they are fully protected against intracerebral injections of paralyzing doses of live virus.

Thomsen[8] reported briefly on a similar experiment in 1912 but in the experience of this author no immunity was produced in the small number of animals employed in the course of his work. However, this work was reported shortly after the virus of poliomyelitis was discovered. We have not determined the mechanism involved in this reaction but we have postulated the possibility of the gradual entrance of poliomyelitis virus into the tissues of the animal through the aid of the concomitant virus infection, vaccine virus, which may open the pathway for the poliomyelitis virus. In effect we may have a situation similar to chemically treated virus, if our hypothesis is correct, where the living virus is slowly absorbed and in insufficient dosage to produce paralysis. It would be interesting to know if, after long residence in the monkey, the virus of poliomyelitis has undergone some change biologically and, if returned to man, will no longer produce paralysis when given subcutaneously or intracutaneously as we have described.

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Effect of Age of a Specific Medium on Morphology of Colonies of Certain Pathogenic Fungi.*

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In this work batches of media were allowed to age at laboratory temperature, planted with organisms and morphological differences observed. In aging, the medium loses water and the nutrient concentrates so that while the ratio of ingredients is probably approximately the same the moisture content, as will be noted below, becomes much less. Our routine W medium, consisting of 4% peptone, 1% dextrose, 1½% agar and adjusted to pH 5.6, was used. The age of media which was placed on laboratory bench tops varied by approximately 10-day intervals from fresh medium to 90-day-old medium.

8 Thomsen, O., *Z. f. Immunitäts.*, 1912, **14**, 198.

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The following representative fungi and 2 non-pathogenic saprophytes, *Lichtheimia sp.* and *Scopulariopsis brevicaulis,* were studied: *Achorion schoenleinii, Acladium castellani, Candida candida, Endodermophyton indicum* (Castellani), *Endodermophyton tropicale, Endomyces capsulatus, Endomyces dermatitidus, Epidermophyton cruris, Epidermophyton inguinale, Epidermophyton rubrum* (Castellani), *Glenospora gammeli, Geotrichum bachmann, Indiella americana, Microsporon audouini, Microsporon felineum, Microsporon gypseum, Monosporum apiospermum, Monilia albicans, Oöspora humi, Sporotrichum schenkii, Trichophyton balcaneum* (Castellani), *Trichophyton crateriforme, Trichophyton decalvans* (Castellani), *Trichophyton granulosum, Trichophyton gypseum, Trichophyton gypseum* asteroides, *Trichophyton gypseum* lacticolor, *Trichophyton interdigitale, Trichophyton japonicum, Trichophyton louisiana* (Castellani), *Trichophyton niveum, Trichophyton purpureum, Trichophyton sulfureum, Willia anomala.*

With age there was retraction and contraction of the medium. This was accompanied by less space for the growth to spread over, with some resultant lessening of growth. In 13-weeks-old medium there was enough shrinkage so that after a month of growth only a dry scale remained. Yet growth occurred in all instances with the exception of *Trichophyton louisiana.* Thirteen-week growths were smaller, much dried yet retained in most instances some of the typical morphology seen on fresh medium. In many instances the growths on 11- and 12-week media were much dried but showed good differentiation. The growths on younger media were good and showed morphology in most instances as good as that on fresh medium. *Trichophyton japonicum* showed more coloring of the mycelia on the older media but most of the other growths showed a color variation which could not be ascribed to age. *Microsporon gypseum* showed a tendency to lose its powder in old media and develop low white aerial mycelium while old media growths of *Epidermophyton inguinale* seemed to show more pigment. The finely waxy radiate border of *Trichophyton purpureum* and the waxy ribbed border of *Trichophyton rubrum* became somewhat less distinct on old media but retained in most instances their yellow color in the early period of growth. In growths which showed frosting on fresh medium there was a tendency on old media to show decrease in this appearance and increase in white mycelium. In the very old media *Microsporon audouini* showed less of the soft mycelium and more the coarser of deeper tan color.

The maintenance of morphology in old media was striking. This

is the converse of the decided effect on morphology of change of the ratio of dextrose to peptone which has been very evident in our studies. Possibly the maintenance of the same ratio of ingredients is the important factor. The reaction which was adjusted to pH 5.6 became as a rule slightly more acid but did not fall below 5.2. In other studies of W medium in which the pH had been varied from 5.2 to 8.0, we found no marked morphological variations. It is logical therefore that this pH change would not be responsible for variation.

In order to visualize the loss of weight we have weighed tubes of W medium over periods of time, each tube containing 10 cc. of medium of a weight of approximately 9.513. Over the period of the study it was found that the medium lost on an average 8.6 mg. per gram in weight per day. For the 90-day period this meant a loss of 7.363 gm. of water. This left a mass of 2.150 gm. in each tube of which 0.65 gm. were the solids added in the form of dextrose, peptone and agar, and 2.085 moisture. This meant a concentration of ingredients of almost 4½ times. In other words, in the oldest medium we were planting our organisms on medium of approximate concentrations of agar of 6.75%, of peptone of 18%, and of dextrose of 4.5%. Nevertheless, the organisms maintained an approximately individually similar morphology as long as this ratio was maintained. This loss of moisture was somewhat less the first few weeks. It is possible that retraction of the medium from the glass and splitting with increased surface accounted for some of the increase. The latter weighings also showed a tendency to slight decrease in weight loss.

This work emphasizes the length of time, namely, approximately 10 weeks, that this medium can be allowed to stand at laboratory temperature and still show good growth with good differentiation. It also suggests the possibility that maintenance of similar peptone-dextrose ratios may mean similar gross colony morphology. The weight loss, which varies with temperature and humidity and which averaged approximately 8.6 mg. per gram of substance per day, was greater than weight loss obtained in the summer with windows open and no artificial heat, which averaged 7 mg. per gram of substance per day. This was due undoubtedly to a higher laboratory temperature and a drier atmosphere in the artificially heated laboratory.