antisera were studied with extracts of several rabbits including the the ones used for antisera preparation, indicating that under the present experimental conditions heteroimmunization of rabbits with rat submandibular gland extract did not elicit the production of auto- or isoantibodies.

Immunoelectrophoretic studies confirmed these observations and demonstrated the presence of both serum albumin and globulins in addition to other antigenic components. The presence of serum constituents was confirmed by studying the gland extract with antiserum to rat serum. The specificity of the reaction of the antiserum was confirmed by immunofluorescent staining. When gamma globulin of absorbed antiserum to rat submandibular gland was used for direct immunofluorescence, specific bright green fluorescence was noticed in cytoplasm of the acinar, tubular and ductal cells.

Résumé. En évaluant par héteroimmunisation les caractères antigéniques de la glande submandibulaire du rat, nous avons découvert plusieurs antigènes spécifiques et autres mis en réaction avec le sérum sanguin et les tissus. Lorsque l'on compare les extraits glandulaires de diverses espèces, on constate que la glande submandibulaire du rat a des caractères hautement spécifiques.

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A Circulating Activator of Granulocytes in Liver Disease

Patients with chronic alcoholism and liver disease have been shown to be associated with increased morbidity and mortality from infections[1–3]. In an attempt to elucidate the cause of this increased susceptibility to infections, granulocyte function in patients with liver disease was investigated, as measured by their phagocytosis-stimulated glucose oxidation (PSGO). No intrinsic granulocyte defect was found, but plasma from these patients was shown to cause an increase in the resting glucose oxidative activity of granulocytes.

Methods. 15 patients with liver disease (10 with alcoholic cirrhosis, 5 with infectious hepatitis) were investigated. Normal blood specimens were obtained from healthy subjects. 20 ml of blood were collected into a heparinized plastic syringe; 10 ml were centrifuged and the plasma was collected. The remaining 10 ml were used for the separation of leukocytes by dextran sedimentation. The white cells were centrifuged and resuspended in either the patient's or normal plasma at a concentration of 1×10^7 to 2×10^7 leukocytes/2 ml plasma. Differential leukocyte counts were performed on these suspensions. The PSGO activity of granulocytes was measured continuously by an ionization chamber method[4]. Base line (resting) metabolism and maximal rate of metabolism after stimulation with 0.05 ml of a 10% suspension of latex particles (0.81 microns – Dow Chemical Co.) were measured for patient cells in patient plasma and in normal plasma, and for normal cells in patient plasma and in normal plasma. Studies were also carried out with normal cells in Krebs-Ringer-Bicarbonate buffer (pH 7.4) with 50 mg/100 ml glucose and either 2 g/100 ml or 4 g/100 ml bovine serum albumin (BSA). Bilirubin (B-grade, Calbiochem) was dissolved in 1 *N* NaOH and the pH was adjusted to 7.4 with 1 *N* HCl. Bilirubin was added in a concentration of 12.5 mg/100 ml or 25 mg/100 ml to appropriate leukocyte-buffer suspensions and PSGO measurements were performed. Glucose in plasma was measured by Glucostat (Worthington Biochemical Corp.).

Table I. Phagocytosis-stimulated glucose oxidation by granulocytes

Number studied		Resting[a] mean ± S.E.	Stimulated[a] mean ± S.E.
15	Normal cells in normal plasma	115 ± 16	414 ± 42
15	Normal cells in patient plasma	185[b] ± 27	364 ± 34
15	Patient cells in patient plasma	145 ± 22	369 ± 55
15	Patient cells in normal plasma	120 ± 21	438 ± 77

[a] Nanomoles CO_2/h per 10^7 granulocytes; [b] $p < 0.02$, compared to normal cells in normal plasma.

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Table II. Summary of bilirubin effect on granulocyte activity

No. of Experiments	Albumin (g/100 ml)	Bilirubin (mg/100 ml)	Resting[a] Mean ± S.E.	Stimulated[a] Mean ± S.E.	Stimulated/Resting Mean ± S.E.
6	4	0	62 ± 10	285 ± 27	5.0 ± 0.7
2	4	12.5	69 ± 12	342 ± 27	5.2 ± 1.2
3	4	25.0	72 ± 27	260 ± 14	5.7 ± 2.6
5	2	0	40 ± 7	255 ± 46	6.6 ± 0.7
6	2	25.0	89[b] ± 14	176 ± 22	2.1 ± 0.1

[a] Nanomoles CO_2/hr/10^7 granulocytes; [b] $p < 0.02$, as compared to 2 g/100 ml albumin and 0 mg/100 ml bilirubin

Results and discussion (Table I). Plasma from patients with liver disease was found to increase significantly ($p < 0.02$) the resting glucose oxidative activity of normal granulocytes. Patients' cells in their own palsma also showed some increase in their resting activity but this was not great enough to be statistically significant ($p > 0.2$) in our series of 15 patients. There was no significant difference in the activity of the stimulated cells in any of the 4 categories. Patient cells in normal plasma demonstrated normal activity.

Patients with liver disease appear to have a plasma factor which causes increased resting granulocyte glucose oxidative activity. All of our patients were jaundiced with 12 of them having a bilirubin concentration of greater than 10 mg/100 ml. We investigated the possible role of bilirubin in this phenomenon (Table 2) and found that high concentrations of bilirubin (25 mg/100 ml) in the presence of low levels of protein (BSA 2 g%) did cause significant stimulation of the resting activity of normal cells ($p < 0.02$). Because the levels of bilirubin required in our in vitro system were higher than those present in some of our patients, and because there are a multitude of plasma changes in liver disease, it is likely that other factors may also be operating in this phenomenon. There does not appear to be any intrinsic abnormality of the PSGO activity of the granulocyte in patients with liver disease. Increased PSGO activity would not explain the increased susceptibility to infections.

Increased resting glucose oxidative activity of leukocytes has been described previously in leukocytes from polycythemia vera patients[5], infected patients[6] and newborns[7]. The significance of this change in these patients is unclear. In our patients, it probably results from stimulation by a plasma factor, which may be bilirubin.

Zusammenfassung. Plasma von 15 Patienten mit alkoholischer Lebercirrhose oder Hepatitis steigert die Glucoseoxydase-Aktivität normaler Granulocyten. Bilirubin hat eine gleichartige Wirkung.

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The Effect of Corticosteroids on the Bactericidal Activity of Leucocytes

Increased susceptibility to infections in patients treated with corticosteroids is a well recognized phenomenon[1]. The exact mechanism by which corticosteroids alter the body's defence against bacterial infection is not well defined. One of the proposed possibilities is that they reduce the phagocytic and/or bactericidal activity of polymorphonuclear phagocytes (PMN).

There are conflicting results from several studies of this matter. ALLISON[2], HIRSCH[3] and DILLARD[4] found normal phagocytosis and intracellular killing, whilst MILLER[5] and CHRÉTIEN[6] reported impaired bactericidal activity by leucocytes of patients treated with corticosteroids, or to whose leucocyte suspension steroids had been added in vitro. The method used by CHRÉTIEN[7] and MILLER[5] was the NBT reduction test as the indicator of bactericidal activity. However, using the same method, MATULA and PETERSON[8] found normal NBT reduction under the same circumstances. These contradicting results led us to investigate the influence of corticosteroids in vivo and in vitro in the system of 'Stimulated NBT reduction test' as described by PARK[9]. In this, test endotoxin is added to the heparinized blood, mixed gently, incubated at room temperature for 5–10 min, and then the NBT test[10] is performed. Approximately half the neutrophils became NBT positive in normal healthy persons, whereas the dye reduction is impaired in conditions with impairement bactericidal activity of the leucocytes.

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Mean values of stimulated and non stimulated NBT reduction test under the influence of corticosteroids

Group	No. of tests	NBT test (%)	NBT stimulated[a]			
			Only endotoxin (%)	Endotoxin + 125γ hydrocortisone (%)	Endotoxin + 50γ hydrocortisone (%)	Endotoxin + 10γ hydrocortisone (%)
Steroid treated children	14	13.3 ± 4.1	28.5 ± 6.2	21.7 ± 5.7	24.1 ± 3.6	26.8 ± 6.0
Normal healthy children	40	9.67 ± 3.4	52.1 ± 2.1	20.6 ± 3.3	39.8 ± 5.9	51.6 ± 2.7

[a] % of positive NBT cells.