.....	76	78

Table VII shows the results of the cultures in these animals. As will be noted, there is no difference between the normal and the depancreatized cats in the frequency with which the blood and the site of inoculation in the skin contain bacteria. However, the liver and spleen of the depancreatized animals contained bacteria with definitely greater frequency than those of the normal controls. Furthermore, as shown in Table VIII, the liver and spleen of the depancreatized

TABLE VIII

Bacteria in liver and spleen in normal and depancreatized cats

LIVER		
34 normal cats.....	35 per cent contained bacteria	27 bacteria per gram of tissue
53 depancreatized cats.	77 per cent contained bacteria	500 bacteria per gram of tissue
SPLEEN		
34 normal cats.....	29 per cent contained bacteria	31 bacteria per spleen
53 depancreatized cats.	81 per cent contained bacteria	520 bacteria per spleen

animals in which bacteria were present contained a much larger number of organisms than did those organs in the normal controls which contained bacteria. No significant difference is found in the chemistry of the blood and tissues in the normal and depancreatized animals. Table IX shows an analysis of the amount of glycogen in the liver and skin, of the sugar of the blood, and of the cholesterol, total protein, albumin and globulin of the serum of the depancreatized cats. It was not possible to find any significant correlation between the liver glycogen, sugar, cholesterol, total protein or albumin and the presence of bac-

TABLE IX

Chemical analyses of blood and tissues of depancreatized cats 24 hours after intradermal inoculation with standard suspension of staphylococcus aureus, grouped according to presence of bacteria

Blood analyses	Cats having no bacteria in liver or spleen	Cats having bacteria present in liver or spleen	Cats with acidosis having bacteria present in liver and spleen
Number of cats	14	44	14
Blood sugar, *mgm. per cent*	259	247	361
Serum cholesterol, *mgm. per cent*	128	136	102
Serum total protein, *per cent*	7.39 ±0.33	6.97 ±0.25	6.32±0.18
Serum albumin, *per cent*	3.09 ±0.22	3.78 ±0.19	2.75±0.15
Serum globulin, *per cent*	4.29 ±0.24	3.15 ±0.28	3.57±0.24
Skin glycogen, *per cent*	0.153±0.016	0.094±0.007	0.082
Liver glycogen, *per cent*	0.892	0.937	0.333

teria in one or another of the tissues. On the other hand, the glycogen of the skin and the globulin of the serum are both found to be significantly decreased in the animals in which the liver or spleen showed the presence of bacteria. The observation in the former experiments that the animals with a larger amount of glycogen in the liver survived a longer time after inoculation might lead one to expect that the organs containing a larger amount of glycogen would be less likely to become infected from the original focus. However, it was not possible to find that the livers of either the depancreatized or normal cats containing a large amount of glycogen were any less liable to be infected than those which contained a small amount. Table X shows the amount of

TABLE X

Amount of glycogen in the skin adjacent to the point of inoculation with bacteria compared with the presence of bacteria at this point 24 hours after inoculation

	Glycogen	
	Normal cats	Depancreatized cats
	mgm. per 100 grams skin	
No bacteria present in skin	149±25.7	116
Decreased number of bacteria in skin	117	102
Increased number of bacteria in skin	87	94

glycogen in the skin of an area adjacent to the point of inoculation with bacteria. It is evident that the presence of bacteria in such a skin focus shows a suggestive but not clearly significant inverse correlation with the amount of glycogen in the skin adjacent to the area of the focus.

It would appear from these experiments that the organs of the depancreatized cats, in which were present several alterations of body chemistry commonly found in the diabetic, were definitely more susceptible to metastatic infection from a focus in the skin than those of normal controls. Furthermore, these organs apparently possessed less ability to curtail the growth of such bacteria as were brought to them by the blood or lymph.

The influence of acidosis on the spread of bacteria from a focus in the skin to the liver, spleen and blood was then investigated. The cats were depancreatized and maintained in the same manner as those in the preceding experiments. For several days before the intradermal inoculation with bacteria no insulin was given, the animals being fed as previously. As soon as the urine showed a strongly positive test for acetone by the sodium nitroprusside reaction they were inoculated. A number of the animals died before the end of 24 hours after the inoculation with bacteria and were not studied further. However, 14 which were alive 24 hours after the inoculation were anesthetized with nembutal and the same determinations made as in the previous experiments. In addition, the carbon dioxide content of the blood serum was measured by the Van Slyke volumetric method.

The cultures showed the presence of bacteria in the skin, liver and spleen of all of the animals. Cultures of the blood showed bacteria in 4 of the 14, a frequency not significantly greater than that found in the depancreatized cats without acidosis. The carbon dioxide content of the serum varied from 44 to 17 per cent by volume.

Table IX shows the chemical determinations on the blood and tissues of these animals. The variations in the amounts of sugar and cholesterol in the individual animals were so large that these figures are not significantly different from those of the other groups shown in this table. There is apparently a significant decrease in the amount of the total protein of the serum in the cats with acidosis. This is especially noteworthy because, until the time when insulin was discontinued and

ketonuria began to develop, these animals were maintained in exactly the same manner as the cats in the other groups shown in this table. The time necessary for the acidosis to become severe, during which time the protein might be expected to have decreased, varied from 3 to 5 days. The serum globulin was significantly lowered in those cats without acidosis in which bacteria were disseminated, as compared with cats without bacteria in the liver and spleen. In the animals with acidosis and dissemination of bacteria the lowering of the serum globulin was less marked. In the analysis of these figures it appears that a lower total protein accompanied a decreased carbon dioxide content of the serum. This was especially clear in a study of the individual observations in which a very low carbon dioxide content of the serum was always accompanied by a total protein below 6 per cent.

In the tissues it will be noted that the glycogen content of both skin and liver is lower in the animals with acidosis and with bacteria in the organs than in those animals without acidosis, in which the liver and spleen contained no bacteria.

In comparing the liver glycogen in columns 2 and 3 of Table IX it is evident that there was no difference in the percentile amount of this substance, whether or not the liver became invaded by bacteria. Furthermore, on a comparison of the liver glycogen in columns 3 and 4 it is evident that, as the livers of both of these groups of animals contained bacteria, the low liver glycogen in column 4 is the result of acidosis, not infection.

Turning to a consideration of the skin glycogen in a control area on the opposite side of the body corresponding in location to the area of inoculation, we find that animals which showed invasion of the organs by bacteria, as shown in columns 3 and 4, had significantly less glycogen than those in which the organs were not infected. If it is reasonable to assume that the glycogen of the control area represents the glycogen of the inoculated area at the time of inoculation, then it is suggested that the low skin glycogen favors the escape of bacteria from the site of infection. However, if one supposes that the low skin glycogen of columns 3 and 4 was the result of a metabolic effect of generally disseminated bacteria, then this argument does not hold.

Part of the investigation was undertaken to determine, if possible, the influence of the sugar of the blood and of the total protein, albumin, globulin, and cholesterol of the serum on the diminished ability of diabetics to form antibodies to as high a titer as do normal controls. B. typhosus vaccine was given to 53 diabetic patients and 32 normal controls according to standard technique. At the time each dose was given, and 2 weeks after the third dose, blood was taken for the above-mentioned examinations, as well as for determination of the agglutinative titer. Table XI

TABLE XI

Agglutinative titer of diabetic patients and non-diabetic controls

	0	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240	1/20480
NON-DIABETIC	0	0	0	0	1	3	8	1	6	6	4	3
DIABETIC	2	4	4	4	6	8	10	13	1	1	0	0

shows the results of the agglutination determinations made 2 weeks after the final injection of vaccine. It is quite evident that there is a marked difference in the titer of the diabetic and non-diabetic sera similar to that found in a previous investigation. Fifty-nine per cent of the non-diabetics agglutinated at a dilution of 1/2560 or more compared with 4 per cent of the diabetics. On the other hand, 13 per cent of the non-diabetics agglutinated at a dilution of 1/320 or less compared with 53 per cent of the diabetics. No correlation, however, was found between the agglutinative titer and the sugar of the blood or the protein, albumin, globulin or cholesterol of the serum in either the diabetic or the non-diabetic groups. As would be expected, the sugar of the blood and the cholesterol of the serum were frequently found to be higher in the diabetics than in the normal controls. Likewise, the total protein, albumin, and globulin of the serum usually were found to be lower in the former. However, these variations did not appear to correlate with the formation of agglutinins.

In view of these observations, it seemed that an investigation of antibody formation in the experimental animal might throw some light on this inability of the diabetic to form agglutinin in

as high a titer as normal controls. In this way it was thought possible to reproduce at least some of the factors commonly found in the diabetic patient and to ascertain, if possible, the influence of these factors on antibody formation. To this end depancreatized and normal cats were given B. typhosus vaccine and the sugar of the blood, the agglutinative titer, total protein, albumin, globulin and cholesterol of the serum, and glycogen of the liver and of the muscle were determined.

Two series of experiments were carried out. In both series normal cats weighing about 2.5 kgm. were depancreatized and, after recovery from the operation, were placed on a diet consisting of pancreas, fish and liver. Regular insulin or protamine zinc insulin was given in sufficient quantity to maintain the animals in as nearly normal a condition as possible. The controls were normal cats of about the same weight maintained throughout the duration of the experiments on a diet of fish and beef heart. The cats in the first series of experiments were given a diet of two and one half times the basal requirement with more insulin, while those in the second series received 50 per cent more than the basal requirement of food and less insulin than those of the first series.

In each experiment 3 control cats and 3 cats depancreatized from 2 to 60 days previously, were generally used. All were given 0.1 cc. of B. typhosus vaccine intravenously. Four depancreatized cats and 1 control cat died less than 24 hours after receiving vaccine.

In the first series of experiments a total of 6 depancreatized and 10 normal cats which survived the inoculation were anesthetized with nembutal 7 days after inoculation, and blood was taken for determination of the agglutinative titer. Table XII shows the agglutinative titer of these animals. It is apparent from these figures that there is no significant difference in the agglutinative titer of the serum of the normal and of the depancreatized animals.

TABLE XII

Agglutinative titer of depancreatized and normal cats

1ST SERIES

Agglutinative titer	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	Died
Normal cats.....	1	2	3	0	2	1	1	1
Depancreatized cats.....	0	0	1	1	1	2	1	3

2ND SERIES

Agglutinative titer	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	Died
Normal cats.....	1	2	2	1	0	0	0	0	0
Depancreatized cats.....	0	1	1	3	1	3	2	0	1

A second series of experiments was done on 6 normal and 12 depancreatized cats. All procedures were the same as those used in the first series except that a different lot of B. typhosus vaccine was used, and the animals were given a lower caloric diet and less insulin. The agglutinative titer of the blood of these animals taken on the 7th day is shown in Table XII. It will be noted that on this day there appears to be a slightly higher titer in the depancreatized than in the normal cats of the second series of experiments.

Table XIII gives the results of the other examinations of the blood and tissues in these animals. The table shows that, although the serum cholesterol is generally lower in the depancreatized animals, there is no correlation between this and the agglutinative titer in the individual animals. However, of the 4 cats with the lowest titer, 2 had no chemical analyses. Of the other 2, both had much lower liver glycogen than any of the normal or other depancreatized cats, and 1 had lower serum protein and serum globulin than the other cats. Examination of the individual protocols indicates that these 4 cats were less well nourished, either through refusal of food or inadequate insulin, than any of the other depancreatized cats in the table. Their average daily intake of meat during the experiments varied between 39 and 69 grams while the other depancreatized cats took between 97 and 130 grams of meat daily.

The elapsed time between the pancreatectomy and the inoculation appears to have no influence on the formation of agglutinin.

It is apparent from the above experiments that depancreatized cats, as compared with normal controls, may lose the ability to form antibody in the blood when their nutrition is impaired. They may thus be compared to the diabetic pa-

TABLE XIII

Chemical analyses of the blood and tissue of depancreatized and normal cats in which the agglutinative titer is shown in Table XII (2nd series)

NORMAL CATS

Cat number	Agglutinative titer	Sugar	Cholesterol	Protein	Albumin	Globulin	Liver glycogen	Skin glycogen
		mgm.	*mgm.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
718	1/320	56–104	188	7.20	3.20	4.00	0.88	0.07
719	1/160	56–154	194	5.90	2.72	3.18	0.64	0.06
714	1/160	56–84	185	5.90	1.12	4.78	1.17	0.19
713	1/160	60–96	162	7.14	3.01	4.13	1.16	0.18
711	1/80	66–80	263	9.60	2.85	6.75	0.93	0.12
712	1/80	68–88	122	8.45	2.22	6.23	0.86	0.12
Average			186	7.36	2.52	4.72	0.94 ±0.08	0.12

DEPANCREATIZED CATS

Cat number	Agglutinative titer	Sugar	Cholesterol	Protein	Albumin	Globulin	Liver glycogen	Skin glycogen	Urine sugar	Days after operation
		mgm.	*mgm.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
691	1/2560	276–291	157	8.25	3.17	5.08	0.65	0.16	++++	29
698	1/2560	92–250	53	8.72	2.22	6.50			++++	24
688	1/640	187–210	77	9.52	3.32	6.20			± to 0	29
689	1/640	84–216		8.45					++++	36
715	1/640	284–288	260	8.00	2.25	5.75				2
693	1/320	292–344	147	6.40	2.40	4.00	0.64	0.13	++++	42
694	1/320	184–276	90	7.38	3.96	3.42	0.66	0.21	++++	35
702	1/320	132–192	118	7.14	2.54	4.60	1.49	0.14	± to ++++	28
716	1/80	200–244	69	5.10	2.25	2.85	0.36	0.05	++++	2
717	1/80	192–340	67	6.70	2.56	4.14	0.15	0.09	++++	2
706	1/40	— Died 7th day							0 to ++++	18
697	1/160	52–206 Died 7th day							± to ++++	24
Average			115	7.57	2.74	4.76	0.66 ±0.19	0.13		

tients and the underfed rabbits reported in previous communications. Of course, little is known concerning the antibody response to a bacterial antigen in cats. They were selected for this work, however, because at the time it had hardly been demonstrated that the pancreas may be safely removed from rabbits, the animals on which most of the antigen-antibody work has been done. It appears that the cats vary considerably in their response to the vaccine, though, on the whole, it would seem that they have less capacity than some other animals to form antibodies following the injection of vaccine.

The fact must not be overlooked that, while we do produce in the depancreatized animal kept under partial control with insulin some of the alterations in blood and tissue chemistry commonly found in the diabetic, we do not produce diabetes mellitus as a definite clinical entity. The long duration of the diabetic state in the patient, as compared with the relatively short time in these experiments between pancreatectomy and inoculation of the animals with the vaccine, may have a significant influence on antibody formation. Furthermore, some endocrine or other metabolic alteration perhaps present in the patient, but not found in the animals under the conditions of these experiments, may account for the difference in the reactions in the two groups.

DISCUSSION

Without attempting to make a final report now, it is, nevertheless, desirable to indicate certain conclusions which may properly be drawn from the foregoing experiments. It would seem that the impaired ability to build agglutinins noted in the diabetic patient is possibly associated with long continuation of the pathologic state. In all of these experiments variations in the blood chemistry, some of which are commonly found in the diabetic patient, appear, with the exception perhaps of globulin, to have no effect on the formation of agglutinins, the survival after intravenous inoculation with bacteria, the growth of bacteria in the blood or the dissemination of bacteria to various organs from a primary focus in the skin. There is a significantly smaller amount of serum globulin in the blood of those depancreatized cats in which the liver or spleen showed bacteria 24 hours after the intradermal inoculation with bacteria. The decreased ability in the patient to form agglutinins may perhaps be caused by an altered nutritional state similar to that in the underfed rabbits and the last 4 of the depancreatized cats in Table XIII.

The ability of the experimental animal to survive after a bacterial infection appears to be definitely increased by the presence of a larger amount of glycogen in the liver or by some meta-

bolic change which accompanies this increased liver glycogen. On the other hand, this larger amount of glycogen does not appear to increase the ability of the individual tissues or organs to inhibit the growth of bacteria coming to them from a primary focus in the skin.

In these experiments it was evident that depancreatized cats exhibited definitely greater frequency of dissemination of bacteria from a focus in the skin to the liver or spleen. These observations in the animals under the conditions of these experiments accord fully with the clinical experience that, in general, the diabetic is less able than the normal person to control adequately even a mild infection in the skin.

Whether this inability to curb the bacteria resides in the tissues of the primary focus or in the organs to which the bacteria are disseminated, or both, is as yet unknown. The abnormal condition which allows the bacteria to invade the organs of the diabetic, as well as of the depancreatized animal, may be in the tissue in which the primary focus occurs. It is suggested from the above experiments that the percentile amount of glycogen in the skin reflects this alteration in the tissue.

CONCLUSIONS

1. A significant correlation is shown to exist in normal rabbits and depancreatized cats between the percentile amount of glycogen in the liver and the survival time after intravenous inoculation with bacteria.

2. A lowered nutritional state, accompanied by decreased liver glycogen, is shown to exist in depancreatized cats in which a low agglutinative titer is found after injection of B. typhosus vaccine.

3. Alterations in the blood of these animals, such as are commonly found in diabetic patients, do not appear to influence the survival time or the ability to form agglutinins.

4. The organs of depancreatized cats 24 hours after an intradermal inoculation with bacteria show the presence of these bacteria with greater frequency than do the organs of normal controls. Alterations in the sugar of the blood and of the cholesterol, protein, albumin and globulin in the serum and glycogen in the liver do not appear to influence this dissemination of bacteria from a focus.

5. Acidosis appears to increase the frequency with which this dissemination of bacteria occurs.

6. The percentile amount of glycogen in the skin of depancreatized cats shows a suggestive, though not clearly significant, correlation with this dissemination of bacteria from a skin focus to the organs of the body.

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