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

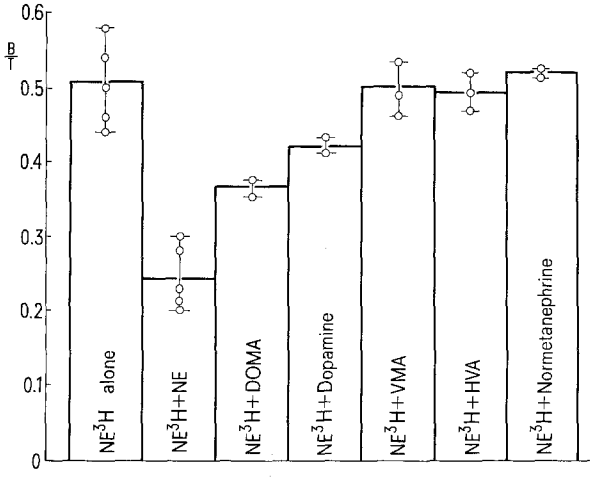
It is well known that many nonpeptide hormones circulate in the blood bound to proteins, but relatively little is known about the nature of catecholamine binding to plasma proteins. That such binding of catecholamines or their derivatives does occur has been suggested by several authors[1–6]. Various 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

(DA), vanilmandelic acid (VMA), homovanillic acid (HVA), and normetanephrine all in a volume of 40 µl. Additional tubes contained diluted serum alone and ^{3}H-norepinephrine alone to ascertain the elution volumes of protein and nonprotein bound ^{3}H-norepinephrine. Incubation was allowed to proceed for 5 minutes at 4 °C. The solution was then placed on a 3.0 ml column of G-50 Sephadex (Pharmacia) made in a 5.0 ml syringe and 0.5 ml fractions were collected in scintillation vials. 10 ml of Aquasol (New England Nuclear) were added and radioactivity was determined by counting in a Nuclear Chicago Unilux Liquid Scintillation Counter. Protein was determined spectrophotometrically at a wave length of 280 nm. All experiments were done in duplicate, and some were repeated several times.

Results. Protein was eluted in 1.0–2.0 ml. The radioactivity was distributed into 2 peaks, the first in 1.0–2.0 ml of eluate and a second in 2.5–4.0 ml of eluate. When no protein was added, a single peak of radioactivity was observed in 2.5–4.0 ml. In each case the peak of radioactivity eluted in 1.0–2.0 ml was designated the 'protein bound fraction'. The figure compares the relative abilities of 6 different catechol derivatives to compete with ^{3}H-norepinephrine for binding sites on serum protein. The results are expressed in terms of bound: total ratios, with and without the addition of the various catechol derivatives. As can be seen in the Figure, dihydroxymandelic acid was found to compete with ^{3}H-norepinephrine approximately half as well as norepinephrine competed with ^{3}H-norepinephrine. Dopamine was found to compete with ^{3}H-norepinephrine approximately $^1/_3$ as well while VMA, HVA and normetanephrine failed to exhibit significant competition with ^{3}H-norepinephrine for binding sites on serum protein.



Relative capacities of various catecholamine derivatives to compete for binding sites for norepinephrine in human serum.

Discussion. It is generally believed that protein-bound hormone is not physiologically active. Therefore, an understanding of the conditions influencing protein binding of catecholamines might be of importance in understanding their metabolic effects. As can be seen from Figure 1, DOMA and DA did compete with NE^{3}H for protein binding sites, though to a lesser extent than did 'cold' NE. This might indicate that minor alterations in the alkyl side chain decrease but do not obliterate binding capacity.

On the other hand, VMA, HVA and NM failed to compete with NE^{3}H for binding sites despite being present in excess (50:1 ratio on a molar basis). This suggests that 3-*O*-methylation obliterates the protein binding of these molecules. This was confirmed by showing that ^{3}H-normetanephrine failed to bind to serum protein when tested in this system.

Zusammenfassung. Bindung der Katecholamine und deren Analogen durch Proteine der Humansera wurde untersucht. Durch Veränderung in der Alkylseitenkette wird die Bindungsfähigkeit der Katecholaminanalogen geschwächt, doch nicht vernichtet. 3-*O*-methylierte Analogen verlieren ihre Bindungsfähigkeit und ist gegenüber Norepinephrin wirkungslos.

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The Glycogen in Some Parts of the Diabetic Skin

We have previously published some preliminary works[1–3] on methods – other than those already in use (carbohydrate metabolism tests) – which could be interesting for the earliest possible diabetes detection. We now attempt to answer whether certain morphological or histochemical changes in epidermal cells are the precursors of clinical manifestations of diabetes.

Material and methods. The material was obtained by the ear lobe biopsy from 100 persons (33 diabetics, 26 borderline cases and 41 healthy persons) aged 21–52 years. The ear lobe biopsy was performed using biopsy-needle and local anesthesia with xylocaine. No complication connected with the ear lobe biopsy was observed. All the material was fixed in Gendre-liquid, put in paraffin wax and cut at thickness of 6–8 µm. The preparations were stained by PAS-method (after Mac Manus), while for the glycogen saliva was used as the control.

Results. The normal epidermis of adult persons practically contains no glycogen, except its upper parts and around pilosebaceous orifices[4]. But in epidermis of our patients (diabetics and borderline cases) we have seen more glycogen than in normal persons, also in the so-called 'free epidermis' between two pilosebaceous orifices. It was present often in all the epidermal layers.