was concentrated tenfold in vacuo was subsequently chromatographed on paper Whatman No. 1.

The author has devised a new solvent system which accomplishes remarkable resolution of fusaric acid (Rf 0.31) using the multiple sectored circular chromatograms (Figure), in contrast to the descending chromatographic technique of PAGE[9] which yields a poor resolution (Rf 0.2). Ethanol:Water (7:3) was used as a solvent. After triple runs, the chromatograms (in duplicate) were dried at room temperature for 24 h. One of the chromatograms was sprayed with 2,7-dichlorofluorescein. In UV-light, the substituted pyrimidines were located as dark absorbing areas against a green fluorescent background[10], thus indicating their presence in days. The other chromatogram was sprayed with 0.1% solution of rubeanic acid in acetone to identify the olive grey spots of copper-fusaric acid complex. To facilitate identification, an authentic sample of fusaric acid was procured and the close agreement of Rf values was the conclusive evidence of its production in vitro. The results are summarized in the Table.

It is apparent from the data that: (1) the uptake of substituted pyrimidines by *F. lycopersici* from irradiated solutions was faster as compared to their non-irradiated counterparts; and (2) the production of fusaric acid was encountered on the solutions supplemented with 5-bromouracil, the response being notable on irradiated solutions.

It has been noted by MANTIONE and PULLMAN[11] that the photoreactivity of a pyrimidine depends on the distribution of the lone electron of the triplet state radical in the pyrimidine ring. They calculated that in the photoreactive pyrimidines the distribution of the odd electron is clustered in the reactive 5 or 6 position. The behaviour of irradiated pyrimidines in the present investigation is in perfect accord with the calculations of these authors.

Concerning the impact of 5-halogenopyrimidines, the author is tempted to hazard the explanation that in this series of substituted pyrimidines, 2 effects counteract each other, viz. the electronegativity and the size of the substituent. In chlorouracil, bromouracil and bromouridine, the steric effect will be a pronounced one and so will the electronegativity of the substituent. Chlorine and bromine (radii = 0.99 and 1.14 Å respectively) will occupy a volume comparable to that of a methyl group (radius = 1.10 Å) in methyluracil[12]. Nonetheless, the difference in the quantum yields of halogenopyrimidines and methyluracil is a notable feature. However, the strong electronegativity of the 2 halogens (3.0 and 2.8 respectively) will give rise to polarization of the reactive 5,6-double bond.

5-Nitrouracil is an interesting compound for various reasons[13]. Although the nitro group is considerably larger than chlorine or bromine, nitrouracil behaves similarly to these compounds in its physical attributes. It is pertinent to emphasize that there was no cleavage of substituents in any of the pyrimidines after UV-irradiation, as solitary dark absorbing areas against a green fluorescent background were located in every case.

Since 5-bromouracil readily replaces thymine in DNA[5,6], an environmentally programmed genetic aberration in the proliferating inoculum of *F. lycopersici* may account for the production of fusaric acid in vitro[15].

Zusammenfassung. Die Bildung von Fusarinsäure durch *Fusarium oxysporum* f. *lycopersici* wird durch die Anwesenheit von 5-Bromuracil in der Kulturlösung unter gleichzeitiger Belichtung gefördert.

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Urease. VIII. Its Interaction with Sodium Dodecyl Sulfate[1]

Determinations of the molecular weight of urease in phosphate buffer of pH 7 (concentration 0.01–1%) have indicated a value of about 480,000[2,3], but there is considerable evidence that units of this weight may undergo either dissociation or association, depending on the conditions[3–6]. Ultracentrifugal analysis of urease dissolved in 6*M* guanidine hydrochloride indicated a mol. wt. of 83,000[7]. This note reports on the interaction of urease with sodium dodecyl sulfate (SDS); this reagent also causes dissociation, the rate and extent of which depend on the absolute concentration of the reagents and quite critically on their ratio. With a sufficient proportion

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of SDS, a subunit is obtained which has a mol. wt. of 50,000–60,000; the dissociation is accompanied by an irreversible loss of activity.

Urease was prepared as previously described[8]. It was assayed by the acidimetric modification of Sumner's method; the specific activity was 2140 U_s^{20}/mg (U_s^{20} denote the units defined as recommended by the International Union of Biochemistry, determined at 20°; 2140 $U_s^{20} \doteqdot 150$ Sumner units[9]). The urease solutions studied in the ultracentrifuge contained about 5 mg of enzyme per ml; the medium was $0.02M$ phosphate containing $10^{-3}M$ ethylenedinitrilotetraacetate, and SDS as needed. The SDS was a specially prepared sample, containing > 98% C_{12}[10].

Figure 1a shows the ultracentrifugal pattern given by native urease; the sedimentation coefficient $s_{20,w}$ was 18 (this and all subsequent values are in Svedbergs). Adding SDS in the proportion 4.5:1 (by weight) resulted in the complete conversion to a product of $s_{20,w} \sim 2$; Figure 1b shows the pattern 6 h after mixing; even after 1 h most of the s-18 peak had disappeared. Very different results were obtained when the SDS/urease ratio was reduced to 0.5; the pattern obtained after 6 h is shown in Figure 2c, i.e. 2 peaks with $s_{20,w}$ 3.3 and 17, respectively. This pattern remained substantially unchanged on longer standing, for as long as 36 h. Figure 2a, b shows the behavior at an intermediate SDS/urease ratio, 1.6; complete conversion to an s-2.7 product occurred, but slowly. Measurements of the activity showed a decrease that approximately parallelled the decrease in the peak of s-17–18; at ratio 4.5, the activity was 30% after 1 h, 3% after 6 h; at ratio 1.6, 70% after 1 h, 22% after 6 h, ~ 0 after 36 h; and at ratio 0.5, 70% after 6 h, 50% after 36 h. Approximately the same activity was found whether or not SDS was present in the buffer used to dilute the urease-SDS stock solution to the assay concentration, i.e. the loss of activity was not reversed by decreasing the SDS concentration.

As may be seen in Figure 2c, d, the ratio 1.2 was sufficient to give almost complete conversion in 36 h. The activity and the viscosity of the solution also were measured at intervals, with the results shown in Figure 3. Qualitatively, it is clear that the gradual conversion of urease from s-18 to s-3 is accompanied by a corresponding decrease in activity and increase in viscosity. The latter attained a constant value in 30–40 h; after 48 h, the solution was diluted and measurements made to determine $[\eta]$. The increase in viscosity indicates change to a more asymmetric molecular shape, which would cause a decrease in s; the observed decrease, however, is much larger than can reasonably be accounted for on this basis, and it must therefore be concluded that dissociation took place.

Molecular weights may be estimated from sedimentation and viscosity data by means of the expression proposed by SCHERAGA and MANDELKERN[11]:

$$M = [s_0 N \eta_0/\beta (1 - \varrho \bar{v})]^{3/2} [\eta]^{1/2}.$$

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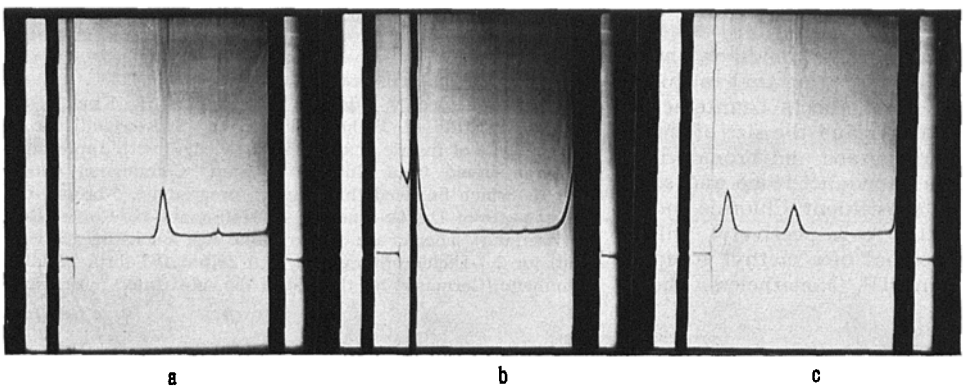

Fig. 1. Ultracentrifugal patterns: (a) native urease; (b) SDS/urease ratio 4.5, 6 h after mixing; (c) ratio 0.5, after 6 h. Speed 59,780, 75° schlieren angle, (a) and (c) taken 20 min after attaining speed, (b) after 40 min.

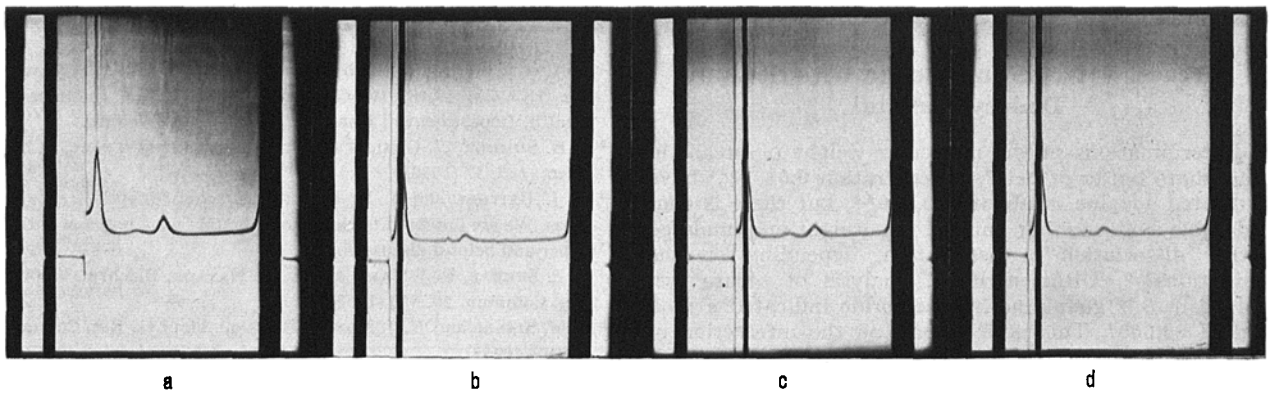

Fig. 2. Ultracentrifugal patterns, 59,780 rpm, 75° angle. SDS/urease ratio 1.6: (a) 1 h after mixing; (b) after 6 h. Ratio 1.2: (c) after 6 h; (d) after 36 h. All pictures taken 20 min after attaining speed.

This equation was applied to native urease, for which the following values were found: $s_0 = 18.6$; $[\eta] = 0.0405$ dl/g; $\bar{v}$ was taken as 0.73[2,3], β as $2.2 \cdot 10^6$[11]. The result is 520,000, within 10% of the value generally accepted.

In order to apply the equations to the urease subunits, one should know how much SDS is bound to the urease. Unfortunately, it is difficult to determine this quantity, especially in this case because the interaction apparently develops gradually. On the other hand, the final result does not depend critically on the amount of SDS bound, owing to compensating effects. Two calculations have been made, which we believe should give the lower and upper limits of the subunit weight, respectively. In one calculation, it is assumed that at ratio 1.2 all the SDS is bound to the urease, thus forming a complex that would contain 55% SDS by weight. The data were: $s_0 = 3.8$; $\eta_0 = 1.01$; $[\eta] = 0.100$; $\bar{v}$ was taken as 0.80, based on the value 0.86 for SDS[12], $\beta = 2.3 \cdot 10^6$. The results are $M = 112{,}000$, the subunit weight 50,000. In the other calculation, it is assumed that only half as much SDS is bound, i.e. the complex contains 37% SDS by weight; this is considered the lower limit since as a matter of fact the ratio 0.5:1 was quite insufficient to affect complete dissociation. The data are: $s_0 = 3.6$, $\eta_0 = 1.05$; $[\eta] = 0.135$; $\bar{v} = 0.78$; $M = 97{,}000$, subunit weight = 60,500.

Although these results are subject to considerable uncertainty, they clearly indicate that SDS causes dissociation of the 480,000 mol. wt. unit to considerably smaller subunits. In view of all the pertinent evidence[13], we presently favor the hypothesis that urease contains 8 subunits of mol. wt. 60,000. There is a definite possibility, however, that the subunits may be smaller; furthermore, it has not as yet been shown whether the units are identical.

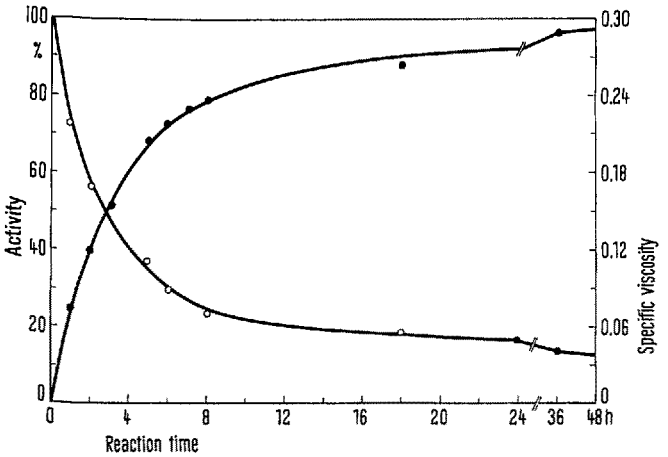

Fig. 3. SDS/urease ratio 1.2. Left ordinate, empty circles: activity, % of control containing no SDS; right ordinate, full circles: specific viscosity.

Riassunto. Il dodecilsolfato di sodio causa la dissociazione dell'urease (massa molecolare 480,000) in una maniera che dipende fortemente sulla concentrazione relativa dei reagenti. Il prodotto ottenuto con un sufficiente eccesso di detergente ha una massa molecolare di 50,000–60,000.

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Feinstruktur von Pilzzellen. 1. Septen bei *Penicillium brevi-compactum*

Im Gegensatz zu anderen Mikroorganismengruppen ist über die Zellfeinstruktur der Pilze noch verhältnismässig wenig bekannt. Verantwortlich hierfür dürften die Schwierigkeiten sein, das Material zu fixieren.

Im Rahmen einer taxonomischen Arbeit über Penicillien[1] wurden die Querwände von *P. brevi-compactum* 151, *P. cyclopium* 114 und *P. nigricans* 80 näher untersucht. Ausgewählte Stämme einer grösseren Sammlung, die unter besonderen Bedingungen dazu neigen, koremienartige Konidiophorenbüschel zu bilden, wurden auf Cellophanfolie (Kalle), die direkt einem Kartoffel-Glucose-Agar auflag, bei Zimmertemperatur kultiviert. Der Bewuchs liess sich mit der Folie leicht abheben und in $n/10$ $KMnO_4$-Lösung mit etwas Detergens überführen. Nach 30–60 min wurden die Objekte in der Alkoholreihe entwässert, in der üblichen Weise in Acrylharz eingebettet, mit einem Ultramikrotom geschnitten und mit dem AEG-Zeiss Elektronenmikroskop EM 8 betrachtet und photographiert.

Der von R. T. Moore[2] beschriebene Aufbau des Ascomycetenseptums ist bei den untersuchten imperfekten Penicillien nur zum Teil bestätigt worden. Ein an den Trennwänden zwischen Phialiden und Metulae gelegentlich beobachteter zentraler Pfropf aus elektronendichtem Material entspricht der Beschreibung von Moore und McAlear[3] an ascogenen Hyphen bei *Dasyscyphus* und *Mollisia*. Die Figuren 1–3 zeigen einen Serienschnitt durch diese Region. Aus der stärker als die beiden anderen vergrösserten Figur 3 geht hervor, dass der median geschnittene Pfropf in beide Zellumina hineinragt und nicht vom endoplasmatischen Reticulum umkleidet wird. Bei Querwänden vegetativer Hyphen, Konidiophoren, Rami oder Metulae fehlten solche «plugs» immer; die Septen hatten stets etwa die gleiche Stärke und Struktur wie die Aussenwände der jeweiligen angrenzenden Zellen (1100–1730 Å). Ein Auskeilen der Querwände nach der

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