tissue as well as to tissue homogenate (Table). Moreover, insulin also potentiated the stimulatory effect of other lipolytic hormones (Figure 1). The stimulation was proportional to insulin concentration in the medium and was demonstrated even in the minutest doses of insulin (1 μU/ml).

This first evidence of lipolytic effect of insulin supposes in aortic tissue quite different properties of tissue receptor. The difference of insulin effect in adipose tissue[5] and in aortic tissue can consist either in different structure of enzyme molecule itself or in its different activation system or combination of both. To characterize hormone-sensitive lipase in aortic tissue we have used some substances which are known to inhibit hormone-sensitive lipase in rat adipose tissue[3], especially monoiodacetic acid and 2-propanol, in our experiments with rat aortic tissue. Figure 2 shows the marked difference of aortic tissue hormone-sensitive lipase: 2-propanol as well as monoiodacetic acid did not inhibit but activated the studied lipase. Inhibition with NaF differed this enzyme from lipoprotein lipase. It seems therefore that in aortic tissue we are dealing with different hormone-sensitive lipase isoenzyme. The mode of insulin action on this lipase in aortic tissue, however, cannot be evaluated from these experiments, and will be analyzed in another paper.

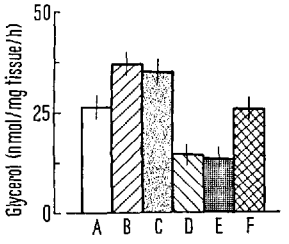

Fig. 2. Effect of inhibitors on rat aortic tissue lipase. Procedure B (homogenate, substrate tristearin). (A) control medium, (B) + monoiodacetic acid $10^{-4}M$, (C) + 2-propanol 1%, (D) + NaF $2 \times 10^{-2}M$, (E) + diethyl *p*-nitrophenyl phosphate $10^{-5}M$, (F) + eserine $10^{-5}M$.

From the theoretical point of view it is important that aortic tissue is sensitive to a number of hormones including insulin, although URRUTIA, BEAVAN and CAHILL[10] and MULCAHY and WINEGRAD[11] concluded that aorta is not sensitive to insulin as they found no potentiation of glucose uptake and metabolism by insulin. The stimulatory effect of insulin on aortic tissue lipase represents apparently another primary effect of insulin[12].

Zusammenfassung. In der Aortenwand von Ratten, Meerschweinchen, Kaninchen und Schweinen wird eine hormonempfindliche Lipase beschrieben, die in vitro durch Katecholamine, ACTH und Glucagon beeinflusst wird. Glucagonfreies Insulin wies eine deutliche lipolytische Wirkung auf und unterschied sich dadurch von der Wirkung im Fettgewebe.

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[12] The authors wish to acknowledge the skilful technical assistance of Miss ZDENA STARÁ.

Polyamines and Nucleic Acids in the Growing Yeast

Spermine and spermidine are widely distributed in animal tissues and their level seems to be particularly high in those organs where protein synthesis is more active[1]. It has also been observed that, at least in the chick embryo, the developmental pattern of nucleic acids is similar to that of polyamines: these appear at the beginning of the incubation and rapidly increase in the first period of differentiation, when the greatest morphological changes are observed[2].

This similarity suggested the existence of a correlation between polyamines and nucleic acids, and recent observations seem to confirm this possibility[3].

Polyamines are also present in a variety of microorganisms; for some of them, such as *H. parainfluenzae*, they represent an essential growth factor[4], for others (lactobacilli) they are factors of cellular stimulation[5].

The presence of spermine and spermidine has been demonstrated also in yeast. However, it is interesting to note that spermine can inhibit the growth of various types of yeast, when present at adequate concentrations in the culture media[6].

All these observations prompted us to study the level of natural polyamines and of nucleic acids in yeast, at various stages of cellular growth.

The experiments were performed with cells of *S. cerevisiae* (strain Fleishman ATCC 7754), cultured in liquid broth according to KLEIN[7], in a 30-l glass shake-fermentor. Air flow rate was 3 l/min.

Samples containing an identical number of cells were taken after different time periods, cooled at 0 °C, centrifuged at 18,000 rpm and lyophylized.

The dry powder was extracted with 0.1 *N* HCl and then with *N*-butanol. The butanol extract was dried and dissolved in 0.1 *N* HCl, and the polyamines were separated by electrophoresis, as described by RAINA[8], using a citric acid-sodium hydroxide buffer at pH 3.5. Spermine and spermidine were stained with ninhydrin and the optical density of eluates was measured at 505 nm.

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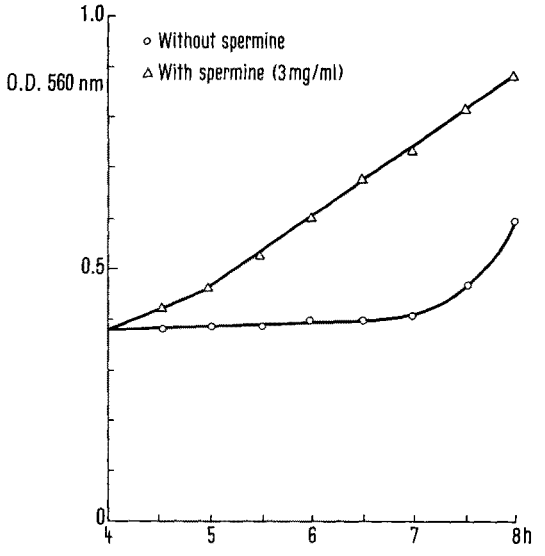
Nucleic acids were determined spectrophotometrically (reading at 260 nm) after extraction and separation according to the method of SCHNEIDER[9].

The results reported in the Table show that the level of both spermine and spermidine greatly increases during the lag time and then decreases in the first exponential period of cell growth. During all this period the level of spermidine is significantly higher than that of spermine. In the following stationary phase, the concentration of both polyamines tends to become constant and is very similar.

Polyamine and nucleic acid content in growing *S. cerevisiae*

	Time (h)						
	0	2	4	6	8	10	12
Spermine (γ/g)	389[a]	1171	281	350	398	700	841
Spermidine (γ/g)	628	1868	976	736	785	956	876
RNA (mg/g)	99.9	102.2	136.3	149.9	122.7	90.9	76.3
DNA (mg/g)	8.1	8.3	10.6	13.1	13.1	15.8	7.3

[a] The values are the means of 3 determinations.



Effect of the spermine on the growing yeast.

RNA increases rapidly in the first period of the exponential cell growth, that is when polyamines have already reached their maximum concentration, and reaches the highest level between the fourth and the sixth hour. The increase of DNA is slower and reaches its peak at the end of the cell growth period.

Parallel experiments in which the lage time of cell growth was increased by about 4 h by subsequent washings of yeast cells with distilled water, showed that the addition of spermine (3 mg/l) to the broth reduces significantly the lag time and stimulates cell growth (Figure).

The results reported indicate that the level of spermine and spermidine undergoes considerable changes during the vital cycle of yeast cells; the highest concentration is reached at the beginning of the logarithmic growth period. Moreover, when the lag time is experimentally prolonged, the presence of spermine in appropriate concentration determines an earlier appearance of the phase of cell multiplication; it is possible to suggest that, also in yeast, polyamines determine a stimulatory effect on the early phases of cell growth. The inhibitory effect observed by other authors might be due to the fact that the polyamine concentration in the culture media used was exceedingly high as compared to the physiological concentration of these compounds in yeast cells.

Riassunto. Sono state studiate le modificazioni del contenuto di spermina, spermidina ed acidi nucleici in cellule di *S. cerevisiae* durante le varie fasi di crescita. Inoltre è stato osservato un effetto di stimolo della moltiplicazione cellulare esercitato dalla spermina sul lievito in cui era stato sperimentalmente aumentato il tempo di latenza.

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Wetting of Fibrin Plate and Apparent Promotion of Fibrinolysis by Surfactin, a New Bacterial Peptidelipid Surfactant

During an investigation to search antifibrinolytic substances in microbial products using the fibrin plate method[1], a dramatic promotion of plasmin-catalyzed fibrinolysis was observed in the presence of the boiled culture filtrates of several strains of *Bacillus subtilis*.

The promoting agent present in the culture filtrate of *B. subtilis* IAM 1213 was crystallized and found further to be endowed with an ability to inhibit fibrin clot formation in the thrombin-fibrinogen system. Descriptions of its purification and characterization as well as determination of its inhibition site in fibrin clot formation have recently appeared in a communication from our laboratories[2]. The agent is a peptidelipid, with the molecular weight around 1050, composed of L-Asp, L-Glu, L-Val, L-Leu, D-Leu and a C_{15}-hydroxy *iso* acid (1:1:1:2:2:1) and from its strong surface active nature, exceeding that of sodium lauryl sulphate (SDS), it was named 'Surfactin'.

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