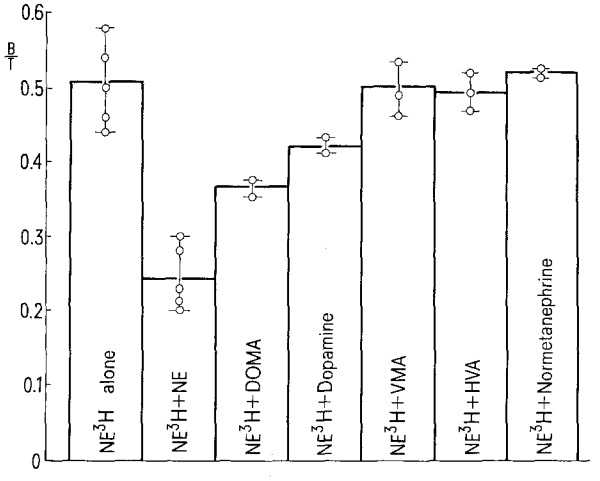
(DA), vanilmandelic acid (VMA), homovanillic acid (HVA), and normetanephrine all in a volume of 40 µl. Additional tubes contained diluted serum alone and ^{3}H-norepinephrine alone to ascertain the elution volumes of protein and nonprotein bound ^{3}H-norepinephrine. Incubation was allowed to proceed 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.

According to findings of PAS reaction in epidermis of our cases, its value ranged from positive (+), uncertain (+ −) to negative (−) as shown in the Table.

By comparing the relation between glycogen in epidermis (PAS reaction) and blood sugar levels, we have shown that PAS positivity of epidermis is directly proportional to the high blood sugar. Satistically, by use the test x^2, we could establish that PAS positivity was significant in cases with high blood sugar ($X^2 = 12.93$ $P < 0.01$).

Discussion. There are in the litterature divergent opinions about relation of diabetes and the skin. Some authors are of the opinion that there is not any connection between the two[5], but others thought that the number of dermatoses in diabetes is augmented[6–8].

We found that in the dermatological praxis it is generally known that the number of diabetics injured from mycosis is augmented. This we could explain by the fact that such skin possesses greater quantities of carbohydrates for better growth of these fungi.

Conclusion. By histological analysis of material from the earlobe of diabetics and border-line cases of diabetes, we established that the PAS positivity in epidermal cells is stronger than in healthy epidermis, and that it is stronger in diabetics than in border-line cases. By comparing the level of blood sugar and PAS-positivity in epidermal cells, we find that the strenght of PAS-positivity is proportional to the level of blood sugar, and that the difference between PAS-positivity in persons with less blood sugar and those with more blood sugar is statistically significant.

PAS reaction in epidermal cells at different diagnoses

Diagnoses	No of diagnoses	PAS reaction (No of cases)
Borderline cases	26	− (6), + − (12), + (8)
Diabetics	33	− (3), + − (9), + (21)
Normals	41	− (14), + − (18), + (9)

Résumé. Après une analyse histologique de la peau du pavillon de l'oreille des diabétiques et cas limites de diabète, nous avons conclu que la positivité PAS de leurs cellules épidermales est beaucoup plus forte que dans l'épiderme sain, et qu'elle est plus forte chez les diabétiques que dans les cas limites de diabète. Nous avons trouvé que l'intensité de la positivité PAS est proportionelle au taux du sucre dans le sang et que la différence entre la positivité PAS chez les personnes ayant un taux bas de sucre et celles qui en ont un taux élevé est statistiquement significative.

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Selective Potentiating Effects of Metal Ions on Vasopressin[1,2]

Extensive literature exists demonstrating the influence of mono- and divalent cations on the sensitivity of various bioassay systems to neurohypophyseal hormones (e.g., [3–6]). These phenomena have usually been associated with a direct effect of the cations on the tissue rather than on the hormones. Neurohypophyseal hormones have been demonstrated to form complexes with paramagnetic ions such as Cu^{++}, Ni^{++} and Co^{++}, involving displacement of specific protons attached to nitrogen atoms[7–9]. However, using the isolated toad urinary bladder as assay system, both the Cu^{++}-vasopressin complex and the free hormone showed identical hydroosmotic activity[10].

In the present study we investigated the possible interactions of Na^+, NH_4^+ and Ca^{++} with arginine vasopressin (AVP) as reflected by selective changes of the rat blood pressure response to the hormone.

Methods. The AVP used had a rat bp activity of 460 ± 13 USP U/mg as determined by four-point assay (n 6). The *stock solution* was comprised of 1 µg AVP/ml water (mean pH 6.6). The water contained less than 0.05 meq/l Na^+. Dilutions used were a) 1 ml stock solution plus 9 ml water equals *control solution*, which contains 100 ng AVP/ml water; b) 1 ml stock solution plus 9 ml electrolyte (Na^+, NH_4^+ or Ca^{++}) solution at 111% final concentration equals *test solution*. The mean pH of the control and test solutions was 6.6. Control and test solutions of AVP were made by using mechanical mixing

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2 Abbreviations used are: AVP, arginine-vasopressin; bp, blood pressure; U, unit; n, number of experiments; $\bar{x}$, mean value; S.E. standard error; p, level of significance; NS, not significant.

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