periods, and by the 25th day following, the entire culture had died out. In another culture, which was started from this one at the 150th generation and kept on a hay infusion medium, conjugation did not occur and this culture is still alive.

75 (600)

The cultivation of tissue in plasma from alien species.

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The present series of experiments have been concerned with the attempt at cultivating in vitro rat sarcoma, rat spleen and mouse carcinoma, in plasma obtained from animals of other species, and at analyzing the factors contributing to the phenomena observed.

In a former note we recorded the fact that mouse and rat plasma could be interchanged as culture media for the tumors of these species, but that growth seemed to be more vigorous when homologous plasma was used. Guinea pig, rabbit, dog, goat, human and pigeon plasmas have been employed in the studies herewith reported.

For determining the viability of tissue under the conditions of the experiment we have made transfers of the pieces to homologous plasma—a rapid and satisfactory test. Animal inoculations have also been made in the case of tumor tissue, but aside from the delay in noting the results the procedure has other objections.

As a culture medium for rat sarcoma guinea pig plasma is only slightly less suitable than rat plasma, the difference consisting chiefly in the extent of the out-wandering of cells. The cells may remain viable in a single drop of plasma for twelve days or more; we have had pieces which showed marked activity after a month's sojourn in several drops of the alien medium. Mouse carcinoma seems to grow almost as well in guinea pig plasma as in rat plasma. Mitoses have been observed after eight days, and cultures nine days old produced tumors when inoculated into mice.

Rabbit plasma is distinctly less suitable for the growth of mouse

and rat tumors. Liquefaction about the pieces of tissue is often quite marked. With sarcoma the growth, though relatively slow, may continue for ten to twelve days.

In dog plasma pieces of sarcoma, after one or two days, present a fairly diffuse radial outgrowth of clear spindle cells, which after this time undergo rapid disintegration. About the pieces there is noted a narrow clear zone (liquefaction). With mouse carcinoma liquefaction is more marked and there is little or no outwandering of cells.

We have not observed any of the phenomena of growth when using goat plasma. After a few hours there is seen surrounding the pieces of tissue a wide granular zone. Liquefaction of fibrin does not occur.

In human plasma the most striking and constant phenomenon is the progressive liquefaction of the fibrin, which is practically complete after six or seven days. In spite of the loss of framework there takes place, however, an active migration of cells, which wander out along the cover glass, reaching often the edge of the medium. As a rule the cells move out separately, but we have observed in several preparations of rat spleen radial out-growths simulating the appearance seen in homologous plasma where the fibrin network is preserved. In the single cells attached to the cover glass the most interesting changes have been noted, especially in the cultivation of rat spleen. After four or five days cells of extraordinary size begin to appear, reaching a maximum size in two or three days. Such a "giant cell" examined in the fresh state shows about its centre a clear nuclear area, with or without knob-like prominences, surrounded by a highly granular zone, which in turn merges into a clear filmy indefinite protoplasm with processes. In their entire extent these cells vary from 100 to 700 mikra in diameter. When stained the knob-like prominences are seen to be nuclei with distinct nuclear membranes and prominent nucleoli. These nuclei are often seen in close apposition arranged about a light hematoxylin staining, reticular or vacuolated area. In the fresh this portion of the cell was interpreted as nucleus. Many large cells varying considerably from these were also seen. Quite large multinucleated cells have been observed in the growth of sarcoma and carcinoma in rat plasma but none that simulated

those just described. Pieces of rat spleen or tumor when transferred to rat plasma after five or six days in human plasma begin to grow very actively. The cells, however, often present extremely ragged outlines. Giant cell formation may be noted after the transfer.

The growth of rat sarcoma in pigeon plasma is especially beautiful and appears to be quite characteristic. During the first four or five days there is a progressive radial extension of large clear spindle cells of strikingly uniform size and morphology. The strings of cells are connected by long slender processes. Fine granular fat accumulations in the protoplasm appear early and increase in size and number with the age of the specimen, just as occurs in rat and guinea pig plasmas. As a rule no further growth takes place after the fifth day, and the cells at this time begin to show signs of disintegration. Intact well stained nuclei, however, may sometimes be seen in preparations eight or nine days old. Transfer of the pieces of tissue in four-day specimens to fresh pigeon plasma does not result in a prolongation of the period of activity.

In summarizing the above findings we notice that goat plasma is the only medium used in which there was no growth; in dog plasma growth was of short duration. Arranged in the order of suitability we have guinea pig, rabbit, pigeon, human, dog and goat. In a study of cytolysins for rat and mouse tissues we found that hemolysins were present in human, dog and goat sera. The sera tested were taken from animals from which plasma had been obtained for tissue cultivation. We have found also that in plasma from guinea pigs immunized against rat corpuscles, growth of rat sarcoma does not occur, or is of an abortive character, and that there is a similar inhibition of growth in plasma from guinea pigs previously treated with large doses of rat sarcoma.