Die Entscheidung zwischen den Formeln VI und VII hat sich bisher noch nicht treffen lassen. Der oxydative Abbau der *des*-Base hat uns eine Reihe von Oxydationsprodukten geliefert, deren Konstitution aber noch nicht geklärt werden konnte; nur Metahemipinsäure konnte identifiziert werden. Es muß also vorläufig noch offen bleiben, ob Pavin der Konstitution VI oder VII entspricht. Hat es die Konstitution VI, so wäre das deshalb von besonderem Interesse, weil dann bei den Abbauprodukten des Pavins, die Derivate des 1,2,5,6-Dibenzo-cyclooctadien-(1,5)-Rings darstellen, neuartige Isomerieerscheinungen zu erwarten sind, die zum Teil den bei den isomeren Disalicyliden beobachteten[1] entsprechen könnten.

Mit dem Übergang zum Pavinskelett sind einige auffallende Änderungen im chemischen Verhalten verbunden. So geht z. B. das Jodmethylat der *des*-Base des Pavins schon beim Kochen mit Wasser bzw. Alkohol unter Abspaltung von Trimethylamin-jodhydrat in einen Alkohol bzw. Äther über, und erleidet keineswegs, wie man erwarten sollte, besonders leicht den HOFMANNschen Abbau. Auch die Bildung eines Neutralkörpers der Summenformel $C_{20}H_{22}O_5$ vom Schmelzpunkt 215–217° schon bei der ersten Stufe des HOFMANNschen Abbaus neben der Bildung der *des*-Base VIII bzw. IX gehört zu den überraschenden Reaktionen dieses Ringsystems, die durch weitere Untersuchungen geklärt werden müssen.

Durch die vorliegende Untersuchung ist der Mechanismus der Bildung des N-Methyl-pavins bei der Reduktion des Papaverin-jodmethylats mit Zinn und Salzsäure geklärt. Offenbar ist das erste Reduktionsprodukt das hierbei nicht faßbare N-Methyl-1,2-dihydro-papaverin (IV), das unter dem Einfluß der Salzsäure sich zum Teil zu dem nicht weiter reduzierbaren N-Methyl-pavin stabilisiert, zum größten Teil aber zu Laudanosin weiter reduziert wird. In analoger Weise muß bei der Reduktion des Papaverins das Pavin über das noch unbekannte 1,2-Dihydro-papaverin (IV; NH statt NMe) gebildet werden. Bei rasch verlaufender Reduktion mit Zinn, das mit 1,6% Antimon legiert ist, entsteht dabei praktisch kein Pavin, da die Weiterreduktion zu Tetrahydropapaverin schneller als die Stabilisierung zum Pavinsystem erfolgt. Bei langsamerer Reduktion mit reinem Zinn tritt dagegen Pavinbildung bis zu 35% der Theorie ein[2].

An der Durchführung der vorstehenden Arbeit waren Frl. M. BORMUTH, Frl. L. SCHOTTENHAMMER und Herr W. VOLLRATH beteiligt.

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Summary

A new way of formation of *N*-methyl-pavine from *N*-methyl-1,2-dihydro-papaverine is described. New structural formulæ for pavine are brought forward as well as the reaction mechanism is cleared up with regard to its formation by the reduction of papaverine with tin and hydrochloric acid.

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[2] Beobachtung von K. FALK.

Dependence, in Yeasts, of Phosphate Uptake and Polymerization upon the Occurrence of Glucose Polymerization[1]

Accumulation of phosphate by phosphate-deficient, non-dividing yeast cells metabolizing glucose has been shown[2] to result in the formation of metaphosphate in the cells. The necessity of a metabolizable substrate for phosphate uptake by yeast cells is well known[3] while inhibition of carbohydrate metabolism by cyanide inhibits both phosphate uptake and metaphosphate formation. The separation of glucose polymerization (aerobic assimilation) from glucose oxidation by the action of dinitrophenol and azide is well known[4]; the inhibitory action of DNP and azide on phosphate uptake is also known[5].

Promotion of aerobic assimilation of glucose by yeasts by addition of riboflavin was found to be accompanied by increased phosphate uptake[6]; the rate of glucose oxidation was not accelerated by the riboflavin addition.

The effects of NaN_3, DNP, and riboflavin on phosphate uptake and polymerization and on glucose consumption and polymerization have been studied concurrently on non-dividing, aerated suspensions of brewer's yeast, *Saccharomyces cerevisiæ* (Carlsberg strain No. 237). The techniques employed were: phosphate uptake (labeled P^{32}), phosphate polymerization (WIAME-BRACHET, toluidine blue, quantitative basophily), glucose consumption (colorimetric analysis), and aerobic assimilation (increase in dry weight of non-dividing cells in a non-nitrogenous medium). The cells were rendered phosphate-deficient by growing an initial inoculum of 4·7 g wet weight in 3 passages through WIAME's phosphate-discharging medium (a glucose, ammonium sulfate, succinate buffer without phosphate) during a period of 3 days; only 10% increase in wet weight occurred during the final passage, and the cells had a smaller percentage dry weight than those of the inoculum.

As shown in the Table, $5 \cdot 10^{-4}$ *M* NaN_3 and $5 \cdot 10^{-4}$ *M* DNP completely suppress phosphate uptake, phosphate polymerization (basophily), and glucose polymerization, but cause only slight decreases in glucose consumption (oxidation). The control phosphate-deficient cells respond to the addition of phosphate by a marked phosphate uptake and polymerization, and aerobic assimilation of glucose. The smaller inhibitions in basophily and glucose assimilation prompted by 10^{-4} *M* DNP are identical, within the accuracies of the methods. Addition of riboflavin (10^{-5} *M*) promoted increases in phosphate uptake, basophily, and dry weight; this concentration of riboflavin is about 100 times that obtainable if all the riboflavin naturally contained in the cells employed were to be released. At the concentrations studied, riboflavin did not antagonize the action of DNP and NaN_3. Before supplying phosphate to the control cells they have a very low basophily; iodine, and other glycogen stains show an almost complete absence of glycogen. Control cells maintained without phosphate during the test showed no increase in basophily or gain

[1] Presented, in part, before the Society for General Microbiology, London, March 24, 1948.

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[6] W. J. NICKERSON and L. J. MULLINS, Nature *161*, 939 (1948).

Effect of DNP, NaN_3, and riboflavin on phosphate uptake, phosphate polymerization, glucose consumption, and glucose assimilation by phosphate-deficient suspensions of *Saccharomyces cerevisiae*.

Total volume of 50 ml per vessel; suspensions agitated during 4-hours' experimental period.

Addition to WIAME Medium $+M/30$ KH_2PO_4	Phosphate polymerization (basophily) (initial extinction) = 0·64/mg)	Phosphate uptake (I^{32} activity)	Glucose assimilation (initial dry wt. yeast = 47 mg/vessel)	Glucose consumption (initial glucose = 106 mg/ml)
	extinction/mg dry wt.	% Standard	mg dry wt. yeast/vessel	mg/ml
None	2·00	247.	63.	13·8
DNP $5\cdot10^{-4}M$	0·64	0	−5.	10·6
DNP $10^{-4}M$	1·50	248.	55.	14·6
NaN_3 $5\cdot10^{-4}M$	0·64	4·8	7.	12·3
Riboflavin $10^{-5}M$	2·15	303.	70.	15·4
Riboflavin $2\cdot5\cdot10^{-6}M$	2·13	314.	70.	17·0
DNP $5\cdot10^{-4}M$ + Riboflavin $2\cdot5\cdot10^{-6}M$	0·57	10·2	−2.	6·0
NaN_3 $5\cdot10^{-4}M$ + Riboflavin $2\cdot5\cdot10^{-6}M$	0·71	7·2	−4.	13·1

in dry weight. The 235% gain in dry weight of the control cells supplied phosphate during the test contrasts sharply with the non-phosphate controls and with the actual loss in dry weight by the cells during their third passage through the phosphate-discharging medium. Thus the actual necessity of phosphate for glucose polymerization is clearly indicated.

The concurrent inhibition of phosphate uptake, phosphate polymerization, and glucose polymerization, without impairment of glucose oxidation, points to an interdependence of the processes inhibited, as already suggested[1]. In view of the participation of the CORI ester in glucose polymerization by yeasts, these results suggest that glycogen and polymerized phosphate may be formed simultaneously from glucose-1-phosphate *in vivo*. The *stimulation* of aerobic assimilation in yeasts by *external* phosphate has long been known[2] but difficult to reconcile with the limiting effect of phosphate on the glucose-1-phosphate $\rightleftarrows$ glycogen + phosphate equilibrium *in vitro*[3]. A coupling between the polymerization of glucose and of phosphate in yeasts is very likely in view of the facts: agents (metabolizable substrate, external phosphate, riboflavin) necessary for, or increasing, the one process, increase the other; agents (DNP, NaN_3) inhibiting the one inhibit the other; the occurrence of one has not been observed in the absence of the other; the ratio of the amounts of the two polymerization products is approximately one.

Polymerization of phosphate from glucose-1-phosphate would constitute an efficient mechanism to account for the accumulation of the ion by non-dividing metabolizing cells against a concentration gradient. In light of the probably very high molecular weight of polymetaphosphate in *Aspergillus niger*[4] and in yeast[5], the production of high energy phosphate bonds of metaphosphate from glucose-1-phosphate assumes thermodynamic possibility–through operation of a BERGMANN principle (polymer product insolubility) reaction[6], and simultaneously provides a way whereby the equilibrium, unfavorable with respect to glycogen *in vitro*, may be driven *in vivo* towards polymerization, as accomplished by the addition of Ba^{++} *in vitro*. Since assimilation of $^2/_3$ of the glucose supplied to yeasts is commonly observed, the cell must possess a mechanism more efficient than Ba^{++} for shifting the equilibrium: glucose-1-phosphate $\rightleftarrows$ glycogen + phosphate. It appears this mechanism may be:—

glucose-1-phosphate = glycogen + polymetaphosphate

Formation of polyhexametaphosphate would agree with the known 6-unit polymerization of glycogen.

The author wishes to thank Prof. Ø. WINGE for kindly extending the use of facilities at the Carlsberg Laboratorium, and to thank Prof. K. LINDERSTRØM-LANG for much valued discussion, especially of the BERGMANN principle reaction.

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Zusammenfassung

Es wird der Nachweis geführt, daß die Hemmung der Glukosepolymerisation (ohne Hemmung der Glukoseoxydation) durch Dinitrophenol und Natriumazid mit einer Hemmung der Phosphataufnahme und -polymerisation parallel geht. Bei Verwendung von phosphatarmen Hefezellen ist die Ingangsetzung der Glukosepolymerisation (nicht der Glukoseoxydation) von der Gegenwart von Phosphat im Nährmedium abhängig. Gleichzeitige Vermehrung von Phosphataufnahme, Phosphatpolymerisation und Glukosepolymerisation konnte durch Zugabe von Riboflavin zu Suspensionen von Hefe, die unter Mangelbedingungen gezüchtet war, erreicht werden. Es wird die Auffassung vertreten, daß Hefezellen, die Glukose in Gegenwart von Phosphat umsetzen, Glykogen (=polymerisierte Glukose) und Metaphosphat (polymerisierte Form) gleichzeitig aus Glukose-I-Phosphat bilden. Für diese Annahme sprechen thermodynamische Überlegungen über den Polymerisationszustand von Metaphosphat.

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