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.

Material and methods. 14 children aged 2–14 years (mean age 6.5 years), receiving Prednisone 2.5 mg/kg for at least 4 days (Rheumatic Fever, Asthma, Nephrotic Syndrome), and 40 normal non-infected children matched for age, were studied. 3 ml of venous blood was drawn into siliconized and heparinized plastic disposable tubes and the following tests were performed: 1. Histochemical NBT reduction test according to PARK's method[10]. 2. NBT stimulated: To 0.5 ml of heparinized blood was added 10 γ endotoxin (B_4 Lipopolysacharide, Difco-Detroit) in 0.05 ml phosphate buffered saline (pH 7.2). The tubes were gently shaken and incubated at room temperature for 10 min, then the NBT test was performed. 3. To 3 samples of 0.5 ml heparinized blood were added respectively 125 γ, 50 γ, and 10 γ hydrocortisone sodium succinate (Upjohn), mixed gently and incubated at room temperature for 10 min. 10 γ Endotoxin was then added to each tube as in paragraph 2. above and the NBT test was performed.

All the tests were performed within 30 min of taking the blood, sterile conditions were carefully maintained throughout. The scoring of the percent of NBT positive cells was made on 4 smears under oil immersion. As proposed by MATULA and PETERSON[8], the number of all phagocytizing cells was scored, as opposed to scoring only the polymorphonuclear cells.

Results. The mean values of the NBT positive cells are summarized in the Table. The steroid treated group showed slightly more elevated mean NBT positive cells but without statistical significance. Stimulation with endotoxin gave a significantly lower value, 28.5 ± 6.2 as compared to the normal controls 52.1 ± 2.1 ($P < 0.005$). Preincubation with hydrocortisone did not influence the cell's NBT reduction ability, since the leucocytes had previously been affected by the pre-administered steroids.

In the normal group there is a significant diminution of the reducing ability of the leucocytes when 125 γ hydrocortisone is added (52.1–20.6, $P < 0.001$). This effect is lessened with 50 γ hydrocortisone. 10 γ hydrocortisone has no influence whatsoever upon the cells.

Discussion. Our results confirm the findings of CHRÉTIEN[6,7], MILLER[5] and others.

Since in our system we did not use any particles for phagocytosis we can only say that we found that corticosteroids inhibit the NBT reducing activity of polymorphonuclear phagocytes both in vivo and in vitro. There are two possible mechanisms for this effect: 1. The stabilizing effect of steroids on the leucocyte or lysosomal membranes inhibits the penetration of endotoxin into the cell, and thus reduces the activation of the enzymatic system which would have lead to NBT reduction.

2. The steroids have a direct influence on the activation of the intracellular enzyme systems. It is known that ingestion of bacteria or other particles by a leucocyte is accompanied by a number of biochemical events within the phagocytes, such as increased O_2 consumption, stimulation of the hexose monophosphate shunt, oxidation of reduced nicotinamide adenine dinudiotide (NaDH), and H_2O_2 production[11]. These biochemical events play a major role in the intracellular killing of bacteria; without this intact system there is no possibility of reducing NBT.

MANDELL et al[12] added hydrocortisone 21-succinate to in vitro phagocytic mixtures. There was no effect on phagocytosis of bacteria but there was inhibition of oxygen consumption and production of hydrogen peroxide. Intracellular killing of bacteria was thus inhibited.

Approximately 100 times greater concentration of hydrocortisone was necessary to demonstrate these results in vitro, than that concentration achieved in human adults by administering 500 mg of hydrocortisone intravenously.

COOPER et al[13] demonstrated that the in vitro addition of various types of steroids inhibited both hexose monophosphate shunt activity and iodination of bacteria. These effects are compatible with the inhibition of bacterial killing. The possibility also exists of interaction between these two above-mentioned mechanisms.

Résumé. L'influence des corticostéroides sur l'activité bactéricide des leukocytes a été étudiée à l'aide de la réaction «NBT stimulated» de PARK. On a démontré par cette méthode que les corticostéroïdes – in vivo et in vitro – abaissent l'activité bactéricide des leukocytes polymorphonucléaires. Nos observations confirment les données antérieures sur les corticostéroïdes étudiés par d'autres méthodes. La réaction «NBT stimulated» semble faciliter l'étude de ces influences.

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Binding of Catechol Derivatives to Human Serum Proteins

It is well known that many nonpeptide hormones circulate in the blood bound to proteins, but relatively little is known about the nature of catecholamine binding to plasma proteins. That such binding of catecholamines or their derivatives does occur has been suggested by several authors[1–6]. Various techniques, such as dialysis[1], electrophoresis[3–5,7], and NMR spectroscopy[6] have been used. One group found no binding between albumin and epinephrine[7].

That the interaction of catecholamines and proteins is of a loose, dissociable nature has been suggested[2]. A recent study has concluded that the active binding site on epinephrine involves the alkyl side chain[6]. The following report provides further evidence that norepinephrine and some of its derivatives bind to plasma proteins and that this activity may depend upon the phenolic hydroxyl groups, as well as the structure of the alkyl side chain.

Materials and methods. Two µl of fresh human serum were diluted in 100 µl of 0.02 *M* barbital buffer, pH 8.6. This solution was added to a series of test tubes containing 2 picomoles ^{3}H-norepinephrine (New England Nuclear, specific activity 59 mC/mg) alone or 2 picomoles ^{3}H-norepinephrine with 100 picomoles of each of the following catechol derivatives (Calbiochem): norepinephrine, dihydroxymandelic acid (DOMA), dopamine