## Properties of Leukocyte Catalase from Normal and Acatalasemic Humans

Apparent lack of catalase in blood of individuals homozygous for Swiss Type Acatalasemia was shown to be due to the synthesis of an enzyme variant of approximately normal specific activity, but of low stability [1,2]. In catalase deficiency of that type, the level of residual enzyme activity in relation to normal mainly depends on the turnover rate of the enzyme. Consequently, the level of residual catalase activity is rather low in cells, such as erythrocytes, whose components have a relatively long half-life. On the other hand, the level of residual catalase is the higher the faster its turnover in that tissue. A suitable example for this latter type, which is easily accessible in humans, is leukocyte catalase. The interest in leukocyte catalase also stems from the observation that it may eventually serve as a diagnostic tool in various respects, such as leukaemia [3], inborn errors [4] and nutritional state [4a].

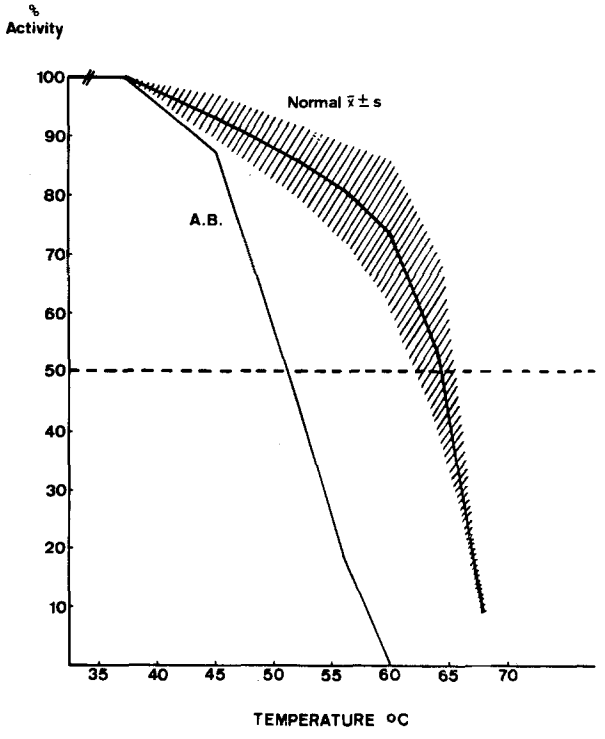


Fig. 1. Stability of leukocyte catalase (heatstability) in normal and acatalasemic humans. Technique of the heat stability test see text. / / / / / / = normal ($\bar{x} \pm s$; $N = 5$); ———— = A. B., homozygous for Swiss Type Acatalasemia. Ordinate: Catalase activity after incubation at given temperature related to initial activity. Abscissa: Temperature at which the sample has been preincubated for 10 min.

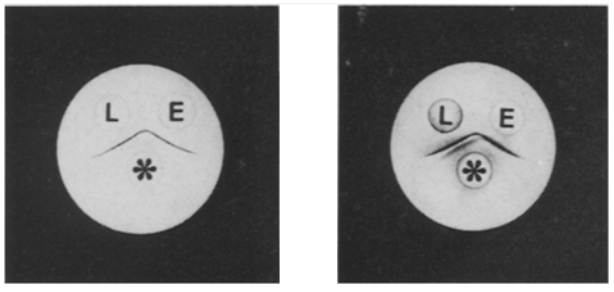


Fig. 2. Demonstration of antigenic identity of erythrocyte and leukocyte catalase in the double immunodiffusion test. Left side: Peroxidatic activity stain (*o*-dianisidine). Right side: Protein stain (amidoblack). L, leukocytes (extract); E, erythrocytes (hemolysate); *, anti-catalase IgG preparation.

*Methods.* For the isolation of leukocytes essentially the procedure of Wyss et al. [5] was followed: Dextran sedimentation was applied to fresh blood samples (20–30 ml) from normal subjects. Contaminating red cells were removed by repeated washing of the leukocyte pellets with cold hypotonic saline. The pellets were then suspended in 0.2 *M* saccharose and the cells disrupted by repeated freezing and thawing followed by ultrasonication. After centrifugation, the supernatant was used in all experiments. Leukocytes from an individual homozygous for Swiss Type Acatalasemia were isolated from a 200 ml blood sample of the propositus A. B.

Catalase activity was measured according to Bonnichsen et al. [6] and expressed as μg catalase/mg protein. For this calculation the formula $k_0/k_1'$, presuming a $k_1'$ of $3.4 \times 10^7\ M^{-1}\ \text{sec}^{-1}$ and a molecular weight of 240,000, was used. The protein content was measured by the method of Lowry et al. [7]. Heat-stability experiments were carried out by incubating samples of normal as well as acatalasemic leukocytes for 10 min at various temperatures, ranging from 4 °C–68 °C. Catalase activity was measured at 37 °C immediately after the pre-incubation period. The anti-catalase IgG-fraction prepared by Shapira [8] was the same as that used in experiments published recently in this journal [2]. Double immunodiffusion tests were done according to Ouchterlony [9], using 1.5% purified Agar (Behringwerke) in 0.05 *M* sodium veronal buffer, pH 8.6. The wells were 6 mm in diameter with a 6 mm distance between. Proteins were stained with Amidoblack 10 B and peroxidase active precipitin lines were detected by applying a substrate mixture to the plates, consisting of 5 ml 0.5% *o*-dianisidine in methanol and 95 ml 0.003% hydrogen peroxide.

*Results.* Total catalase activity in leukocyte preparations of normal subjects was found to be equivalent to $3.09 \pm 0.73$ μg catalase/mg protein. The average residual activity measured in 2 leukocyte preparations from A.B., homozygous for Swiss Type Acatalasemia, was 0.41 μg/mg protein, which amounts to approximately 13% of the normal. In comparison, the residual activity found in erythrocytes of A.B. was only about 1% of the red-cell catalase found in normal subjects (Table). Heat stability experiments were carried out with normal and acatalasemic leukocyte preparations. The remaining relative activities after incubation at various temperatures are shown in Figure 1. A distinct difference in thermolability of leuko-

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cyte catalase from either source can be noticed. Normal catalase retained as much as 50% of its original activity after preincubation at 64 °C, whereas – under identical conditions – complete inactivation had occurred in the acatalasemic leukocyte preparation. In this instance, the 50% activity level was reached after incubation at 51 °C already. Immunological antigenic properties of normal leukocyte catalase have been compared with those of erythrocyte catalase in the double immunodiffusion test (Figure 2). A strong precipitin line showing complete identity is formed between the wells containing the preparations of normal leukocytes, normal erythrocytes and anti-catalase IgG (Figure 2, right side). This line exhibits strong peroxidatic activity (Figure 2, left side). A second, as yet unidentified weaker line exerting no peroxidatic activity is precipitated in the range of the leukocyte preparation only. Experiments of the same type performed with analogous preparations from A.B. suggest that there is also antigenic identity between normal and acatalasemic leukocyte catalase.

*Discussion.* Evidence is presented that leukocytes of an acatalasemic subject (homozygous for Swiss Type Acatalasemia) contain appreciable amounts of catalase. In A.B. the level corresponds to about $^1/_7$ of that of normal, which is distinctly higher than that found in the erythrocytes of the same individual (1–2% of normal). Like the mutant enzyme in the erythrocytes[1], its antigenically identical counterpart in leukocytes also exerts the same unusually low degree of thermostability. This observation is compatible with the assumption that, in an enzyme deficiency, two different types of structural gene mutations may lead to an apparent loss of enzymatic activity: 1. Enzyme variants of low specific activity, 2. variants with approximately normal specific activity, but of low stability. Eventually, both possibilities may lead to the same type of disorder. Examples are the different forms of glucose-6-phosphate-dehydrogenase-deficiency or of acatalasemia[1,10]. In the unstable mutant-type, the low level of residual activity in red blood cells must be considered as a poor indicator for the general situation, since in most other cells and tissues distinctly higher levels are found. In conclusion, there is a close analogy as to catalase stability and activity distribution pattern between acatalasemic mice and individuals homozygous for this particular type of acatalasemia[11,12].

Catalase activity in leukocytes and erythrocytes of normal and acatalasemic humans (Family B.)

| Material analyzed | | Catalase activity |
|---|---|---|
| Leukocytes | Normal ($N = 15$) | 3.09 ± 0.73 μg/mg Protein |
| | Acatalasemic ($N = 2$) | 0.41 (= 13% of normal) |
| Erythrocytes | Normal ($N = 15$) | 2.38 ± 0.36 μg/mg Hb |
| | Acatalasemic ($N = 2$) | 0.02 (~1% of normal) |

*Zusammenfassung.* Beim Akatalasie-Fall A.B. ist die in den Leukozyten vorhandene Katalase-Restaktivität wesentlich höher (13%) als diejenige in den Erythrozyten (etwa 1% der Norm), wie dies beim Vorliegen einer instabilen Enzymvariante zu erwarten ist. Die Enzyme beider Zelltypen sind antigen-identisch und zeigen denselben Grad von Thermolabilität.

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## The Detection of 5α-Androst-16-en-3α-ol in Human Male Axillary Sweat

The 16-dehydro $C_{19}$ steroid 5α-androsten-3α-ol (androstenol) is excreted in the urine of adult human males in substantial quantities (of the order of 1 mg/24 h urine) and in lesser amounts in adult female urine[1]. The physiological function, if any, of the 16-dehydro $C_{19}$ steroids in man is unknown, but androstenol secreted in the saliva of the boar acts as a releaser sex pheromone in eliciting the characteristic immobilization response of the oestrous sow to the advances of its mate[2–4].

There are, however, circumstantial grounds for believing that pheromones may play a part in human behaviour[5,6]. Androstenol, which has a musk-like odour detectable by human subjects at extremely low levels (1–5 ng on water at 20 °C, 10 cm from the nose; BROOKSBANK, unpublished) is a likely candidate as a pheromone in man, though various other steroids can be smelt at much higher concentration[7]. External secretion, onto the skin surface, would be the most probable way in which human pheromones are exhibited. In view of the evident capacity of axillary glands and hairs for preferential uptake and release of steroids[8,9] and the similarity of axillary apocrine glands in morphology and in androgen-dependence to apocrine glands specialized in lower mammals for pheromone secretion[10–12], we believed that axillary sweat might contain sufficient quantities of 16-dehydro $C_{19}$ steroids to be detectable by gas chromatography-mass spectrometry (GC/MS). Previous efforts, using GC alone

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