gegeben. Es zeigt sich, dass die beiden Verfahren nur *beschränkt vergleichbare Ergebnisse erzielen.* So waren 7 der 19 im Ouchterlony-Test positiven Seren in der Haemagglutination negativ. Umgekehrt liessen sich bei 68 der im Ouchterlony-Test negativen Seren mittels der Haemagglutinationsreaktion Antikörper nachweisen. 10 dieser 68 in der Haemagglutination positiven Seren wurden mit «Carbowax» (Polyaethylen-Glycol) 3–10fach konzentriert. Aber auch in den konzentrierten Seren waren mit dem Ouchterlony-Test keine praezipitierenden Antikörper nachweisbar. Wodurch der verschiedene Ausfall der beiden Reaktionen bedingt wird, ist bis jetzt unbekannt. Es wäre denkbar, dass die durch die beiden Reaktionen erfassten Antikörper nicht gänzlich identisch sind, oder dass Hemmstoffe unbekannter Art interferierend bei der Antigen-Antikörperreaktion wirken.

Der Einfluss des Lebensalters. Wie Tabelle II zeigt, stammen die meisten der im Ouchterlony-Test *positiven Seren* von Kindern des *1. und 2. Lebensjahres.* Nur einmal konnten von 78 untersuchten älteren Kindern und Erwachsenen caseinpraezipitierende Antikörper gefunden werden. Der Unterschied zwischen den beiden Altersgruppen hinsichtlich des Auftretens von Casein-Antikörpern ist auffällig. Die Differenz lässt sich jedoch nicht statistisch berechnen, da bei der Gruppe der 1- bis 2jährigen eine Auslese vorliegt. Fanden sich doch bei den untersuchten Kindern vornehmlich Probanden mit Allergieverdacht, während die Wahl der Erwachsenen zufällig erfolgte.

Tab. II. Der Einfluss des Lebensalters auf die Nachweisbarkeit von Casein-Antikörpern

Alter	positiv im Ouchterlony-Test	negativ
1. und 2. Lebensjahr	11 Kinder (12,5%)	77 Kinder (87,5%)
Kinder vom 3. Lebensjahr an und Erwachsene	1 Kind (1,26%)	78 Kinder (98,74%) und Erwachsene

Diese Untersuchungen decken sich mit denen von LIPPARD, SCHLOSS und JOHNSON[7], die nach der Verabreichung von Milch mit der Komplementbindungsreaktion fast bei allen Probanden zwischen dem 1. und 15. Lebensmonat Antikörper gegen *Kuhmilch* fanden, während zwischen dem 2. und 5. Jahr nur gelegentlich Antikörper nachzuweisen waren. Die grosse Ausbeute an positiven Resultaten bei LIPPARD, SCHLOSS und JOHNSON ist wahrscheinlich dadurch bedingt, dass die genannten Autoren auf Antikörper gegen die *gesamte Kuhmilch* prüften, während wir bei der Untersuchung auf Antikörper gegen Casein nur einen *Teil des Kuhmilch-Antigens* erfassten.

Summary. It is possible to demonstrate antibodies against casein in the serum by the Ouchterlony technique. It is a simple technique and needs only a small amount of serum. In a few cases we could demonstrate antibodies against casein in sera, in which the hemagglutination-technique did not show positive results. In some cases, however, sera which were positive in hemagglutination-tests gave negative results by the Ouchterlony technique. The possibility is discussed that by the two techniques different kinds of casein-antibodies can be detected.

E. BERGER, A. BÜRGIN-WOLFF und M. JUST[8]

Serologisches Laboratorium des Universitäts-Kinderspitals, Basel (Schweiz), 16. Oktober 1961.

[7] V. W. LIPPARD, O. M. SCHLOSS und P. A. JOHNSON, Amer. J. Dis. Child. *51*, 563 (1936).

[8] Für die Durchführung der serologischen Untersuchungen sind wir Fräulein CHRISTINE DYBALLA und Fräulein IRENE v. SCHWEINICHEN zu grossem Dank verpflichtet.

Size of Inoculum and Growth Kinetics of Moulds

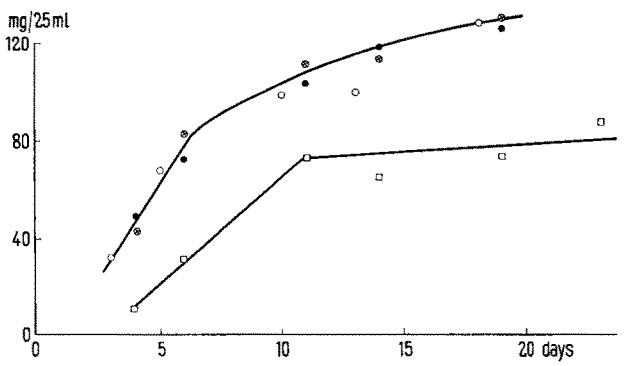
Growth rate and maximum yield of mycelium of various strains of *Aspergillus* proved to be markedly influenced by the size of the inoculum when grown in substrates poor in trace elements. Using *Aspergillus oryzae* in stationary cultures, linear growth rates of 2.52 and 0.45 mg/100 ml/h were obtained in a synthetic substrate (glucose 10 g, $(NH_4)_2SO_4$, 1 g; $MgSO_4 \cdot 7\,H_2O$, 0.3 g; KH_2PO_4, 4.54 g; Na_2HPO_4, 4.73 g; $FeCL_3 \cdot 6\,H_2O$, 15 mg; H_2O, 1 l) when 4.10^7 and 4.10^3 washed conidia respectively were inoculated per 100 ml of substrate. Under the same conditions except for the addition of the following trace elements, $CaCl_2 \cdot 6\,H_2O$ 1 mg; $ZnSO_4 \cdot 7\,H_2O$ 0.5 mg; $CuSO_4 \cdot 5\,H_2O$ 0.05 mg; $MnSO_4 \cdot 4\,H_2O$ 0.05 mg; H_2O 1 l; the corresponding values were 6.68 and 3.12 mg/100 ml/h, thus indicating a much smaller relative difference. When certain other substrates with added trace elements were used, such as increasing the concentration of phosphates, the difference in rate of growth disappeared when the sugar concentration was relatively small. The maximum yields of mycelium obtained in the previously mentioned substrates were 260 and 180 mg/100 ml for the large and the small inoculum respectively when no trace elements were added, whereas the maximum yield was not significantly influenced by the size of the inoculum in the presence of added trace elements, since yields of 330 and 325 mg/100 ml were obtained respectively. Similar effects could be observed with mycelium as inoculum.

A reversal of the above mentioned results was noted in experiments using the substrate poor in trace elements and inoculated with mycelium or conidia to give 4 to 20 viable units per 100 ml, i.e., growth rates and maximum yields increase over those observed with cultures inoculated with much larger inocula.

Experiments have been carried out in order to account for the above phenomena. The assimilating activity of mycelium has been tested after various stages of cultivation, since the age of the culture may have a relation to the effects caused by the size of the inoculum, and cultures originating from inocula of differing sizes have a different age at a certain stage of mycelial development. Dispersed mycelium has been produced under submerged conditions in a substrate without added trace elements which had been inoculated with 4.10^6 conidia per 100 ml. Samples were taken in the linear phase of growth (after 2 and 3 days), at the stage of maximum mycelial development (4 days), and in the phase of autolysis (6 days). The mycelial content was 164, 240, 362 and 268 mg/100 ml at the corresponding days of sampling. The mycelium of these samples was washed and inoculated into fresh medium (of the same composition as has been used for producing the inoculum) at a rate equivalent

to 4 mg dry weight per 100 ml of substrate and incubated in a stationary state. A duplicate series of tests was set up, to each of which 4 ml of culture filtrate were added from the test from which the inoculum was derived. Similar tests were set up using the above media, but the inoculum was washed conidia added at a rate of 4.10^6 (0.08 mg dry weight) per 100 ml of substrate with, as well as without addition of the various culture filtrates. The growth rates in the approximately linear range were taken as a measure for the assimilating activity and are represented in the Table.

It is apparent that filtrates from the initial culture contain substances which have been excreted by the mould and that these substances when added to fresh substrate at the stage of inoculation increase markedly the rate of growth of cultures inoculated with conidia as well as those inoculated with mycelium from 2 and 3 day cultures. With the amount of culture filtrate used in the present experiments there was only a slight increase in rate of growth with mycelium of the four-day culture and no increase with the mycelium from the six-day culture. The stimulation is therefore more pronounced when these excreted substances act on germinating conidia or on young mycelium. The stimulation due to these substances is also reflected in the total yield of mycelium (Figure).



Influence on growth of *Aspergillus oryzae* of small amounts of filtrates from cultures of varying ages added at the stage of inoculation. Inoculum: 10^6 conidia per 100 ml of substrate. Stationary cultures at 25°C. □ No culture filtrate added. ● Filtrate from two-day culture added. ⊗ Filtrate from three-day culture added. ○ Filtrate from four-day culture added.

It is interesting to note that although the initial inoculum of conidia was less than that of mycelium on the basis of dry weight the rate of growth of the former was always greater than the latter when the medium contained no added filtrate. Further it is obvious that as the age of mycelium inoculum increases from 2 to 6 days, so does the rate of growth. As it appears reasonable to assume that the rate of growth of a washed inoculum in a fresh substrate is a measure for the assimilating activity of the mycelium in the initial culture, we can conclude that, in the initial culture of our experiment, the assimilating activity of the mycelium decreases in the early stages of the culture cycle (columns 1 and 3 of the Table), then increases (columns 3, 5, 7 and 9) even if the stage of autolysis is reached. This increase is likely to be due to a conditioning by prolonged action of stimulating substances so that eventually an addition of these is without further effect.

The decrease in synthesizing activity of the mycelium in early culture stages can probably best be explained

Rates of growth (mg/100 ml/h) in the approximately linear range of cultures inoculated with 10^6 conidia as well as of those inoculated with 1 mg mycelium from a culture of various ages with and without addition of culture filtrate.

Inoculum									
Conidia		Mycelium							
		Age of inoculum culture (days)							
		2	2	3	3	4	4	6	6
	+ c.f.		+ c.f.		+ c.f.		+ c.f.		+ c.f.
1.46	2.92[a]	0.189	0.742	0.303	1.43	0.972	1.166	1.39	1.39
1	2	3	4	5	6	7	8	9	10

c.f. = Culture filtrate. [a] Average growth rates of cultures supplemented with culture filtrates from 2, 3, 4, and 6 days; see also Figure.

by the formation of adversely acting substances leading to characteristics of the mycelium which are neither easily nor completely reversed by growth-stimulating substances formed during cultivation. Such adversely acting substances have recently been demonstrated in cultures from small inocula.

Since there are stimulating products, which by acting at early stages of growth influence markedly the characteristics of the mycelium of later stages, it is easy to understand that a large inoculum leads to higher assimilating activity because then a critical concentration of these stimulating products is built up *early*, whereas in the case of small inocula adversely acting substances apparently are not as easily overcome by stimulating ones, due to a strong dilution of the latter.

The increase of rate and yield with extremely small inocula can be explained if we assume that below a certain small inoculum the dilution effect of excreted growth-inhibiting substances becomes so pronounced that they have little influence, and later excreted growth-inducing substances can then have a stronger effect.

One of the reasons why these effects of the size of the inoculum have not been observed more generally is probably that they are counteracted by trace elements.

Further investigations on the influence of growth-promoting and growth-inhibiting substances produced during cultivation of *Aspergillus oryzae* are in progress[1].

Zusammenfassung. Wachstumsstimulierende Substanzen werden in Kulturen von *Aspergillus oryzae* gebildet und ins Substrat ausgeschieden. Wenn diese Substanzen während frühen Stadien der Kulturentwicklung einwirken, steigern sie Wachstumsgeschwindigkeit und maximalen Ertrag beträchtlich. Dieses Verhalten erklärt teilweise die bei verschiedenen Schimmelpilzen festgestellte erhöhte Wachstumsgeschwindigkeit und maximale Ausbeute an Mycel durch grössere Impfmengen, da hierdurch eine gewisse kritische Konzentration der betreffenden Substanzen früher erreicht wird als mit kleineren Impfmengen.

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