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Effects of Guinea Pig Serum on 6C3HED and AKRL1 Lymphoma Cells Implanted in Various Hosts.* (22200)

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Cells of Lymphoma 6C3HED promptly begin to die when serum from normal guinea pigs is injected into C3H mice in which they are growing(1); yet the lymphoma cells remain viable when they are held in contact with guinea pig serum during several hours at 37°C *in vitro*(1,2). The facts suggest that the animal host somehow participates in the process whereby these lymphoma cells are killed *in vivo,* perhaps by supplying special conditions essential for the process, or by furnishing some substance—an isoantibody, for example, or one or another of the components of complement—that might work with guinea pig serum in producing the effect(1,2). A number of questions related to the facts just mentioned are raised by a more recent observation—namely that cells of Lymphoma AKRL1 continue to proliferate without restraint when normal guinea pig serum is given to AKR mice carrying them(3). Do the AKR mice fail to supply such conditions as might be required for the effectiveness of guinea pig serum *in vivo,* or are they devoid of some essential host factor? Is there an intrinsic difference in cells of Lymphoma 6C3HED and those of Lymphoma AKRL1, the former being susceptible to the effects of guinea pig serum *in vivo* while the latter are insusceptible? The findings of several experiments, now to be given, bear on these questions.

Results. The experiment of Fig. 1 shows that the cells of Lymphoma 6C3HED were inhibited quite conspicuously when guinea pig serum was given intraperitoneally to AKR mice into which 10 million lymphoma cells had been implanted in each of 2 subcutaneous sites by a technic already described(1), and the finding was confirmed in a subsequent experiment done in precisely the same way. Furthermore, in the experiment of Fig. 1 the inhibitory effect of guinea pig serum was even more marked in the alien and resistant AKR hosts than in the C3H hosts of the sort in which this lymphoma had originated (and which had been implanted with 1 million 6C3HED lymphoma cells in each of 2 subcutaneous situations). This result suggests that the effects of guinea pig serum might depend upon, or be enhanced by, some resistance manifested by the animal

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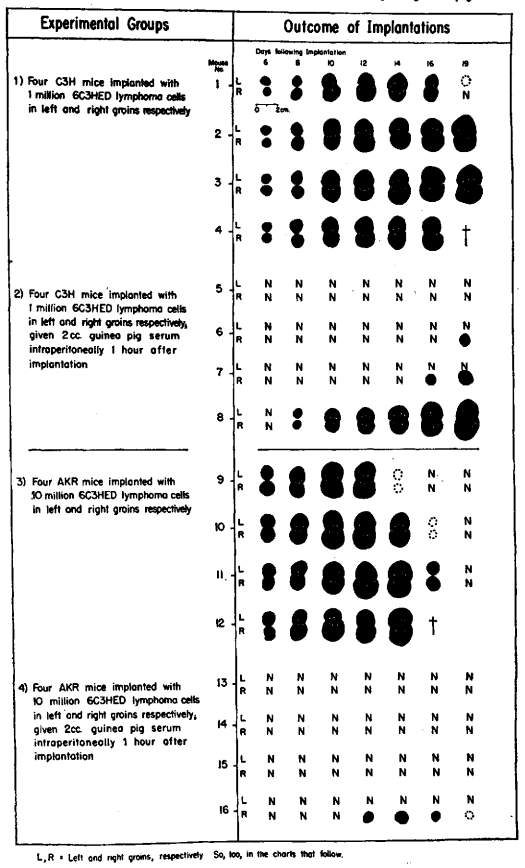

FIG. 1.

hosts, this being the greater in the mice of alien breed. More will be said about this further on.

Next the converse experiment was done. Ten million cells of Lymphoma AKRL1 were implanted in each groin of 8 C3H mice (which were presumably resistant to them since the cells had originated in AKR mice), and 1 million cells were implanted in each groin of 8 AKR mice of the sort in which the lymphoma had originated(3). Four mice of each breed were left untreated as controls and the others were given, approximately 1 hour after implantation, 2 cc of normal guinea pig serum from the same pool as that used in the experiment of Fig. 1. The guinea pig serum had no inhibitory effect whatever on subsequent growth of lymphoma cells in either native or alien hosts; for the cells implanted in treated and untreated AKR mice grew and formed palpable tumors of approximately equal size, and these all enlarged until death of the animals, as careful chartings showed. Furthermore, the AKRL1 cells that had been implanted in the alien C3H hosts regularly grew to form tumors 10-16 mm across on the 10th day following implantation; but these tumors dwindled and disappeared in 5 of the 8 mice between the 10th and 19th days, while growing progressively in one of the untreated and 2 of the treated animals. The process of regression, although essentially similar in treated and untreated animals, was somewhat more abrupt in the untreated animals, the finding suggesting either that the guinea pig serum might have interfered to some extent with the process of regression or that it might have enhanced growth of these lymphoma cells under these conditions.

In a further test of this sort 2 additional lymphomas of AKR mice — AKR13 and AKR17(3)—were used. Ten million cells of each type were implanted in both groins of 8 C3H mice, 4 of which were left untreated as controls while the others were given 2 cc of guinea pig serum intraperitoneally one hour after the implantations. During the next 6-10 days, tumors measuring up to 17 mm across appeared at every implantation site, the growths in treated animals being precisely like those in untreated controls; within the following 10 days, however, the growths all dwindled and disappeared (Fig. 2), this process too being essentially the same in the treated and untreated groups. In concurrent tests, the guinea pig serum did not influence the outcome of implantations of these cells in AKR mice, the findings confirming observations already published(3).

F1 hybrid C3H x AKR mice were employed in the several further experiments. In the most definitive of these, 500000 6C3HED lymphoma cells were implanted in one groin of 8 hybrid mice, and 500000 AKRL1 lymphoma cells were implanted in the opposite groin; half of the implanted mice were then kept as controls and the others were given normal guinea pig serum intraperitoneally, 2 cc

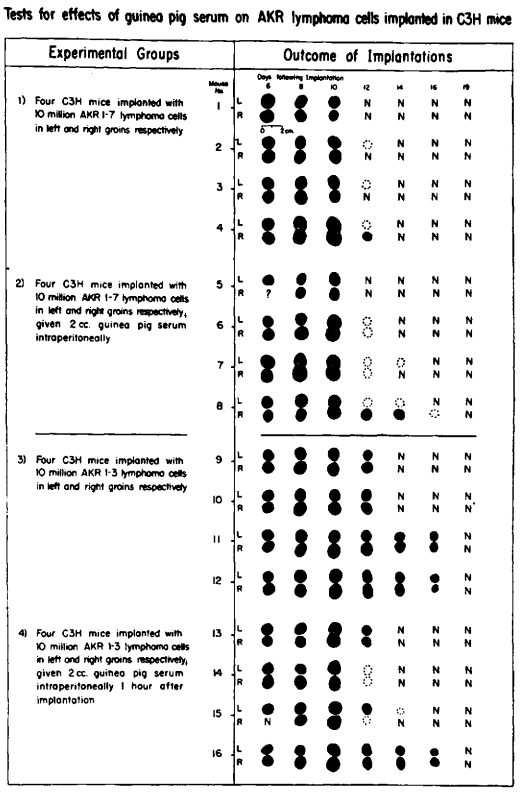

FIG. 2.

approximately 1 hour after the implantations had been made and 2 cc on each of the following 2 days. Fig. 3 shows the outcome. In the untreated control mice, tumors 10 to 14 mm across were palpable in both groins on the 8th day following the implantations, and these enlarged progressively until death of the hosts between the 18th and 19th days. In the 4 treated mice, tumors similar to those of the control animals appeared in the groins which had been implanted with AKRL1 lymphoma cells, and these growths also enlarged progressively; but the sites implanted with 6C3HED cells remained devoid of tumors. In a further experiment 500000 6C3HED cells were implanted in both groins of 8 hybrid mice and the same number of AKRL1 cells were implanted in each groin of 8 additional animals; half of the mice of each implantation were then given 3 injections of guinea pig serum as in the preceding test. The 6C3HED cells grew as before in the untreated animals, but failed to form palpable tumors in those given guinea pig serum; the AKRL1 cells formed tumors in the treated and untreated hosts which appeared promptly and grew rapidly and progressively in all instances.

Mention was made above of the possibility that the effects of guinea pig serum *in vivo* might depend upon or be enhanced by some resistance provided by the animal hosts and directed against the lymphoma cells as foreign tissue. If this should prove to be the case, then the effects should be more marked against lymphoma cells that had grown for some days in alien hosts; for under these circumstances such immune responses as the hosts were capable of making should have been stimulated. An experiment was done next to test the possibility. Ten million cells of each of 3 AKR lymphomas previously employed—AKRL1, AKR13, and AKR17—were implanted in both groins of 8 (alien) C3H mice. Nothing further was done to any of the animals until 6 days later, at which time palpable tumors measuring approximately 1 cm across were present at all implanted sites. On the 6th, 7th, and 8th days, 2 cc of normal guinea pig serum were given to 4 of the mice that

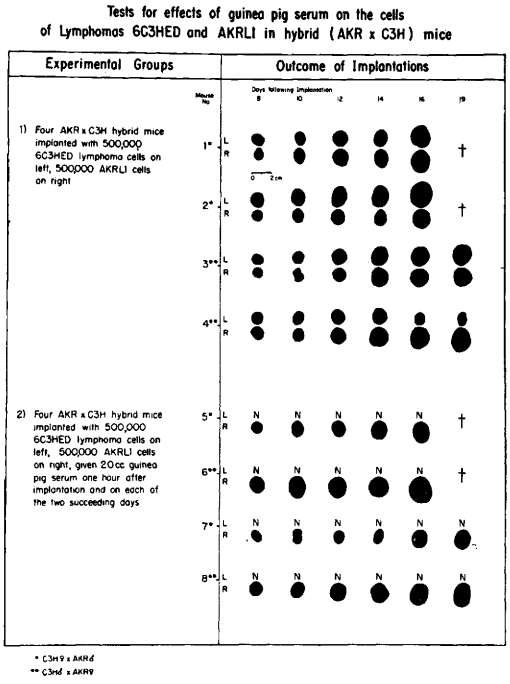

FIG. 3.

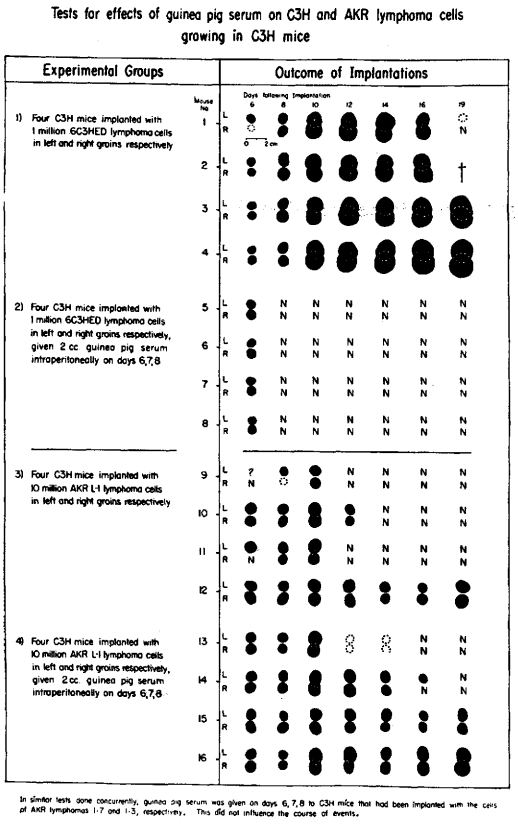

FIG. 4.

had been implanted with each type of lymphoma. The outcome in the mice implanted with 6C3HED and AKRL1 cells is shown in Fig. 4. It can be seen that the injected guinea pig serum promptly brought about regression of the 6C3HED lymphomas but had no such effect on the AKRL1 growths. The guinea pig serum given in this way to the C3H mice carrying Lymphomas AKR13 and AKR17 was likewise devoid of effect, though to conserve space the findings are not given in the chart.

The findings just described were extended in several further experiments. In the first of these 6 million cells of lymphoma 6C3HED were implanted in 6 mice of each of 2 alien strains heretofore unused (strains A and DBA), and in 6 C3H mice also; half of the animals were kept untreated as controls while the others received 2 cc of guinea pig serum on the day of implantation and on each of the next 2 days as well. Tumors appeared promptly in the untreated mice of all 3 breeds and grew progressively to bring about death of the hosts between days 11 and 18, except in one mouse of strain A in which the growths regressed between the 10th and 12th days. Palpable tumors did not appear in any of the mice given guinea pig serum. In a similar experiment, 6 million AKRL1 cells were implanted in both groins of 6 strain A and 6 DBA mice, half of the animals of each breed being treated with guinea pig serum as before. In the strain A mice, palpable tumors 9 to 11 mm across appeared in all treated and untreated animals 7 to 8 days following implantation; the growths in both groups of mice enlarged during the ensuing 4-6 days but thereafter dwindled and disappeared, the process of regression again being essentially the same in treated and untreated animals. In all 3 untreated DBA mice and in one of those given guinea pig serum the AKRL1 cells failed to form palpable tumors, but in 2 of the treated animals they gave rise to bilateral growths that appeared between 7th and 11th days and enlarged progressively until the 20th day when the animals were discarded. A further indication that guinea pig serum can enhance growth of AKRL1 cells *in vivo* was provided by an additional experiment. Eight C57 mice were implanted with 1.5 million AKRL1 lymphoma cells and half of the animals were then given 2 cc of guinea pig serum. Again, the untreated animals failed to develop palpable tumors, but all 3 of those given guinea pig serum developed growths, which measured 12-14 mm across on the 7th day following implantation, enlarged during the ensuing 2 days, and thereafter abruptly disappeared. It remains to be seen whether these findings have a relation to the observations of Kaliss which indicate that certain antisera may enhance growth in alien hosts of transplanted cancers of other types (4,5).

Summary. In the experiments here given, serum from normal guinea pigs acted regularly and powerfully against cells of Lymphoma 6C3HED when these were implanted in mice of various breeds (C3H, AKR, A, DBA, and hybrid C3H x AKR). But it did not inhibit

the cells of Lymphoma AKRL1 in hosts of the above mentioned sorts, and it likewise failed to inhibit growth of cells of 2 additional lymphomas (AKR13 and AKR17) in C3H mice. The findings make it plain that guinea pig serum does not act against the 3 types of AKR lymphoma cells even when these are implanted in alien hosts; indeed in some of the experiments guinea pig serum seemed to enhance the growth of AKRL1 cells in alien hosts. Considered together the observations weigh heavily against the supposition that the mouse host provides natural or induced isoantibodies which act in conjunction with guinea pig serum in killing the cells of Lymphoma 6C3HED *in vivo*, and they support the inference that cells of Lymphoma 6C3HED differ intrinsically from those of Lymphoma AKRL1, the former being susceptible to the effects of guinea pig serum *in vivo* while the latter are insusceptible.

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Effect of Tumors on Level of Plasma Amino Acids of Eviscerate Rats.* (22201)

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It was expected that the presence of a growing tumor would suppress the rise in plasma amino acids which occurs following evisceration. The contrary is true. The rise is more rapid in eviscerate rats which are hosts to a Walker carcinoma.

Methods. Male rats of the Sprague-Dawley strain were fed Rockland rat diet. Suspensions of tumor were implanted intramuscularly into each thigh near the trunk and permitted to grow for periods of 4 to 14 days. At a weight of approximately 250 g non-fasted rats were anesthetized with cyclopal sodium and functionally eviscerated. Some of the animals were kept without infusions and others were given continuous intravenous infusions of glucose with and without insulin for periods of either 3 or 6 hours. Heparinized blood was collected by cannula from the abdominal aorta and the level of plasma amino acids determined by the method of Hamilton and Van Slyke(1).

Results. The conditions of individual experiments and the results are shown in Table I. The values are grouped according to the combined weights of tumors of each rat at autopsy. The level of plasma amino acids is not elevated in non-eviscerated tumor hosts. The average values for plasma amino acids increased more rapidly in tumor-bearing rats, the extent of rise showing a positive relationship to the weight of the tumors. Although insulin suppresses the rise in plasma amino acids in all eviscerate animals(2) it does not prevent a more rapid rise in tumor hosts. Whether the extra plasma amino acids arise from the tumor itself, or from normal tissues which are catabolized more rapidly in the presence of a tumor, is not known to us. We have no information about changes in tumor metabolism following evisceration.

Summary. Following evisceration the level of plasma amino acids rises more rapidly in rats which are hosts to a Walker carcinoma

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