Carbon source (carbon content 2%, equivalent to 5% glucose in Richards medium)	Mycelial weight in mg	Total fusaric acid in mg	mg fusaric acid per mg dry mycelial weight[a]	Comparison of efficiency with the control (taken as 100%)
1. Glucose (Richards medium)	156.8	3.38	0.022	100
2. Starch	192.2	5.38	0.028	127
3. Cellulose	74.6	1.88	0.025	114
4. Casein	162.4	10.10	0.062	282
5. Egg albumin	172.0	11.22	0.065	295
6. Coconut oil	60.8	1.88	0.030	136
7. Olive oil	63.6	2.20	0.035	159
8. Yeast ribonucleic acid	13.0	0.32	0.024	109
9. Calf-thymus deoxyribonucleic acid	60.0	1.37	0.023	105
10. Citric acid	258.8	4.65	0.018	82
11. Succinic acid	374.6	4.95	0.013	59
12. Fumaric acid	366.2	4.95	0.014	64
13. Susceptible host extract	60.0	4.05	0.068	309
14. Resistant host extract	58.0	3.025	0.052	236

[a] Efficiency of fusaric acid production

evaporated using a current of air at room temperature and the residue dissolved in 10 ml of 80% ethanol. 0.2 ml of the samples were chromatographed for 18 h on Whatman No. 3 MM filter paper using the solvent system *n*-butanol, acetic acid and water (in the ratio 4:1:5) along with a set of standard solutions varying in concentration from 40–100 μg. The spots were marked under UV and eluted with 5 ml of 80% ethanol whose absorbancies were measured at 268 mμ, in a Beckman spectrophotometer (ZAHNER[4]).

It is interesting to note (Table) that the proteins support good growth and also fusaric acid production. The total fusaric acid production was maximum in their presence. This may be due to the fact that metabolic products of proteins available in high concentration probably promote the synthesis of fusaric acid, as it has been shown earlier that α- and β-alanines, glutamic acid, γ-amino butyric acid and serine stimulate fusaric acid production[5]. It may be that tryptophan contributes to the pyridine ring. No appreciable variation is observed in the efficiency of toxin production among the carbohydrates and nucleic acids. High growth in the presence of starch results in good yield of fusaric acid. Poorest toxin production is observed with the nucleic acids, probably due to poor growth. A slight increase in the efficiency of fusaric acid production observed in the presence of lipids may be due to the free availability of acetate, which was shown to be incorporated into fusaric acid by using labelled acetate[5]. Excellent growth in the presence of the Krebs cycle intermediates offers good yields of the toxin. However, the efficiency of production is found to be poor, thus indicating that these organic acids are preferentially utilized for growth purposes only.

The efficiency of fusaric acid production in the presence of autoclaved host extracts approaches that of the proteins. In spite of the poor growth, fusaric acid yield approaches that of the control, showing a high efficiency in toxin synthesis. Fusaric acid production in the presence of autoclaved extracts of both the resistant and susceptible hosts may be due to the denatured proteins that are available in these extracts. However, the difference observed may be due to the variation in their constitution which may have a relation to the resistance.

Zusammenfassung. Die Bildung von Fusarinsäure durch *Fusarium vasinfectum* Atk., bezogen auf das Trockengewicht, ist am grössten, wenn Proteine oder Auszüge aus Wirtspflanzen und am geringsten, wenn Säuren des Krebszyklus als Kohlenstoffquelle geboten werden.

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Heterogeneity of Catalase in Blood of Heterozygous Cases of Acatalasia

Catalase can be isolated from small samples of hemolysate by gel-filtration on Sephadex[1] or by column chromatography on calcium phosphate-DEAE-cellulose complex gel[2]. Using the latter technique, 2 fractions can be obtained by extraction with 0.15 *M* sodium chloride solution (= fraction with index number 1) and 0.2 *M* secondary sodium phosphate solution pH 8.2 (= fraction with index number 2).

By applying this technique to samples of (a) normal human blood, (b) blood of a heterozygous, and (c) blood of a homozygous case of acatalasia, 6 different preparations of purified human red cell catalase are obtained. Analysis of their electrophoretic mobility on a mixture of

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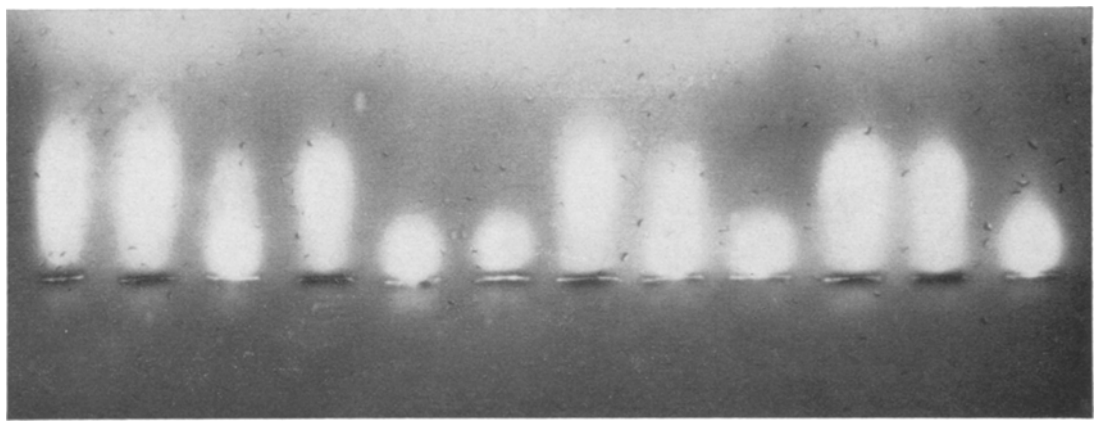

A_1	A_2	B_1	B_2	C_1	C_2	A_1 + A_2	B_1 + B_2	C_1 + C_2	A_1 + B_2	A_2 + B_2	B_1 + C_1

Electrophoretic mobility of red cell catalase isolated from (a) normal human blood, fractions A_1 and A_2, (b) a heterozygous case of acatalasia ('hypocatalasaemia') P.V., fractions B_1 and B_2, (c) a homozygous case of acatalasia M.V., fractions C_1 and C_2. From left to right: run No. 1–6: Single fractions A_1, A_2, B_1, B_2, C_1, C_2; run No. 7–12: $\overline{aa}$ mixture of fractions 7 = $A_1 + A_2$, 8 = $B_1 + B_2$, 9 = $C_1 + C_2$, 10 = $A_1 + B_2$, 11 = $A_2 + B_2$, 12 = $B_1 + C_1$. Solutions analysed: approx. 10 μl containing 0.1–0.5 μg protein. Electrophoresis was run at pH 9.0 (*Tris*-buffer 0.11 *M*) at 4 °C for 8 h (300 V; 60 mA; plate 13 · 20 cm). Detection of spots according to THORUP[4] by means of subsequent treatment of the plate by solution 1 (= 1.5% H_2O_2 + 1.5% $Na_2S_2O_3$) and 2 (= 1.5% Na I).

starch, agar and polyacrylamide-gel[3], and subsequent staining of the plate by means of THORUP's technique[4,5] gave us the following result (see Figure): The mobility of either fraction isolated from normal human red cells (A_1, A_2) and from acatalatic cells (C_1, C_2) is the same, but there is a distinct difference in mobility between catalase of normal and of acatalatic red cells ($A_1 = A_2 > C_1 = C_2$).

However, from the blood of a heterozygous individual, 2 fractions of different electrophoretic mobility are obtained ($B_1 < B_2$). Fraction B_1 migrates approximately as little as the catalase active material obtained from acatalatic cells ($B_1 \sim C_1$ and C_2). Fraction B_2, however, exerts the same mobility as the catalase obtained from normal subjects ($B_2 \sim A_1$ and A_2). If fractions are combined and analysed electrophoretically, the mobility of the single components is not affected by the presence of the other fraction (see Figure; runs Nos. 7–12). According to the terminology for denomination of heterogenous enzyme fractions, the faster moving catalase may be termed I and the slower moving II. Thus, the formula of catalase in blood of normal humans, heterozygous and homozygous acatalasia cases might be expressed as I I, I II and II II respectively.

Catalase active material isolated previously from normal and acatalatic human red cells by gel filtration proved to be identical with respect to kinetics, mobility in gel-filtration, azide sensitivity and precipitation by anticatalase[6,7]. This led to the assumption that acatalasia – at least, in so far as the Swiss cases are concerned – may be considered as a pure form of a control-gene mutation[6,8]. From the observation shown in the Figure, one must conclude, however, that catalase from these two sources – in spite of its apparent enzymatic and antigenic identity – is different in its physico-chemical properties. Catalase in blood of homozygous acatalatic subjects is mainly localized in reticulocytes[9,10]; therefore it will be of particular interest to investigate whether this atypical form of catalase is chemically or biologically less stable than catalase normally present in the red cell. The fact that catalase from red cells of normal humans and of homozygous acatalatic subjects seem to differ in their structure, offers an explanation for the finding that blood catalase in heterozygous cases of acatalasia (= 'hypocatalasaemia') is heterogenous in nature[11].

Zusammenfassung. Mittels Chromatographie auf Calciumphosphat-DEAE-Cellulose lassen sich aus Hämolysat von Menschenerythrocyten zwei Fraktionen gereinigter Katalase darstellen. Im Gegensatz zu den Fraktionen aus normalen und homozygot akatalatischen Zellen zeigen die aus Blut heterozygoter Defektträger isolierten Katalasefraktionen ein elektrophoretisch verschiedenes Verhalten. Die Beweglichkeit der rascher wandernden Fraktion entspricht jener des normalen Enzyms, während die langsamer wandernde Fraktion sich gleich verhält wie das aus Akatalasieerythrocyten isolierte Enzym. Daraus folgt, dass zwischen Katalase in normalen und Akatalasiezellen trotz nachgewiesener Antigenidentität gewisse Strukturunterschiede zu bestehen scheinen.

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