a sign of an inflammatory reaction, the increase of γ-globulins could be interpreted as related to antibody formation. Experiments to detect precipitating antibodies gave negative results without exception. On the contrary, with the hæmagglutination technique of Boyden[5] individual specific antibodies could be demonstrated, but only of a low titre. It is interesting that these antibodies were present as early as 7 days after the beginning of sensitization. In our investigation the properdin content of the sera determined by an inulin adsorption method[6] rose significantly 14 days after the first sensitization and 7 days after the grafting of homologous skin, respectively. Later on, the properdin values returned to normal level.

To demonstrate transplantation immunity, a gross and microscopic examination of the homografts on the sixth day after grafting was performed. Whereas in group *A* (sensitization by cells injected intravenously) no sign of graft breakdown could be observed, the grafts in group *B* and *C* (sensitization by fresh and lyophilized tissue extracts by the intravenous route) were almost or completely destroyed at this date.

For the detection of sessile antibodies we used the regional lymph nodes of the grafting site and the spleen of rabbits of group *B* (25 specimens altogether) obtained on the fourteenth day after beginning of sensitization and on the seventh day after skin grafting, respectively. The organs were pressed through a stainless-steel sieve and, so isolated, the cells were tested for the existence of sessile antibodies following three different methods: (1) The cells were transferred to untreated animals[7]. In no case could transplantation immunity be shown. These observations are in contrast to the findings of Mitchison. An explanation might be the genetic difference of the rabbits causing the destruction of the transferred lymph cells by homograft reaction, whereas Mitchison used inbred strains of mice.

(2) Investigations of the passive cutaneous hypersensitivity reaction[7] were performed. Here the cells were injected intracutaneously into untreated rabbits and 12 h later the corresponding antigen was given. On the average 24–48 h later a positive reaction with induration, erythema in every case and sometimes even necrosis could be observed. Controls with a non-corresponding antigen or with saline showed mostly a negative result. Experiments with non-sensitized lymph cells were also negative.

(3) In order to detect sessile antibodies by an *in vitro* method we utilized the technique described by Warnatz *et al.* and Steffen[7,8]. Here lymph node and spleen cells of sensitized rabbits were incubated with the corresponding antigen *in vitro* and the washed cells mixed with a heterologous antiserum (dog, rat) of a known titre and specifically directed against that antigen. The decrease in the titre of the antiserum after incubation was determined. It is assumed that the antigen reacts with the antibodies which are fixed at the investigated cells. The decrease in titre of the heterologous antiserum after incubation with cells so prepared indicates the existence of cellular fixed antibodies. In our investigation the titre decreased 1–3 steps of an antiserum dilution row. As compared with results of the controls these findings suggest that cellular fixed antibodies exist.

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β-Carotene and Sexuality in the Mucoraceae

There have been frequent reports of an association between β-carotene and reproduction in the Phycomycetes[1]. Recently, a stimulation of β-carotene production in mixed + and − cultures of four heteromictic[2] species of the Choanephoraceae has been reported[3,4]. It is not known whether this is a general or an isolated phenomenon in the Mucorales.

The following strains of heteromictic species were tested for β-carotene accumulation in intra- and inter-specific mixed cultures:

1	*Mucor hiemalis* +1	10	*C. elegans* −1
2	*M. hiemalis* −1	11	*Absidia glauca* +1
3	*Phycomyces nitens* +*H*102	12	*A. glauca* −1
4	*P. blakesleeanus* +1	13	*Mucor rammanianus*
5	*P. blakesleeanus* −1	14	*Circinella* sp.
6	*P. blakesleeanus* −6	15	*Rhizopus nigricans* +1
7	*P. blakesleeanus* −*E*45	16	*Pilobolus sphaerospora*
8	*Helicostylum pyriforme*	17	*Conidiobolus villosus*
9	*Cunninghamella elegans* +1	18	*Thamnidium elegans*

In addition, three homomictic species were tested:

19 *Rhizopus sexualis*
20 *Zygorhynchus moelleri*
21 *Z. vuilleminii*

All were originally obtained from the Centraalbureau voor Schimmelculture, except 13, which was isolated in this laboratory from soil. The homomictic species formed abundant zygospores on solid medium, as did the heteromictic strains in the appropriate combinations.

An inoculum of 10^7 asexual spores, which was found to be optimal, was added to 25 ml. of modified Schopfer's[5] medium in 150-ml. conical flasks plugged with gauze-covered cotton wool. The flasks were mounted on an illuminated reciprocating shaker at 25° C and the submerged shake cultures grown for up to four weeks. Cultures were triplicated at least.

The washed and partially dried mycelium was extracted with acetone and the principal pigment, β-carotene, characterized and determined spectrophotometrically[6,7].

Strains 1–8 grown singly did accumulate β-carotene to varying degrees. However, in no mixed culture was the amount of β-carotene significantly different from the expected mean of the values accumulated by the component strains grown alone (Table 1). Moreover, none of strains 9–21 accumulated β-carotene to a detectable degree, either in isolation or in mixed cultures although the homomictic cultures formed zygospores.

Table 1. β-Carotene Accumulation by Mixed Cultures.
(Values are in μg β-carotene/g dry weight of mycelium)

Strains	1 × 2	3 × 5	3 × 6	3 × 7	4 × 5	4 × 6	4 × 7	5 × 6	5 × 7	6 × 7
Expected	406	148	110	147	175	137	174	112	149	111
Observed	457	137	133	152	166	128	162	120	152	123
S.D.	39	10	12	13	15	11	12	12	4	13

These results confirm and extend those of Reichel and Wallis, who found no stimulation of 'carotene' accumulation in mixed cultures of their strains of *P. blakesleeanus*[8]. Such a stimulation appears at present, therefore, to be confined to the Choanephoraceae.

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