cate two possible mechanisms for inhibition; one where a specific block of the ADP reaction is involved and the other possibly a general surface action on the platelet membrane.

The PRP-N of one rabbit was found to be about 100 times less sensitive to adenosine inhibition of ADP induced aggregation than that normally observed. On the other hand, the inhibitory effect of methapyrilene hydrochloride was in the normal range. A possible explanation for this appears to be that the plasma of this particular rabbit had a very high adenosine desaminase activity.

Summary. The inhibitory activity of adenosine derivatives on ADP induced platelet aggregation appears to be quite specific. Only one derivative, 5'-adamantoyl adenosine, retained the potency of adenosine. Isoadenosine was 1/100 as active as adenosine. Isoadenosine diphosphate did not induce platelet aggregation at a dose as high as 2.6×10^{-4} M as compared to ADP which produces extensive aggregation at 4.2×10^{-7} M. All of the 6 antihistaminics tested inhibited ADP induced aggregation, but there was no correlation between antihistaminic and aggregation inhibitory potency. Both classes of compounds also inhibited collagen induced aggregation.

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Phagocytic and Bactericidal Capacity of Polymorphonuclear Leucocytes Recovered from Venous Blood of Human Beings.* (31570)

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The phagocytic efficiency of polymorphonuclear granulocytic leucocytes (PMNG) derived from diabetic but non-ketotic animals and man has been found equal to that of cells derived from control subjects(1,2,3). Metabolic acidosis due to the ketosis of poorly regulated diabetes mellitus, however, has been found to depress phagocytic vigor(3).

Since phagocytosis results in death or destruction of many types of ingested micro-

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organisms, it was of interest to determine whether leucocytes recovered from the peripheral blood of non-ketotic diabetic patients were able not only to ingest but also to kill a bacterium of known susceptibility with a vigor equal to that shown by PMNG from non-diabetic humans.

Materials and methods. Human donors. Seven non-diabetic, healthy males from 23 to 28 years of age served as donors for control leucocytes. Their fasting blood sugar values were below 100 mg%. Nineteen diabetic patients of both sexes from the medical wards of the University Medical Center and the Veterans Administration Center provided the special cells for these experiments. Their ages ranged from 37 to 70 years and 15 patients were over 50 years of age. Twelve of the patients were known to have been diabetic for more than 3 years. Control of diabetes was maintained in 9 patients by diet alone whereas the other 10 required either isophane insulin (NPH) or regular insulin for regulation of the disease. None of the patients had keto-acidosis or an infectious disease when first hospitalized or during the time of study. Likewise, there were no other illnesses recognized to complicate the primary diabetic state.

Glassware and reagents. All glassware was carefully washed to eliminate antibacterial substances and was then sterilized at 170°C for 2 hours to destroy pyrogens. The solution used for washing harvested leucocytes was prepared by adding 2.0 ml 2 M KCl to 1000 ml isotonic, pyrogen free saline (Baxter Laboratories, Morton Grove, Ill.).

Preparation of leucocytes. Twenty-four hours before leucocytes were harvested, serum from each potential donor was separated aseptically from clotted venous blood and stored at 4°C. The glucose level of each specimen was determined by automated techniques. As a part of these experiments, as outlined in Table II, extra carbohydrate in the form of 50% glucose (Baxter Laboratories, Morton Grove, Ill.) was added to each serum sample in order to produce high levels. Osmolarities of all serum samples were measured in a Fiske osmometer, Model G.

On the day of experimentation, venous blood was drawn by sterile procedure and anticoagulated with ethylene diamine tetraacetate (EDTA)(4). Clinical dextran was added to each 10 ml of blood to hasten sedimentation of erythrocytes(4). The supernatant plasma was harvested and spun down at 800 r.p.m. for 10 minutes to separate white blood cells from platelets. After 2 washes in isotonic Na/K saline, the cellular sediment from 8.0 ml of blood was resuspended in 1.0 ml autogenous serum for enumeration. Smears were then made to determine cellular viability by supravital staining with trypan blue and to determine the extent of spontaneous aggregation. Wright stained smears were used to identify the types of cells present.

Bacterial culture. A stock culture of rough pneumococcus (R 36 NC) grown for 14 hours in beef heart infusion medium was transferred to fresh medium for an additional 4 hours of growth. The bacteria were then handled as described previously(4).

Phagocytic system. Sufficient pneumococci were added to the white blood cells suspended in serum to give a bacterium-cell ratio of 10 to 1. The mixtures were mixed on a horizontal rotator at a rate of nine revolutions per minute at 37°C for 30 minutes. Smears were then made for quantitating the viability of cells, the phagocytic efficiency, and intensity of the clumping reaction.

Phagocytosis estimates. The number of PMNG found to contain ingested bacteria was expressed as the per cent of phagocytosis. Only leucocytes that were intact and not enmeshed in clumps were counted.

Assay of bactericidal efficiency. After the pneumococcus-leucocyte mixture was removed from the rotator, slide cultures were made by a technique described previously(5). After cultures were incubated for 2 hours, they were stained with methylene blue, examined under an oil 97X objective and viability of the pneumococcus was estimated by counting 100 consecutive extracellular organisms that occurred either singly or as isolated microcolonies. Next, the incidence of microcolony formation within 100 consecutive leucocytes was determined. Ingestion of multiples of as many as 6 diplococcal forms were counted as dormant organisms whereas more than 6 organ-

TABLE I. Rates of Phagocytosis, Phagocytic Indices, and Bactericidal Efficiency of Human PMNG Recovered from Venous Blood When Mixed for 30 Minutes at 37°C with Rough Pneumococcus in Autologous Serum.

Source of PMNG	No. of experiments	Range of fasting blood sugar values, mg %	Range of serum osmotic pressures, mOsm/l	Rate of phagocytosis (mean), %	Phagocytic index* (mean)	Bactericidal efficiency (mean), %
Healthy donors	7	75– 84	292–301	85 ± 9	10.1 ± 1.9	84 ± 4
Diabetic patients Group I	8	65–113	276–301	91 ± 4	8.3 ± 2.4	89 ± 3
Diabetic patients Group II	8	121–193	245–301	88 ± 8	9.3 ± 2.3	89 ± 3
Diabetic patients Group III	7	222–372	280–312	88 ± 5	8.6 ± 1.0	91.0± 3

* Phagocytic index = No. of bacteria ingested by one phagocytic PMNG expressed as mean.

TABLE II. Rates of Phagocytosis, Phagocytic Indices, and Bactericidal Efficiency of Human PMNG Recovered from Venous Blood, When Mixed for 30 Minutes at 37°C with Rough Pneumococcus in Autologous Serum Fortified with Exogenous Glucose.

Source of PMNG and serum	No. of experiments	Range of serum glucose levels after addition of glucose, mg %	Range of serum osmotic pressures, mOsm/l	Rate of phagocytosis (mean), %	Phagocytic index* (mean)	Bactericidal efficiency (mean), %
Healthy donors	5	610–1040	323–334	85 ± 8	8.8 ± 2.0	80 ± 8
Diabetic patients Group I	9	357– 750	292–399	91 ± 5	8.8 ± 2.8	87 ± 5
Diabetic patients Group II	7	410– 753	298–319	86 ± 7	8.2 ± 1.8	89 ± 5
Diabetic patients Group III	7	408– 687	308–328	88 ± 5	9.0 ± 2.2	89 ± 4

* Phagocytic index = No. of organisms ingested by 100 PMNG expressed as mean value.

isms were enumerated as microcolonies. Superimposition of microcolonies upon leucocytes did not cause confusion since extracellular colonies did not distort PMNG.

Phagocytic indexes. The number of bacteria contained within the cytoplasm of each of 100 consecutive phagocytic PMNG was averaged and recorded as the phagocytic index.

Grouping of experiments. Values from experiments performed with PMNG from healthy, human donors were grouped together in order to derive baseline ranges for the efficiency of phagocytosis, phagocytic index, and the bactericidal efficiency of the cells (Table I). Likewise, similar values from experiments performed with PMNG from diabetic patients were subdivided arbitrarily according to levels of fasting blood glucose to give 3 separate categories containing 7 or 9 individuals (Table I). Finally, results obtained from experiments where extra glucose was added to serums from both controls and diabetic patients were grouped for comparison (Table II).

Statistical evaluation. The method of Croxton was used to determine the statistical significance between mean values for the different groups of experiments(6).

Results. Phagocytic efficiency of cells from healthy and diabetic individuals. From Table I it is clearly evident that PMNG from healthy donors exhibited high levels of competency for ingestion and killing of rough strain pneumococcus when suspended in autologus serum. When these values are compared with results obtained with PMNG from diabetics (Table I) it is apparent that the phagocytic cells functioned with equivalent efficiency. This was so for cells recovered from patients with fasting blood sugar values very near that of the controls (Table I, Group

I) and for leucocytes obtained from donors with grossly elevated fasting blood sugar levels (Table I, Group III). Comparison of these values by statistical analysis with those obtained from the healthy individuals failed to reveal evidence that the differences were sufficiently large to be considered significant.

Phagocytic efficiency of cells from healthy and diabetic individuals suspended in autologous serum containing exogenous glucose. The results of these experiments are summarized in Table II. Once again the phagocytic and killing capacities of PMNG from healthy donors proved to be vigorous and unmodified by the artificially high levels of serum glucose. Similarly, (Table II, Groups I, II, and III) the same functions for phagocytes recovered from blood of diabetic patients were not altered substantially when the serum glucose was set at higher levels.

In a final series of experiments but not outlined in the Table, the influence of exceedingly high glucose levels upon the phagocytic efficiency of PMNG from healthy donors was studied. The values of serum glucose ranged from 1050 mg% to a high of 1620 mg%. There was a corresponding elevation of serum osmolarity to levels from 358 to 373 mOsm/l. These results can be summarized briefly by saying that the cellular phagocytic functions were completely unaltered by exposure to this exereme environment. Viability as measured by the uptake of trypan blue stain remained quite high.

These latter data were compared to results from the first group of experiments (Table I) and statistical treatment indicated that the differences in the mean values of the various experiments were not significant.

Discussion. From these results it can be stated that no evidence was found for an impaired efficiency in the phagocytic and bactericidal function of PMNG recovered from venous blood of diabetic humans. When these cells were suspended in a fluid system of autologous serum and mixed with a rough strain pneumococcus, phagocytosis was not only brisk but the intensity equaled that for leucocytes studied from healthy donors. The capacity of cells from diabetic patients to kill ingested pneumococcus was also comparable to that of PMNG from healthy donors. These findings were true no matter how poorly the diabetes of the donor had been controlled short of the development of ketoacidosis. These findings are quite consistent with results reported previously by Bybee and Rogers(3) and Marble *et al*(6) who used different techniques to show that venous leucocytes recovered from uncomplicated diabetics were efficient phagocytes but that ketoacidosis impaired substantially the phagocytic efficiency and killing capability of these cells.

Under different circumstances, Drachman found that exudate cells recovered from diabetic and normal rats were sensitive to the hypertonic effects of high levels of glucose. Leucocytes harvested from blood of alloxanized rats did not exhibit this defect and in his hands were vigorously phagocytic(2). Contrary evidence was reported by Wertman and Heney(7) and by Cruickshank and Payne (8) who noted that leucocytes and whole blood from alloxanized diabetic rabbits ingested and destroyed bacteria less efficiently than did blood from healthy rabbits. These results probably stem from differences in experimental design and technique.

The levels of glucose in these experiments were consistent with values commonly seen in diabetic patients with problems of infection. Although the serum osmolarity values were modestly elevated in some instances, it should be pointed out that in no instance did the values approach levels reported to inhibit phagocytosis *in vitro*(9,10). Such extremes of osmolarity as studied by Chernew and Braude(9) may be encountered in the medullary portion of the kidney, an area of high incidence of bacterial infection in diabetic humans.

Summary. The phagocytic and bactericidal efficiency for rough strain pneumococcus by leucocytes recovered from venous blood of diabetic patients was compared to cells from healthy controls. Both cells and bacteria were suspended in autologous serum and subjected to gentle rotational mixing. It was found that the phagocytic activity, the phagocytic index, and the killing capacity of white blood cells from diabetics equaled that of the controls. This was so even when glucose levels of the

serum used for resuspending the mixture were artificially increased by introducing sugar from exogenous sources. It was of interest that no matter how poor had been the control of the diabetes, patients with ketoacidosis and complicating infection were excluded from the study, no evidence was found for an impaired function by these cells.

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Induction of Paradoxical Sleep by Lights-Off Stimulation.* (31571)

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Sleep cycles have been studied in man and a number of other mammalian species, including the rat(1). Each cycle consists of 3 discrete phases classified on the basis of electroencephalographic (EEG) activity: alertness (A), slow wave sleep (SS) and paradoxical sleep (PS). The paradoxical phase is a recent discovery, and much effort has been expended to unravel its physiology. That PS is required for the homeostasis of the organism appears well established, since depriving the animal of PS leads to a compensatory increase in time spent in this sleep state(2) and extreme deprivation of PS can lead to hallucinations(3). When the pontine center which appears to initiate PS is destroyed in the cat(4), the animal may be left with a 2-stage sleep cycle (A and SS) or it may suffer insomnia to the point of death. However, almost nothing is known concerning discrete functional mechanisms which may be regulated *via* PS. This report presents evidence that PS in the rat can be triggered by change in lighting, and that the PS so induced may be involved in the maintenance of various physiological mechanisms which are of a circadian nature and light sensitive.

Methods. Mature cycling female Sprague-Dawley rats maintained on a diurnal light cycle of 14 hours light and 10 hours dark were used in our experiments. Both cortical and subcortical electrodes were implanted chronically, the latter in the following brain regions: preoptic, arcuate and ventromedial hypothalamus, amygdala, reticular formation of hippocampus. Each animal received 3 deep concentric bipolar electrodes and 2 superficial silver ball electrodes on the frontal and parietal cortex. Recording was done with Grass EEG equipment with the subjects in a sound-proof recording room, in which short (30 minutes) light cycles were controlled automatically with a programmable Gerbrand timer. For most experiments a cycle of 25 minutes "lights-on" and 5 minutes "lights-off" was employed. Recordings were made simultaneously from 2 rats in cylindrical glass chromatography jars separated by an opaque partition. The rats did not appear to influence each other. Each experimental session lasted approximately 8 hours, 10:00 a.m. to

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