red was added (up to 100 µg/ml) and the cultures reincubated for 30 min. The medium was then discarded and the monolayers, after 5 washings with phenol red free Hank's BSS, were solubilized with 8 ml of natrium deoxycholate (2% in H_2O, pH 7.3). The neutral red incorporated was measured on the basis of percent transmission at 530 nm.

Results. Data of the Table show that the incubation of poliovirus infected cells at 41.5 °C causes a complete but reversible inhibition of virus growth. At lower temperatures only a partial inhibition is observed, while at higher ones virus recovery is not complete.

Figure 1 shows that the incorporation of H^3 Uridine in infected cells is very low, both at 37 °C and at 41.5 °C within $2^1/_2$ h after infection; thereafter a sharp increase is observed at 37 °C (up to a maximum at the 5th h) while no further increase can be detected at 41.5 °C.

Viral protein synthesis is, on the contrary, scarcely influenced by supraoptimal temperatures, at least in the early stages of infection (Figure 2). Both at 37 °C and 41.5 °C a significant amount of H^3 Leucine is incorporated in viral antigens within the first $3^1/_2$ h; only later at 41.5 °C the uptake ratio declines, to stop completely $4^1/_2$ h after infection.

The possibility of a continuous breakage of viral RNA at 41.5 °C has been investigated. Data of Figure 3 show that at 41.5 °C no H^3 Uridine is incorporated later than $2^1/_2$ h after infection and that the small amount of H^3 Uridine incorporated in acid-insoluble form within $2^1/_2$ h after infection is firmly retained for at least 2 more h, that is up to the first appearance of the cytopathic effect.

Data of Figure 4 finally demonstrate that both the large amount of viral RNA synthesized at 37 °C and the scarce quantity made at 41.5 °C within 3 h from the infection are mostly represented by RNAase sensitive (monohelix?) molecules; they therefore cannot be considered as a residual of an intracellular breakage by RNAases.

Discussion and conclusion. The results reported here can be summarized as follows: 1. At certain supraoptimal temperatures (41.5 °C) the incorporation of labelled precursors into poliovirus RNA is strongly reduced. This is not due to a continuous digestion of neosynthesized RNA molecules by nucleases but, rather, to an inhibition by supraoptimal temperatures of the de novo synthesis of viral RNA. 2. Under the same conditions the overall synthesis of poliovirus proteins is scarcely influenced, at least for $3^1/_2$ h after infection. It can therefore be assumed that most of the viral proteins made at 41.5 °C (including those responsible for metabolic blockade and cytopathic effect) have been built up in polyribosomes organized by the infecting virus RNAs. This gives the opportunity to use supraoptimal temperatures for studying the direct action of antiviral compounds on the synthesis of poliovirus proteins, ruling out a possible influence of the viral RNA replication.

Riassunto. La scarsa incorporazione di precursori dello ARN in cellule infette da poliovirus ed incubate a 41,5 °C è dovuta ad una inibizione della sintesi dello ARN virale più che non ad una sua digestione ad opera di nucleasi.

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[13] P. G. W. Plagemann and H. E. Swim, Bact. Rev. *30*, 288 (1966).

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Caffeine Removal from Growth Media by Microorganisms

The protean manifestations of the biological effects of caffeine (and other methylated xanthines) is exemplified by its toxicity to microorganisms[1], plants[2] and animals[3]; its roles as mutagen[4] and enzyme inducer[5]; its inhibition of cyclic AMP hydrolases[6] and the dimer excizing enzyme in repair of DNA[7]; and its ability to release Ca^{++} from the sarcoplasmic reticulum[8]. Its most well-known and pleasant attribute, the mild stimulation occasioned by intake of beverages, is not universally enjoyed. Thus, 10% of the coffee consumed in the United States and Europe is decaffeinated[9].

We have considered biological alternatives to the present use of chlorinated hydrocarbons for extraction of caffeine from coffee. There exists a paucity of information in this regard. *Fusarium oxysporum* apparently resists the toxicity of and grows, albeit poorly, on caffeine[10]. *Pseudomonas aeruginosa* appears to possess xanthine dehydrogenase and uricase of rather broad specificity capable of degrading methyl xanthines to methyl allantoins[11]. In the present communication we show that caffeine can be utilized as sole source of either carbon or nitrogen for growth of a bacterium, *Bacillus coagulans*, and two fungi, *Penicillium roquefort* and a *Stemphylium* species.

In addition to these three species, other colonies grew on our agar plates, some of which were identified as actinomycetes. However, prolonged exposure of the isolation medium was necessary to cause the appearance of relatively few colonies. Furthermore, neither the purine-utilizing *Sordaria brevicollis*[12] nor an authentic culture of *B. coagulans* (NCA 43P) grew in caffeine at the levels used in this investigation. Thus, although not restricted to a narrow group of microorganisms, the ability to utilize caffeine is not ubiquitous.

[1] N. Fries and B. Kihlman, Nature, Lond. *162*, 573 (1948).

[2] R. H. Kurtzman jr., A. C. Hildebrandt, R. H. Burris and A. J. Riker, Virology *10*, 432 (1960).

[3] C. F. Poe and C. C. Johnson, Acta pharmac. tox. *9*, 267 (1953).

[4] M. Demerec, Am. Naturalist *85*, 119 (1957).

[5] H. Nishikawa and I. Shiio, J. Biochem., Tokyo *65*, 523 (1969).

[6] J. M. Streeto and W. J. Reddy, Analyt. Biochem. *21*, 416 (1967). – R. Alverez jr., Pl. Physiol., Suppl. *44*, 36 (1969). – R. L. Singhal, R. Vijayvargiya and G. M. Ling, Science *168*, 261 (1970).

[7] A. S. Sideropoulos and D. M. Shankel, J. Bact. *96*, 198 (1968).

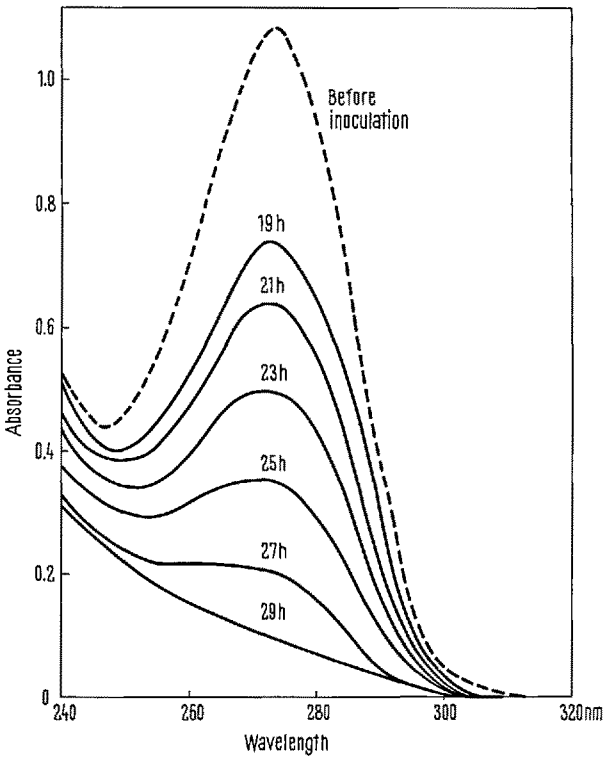
[8] A. Weber, J. gen. Physiol. *52*, 760 (1968).

[9] M. Sivetz, *Coffee Processing Technology* (Avi Publishing Co., Westport, Conn. 1963), vol. 2.

[10] F. T. Wolf, Bull. Torrey bot. Club *82*, 342 (1955).

[11] W. Franke and G. E. Hahn, Z. physiol. Chem. *301*, 90 (1955).

[12] A. H. Roush and J. W. Snyder, (Abstr.) XI International Botanical Congress (Seattle 1969), p. 184.



Removal of caffeine by *Stemphylium* from culture medium. Fungal inoculum, ca. 100 mg dry weight, was incubated in the dark with shaking at 20 °C in 50 ml of medium containing 0.001 *M* caffeine, 0.088 *M* sucrose, 0.024 *M* K_2HPO_4 and Hoagland's salts (Cl^- replacing NO_3^-). One ml aliquots of medium were withdrawn for UV-spectroscopy using a 1 mm light path. The number over each curve denotes h after inoculation. Spectra from 220 to 240 nm, run but not shown, also exhibited progressive temporal diminution of absorbance values at all wavelengths and hence no buildup of UV-absorbing intermediates.

Table I. Growth of fungi on caffeine

	Dry weight mg/50 ml	
Nitrogen source[a]	*Stemphylium*[b]	*P. roqueforti*[c]
Nitrate, 0.03 *M*	446	459
Caffeine, 0.01 *M*	449	397
Caffeine, 0.02 *M*	311	370
Caffeine, 0.04 *M*	217	279
Caffeine, 0.10 *M*	0	0

[a] Hoagland's salts in 0.088 *M* sucrose with indicated concentrations of nitrogen source in 250-ml Erlenmeyer flasks (shake rate = 200 × g) were used for growth of isolates at 20 °C in the dark for 10 days. Each flask was inoculated with ca. 25 mg (dry weight) of the isolate. [b] *Stemphylium* was isolated from air on agar containing Czapek's medium in 0.01 *M* caffeine. [c] *P. roqueforti* was isolated from air on agar containing Hoagland's salts (Cl^- replacing NO_3^-) in 0.01 *M* caffeine and 0.08 *M* sucrose.

Table II. Rate of purine removal from fungal culture media

	Loss of purine, μmoles/h/50 ml[a]	
Purine	*Stemphylium*	*P. roqueforti*
Xanthine	24.3	15.5
Caffeine	11.9	13.3
Theobromine	13.1	13.7

[a] Fungal inocula of 25–75 mg dry weight were incubated in the dark with shaking at 20 °C in 50 ml media containing 0.01 *M* purine in 0.088 *M* sucrose and Hoagland's salts (Cl^- replacing NO_3^-). After 24 h aliquots of media were removed every 2 h and the absorbance at 273 nm (caffeine and theobromine, Aldrich Chemical Co.) and at 267 nm (xanthine, Schwarz Bioresearch, Inc.) were determined. Purine losses were linear over 6 h. Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

Table I illustrates the growth of the fungi in culture media containing caffeine as sole source of nitrogen. *Stemphylium* grew as well in 0.01 *M* caffeine as in 0.03 *M* nitrate. The survival observed here is in contrast to the toxicity of caffeine at this level to *Ceratocystis* (*Ophiostoma*)[1] and the growth of *Stemphylium* (449 mg) is much greater than the borderline growth (25 mg) of *Fusarium* under comparable conditions[10].

Disappearance of caffeine from a culture medium of actively growing *Stemphylium* is illustrated in the Figure. The spectra reveal no evidence of accumulation of UV absorbing catabolites. The data of Table II show that methyl xanthines are as efficaciously consumed by *P. roqueforti* as is xanthine. The caffeine concentration is about 5 times the concentration present in beverages[9]. We detected neither caffeine nor UV-absorbing caffeine catabolites in homogenates of washed fungi. Such complete degradation is consonant with requirement of nitrogen for growth and provides a mechanism for protection from genetic damage.

B. coagulans was isolated from soil on an agar containing 0.01 *M* caffeine in Czapek's medium. Although subsequent cultures of the isolate removed caffeine readily (23 μmoles/h), we found growth to be rather sluggish and variable. Growth requirements appear to be rather fastidious although we have maintained viable cultures for at least 7 months. Furthermore, the size of the bacterium in the isolates was smaller than the NCA 43P strain bacterium grown routinely in this laboratory. This latter observation, along with the extended exposure time previously mentioned suggest a degree of selective adaptation[13].

Zusammenfassung. *Bacillus coagulans* sowie 2 Pilze, *Penicillium roqueforti* und eine *Stemphylium*-Spezies wurden auf Nährböden isoliert, welche Kaffein als einzige Quelle für Kohlenstoff und Stickstoff enthalten. Diese Mikroorganismen vermehren sich mit derselben Geschwindigkeit auf Kaffein- wie auf Nitratmedien. Dieser Kaffein-Katabolismus wäre möglicherweise der Weg, um die Giftigkeit und mutagenen Wirkungen von Kaffein zu verhindern.

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[13] We thank Dr. A. H. ROUSH for a culture of *Sordaria brevicollis*.