STUDIES ON THE EFFECT OF EXPERIMENTAL NONKETOTIC DIABETES MELLITUS ON ANTIBACTERIAL DEFENSE

I. Demonstration of a Defect in Phagocytosis*

By ROBERT H. DRACHMAN, M.D., RICHARD K. ROOT,‡ M.D., and W. BARRY WOOD, Jr., M.D.

(From the Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore)

Plates 17 and 18

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Diabetes mellitus is often cited as a metabolic disease that depresses the antibacterial defenses of the host (1). Many clinical investigators have reported that patients with uncontrolled diabetes are unusually susceptible to bacterial diseases, particularly of staphylococcal etiology (2). Attempts to confirm this relationship experimentally, however, have been only partially successful.

No impairment of immunological competence has been detected in diabetic patients (3), and deficiencies in cellular defense have been demonstrated only in the presence of ketoacidosis (4–6), even though an insulin-correctable depression of glycolysis has been noted in the leukocytes of nonacidotic diabetics (7, 8). Similar results have been reported in animals with diabetes induced by pancreatectomy or treatment with alloxan; i.e., only when the relatively complicated metabolic and circulatory derangements of ketoacidosis have been present, has increased susceptibility to bacterial (9) and mycotic (10) infections been consistently demonstrated. Although nonacidotic rats with chronic alloxan diabetes have been reported to be more susceptible to experimental staphylococcal peritonitis than nondiabetic rats (11), no such difference was demonstrable in either mice (12) or rabbits (9).

The present studies were undertaken in order to determine experimentally whether chronic hyperglycemia per se impairs antibacterial resistance and to define the mechanism involved, if it does so.

In Vivo Experiments

Choice of Animal Model.—Male albino rats weighing 160 to 200 g[1] were used in all experiments because of (*a*) the ease with which they are made chronically diabetic with alloxan (13),

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‡ Present address: National Institutes of Health, Bethesda, Maryland.

[1] Purchased from Charles River Breeding Laboratories, Brookline, Massachusetts, and from Sprague-Dawley Farms, Madison, Wisconsin.

and (*b*) the extent to which their responses have been studied in experimentally induced bacterial infections (14).

Alloxan monohydrate[2] was injected subcutaneously in a dose of 200 mg per kg of body weight. The animals were allowed Rockland mouse-rat pellets[3] and water ad lib., both before and after the injection. Within 24 hr, they developed ruffled fur, polydipsia, polyphagia, diarrhea, polyuria, glycosuria, and ketonuria. Whereas the polydipsia, polyuria, hyperglycemia (300 to 900 mg/100 ml),[4] and glycosuria persisted, the ketonuria subsided on about the 4th day.[5] Approximately 25% of the injected animals died within the 1st wk; the survivors usually lost 20 g in weight during the next 2 wk, and thereafter gained weight more slowly than controls. Infection was not induced until the ketonuria had subsided and hyperglycemia (>300 mg/100 ml) and a heavy glycosuria (4+) had persisted for at least 3 wk.

On account of the data already available on its pathogenesis (14), pneumococcal pneumonia was selected for study in the diabetic rats. The infecting organisms, suspended in 10% mucin, were inoculated intrabronchially under light ether anesthesia.[6] When the pneumonia was thus produced with a highly virulent strain of Type 1 pneumococci, all of the nondiabetic animals died from even a minimal inoculum. Conversely, when an organism of very low virulence, e.g. *Escherichia coli*, was injected in maximum doses, barely discernible lesions were produced. Accordingly, a bacterial strain of intermediate virulence was sought that would kill all of the control animals at high dosage and none of them at low dosage. These criteria were met by a Type 25 pneumococcus, kindly provided by Dr. Robert Austrian of the University of Pennsylvania.

Mortality Studies.—As shown in Text-fig. 1, the cumulative mortalities in the diabetic rats at each of four intrabronchial doses of Type 25 pneumococci were significantly higher than in the nondiabetic rats. In addition, the LD_{50} in the diabetic group was less than one twentieth of that in the nondiabetic group.

Bacterial Counts in Lesions.—Since neither ketonuria nor a significant increase in hyperglycemia developed in the diabetic animals during the infection, it seemed unlikely that the mortality differences were due solely to an exacerbation of the diabetes. To determine whether a loss of antibacterial resistance was involved, the kinetics of bacterial growth in the pulmonary lesions were compared in the two groups (Text-fig. 2). The average numbers of viable pneumococci in the lesions of the diabetic animals at 24 and 36 hr were nearly ten times as great as in the nondiabetics.

Histologic Observations.—Further evidence that the antibacterial defenses of the diabetic rats were compromised is presented in Fig. 1. Histologic examinations of the 24- and 36-hr lesions revealed many more bacteria and much less phagocytosis in the diabetic animals than in the controls. Inasmuch as the blood

[2] Mann Research Laboratories, Inc., New York.

[3] Teklad, Inc., Monmouth, Illinois.

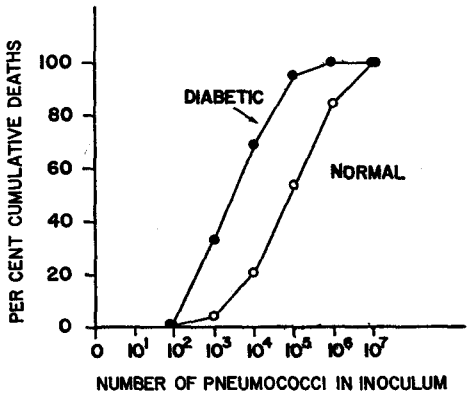
[4] All glucose levels were measured by the glucose oxidase method (15).

[5] Blood pH determinations also revealed that there was no persistent acidosis.

[6] The granular mucin (Type 1701, Wilson Laboratories, Chicago) was added to 0.15 M NaCl, and the mixture was autoclaved and brought to pH 7.4 with 1 N NaOH. Details of the methods used in handling the cultures, producing the pneumonia, and following its progress in the lungs are described in reference 16.

leukocyte counts were, if anything, higher in the diabetic than in the nondiabetic animals (Table I), it was assumed that the overgrowth of bacteria in the diabetic lesions was not due to an inadequate supply of circulating leukocytes. Indeed, the histologic findings indicated no impairment of the inflammatory response in the diabetic animals, but rather a failure of the mobilized leukocytes to ingest the invading organisms.

Intraperitoneal Phagocytosis.—The apparent depression of phagocytic activity was confirmed in the following experiment.



TEXT-FIG. 1. Comparative dose response curves of diabetic and nondiabetic (normal) rats inoculated intrabronchially with Type 25 pneumococci suspended in mucin. Ten rats in each group were inoculated at each dosage level, except at 10^2 and 10^7 where only 5 rats in each group were inoculated. All deaths occurred within 5 days. The LD_{50} calculated by the Reed-Muench method (17, 18) was 5×10^3 for the diabetic animals and 9×10^4 for the nondiabetic. A statistical test (19) comparing the weighted mean differences in mortality at the 4 intermediate dosage levels indicated differences in mortality significant at the 0.01% level.

Acute peritonitis was produced in both diabetic and nondiabetic rats by the injection of 5 ml of autoclaved 3% starch and 5% aleuronat solution diluted 1:1 with tryptose phosphate broth (20). After 20 hr such induced exudates were found in the nondiabetic rats to contain 0.9 to 1.2×10^8 leukocytes (ca. 70% granulocytes) and in the diabetic rats to contain 1.9 to 2.0×10^8 leukocytes. To keep the multiplicity of cells to pneumococci approximately the same in both sets of 20-hr exudates (21), the number of pneumococci injected intraperitoneally in the two groups of rats was 8×10^8 and 16×10^8 respectively.[7] 30 min later each rat was killed with chloroform and its peritoneal exudate was removed, concentrated by centrifugation in the cold (150 *g* for 5 min), and stained with methylene blue.

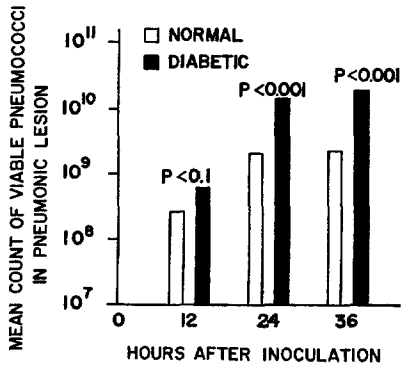
The comparative phagocytic indices obtained clearly showed an impairment of phagocytosis in the exudates of the diabetic animals (Table II).

[7] The organisms were injected into each animal in 0.5 ml of broth.

In Vitro Experiments

From the collective results of the foregoing in vivo experiments it was concluded that chronically hyperglycemic, nonacidotic, diabetic rats are less resistant to acute pneumococcal pneumonia than nondiabetic rats, and that the lowered resistance is due primarily to a defect in the phagocytic defense. In vitro experiments were therefore undertaken to investigate the nature of this defect.

Design of Phagocytic Test.—The results of in vitro phagocytic tests are often misleading because the conditions under which the cells must operate are not similar enough to the conditions that obtain in vivo. One of the most frequent



TEXT-FIG. 2. Comparative numbers of viable pneumococci in pneumonic lesions of nondiabetic (open columns) and diabetic rats (solid columns) sacrificed 12, 24, and 36 hr after intrabronchial inoculation. All lesions were confined to the left lung, which was removed en bloc by sterile techniques, ground in a mortar with sterile sand and 9 ml of trypticase soy broth, and diluted in 10-fold increments with broth for counting by the pour plate technique. The statistical significance of the difference noted in the two groups of animals at each time interval is indicated by the *P* value recorded above the columns.

dissimilarities relates to the amount of fluid in the system; most in vitro preparations of cells and bacteria are far more dilute than infected inflammatory exudates in the host (14, 22). The phagocytic test used in the present studies was therefore designed to simulate as closely as possible a concentrated inflammatory exudate (21, 22).

Pneumococci harvested from 4-hr cultures in serum-beef infusion broth (21) were washed twice in cold HBG,[8] concentrated by centrifugation, counted in a Petroff-Hausser counting chamber, and diluted in HBG to the point where each 0.025 ml of suspension contained 20×10^8 pneumococci. Leukocytes were obtained from acute peritoneal exudates induced in rats by the starch-aleuronat method already described. After 21 hr each rat was killed with

[8] Modified Hanks' solution (120 mM NaCl, 10.1 mM KCl, 5.6 mM Na_2HPO_4, 0.9 mM KH_2PO_4, 2.9 mM K_2HPO_4) containing 0.01% crystalline bovine serum albumin and 0.1% glucose (23).

chloroform, its abdominal cavity was opened, and the exudate was removed with 15 to 25 ml of cold HBG containing 0.005% heparin. The exudates from several rats were pooled, and the cells were centrifuged twice in HBG at 150 *g* for 5 min. Aliquots containing 2.5×10^8 cells were centrifuged, the supernatant was decanted as completely as possible, and 0.025 ml of the bacterial suspension and 0.1 ml of fresh rat serum were added to the packed cells. After thorough mixing, 0.03 ml of the combined suspension was spread evenly over an area of 1×2 cm on a glass slide and incubated for 30 min at 37°C in a sealed Petri dish lined with moistened

TABLE I

Comparative Blood Leukocyte Counts in Diabetic and Nondiabetic Rats with Type 25 Pneumococcal Pneumonia*

Metabolic state	Rat No.	Time after inoculation		
		2 hr	10 hr	24 hr
Diabetic	1	9,100	20,800	2,600
	2	7,200	15,800	2,100
	3	8,300	21,500	4,900
	4	14,100	23,800	10,200
Nondiabetic	5	7,500	15,600	4,400
	6	8,800	11,100	2,300
	7	7,500	13,800	2,700
	8	13,900	10,400	3,000

* Counts made on blood from tail.

TABLE II

In Vivo Phagocytosis of Type 25 Pneumococci in Preformed Peritoneal Exudates of Diabetic and Nondiabetic Rats

Metabolic state	Phagocytosis*
	%
Diabetic	44, 38, 39, 44
Nondiabetic	75, 73, 79, 81

* Each count was made on the exudate of a different animal. Multiplicity of cells to pneumococci in exudates $\cong$ 1:8.

filter paper. The mixture was then washed from the slide with cold HBG and centrifuged at 80 *g* for 3 min. Most of the unphagocytized bacteria remained in the supernate, which was discarded. Smears made from the centrifugate were air-dried and stained with methylene blue. The percentage of cells containing one or more ingested pneumococci (per cent phagocytosis) was determined by counting 400 to 600 cells.

Demonstration of Defect in Serum.—Since polymorphonuclear leukocytes derive energy for phagocytosis from the metabolism of glucose (24), and since glycolysis has been found to be depressed in the granulocytes of diabetic patients

(7), it was presumed a priori that the factor impairing phagocytosis in the chronically diabetic rats would be found in the cells rather than in the serum. That this was *not* the case, however, is shown in Table III. In normal rat serum, the phagocytic activity of leukocytes from the diabetic rats was just as great as that of leukocytes from the nondiabetic controls. In the diabetic rat serum, on the

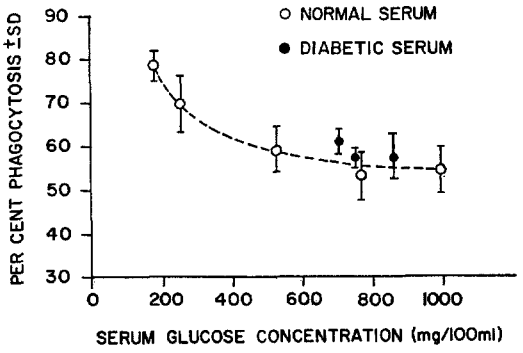
TABLE III

In Vitro Phagocytosis of Type 25 Pneumococci in Serum-Cell Systems from Diabetic and Nondiabetic Rats

Exudate leukocytes	Incubation medium	
	Nondiabetic serum	Diabetic serum
From nondiabetic rats	75.6 (±3.6)*	54.8 (±7.0)*
From diabetic rats	76.0 (±3.8)‡	57.8 (±6.3)‡

* Mean per cent phagocytosis (± SD) in 4 experiments.

‡ Mean per cent phagocytosis (± SD) in 5 experiments.



TEXT-FIG. 3. Effect of increasing concentrations of glucose on in vitro phagocytosis of Type 25 pneumococci in concentrated serum-leukocyte suspensions. Open circles refer to preparations containing nondiabetic serum to which extra glucose was added; solid circles refer to comparable preparations containing diabetic serum. Bars indicate ± 1 standard deviation of per cent phagocytosis.

other hand, phagocytosis by both the normal and the diabetic leukocytes was depressed. Similarly, the serum from the diabetic rats was found to impair the motility of both the normal and the diabetic cells (Fig. 2).

Identification of Depressive Factor.—The combined results of the following two experiments indicate that the depressive factor in the diabetic serum was its abnormally high content of glucose.

1. Using leukocytes and serum from nondiabetic rats, phagocytic tests were performed in which increasing concentrations of glucose were added to the se-

rum. As shown in Text-fig. 3, the depression of phagocytosis that resulted was approximately equal to that caused by diabetic serum containing about the same amount of glucose.

2. To make certain that the glucose, rather than some other component of the diabetic serum, was the primary factor involved, different aliquots of the same lot of diabetic serum were dialyzed against normal nonhyperglycemic serum and normal serum which had been made hyperglycemic by the addition of glucose. The results recorded in Table IV show that dialysis against the nonhyperglycemic serum removed most, if not all, of the depressive factor from the diabetic serum, whereas dialysis against the hyperglycemic serum removed none of it.

TABLE IV

Effect on Phagocytosis of Removing Glucose from Diabetic Serum by Dialysis*

Serum in phagocytic test	Final glucose concentrations	No. of experiments	Phagocytosis (± SD)
	mg/100 ml		%
Nondiabetic	156	5	65.5 (±4.7)
Diabetic, undialyzed	821	4	38.3 (±0.7)
Diabetic, dialyzed vs. normal serum‡	185	3	60.0 (±6.6)
Diabetic, dialyzed vs. hyperglycemic serum§	911	3	28.5 (±8.0)

* Of type 25 pneumococcus.

‡ 1 ml of diabetic serum was dialyzed for 20 hr at 4°C against 2 changes of 10 ml each of normal serum containing 156 mg/100 ml of glucose.

§ Dialyzed in same manner against normal serum to which glucose had been added to make a final concentration of 944 mg/100 ml.

Clearly the principal depressive factor in the diabetic serum was its high content of glucose.

Mechanism of Glucose Effect.—Since the glucose depressed phagocytosis of normal and diabetic leukocytes to about the same degree (Table III), it seemed unlikely that the effect was due to a direct interference with energy metabolism. The phagocytic activity of granulocytes is known, on the other hand, to be impaired in hypertonic media (26–29). The possibility that the hyperosmolarity of the hyperglycemic serum was responsible for the depression of phagocytic activity was therefore tested.

To individual samples of pooled normal rat serum different sugars were added to make the final concentration of total sugar[9] in each sample 43 mM, i.e. the equivalent of 775 mg/100 ml of glucose. The sugars added were glucose, fructose, sucrose, mannose, arabinose, and xylose. At least the last two of these are not metabolized by granulocytes (30).

[9] Endogenous plus exogenous; see Table V.

All of the sugars depressed the phagocytosis of pneumococci in vitro to about the same extent (Table V), suggesting that their effect on the leukocytes was osmotic rather than metabolic.

Limitations of Glucose Effect.—When the phagocytic tests were performed using a more dilute system in which the same number of exudate leukocytes as

TABLE V

Phagocytosis of Type 25 Pneumococcus in Normal Serum Made Hypertonic with Various Sugars

Sugar added*	Phagocytosis
	%
—‡	72 (±3.5)§
Glucose	40, 36, 45‖
Fructose	46, 40, 52
Sucrose	50, 50
Mannose	47, 47
Xylose	44, 46
Arabinose	50, 50, 48, 50, 47

* To make final concentration of total sugar 43 mM.

‡ Endogenous glucose ca. 10 mM (180 mg/100 ml).

§ Standard deviation of mean for 10 experiments.

‖ Each figure is from a different experiment.

TABLE VI

*In Vitro Phagocytosis of Type 25 Pneumococci in Dilute Phagocytic System**

Serum	Sugar added‡	Phagocytosis
		%
Normal	—	76, 78, 75§
"	Glucose	70, 73, 77
"	Sucrose	70, 80

* The same number of leukocytes (2.5×10^8) and one fourth the number of bacteria (5×10^8) were used as in the standard concentrated system (Tables II to V), and the volume of serum in which they were suspended was 1.0 ml rather than 0.1 ml. Tubes were rotated end-over-end at 12 RPM for 30 min at 37°C.

‡ To make final concentrations of total sugar 43 mM (see Table V).

§ Each figure indicates the result of a different experiment.

in the concentrated system were suspended in 1 ml (instead of 0.1 ml) of serum in a closed tube rotated end-over-end for 30 min at 12 RPM and 37°C,[10] neither

[10] Unless the mixture is agitated to increase the contacts of bacteria with leukocytes, very little phagocytosis occurs in such a dilute system (21). With agitation in this volume of serum (1.0 ml), however, the per cent phagocytosis in the control is >90%. Since at this maximal level of phagocytosis, the test is relatively insensitive to minor changes in phagocytic function,

TABLE VII

*In Vitro Phagocytosis of Type 25 Pneumococci in Normal and Hyperglycemic Rat Blood**

Blood	Multiplicity of pneumococci to leukocytes	No. of experiments	Phagocytosis (± SD)
			%
Normal‡	8:1	5	92.7 (±2.4)
Glucose added§	8:1	5	95.2 (±1.3)
Diabetic‖	8:1	4	95.5 (±0.8)
Normal	1:1	8	47.9 (±3.4)
Glucose added	1:1	8	44.3 (±1.7)

* 1 ml of heparinized whole blood, to which pneumococci were added at the pneumococcus-leukocytic multiplicities indicated, were rotated end-over-end at 12 RPM for 30 min at 37°C. Counts were made as in other phagocytic tests (see text).

‡ Range of glucose concentrations 95 to 157 mg/100 ml.

§ Range of glucose concentrations 505 to 757 mg/100 ml.

‖ Glucose concentration 505 mg/100 ml.

TABLE VIII

In Vitro Phagocytosis of Unencapsulated (R) Pneumococci in Normal and Hyperglycemic Rat Serum‡*

Multiplicity of pneumococci to leukocytes	Phagocytosis	
	Normal serum§	Hyperglycemic serum‖
8:1	95, 94	92, 96
4:1	77, 75	70, 72
2:1	67, 64	63, 64
1:1	50, 54	56, 47

* Strain R36NC (21).

‡ Standard concentrated system (see text and Tables II to V).

§ Range of glucose concentrations 198 to 208 mg/100 ml.

‖ Range of glucose concentrations 684 to 772 mg/100 ml.

glucose nor sucrose added to the serum (as in the preceding experiment) depressed the phagocytosis (Table VI). Likewise, in analogous experiments done with whole blood, the per cent phagocytosis was depressed only slightly, if at all, in blood to which glucose had been added (Table VII); and finally, even in the original concentrated system containing exudate leukocytes, serum to which

the multiplicity of bacteria to cells was decreased from 8:1 to 2:1. This change lowered the per cent phagocytosis to the same sensitive range (ca. 70%) that obtained in the tests performed with the standard concentrated system (Tables II to V).

glucose had been added failed to exert a significant depressive effect when the encapsulated Type 25 pneumococci were replaced by a more easily phagocytized unencapsulated (R) strain of the same species (Table VIII).

These findings indicate that the depressive action of the hyperglycemic serum can be clearly demonstrated in vitro only under conditions that (*a*) simulate the cellular crowding of full-blown inflammatory exudates, and (*b*) extend the phagocytic capabilities of the cells (see Discussion).

DISCUSSION

Impairment of antibacterial defense is most likely to be detected when the host-parasite relationships are in a state of delicate balance. The type of bacterial disease selected for study, be it naturally acquired or experimental, is therefore critical. In the present experiments a model bacterial disease was employed in which it was possible, by varying the infecting dose, to achieve the necessary balance between the host and the organism. Under these conditions, the depressive effect of prolonged hyperglycemia on the defenses of the host was readily demonstrated. Failure of others (9, 10, 12) to observe the effect in experimental infections was doubtless due to the nature of the model studied.

Evidence was obtained both in vivo and in vitro that the phagocytic capabilities of polymorphonuclear leukocytes are affected by the high levels of glucose in the tissues of chronically diabetic rats. In the intact diabetic animal, not only did the histological and quantitative bacteriological findings suggest a depression of phagocytosis, but an impairment of in vivo phagocytic activity was also demonstrated in a preformed inflammatory exudate. The latter finding was of particular significance, since it indicated that the relationships between high glucose levels and phagocytosis, that were defined more precisely in vitro, were also applicable in vivo.

Although phagocytosis by granulocytes has long been known to be impaired in hypertonic media (26–29), the degree of hypertonicity required to produce the effect has usually been somewhat larger (40 to 100 milliosmols) than that provided by the hyperglycemic serum (ca. 10 to 40 milliosmols). The cells employed in these earlier studies, however, were not subjected to the kind of phagocytic stress that was investigated in the present experiments, where it was found that sugar elevations of the degree encountered in chronic diabetes affected phagocytosis only when the leukocytes were operating in crowded exudates and were ingesting pneumococci with antiphagocytic capsules.

While the present studies were in progress, Briscoe and Allison (31) reported that Type 1 pneumococci were phagocytized and killed just as effectively by exudate leukocytes from nonketotic diabetic rats as from normal rats. The results, therefore, confirmed the observation that there is no phagocytic defect in the diabetic leukocytes. No experiments, however, were included to detect a possible defect in the diabetic serum. Because the phagocytic tests were carried out

in a concentrated cell-pneumococcus system, simulating the conditions of an in vivo exudate, it was predicted from the present studies that the addition of hyperglycemic serum, or merely of sufficient glucose, to the system would cause a depression of phagocytosis by both normal and diabetic leukocytes. When the experiments were performed, the prediction was fully confirmed (Table IX).[11]

The limitations of the glucose effect on phagocytosis raise several interesting questions. Why, for example, is phagocytosis not significantly impaired in the whole blood of diabetic rats when it is in their peritoneal exudates? Is it merely because the phagocytic system in blood is more dilute than in most inflammatory exudates, or do blood granulocytes differ in some way from exudate granulocytes? Why also are exudate cells resistant to the depressive effect of

TABLE IX

*Effect of Added Glucose on Phagocytosis of Type I Pneumococci in Concentrated Systems Studied by Briscoe and Allison**

Surface	Medium	Phagocytosis
		%
Filter paper	Serum	68, 62
	Serum + glucose‡	52, 43
	Saline	56, 53
	Saline + glucose‡	38, 40
Glass§	Serum	45, 53
	Serum + glucose‡	25, 37

* See reference 31.

‡ Final concentration of glucose: 750 mg/100 ml.

§ On glass virtually no phagocytosis (<3%) occurs in isotonic saline, even in the absence of added glucose.

glucose when tested in a phagocytic system containing more serum than is usually present in established exudates? Are nonspecific serum opsonins involved, or does incubation in the larger volume of serum somehow modify the resistance of the cells to the effect of the glucose? Experiments designed to answer these questions are now in progress.

The clinical implications of these studies relate to the importance of controlling hyperglycemia in patients with diabetes mellitus (2). Although most authorities agree that the objective of insulin therapy is to return the carbohydrate metabolism as nearly as possible to normal, some clinicians still contend that only ketosis and ketonuria need be avoided (32). The present results indicate

[11] These additional experiments were kindly performed by M. R. Smith, who originally designed the phagocytic test employed by Briscoe and Allison (20, 21).

that the severity of an experimental bacterial infection in rats may be significantly enhanced by sustained hyperglycemia.

SUMMARY

Chronically diabetic nonketotic rats were shown to be more susceptible to experimental Type 25 pneumococcal pneumonia than nondiabetic rats. The cumulative mortality in the diabetic group was significantly higher at infecting doses of 10^3, 10^4, 10^5, and 10^6 organisms, and the LD_{50} was less than one twentieth of that for the nondiabetic group. More than ten times as many viable pneumococci were found in the pneumonic lesions of the diabetic animals at 24 and 36 hr as were present in the lesions of the nondiabetic controls, and serial histologic studies revealed that phagocytosis was strikingly depressed in the alveolar exudates of the diabetic animals. The diabetic state was also found to cause a similar depression of in vivo phagocytosis in preformed peritoneal exudates.

The results of in vitro experiments indicated that the principal defect in the diabetic animals resided in their serum rather than in their polymorphonuclear leukocytes. The depressive factor in the serum was identified as the abnormally high concentration of glucose. Since equivalent molar concentrations of unmetabolized sugars added to normal serum caused a similar depression of phagocytosis, it was tentatively concluded that the action of the glucose on the leukocytes was primarily osmotic. The sensitivity of the granulocytes to the glucose effect, however, depended upon the conditions of the phagocytic test. Only when the pneumococci were encapsulated and the leukocytes derived from inflammatory exudates were crowded together, as in vivo, was the depressive action of the glucose clearly demonstrable.

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EXPLANATION OF PLATES

PLATE 17

FIG. 1. Comparable histologic sections of 24-hr pneumonic lesions in nondiabetic (upper) and diabetic rats (lower). Lungs were fixed and stained (Gram-Weigert method) as described in reference 16. Photographs (left to right) are of edema zone, zone of early consolidation, and zone of advanced consolidation, respectively (16). × 844.

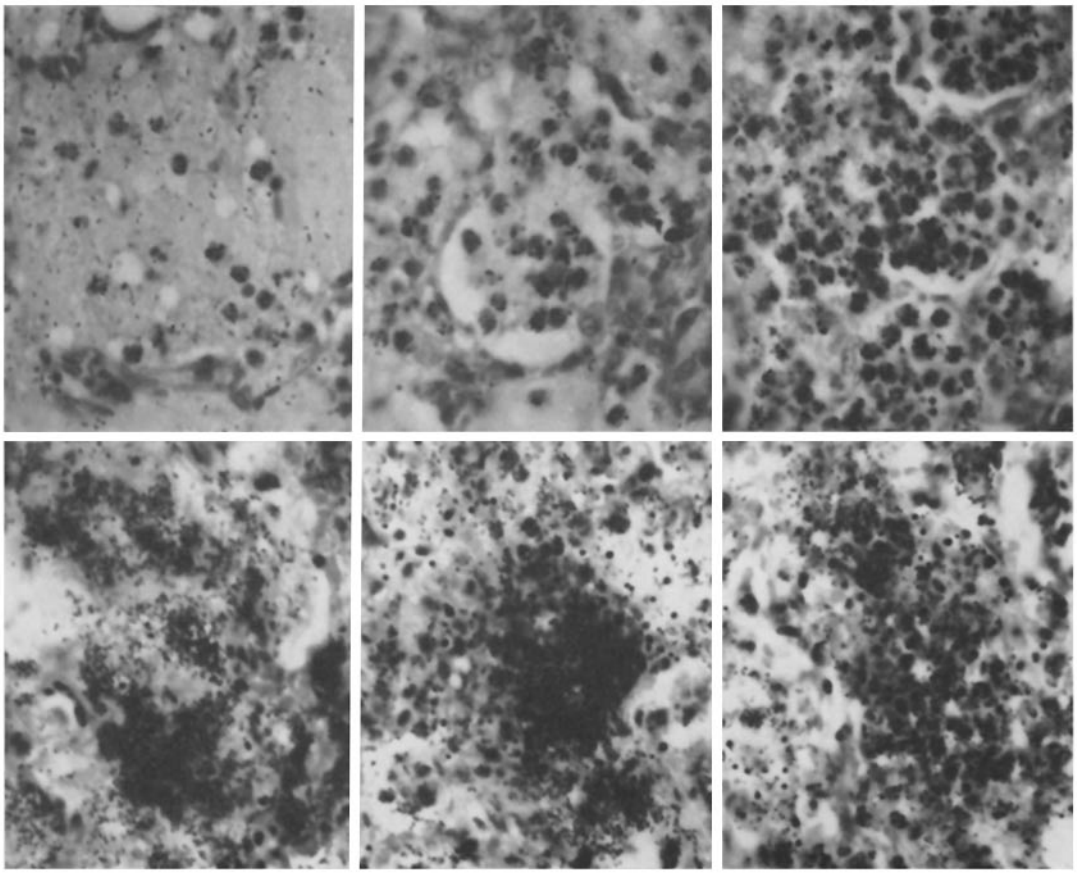

FIG. 1

(Drachman et al.: Diabetes and antibacterial defense)

PLATE 18

FIG. 2. Motility of leukocytes in diabetic (left) and nondiabetic (right) serum as indicated by their migration at margin of hanging drop preparations incubated for 30 min at 37°C. The suspensions contained the same concentrations of cells and bacteria as in the phagocytic tests (see text). This method of estimating leukocytic motility was designed by M. R. Smith (25). × 345 (upper), and × 776 (lower).

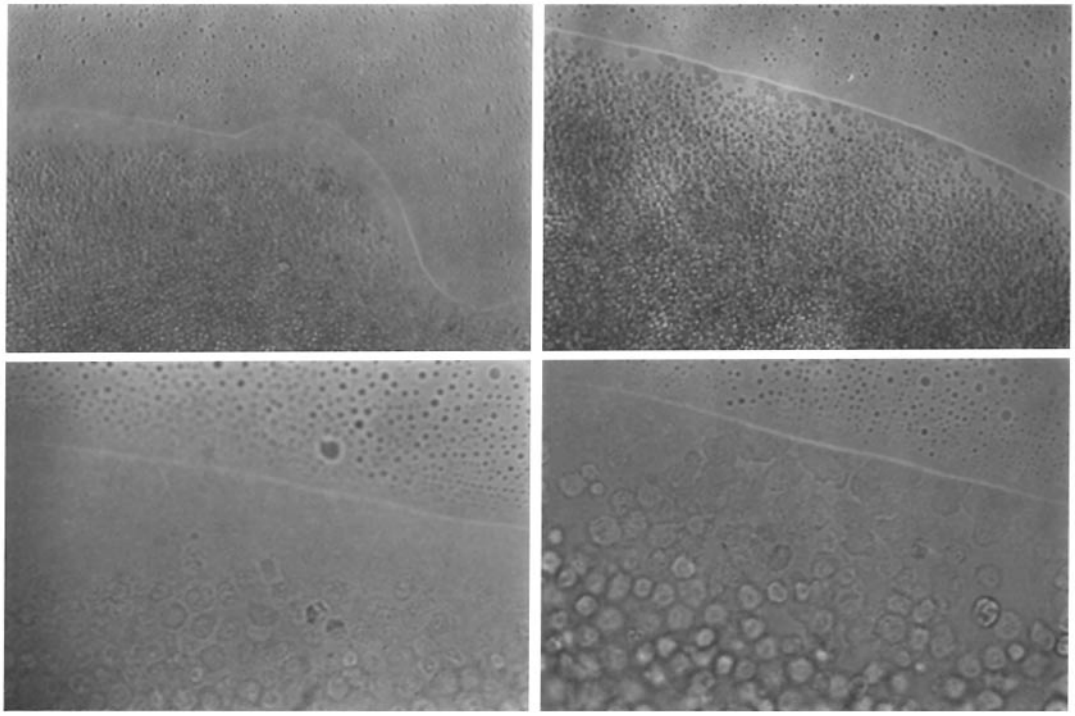

FIG. 2

(Drachman et al.: Diabetes and antibacterial defense)