Tabelle II. Anti-A-IgG bei kompatibler und inkompatibler Blutgruppenkonstellation von Mutter und Kind

	Anti-A-IgG[a] positiv		Anti-A-IgG[a] negativ	
Blutgruppenkonstellation		Bilirubin (mg/100 ml)		Bilirubin (mg/100 ml)
Kompatibel (43 Seren)	8/43		35/43	
		> 10: 5		> 10: 8
		< 10: 3		< 10: 27
Inkompatibel 0/A, B/AB (58 Seren)	22/58		36/58	
		> 10: 11		> 10: 8
		< 10: 11		< 10: 28

[a] Anti-A-IgG-Titer: $\geqq 1/20$ = positiv; $\leqq 1/10$ = negativ.

und Kind sowie die Bilirubin-Werte des Kindes entnommen. Alle Fälle mit mütterlichen Antikörpern ausserhalb des AB0-Systems wurden ausgeschlossen.

Resultate. Mit beiden angewendeten Methoden zur Bestimmung des Anti-A-IgG-Gehalts erhält man vergleichbare Resultate. Die Merkaptoäthanolmethode ergibt etwas höhere Titer (Tabelle I), ist jedoch mit dem Nachteil behaftet, dass häufig eine Gelbildung auftritt, welche im Coombstest stört und zu falscher Interpretation führen kann. Um die Eignung der Anti-A-IgG-Bestimmung für die Erfassung einer Erkrankung des Kindes infolge 0/A-Unverträglichkeit zu beurteilen, wurden die mütterlichen Seren aufgeteilt nach Vorhandensein und Fehlen des IgG-Agglutinins und einer Hyperbilirubinämie des Kindes, wobei ein Wert von mehr als 10 mg/100 ml Bilirubin als pathologisch bewertet wurde (Tabelle II). Bei einer inkompatiblen Blutgruppenkonstellation wurden in rund 40% aller Fälle Anti-A-Antikörper vom IgG-Typ gefunden, davon zur Hälfte zusammen mit einer Hyperbilirubinämie.

Auch bei kompatiblen Schwangerschaften war jedoch mehrfach Anti-A-IgG mit und ohne Hyperbilirubinämie nachweisbar. Somit führt nicht jede 0/A-Inkompatibilität von Mutter und Kind zur Bildung von Anti-A-IgG und das Vorhandensein von Anti-A nicht stets zu einer Hyperbilirubinämie des Kindes. Häufig tritt aber auch eine Hyperbilirubinämie bei 0/A-Inkompatibilität auf, ohne dass Anti-A-IgG dafür verantwortlich gemacht werden kann. Da Anti-A-Agglutinin vom IgG-Typ auch bei kompatiblen Schwangerschaften nachgewiesen werden kann, hat die Bestimmung von Anti-A-IgG im mütterlichen Blut höchstens ergänzenden Wert.

Summary. Anti-A agglutinins of immunoglobulin G type were estimated in mothers of newborns with compatible and incompatible blood group constellations. Removal of IgM agglutinin by adsorption on DEAE cellulose proved to be a reliable method. Clinical data of hemolytic disease due to blood group incompatibility do not agree with the presence or absence of IgG agglutinins.

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Specific Induction of Chlamydospore Formation in *Candida albicans* by N-Acetyl-D-Glucosamine

Chlamydospores constitute an important morphological and physiological differential characteristic of *Candida albicans*. Many different media and conditions promoting chlamydospores production as an aid for laboratory diagnosis of this yeast were proposed. However, the formation of chlamydospores usually requires prolonged cultivation and is generally inconstant, mainly because the precise nature of the factors determining chlamydospore formation is unknown.

In a previous note[1], we described a serum medium for a rapid and abundant production of chlamydospores in *C. albicans*; however, this medium, which proved useful for diagnostic purposes, was too complex for biochemical studies. In this communication we describe a specific induction of chlamydospores in *C. albicans* by N-acetyl-D-glucosamine and a reproducible way for obtaining a large quantity of typical chlamydospores, suitable for morphological and biological studies.

Various strains of *C. albicans* (Robin) Berkhout, of fresh isolation in our laboratory or from Type Collection of the Institute of Microbiology, Rome, Italy, were used throughout this study. When 16 h old blastospores obtained by cultivation in Agar Sabouraud (BBL) at 37 °C, were incubated at a concentration of 8×10^5 to 1.1×10^6 cells/ml in media containing N-acetyl--D glucosamine (Fluka, Switzerland), all the strains examined gave chlamydospores but to a different rate and extent. For convenience, the results further referred to in the text will refer to the strain A_{1F}[2], taken as a standard reference strain.

In preliminary experiments we found that N-acetylglucosamine remarkably stimulates chlamydospore formation in serum media. As shown in Figure 1, blastocells of *C. albicans* incubated in a mixture of swine serum diluted 1:10 with N-acetylglucosamine (1 mg/ml) give a massive production of chlamydospores after incubation at 37 °C (4 h) followed by 24–48 h at room temperature.

[1] N. SIMONETTI and V. STRIPPOLI, Experientia *27*, 985 (1971).
[2] N. SIMONETTI and V. STRIPPOLI, Mycopath. Mycol. appl. *51*, 19 (1973).

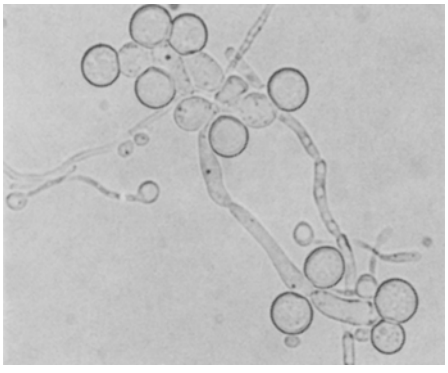

Fig. 1. Chlamydospores of *C. albicans* obtained after incubation (4 h at 37 °C plus 48 h at room temperature) of blastocells in swine serum diluted 1:10 containing 1 mg/ml N-acetylglucosamine. Leitz Ortholux-Orthomat microscope. ×500.

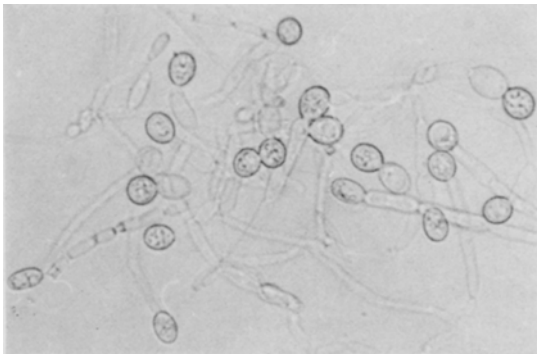

Fig. 2. The same chlamydospores as in Figure 1, but without serum. Acetylglucosamine was dissolved in water or MES buffer. ×380.

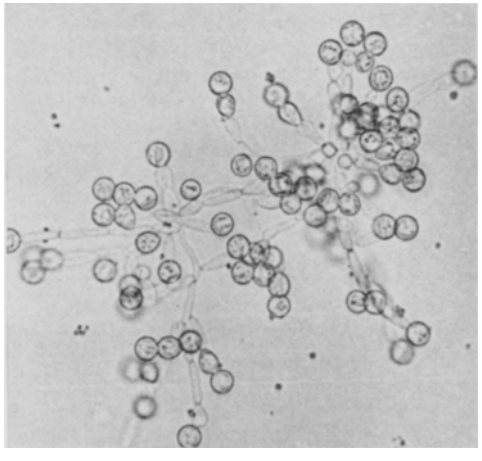

Fig. 3. Massive production of chlamydospores of *C. albicans* in N-acetylglucosamine-agar. Note the marked difference in contrast between the chlamydospores and the supporting pseudomycelium. For other experimental details see text. ×300.

Subsequently, we found that serum can be omitted from incubation mixture and that the simplest medium for obtaining chlamydospores are made up of N-acetylglucosamine dissolved in imidazole-HCl 0.01 *M* pH 6.6 buffer, or in 2-morpholino-ethanesulfonic acid (MES, Fluka) buffer 0.05 *M* at a pH of 6.2 or also in simple water. As shown in Figure 2, many typical chlamydospores are found after 4 h at 37 °C followed by 48–72 h at room temperature in N-acetylglucosamine-containing MES buffer.

It is possible to add 2% agar base (Special Agar Noble, Difco) to N-acetylglucosamine-medium, so obtaining an excellent solid medium for chlamydospore production. In this case, the purification of the agar is not important (as it is in the medium proposed by JANSONS and NICKERSON[3]) and the chlamydospores are obtained by simple streaking 0.1 ml of a suspension containing 10^4–10^6 blastospores (16 h old) on the surface of the agar itself or on a slice of dyalisis tube (The Scientific Instrument Centre Ltd., London, England) put onto the surface of the agar. After streaking, the medium was left to dry for 70 min at 37 °C in Petri dishes, then incubated for 24 h at 26 °C. Maximal yeld of chlamydospores was observed after 48 h. These chlamydospores were typical in morphology and size (Figure 3).

Glucose, glucosamine, galactosamine, N-acetylgalactosamine, fructose, glucose-1-phosphate could not replace N-acetylglucosamine in chlamydospore induction, which was only slightly stimulated by $MgSO_4$ (0.1 m*M* Merck, Germany) or $MnCl_2$ (0.1 m*M*, Merck, Germany); the optimal pH ranges from 6.2 in MES buffer to 6.6 in imidazole HCl buffer.

In general the experimental conditions of chlamydospore formation seem very similary to those specifically inducing germ-tube formation from blastospore of *C. albicans*[4] and in this respect N-acetyl-D-glucosamine can be considered a 'key' substance for dimorphism and morphological variations in *C. albicans*.

The possibility of obtaining high yeld of typical chlamydospores of *C. albicans* with N-acetyl-D-glucosamine by a simple and reproducible method offers also a new reliable approach to a simple laboratory diagnosis of *C. albicans*.

Riassunto. La N-acetil-D-glucosamina in acqua, in alcuni tamponi o in siero, induce specificamente la formazione di clamidospore in *Candida albicans*. Esse mostrano una tipica morfologia e sono idonee per studi biochimici e per la diagnosi di questo micete.

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[3] V. K. JANSONS and W. J. NICKERSON, J. Bact. *104*, 910 (1970).
[4] N. SIMONETTI, V. STRIPPOLI and A. CASSONE, Nature, Lond., *250*, 344 (1974).

Ein neues Verfahren zur Peptidsynthese: die alternierende Fest-Flüssigphasen Methode

Die bisher beschriebenen Verfahren zur Peptidsynthese unter Verwendung unlöslicher oder löslicher Träger basieren alle auf der von MERRIFIELD[1] in die Peptidchemie eingeführten Grundidee, d.h. die im schrittweisen Verfahren wachsende Peptidkette ist entweder über die Carboxylgruppe[1,2] oder die Aminogruppe[3] mit einem polymeren Träger verknüpft. Hierdurch soll die Abtrennung der zur Kettenverlängerung im