Iproniazid verhindert die durch Reserpin verursachte Erniedrigung des γ-Aminobuttersäuregehaltes des Gehirns und der Krampfschwelle (siehe Methodik)

	Versuchszahl	γ-Aminobuttersäure µMol/g	Elektroschock 100 Hz, 5 msec, 5 sec
Kontrollen	60	3,49 ± 0,14	4,4 mA (4,2–4,6)
Iproniazid 100 mg/kg (20 h)	7	3,92 ± 0,19	5,0 mA (4,8–5,2)
Reserpin 5 mg/kg (3 h)	20	2,45 ± 0,22	1,2 mA (1,0–1,4)
Reserpin 5 mg/kg (3 h) + Iproniazid 100 mg/kg (20 h)	12	3,48 ± 0,23	5,9 mA (5,5–6,3)

Methodik. Weisse Mäuse (18–20 g). *Reserpin* (5 mg/kg) wurde subkutan, in den Versuchen, in denen schon 15 min nach der Injektion GABA-Gehalt und Krampfschwelle bestimmt wurden, intraperitoneal injiziert. *Iproniazid* (100 mg/kg) intramuskulär 20 h vor Tötung der Tiere (GABA-Bestimmung) bzw. vor Elektroschock.

GABA-Bestimmung. Äthylalkoholische Extrakte aus je 3 Gehirnen. Nach Eindampfung zur Trockne Aufnahme in 0,7 ml Aqua dest. + 0,2 ml $HClO_4$ (1,2 *n*). Hochspannungselektrophoretische Auftrennung des in der Ultrazentrifuge gewonnenen klaren Überstandes. Quantitative Bestimmung mit dem Spinco-Analytrol (Beckman Instr.) nach Anfärbung mit Ninhydrin und Stabilisierung der Farbflecke mit Cu-Reagens.

Elektroschock. Cornealelektroden. Reizung mit 100 Hz, 5 msec., Reizdauer 5 sec. Ermittlung der Stromstärke (mA), bei der 50% der Tiere krampften, an Gruppen von mindestens je 15 Mäusen. Berechnung nach LITCHFIELD und WILCOXON[12].

Summary. 1. In mice, *reserpine* (5 mg/kg) causes a long-lasting depletion of the brain for γ-aminobutyric acid (GABA), which corresponds temporally to the lowered seizure threshold (electroshock).

2. *Iproniazid* prohibits both the depletion of GABA and the lowering of seizure threshold.

3. Relations between amine- and GABA-metabolism of the brain are pointed out.

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On the Inhibition of Alcoholic Fermentation in *Brettanomyces* Yeasts under Anaerobic Conditions

As was shown by CUSTERS[1], the yeast *Brettanomyces claussenii* ferments glucose at a higher rate under aerobic than under anaerobic conditions. CUSTERS introduced the term 'negative Pasteur effect' (neg. P. E.) for this phenomenon. As WIKÉN and RICHARD[2,3] observed a similar effect in *Saccharomyces* strains under particular conditions, a closer study of the effect seemed to be of interest. As the neg. P. E. is characteristic of all *Brettanomyces* species[4], efforts were concentrated on *Br. claussenii*, strain CBS 76.

In Warburg experiments (direct method at 30°C) anaerobiosis was obtained by flushing for 30 min with pure N_2, freed from traces of O_2 by means of a Pd-asbestos catalyst saturated with H_2. Assuming a respiratory quotient (R. Q.) of 1 for the oxidation of carbohydrate, gas production under aerobic conditions essentially represents the aerobic fermentation. If R. Q. would be lower than 1, e.g. in oxidation of ethanol, the actual aerobic fermentation would be even higher than that directly observed.

Cells of *Br. claussenii*, harvested from aerobic shake cultures on various liquid media after different times of incubation at 30°C, invariably showed a neg. P. E. (Fig. 1, curves A and B) in fermentation of all sugars examined (galactose after adaptation), in distilled water as well as in phosphate solution at pH 3.5 to 6.5 and in buffers at pH 4.5, prepared with a large number of organic acids[5]. The rate of aerobic fermentation strongly depends on the concentrations of K^+ and Na^+ present[5].

Studies on the elimination of the neg. P. E. were performed in KH_2PO_4 buffer at pH 4.5 with yeast, harvested from a synthetic substrate containing glucose, minerals, biotin, and thiamine, at a culture age (42–48 h) characterized by maximum fermentation activity per unit of cell dry weight.

The anaerobic fermentation is appreciably stimulated even by minute amounts of O_2 (e.g. 0.1% in the gas phase) or by aerobic addition of glucose previous to flushing with N_2 (as in *Saccharomyces* ssp.[6]). This cannot be due to storage of fermentable substrate within the cells under aerobic conditions, because on anaerobic addition of glucose after completed fermentation of this sugar, added aerobically, the anaerobic fermentation rate also approaches the aerobic one. In addition, a stimulating effect on anaerobic fermentation may be seen on anaerobic addition of the clear supernatant, obtained by shaking washed cells of *Br. claussenii* or baker's yeast aerobically in glucose solution at 30°C for a few hours and centrifuging the suspension. When suspensions of *Br. claussenii* and baker's yeast are mixed in the Warburg vessels, the rates of the aerobic fermentations appear to be additive, but the rate of the anaerobic fermentation of the mixture equals or even surpasses the sum of the anaerobic rate of baker's yeast and the aerobic rate of *Br. claussenii* (Fig. 1). These results suggest that some substance(s), formed in fermentation of glucose, may replace O_2 in eliminating the neg. P. E. This stimulation is not caused by CO_2, ethanol, or acetic acid, but can be produced by acetaldehyde (10^{-2} *M*) and by pyruvic acid (5×10^{-3} *M*).

In order to study a possible effect of lipid components, acetone-ether extracts of *Br. claussenii* and baker's yeast were mixed with water and the solvents evaporated *in vacuo*, leaving emulsions of the extracted material, which strongly stimulated the anaerobic fermentation in *Br. claussenii*. However, in control experiments, acetone (10^{-2} *M*) produced the same effect. Ether (5×10^{-2} *M* or even less) also stimulates to an appreciable extent. In addition to acetaldehyde, acetone, and pyruvic acid, also other carbonyl compounds are active in eliminating the

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neg. P. E., e. g. formaldehyde (10^{-3} *M*), acetoin (10^{-3} *M*), dihydroxyacetone (10^{-2} *M*), methylethylketone (10^{-2} *M*), and α-ketoglutaric acid (5×10^{-2} *M*). On the other hand, ethanol, *iso*-propanol, lactic acids, methanol, 2, 3-butanediol, and glycerol do not stimulate. Oxidizing agents such as ferricyanide (10^{-2}–10^{-1} *M*) and quinone (2×10^{-4} to 10^{-3} *M*) have no stimulating effect, whereas methylene blue (5×10^{-4}–5×10^{-3} *M*) has only a moderate one; at the higher concentrations mentioned, these agents already considerably reduce the rate of aerobic CO_2 production.

The effect on anaerobic fermentation by pyruvic acid (5×10^{-3} *M*) or acetoin (10^{-3} *M*) is abolished by previous, but not by subsequent addition of KF (10^{-4} *M*).

The results suggest an action of the carbonyl compounds as H-acceptors in enzymatic dehydrogenation, comparable with the so-called phytochemical reduction[7]. Oxidized coenzyme I (DPN) enhances anaerobic fermentation to an extent depending on its concentration (Fig. 2). Reduced coenzyme I (DPNH) has only a small effect, corresponding to the known amount of DPN present as an impurity.

In connection with these results, it may be mentioned that it was recently demonstrated[8] that cell-free extracts of *S. cerevisiae* as well as crystalline yeast alcohol dehydrogenase oxidize DPNH on addition of lower aliphatic aldehydes.

As supposed by several authors[1,2,9], the inhibition of fermentation found under anaerobic conditions in different yeasts might be due to a too much reduced state of one or more catalytic components in the enzyme system concerned. In view of the above findings, it is tentatively suggested that the inhibition of the start of fermentation in *Br. claussenii* under anaerobic conditions is, at least in part, due to a shortage in DPN. This inhibition is abolished on addition of O_2 or of other substances able to oxidize DPNH enzymatically.

A respiration-deficient mutant of *Br. claussenii* was studied in order to determine if respiration is involved in the above oxidation by O_2, as might be expected. In this mutant the rates of aerobic and anaerobic fermentation are nearly equal, and both processes are stimulated by acetoin or DPN (Fig. 3), whereas DPNH has practically no influence.

The present investigation is being continued.

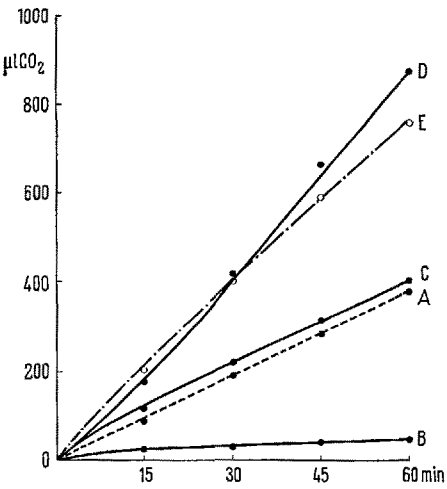

Fig. 1. *Brettanomyces claussenii* and baker's yeast, potassium phosphate buffer, 0.18 *M*, pH 4.5, glucose 0.1 *M*.

●---● aerobic fermentation. ●——● anaerobic fermentation.

A) *Br. claussenii*, 20 mg wet weight/vessel. B) idem. C) baker's yeast, 5 mg wet weight/vessel. D) *Br. claussenii*, 20 mg wet weight/vessel + baker's yeast, 5 mg wet weight/vessel. E) calculated: A + C.

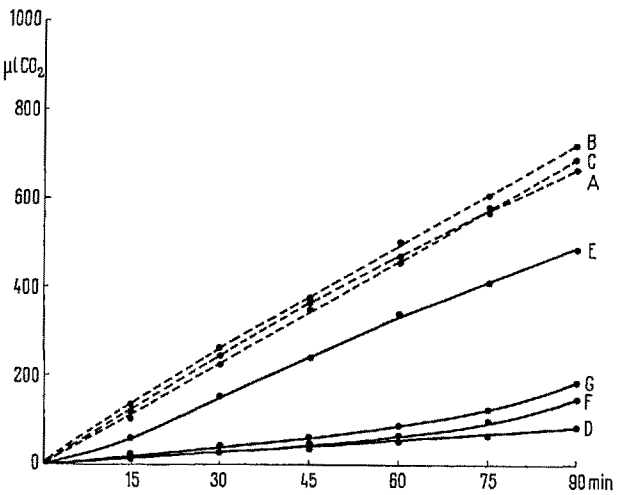

Fig. 2. *Br. claussenii*, 20 mg wet weight/vessel, potassium phosphate buffer, 0.05 *M*, pH 4.5.

●---● aerobic fermentation. ●——● anaerobic fermentation.

A) glucose, 0.05 *M*. B) idem + DPN, 0.02 *M*. C) glucose, 0.05 *M* + DPNH, 0.02 *M*. D) glucose, 0.05 *M*. E) idem + DPN, 0.02 *M*. F) glucose, 0.05 *M* + DPN, 0.001 *M*. G) glucose, 0.05 *M* + DPNH, 0.02 *M*.

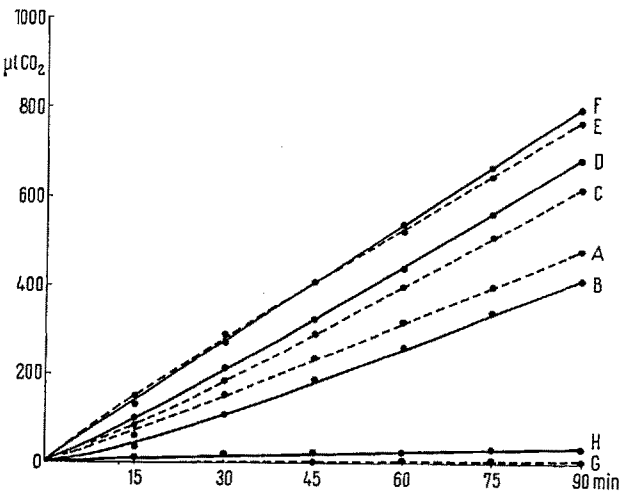

Fig. 3. *Br. claussenii*, respiration-deficient mutant, 20 mg wet weight/vessel, potassium phosphate buffer, 0,05 *M*, pH 4.5.

●---● aerobic fermentation. ●——● anaerobic fermentation.

A) glucose, 0.05 *M*. B) idem. C) idem + DPN, 0.02 *M*. D) idem + idem. E) glucose, 0.05 *M* + acetylmethylcarbinol, 0.05 *M*. F) idem + idem. G) acetylmethylcarbinol, 0.05 *M*. H) idem.

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[10] The author expresses his thanks to Prof. T. WIKÉN for stimulating interest, to Mr. C. J. E. A. BULDER for kindly supplying the respiration-deficient strain of *Br. claussenii*, and to Miss H. BRAKMAN for valuable technical assistance. The author is indebted to the Koninklijke Nederlandsche Gist- en Spiritusfabriek N. V., Delft, for a gift of pure DPN and DPNH. Part of the work was supported by the Delfts Hogeschoolfonds and by the Netherlands Organization for Pure Research (Z. W. O.).

Zusammenfassung. Der «negative Pasteureffekt» in *Brettanomyces*-Hefen kann durch O_2, Gärprodukte der Hefen, Diäthyläther, Aldehyde, Ketone und andere Carbonylverbindungen, sowie durch DPN beseitigt werden.

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Die Wirkung von Hybridenwein auf die Leber

Unter Hybridenwein versteht man das Gärungsprodukt des Traubensaftes von Kreuzungen aus europäischen, sogenannten Edelreben und resistenten amerikanischen Reben. Mehrere Autoren (PAGES, LÉOBARDY, BREIDER et al.) behaupten, dass nach Genuss solcher Weine bei gesunden Organismen Leberschädigungen auftreten würden. Diese Frage ist aber aus manchen Gründen noch keineswegs abgeklärt. Über die Wirkung von Äthanol auf eine vorgeschädigte Leber liegen bereits zahlreiche Untersuchungen vor (Übersicht KLIEWE[4]). Da aber noch unbekannt ist, ob und warum Leberschäden nach Trinken von Hybridenwein auftreten können, wurde unter anderem zunächst untersucht, ob eine durch Allylalkohol vorgeschädigte Leber im Vergleich zu Äthanol und Edelwein durch Hybridenwein unterschiedlich ungünstig beeinflusst wird.

Weisse männliche Ratten in den engen Gewichtsgrenzen von 145–155 g erhielten 3 Tage lang das hochwertige Standardfutter «Altromin», am Tag vor Versuchsbeginn Gerste. Unmittelbar vor dem Versuch wurden sie nach dem Gewicht aussortiert. Alle Lösungen verabreichten wir mit der Schlundsonde. Jedes Tier erhielt 2 ml der zu testenden Lösung (Kontrolle: Wasser). 1 h danach wurden 0,6 ml eines frisch hergestellten, 3,5%igen Allylalkohols («Merck») appliziert. Danach blieben die Tiere 10 h lang ohne Futter und Flüssigkeit; nach weiteren 38 h wurden sie nach Kopfschlag und Entbluten – jeweils ein Kollektiv einzeitig – aufgearbeitet.

In geringer Abweichung von den Angaben von EGER[5] wurde die Oberfläche der gesamten Leber (Ober- und Unterseite) sowie die der nekrotischen Stellen nach flächenhafter Projektion ohne Berücksichtigung der Lappenwölbungen planimetrisch erfasst und der prozentuale Anteil der Nekroseflächen an der Gesamtoberfläche zur statistischen Auswertung herangezogen.

Als Testlösungen dienten ein weisser Hybridenwein von einer FS_4-Hybride (Äthanol nach der ADH-Methode = 12,79 Vol.%, Invertzucker = 2,6 g/l, freie SO_2 = 49 mg/l), ein Edelwein (Siebeldinger Mönchspfad, Äthanol nach der ADH-Methode = 11,65 Vol.%, Invertzucker = 1,7 g/l und freie SO_2 = 50 mg/l. Zucker- und Alkoholgehalt wurden an den Hybridenwein angeglichen. Zur Kontrolle diente unvergälltes Äthanol (12,79 Vol.%) und Wasser (Ergebnisse siehe Tab. I und II).

Daraus ist zu ersehen, dass der Allylalkoholschaden der Leber durch Äthanol, Edelwein und Hybridenwein bei gleichem Volumen und gleichem Alkoholgehalt in dieser Reihenfolge zunehmend verstärkt wird. Dieser Befund ist mit einer hohen Wahrscheinlichkeit statistisch gesichert. Der Versuch mit Äthanol allein bestätigt gleichzeitig die Ergebnisse von EGER[5].

Summary. Experiments on rats revealed that liver damage caused by allyl alcohol (measured by the rate of necrosis) is enhanced by alcoholic beverages such as ethanol and European or hybride wine. In this respect, wine seems to cause more damage than mere alcohol.

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Hygiene-Institut der Universität Mainz, 19. Oktober 1960.

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Tab. I. Der Einfluss alkoholischer Getränke auf den Allylalkoholschaden der Leber

Testlösung	*n*	Mittlere Nekroserate in %	s. d.
Allylalkohol allein	9	27,5	± 5,6
Äthanol	9	38,3	± 3,4
Edelwein	7	50,9	± 7,5
Hybridenwein	9	78,1	± 13,7

Tab. II. Statistische Vergleiche

Vergleich der Versuchsgruppen	*t*	*P*
Allylalkohol: Äthanol	5,070	< 0,001
Allylalkohol: Edelwein	7,228	< 0,001
Allylalkohol: Hybridenwein	10,257	< 0,001
Äthanol: Edelwein	4,628	< 0,01 (> 0,001)
Äthanol: Hybridenwein	8,487	< 0,001
Edelwein: Hybridenwein	4,666	< 0,01 (> 0,001)

The Blood Flow of the Cerebral Cortex Determined by Radioactive Krypton^{85}

A quantitative method for the determination of regional blood flow in the brain has been developed by KETY et al.[1]. The method was based upon measurement of the incorporation rate of an inert radioactive gas, and the technique required decapitation of the experimental animal. Hence only one measurement per tissue could be made in each animal. The method described below is a modification of the method of KETY et al.[1] in which tissue sampling has been replaced by external counting over the exposed surface of the brain, enabling multiple measurements of the blood flow in the same area of the cerebral cortex to be carried out.

The radioactive inert gas used was krypton^{85} (Kr^{85}) which emits practically only low energy β-radiation. Saline equilibrated with Kr^{85} was injected into one common carotid artery in a cat under light nembutal anesthesia. A gyrus of the parietal cortex of the same side was exposed by a small craniotomy and resection of the dura.

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