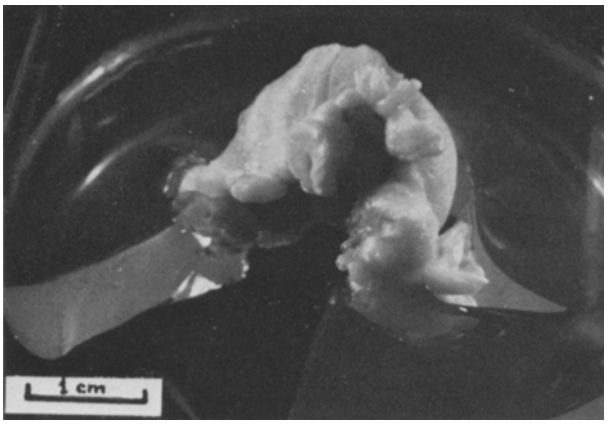

Fig. 2. A 51-day-old, in vitro grown ovary of hyacinth on the MS medium supplemented with 1 ppm of NAA and 10 ppm of BA: the formation of small bulblet-like formations from the ovary wall can be observed.

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Organ regeneration of *Alstroemeria* was attempted in various floral parts through tissue culture by Ziv et al.[6]. Callus or root regeneration was obtained from ovaries larger than 5 mm in diameter and branched sections of flower pedicels, but no bud differentiation occurred. All other parts of the inflorescence failed to differentiate. Best callus development was obtained on the cut surface of the ovary planted upright on White medium containing 2.0 ppm kinetin and NAA.

Our study has demonstrated the possibility of differentiation of bulblet-like formations and roots from the ovary wall of *Hyacinthus orientalis* L., and detailed studies of the regeneration processes of this species are in progress.

Zusammenfassung. Isolierte Fruchtknoten von *Hyacinthus orientalis* L. auf Murashige und Skoog-MS-Nährlösung kultiviert, zeigen nach Zugabe von Phytohormonen Ansätze zu Organdifferenzierungen.

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Inhibition of Growth and Respiration of Yeast (*Saccharomyces carlsbergensis*) by Tobacco Smoke Condensate and its Fraction

Extensive experimental work has been conducted in order to develop a quantifiable bioassay for the evaluation of cigarette smoke toxicity[1,2]. The various bioassay procedures that are being used at present involve laborious and expensive methods which at times need to be carried out for a considerable time period in order to obtain any meaningful information[3]. Since yeast cells are relatively easy to grow under various conditions, we have utilized these cells to determine the biological toxicity of the tobacco smoke condensate (TSC) and the water soluble (WS) fraction extracted therefrom. This report describes investigations conducted to determine the effect of (TSC) and its (WS) fraction on the aerobic growth and the respiration of isolated yeast mitochondrial particles.

Materials and methods. All studies were carried out with the yeast, *Saccharomyces carlsbergensis*, grown aerobically in a medium containing 0.2% $MgSO_4$, $7H_2O$, 0.6% $(NH_4)_3PO_4$, 0.5% yeast extract and the growth substrate (2% sodium lactate or 2% glucose), with the final pH adjusted to 5.0 with phosphoric acid.

The growth studies were conducted by growing the organism in 500 ml triple-baffled Bellco Erlenmeyer flasks containing 100 ml of the growth medium. The TSC or the WS fraction was incorporated into the medium to obtain the desired concentration. After 18 to 24 h of growth the cells were harvested by centrifugation, washed once with cold distilled water and the growth was recorded as dry weight.

Mitochondrial particles were prepared from yeast cells grown in lactate medium for 18 h with vigorous aeration. Harvested cells were washed, suspended in a medium containing 0.25 *M* mannitol, 0.02 *M Tris* HCl (pH 7.5), 0.0001 *M* EDTA, 0.2% BSA and broken in a Mini Colloid Mill (Gifford Wood Inc., Hudson. N.Y.). After removal of unbroken cells and cell debris at $1000 \times g$ (5 min), mitochondrial particles were obtained by centrifuging the supernatant at $20{,}000 \times g$ (10 min). The pellet thus obtained was washed once and constituted the mitochondrial preparation. Respiration of cells and the mitocondrial particles was measured polarographically with a Clark-type oxygen electrode and a YSI oxygen monitor (Yellow Springs, Ohio). The proteins were determined by biuret reaction.

The TSC and its WS fractions were prepared by the core services facility of the University of Kentucky Tobacco and Health Research Institute. The U.K. reference cigarettes, (1R1) were maintained at 60% relative humidity and 24 °C temperature. After conditioning for 24 h, the cigarettes were smoked at a rate of 35 ml puff volume for 2 sec/min. Whole smoke was condensed by freezing in glass traps maintained at −60 to −80 °C with dry ice acetone bath. The condensate so trapped was suspended in acetone by sonic vibrations. The WS fraction was extracted from TSC after drying in a water bath at 35 °C under 25″ Hg vacuum, and the resultant condensate was extracted with water and filtered to remove the water insoluble residue.

Results. Aerobic growth of yeast on lactate medium was inhibited by the TSC as well as the WS fractions (Table I).

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Table I. Effect of tobacco smoke condensate (TSC) and the water soluble (WS) fraction extracted therefrom on the aerobic yeast growth on lactate

Concentration	Inhibition (%) of yeast growth by	
(mg/ml of medium)	Tobacco smoke condensate	WS fraction
0.75	20.0	43.0
1.0	31.0	80.0
2.0	74.2	100.0

Table II. Effect of autocalving on the inhibitory properties of the WS fraction from tobacco smoke condensate

Concentration of WS fraction in growth medium (mg/ml)	Inhibition of yeast growth (%)
0.75 (autoclaved)	40.0
0.75 (non-autoclaved)	34.0
1.0 (autoclaved)	80.0
1.0 (non-autoclaved)	66.0

The inhibition increased with the increase in the concentration of the TSC or WS fraction in the growth medium. When lactate was replaced with glucose as the growth substrate in the medium, the growth of yeast remained relatively unaffected by WS fraction. Since nicotine, phenols, and cyanide are some of the important components present in the WS fraction; their individual effect on the aerobic growth of yeast was determined. Added nicotine to the growth medium did not affect the aerobic yeast growth. There was, however, a 15–20% inhibition of growth when the medium contained 0.1 mg of phenol per ml. Cyanide at 1.5 µg/ml of medium also caused a 15–20% inhibition of yeast growth on lactate.

Additions of WS fraction to the growth medium before autoclaving slightly increased its inhibitory property. Addition of the fraction to the cooled medium after autoclaving produced slightly less inhibition of growth (Table II).

Whole cell respiration of yeast initiated with glucose, lactate or ethanol was inhibited significantly by the WS fraction. A 20–75% decrease of the cellular respiration occurred in the presence of 50–200 µg of dryweight water soluble residue/ml of reaction mixture.

Studies with isolated respiratory particles from yeast also indicated a strong inhibition of O_2 uptake when NADH, succinate and ascorbate served as the oxidizable substrates. A progressive increase in the degree of inhibition occurred concomitant with the increase in the concentration of the WS fraction (Table III).

Discussion. Yeast cells are versatile organisms and are capable of growth under a variety of conditions. In presence of glucose, yeast derives its energy for growth via glycolytic pathway, as is evident from enhanced rate of fermentation[4] and higher aldolase activity[5] in glucose-grown cells. It is known that glucose inhibits the respiratory pathway[4] via repression of the associated oxidative enzymes[6–9]. In contrast, during its growth on a non-fermentable carbon and energy source, the respiratory enzymes are derepressed[5], and the energy requirements for growth are generated via respiration[4]. Our studies showing inhibition of aerobic yeast growth on lactate, a nonfermentable growth substrate, but not on glucose, would suggest an adverse affect of tobacco smoke condensate, especially the WS fraction, on respiratory metabolism. Certain of the suspected constituents of the WS fraction (e.g. nicotine, phenol) either did not affect yeast growth on lactate or produced slight inhibition, which could not account for the strong inhibition caused by the WS fraction. Thus, nicotine at as much as 25 times greater concentration than present in the WS fraction did not affect growth. The contribution of phenol is also doubtful since only 15–20% inhibition of growth occurred at 100 µg phenol/ml of medium. It may be pointed out that a concentration of the WS fraction (1 mg/ml) which produced almost 80% inhibition of aerobic yeast growth contributed only 5 µg of phenol and 15 µg of nicotine/ml growth medium. In addition, since both these components are relatively volatile, it would be expected that they are unstable upon autoclaving. However, an increase in the degree of inhibition after autoclaving indicated otherwise; such observations suggest the presence of other constituents in the WS fraction which are potent inhibitors of aerobic yeast growth.

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Table III. Inhibition of respiratory activity of the yeast mitochondrial particles by the WS fraction[a] from TSC

Substrate	Concentration of (WS) fraction (µg/ml RM)[b]	Respiration (nmole O_2/min/mg protein)	Inhibition (%)
NADH (2 m*M*)	0.0	59.0	0.0
	6.0	53.0	10.0
	15.0	30.0	49.0
	30.0	25.0	59.0
	45.0	14.0	78.0
Succinate (10 m*M*)	0.0	23.0	0.0
	6.0	21.0	10.0
	15.0	18.0	20.0
	30.0	16.0	30.0
	45.0	15.0	35.0
	60.0	10.0	55.0
Ascorbate[c] (10 m*M*)	0.0	21.0	0.0
	50.0	11.0	50.0
	100.0	10.0	50.0
	150.0	9.0	68.0

[a] WS fraction was used within a week of its preparation. [b] Reaction mixture (RM) contained 0.65 *M* mannitol, 0.1 m*M* EDTA, 0.1 *M* phosphate buffer (pH 6.5). [c] Ascorbate oxidation was recorded in the presence of added N.N.N.N.-tetramethyl-*p*-phenylene-diamine (TMPD) 10^{-5} *M*.

Inhibition of whole cell respiration by tobacco smoke fractions is in agreement with other reports[10,11]. Adverse effects of microgram quantities of tobacco smoke fractions on mitochondrial oxidases suggest an interaction of tobacco smoke constituents with the mitochondrial electron transport chain components as well as associated enzymes. Complexity of similar interactions of tobacco smoke with purified yeast alcohol dehydrogenase has been reported[12,13]. Recently, fractions have also been isolated from tobacco smoke condensate which cause enzymatic inhibition by binding the essential metals[14].

Our preliminary studies indicate that the WS fraction from tobacco smoke condensate inhibits yeast growth by interference with the respiratory metabolism of the cells. Since the tobacco smoke and its fractions have complex chemical composition, the mechanism of their inhibitory action can be better understood only when subfractionation and identification of the constituents have been achieved.

Zusammenfassung. Das aerobische Wachstum der *Saccharomyces carlsbergensis* wird bei Anwesenheit des wasserlöslichen Tabakrauchkondensates im Laktat Nährmedium stark gehemmt, obwohl eine Wachstumshemmung im Glukosemedium nicht stattfindet. Mikrogrammengen des Kondensatanteils hemmen die Atmung intakter Zellen sowie diejenige isolierter Mitochondrien der Hefe.

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Peripheral Inhibition in Sustained and Transient On-Center Ganglion Cells in Cat Retina

Over the past 8 years research from several laboratories[1–7] indicates that on-center retinal ganglion cells of the cat can be divided into 2 groups. The 2 groups have been referred to as X and Y cells[1,7], Type I and Type II cells[3,5], Group I and Group II[6,8,9] or as sustained and transient cells[2,4]. We shall adopt the language of Cleland, Dubin and Levick[2] and refer to these units as *transient* and *sustained* cells. The criteria that we use for categorizing these cells have been described in detail elsewhere[6,8,9].

The purpose of the present study was to assess interactions between the central (on) and peripheral (off) regions of the receptive fields of on-center transient and sustained cells. The study differs from other center-surround interaction investigations[6,10] in that it deals specifically with the temporal characteristics of the interactions.

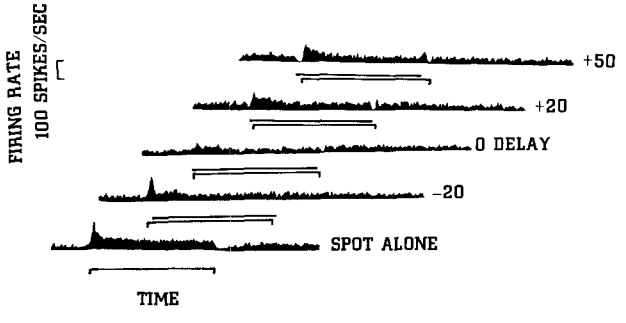

Fig. 1. Time series for typical sustained cell. Average response histograms are generated from 15 stimulus repetitions. Negative numbers indicate that the onset and termination of the center spot preceded that of the annulus; positive numbers signify that the annulus was presented first. All times are in msec. The lowest trace shows the response to the center spot presented alone. Background illumination is 0.3 candles/m^2. All targets are 1.2 log units above threshold.

Single cell recordings were made from 97 optic tract fibres of lightly anesthetized cats. Details of the recording system, optical system, and animal preparation can be obtained by referring to Winters, Hickey and Pollack[8]. The targets for the study were a 0.8° spot flashed in the receptive field center and a concentric 4.0° × 10.5° annulus. The temporal relation between these 2 targets was varied over a 150 msec range. Both the spot and annulus were square wave modulated (in time) with a duration of 1 sec and frequency of 0.3 cycles/sec. The targets were superimposed upon a diffusely lit tangent screen located 80 cm from the cat's eyes. Thresholds for the spot and annulus were determined separately and the intensity of each target was adjusted so that it was 1.2 log units above threshold.

Average response histograms for a typical sustained cell are shown in Figure 1. The lowest trace in the figure shows the response of the cell to 0.8° spot in the receptive field center. The other 4 traces give the responses to the same spot presented in conjunction with a 4.0° × 10.5°, concentric, annulus flashed in the receptive field periphery.

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