## Agglutination of established cell lines of human leukocytes by human transplantation BUFFALO, N. Y.

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he usual serum antibody response of the normal untreated human to allografts remains largely unknown. Antibodies stimulated by allografts have been detected in human serum by hemagglutination,<sup>2</sup> leukoagglutination,3 cytotoxicity,13 antiglobulin consumption,3 and mixed agglutination on tissue cultures.<sup>8, 9</sup>

These studies, however, were performed on small numbers of subjects who were in varying states of health and who were immunized by a variety of methods. More comprehensive studies are needed to clarify this subject, since the results may have real clinical significance.

This paper presents data on the leukoagglutinin response of the healthy adult male to repeated skin allografts. A modification of the leukoagglutination technique is presented in which malignant leukocytes maintained in suspension cell cultures were used as target cells. With this technique it has been possible to detect leukoagglutinins with a considerably higher frequency than was expected and to relate the appearance of these antibodies to the way in which the host rejected the allograft.

## METHODS

Preparation of human immune transplantation sera. Fifty healthy adult male volun-

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teers were divided into 25 pairs. Each member of each pair was expected to donate to and receive from his partner 3 successively applied full-thickness allografts of skin, 6.25 cm.2 in size: Second-set allografts were applied 6 weeks after the first-set, and the third-set allografts were applied 4 weeks after the second-set allografts. Serum samples were obtained from each subject prior to the first allograft and at weekly intervals throughout the experiment until 14 days after the third-set allograft. Ultimately, serum was obtained from 46 volunteers who had received 3 successive allografts, from 2 subjects who received only 2 allografts, and from 2 individuals who received only one allograft. The volunteers elected to call this study the Skingraft, Attica Group, Experiment. Therefore, the sera will be referred to as the SAGE sera.

Sera from multiparous females. These were obtained from 46 mothers during their postnatal hospitalization. Each donor had experienced three or more pregnancies.

Cell cultures. Sixteen established cell lines of leukocytes were obtained from the Roswell Park Memorial Institute and maintained in suspension cultures according to the method of Iwakata and Grace.<sup>5</sup> Eight lines were of malignant origin and were used in the studies reported. Eight lines originated from normal subjects and were used as controls.

Normal human leukocyte preparation. These were prepared for conventional leukoagglutination according to the method of van Rood and van Leeuwen<sup>12</sup> and used promptly.

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