of an *in vivo* photodynamic effect on the ground substance. The phenomena of increased diffusion here described should be therefore considered as being of the same type as those observed whenever the degree of polymerization of the ground substance components, particularly hyaluronic acid, had been altered by either chemical or enzymic agents.

Summary. As a result of a photodynamic effect, a constant notable increase has been observed in the diffusion of India ink and diphtheria toxin inoculated in the dermis. Since the spreading test may be assumed, to a certain extent, an indication of the conditions of the ground substance of dermal connective tissue, these results seem to demonstrate that after a photodynamic effect this substance is modified.

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Tests for Isoantibodies Active in Conjunction with Guinea Pig Serum Against 6C3HED Lymphoma Cells.* (21258)

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In previously reported experiments, normal guinea pig serum brought about the death of transplanted 6C3HED lymphoma cells when injected intraperitoneally into mice carrying them but had no effect on the lymphoma cells during many hours' contact at 37°C *in vitro,* the findings showing plainly that the effect *in vivo* was the result of some reaction in which the guinea pig serum and the animal host both participated(1). Since the C3H mice used as host animals occasionally manifested some resistance against the transplanted 6C3HED lymphoma cells—this being engendered presumably by antigens present in the long-transferred lymphoma cells but absent from the tissues of the current hosts, and related perhaps to the presence of isoantibodies in the blood of the resistant animals (2,3)—the possibility was considered that the resistant animals might provide isoantibodies which, acting in concert with the guinea pig serum, killed the transplanted 6C3HED lymphoma cells *in vivo*(1). The fact that mouse serum is normally devoid of one or more of the constituents of hemolytic complement(4) has interest in relation to this possibility, and a recent observation seemed to lend additional weight to it. For guinea pig serum, while inhibiting to some extent the cells of an AKR lymphoma that had been transplanted for some time in AKR mice of the line in which it originated, with transfer later to AKR mice of another (presumably to some extent resistant) line, had little or no effect *in vivo* on the cells of several AKR lymphomas that had been transferred only a few times in mice of the inbred line in which they had arisen (and which were presumably not resistant to the cells of the respective lymphomas)(5).

If isoantibodies were the host factors that participated with the injected guinea pig serum in bringing about the *in vivo* effects referred to above, their presence should be disclosed directly by experiments in which mouse serum containing the isoantibodies is mixed with active guinea pig serum, the mixture then being held in contact with the lymphoma cells during suitable periods of time *in vitro,* with tests made later for viability of the exposed cells. Such experiments are feasible and a number of them have been done, as will now be described.

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Experimental. The hypothetical isoantibodies might conceivably be found in the serum of normal C3H mice of the inbred line used, though previous work done by others does not make this seem likely(2,3). If such antibodies are actually the responsible host factors, they would have to be present in the serum of all the C3H mice carrying 6C3HED lymphomas or implanted with the lymphoma cells—or else be very rapidly engendered in such animals following injection of the normal guinea pig serum; for much experience has shown that the 6C3HED lymphoma cells regularly begin to die within a few hours after normal guinea pig serum has been injected into animals carrying them, and this proves true even in animals in which a single injection of guinea pig serum is given one hour after implantation with the cells(1). Furthermore, the hypothetical isoantibodies might be expected to be present in high titer in the serum of mice that had recently overcome the growths, especially if these resistant hosts were of an alien breed(2). Hence in the present work tests were made with 1 or more specimens of pooled serum procured from mice in each of the following categories: normal C3H mice (bred in this laboratory), C3H mice with enlarging 6C3HED lymphomas, C3H mice from which 6C3HED lymphomas had regressed and which had recently proved negative to challenge reimplantations with large numbers of the lymphoma cells, normal strain A mice (regularly resistant to the 6C3HED lymphoma cells), and strain A mice in which 6C3HED lymphomas had regressed and which had recently proved negative upon challenge re-implantation.

In each experiment, mixtures were set up containing three ingredients, as follows: A) 6C3HED cells, freshly suspended as individuals in buffered-glucose-Ringer's solution, as described in detail elsewhere(1), in a final concentration of 4,000 cells/mm^3. B) Specimen of pooled mouse serum to be tested for isoantibodies. This was regularly procured from 9 or more mice; usually the specimen was tested the day of bleeding, though occasionally it had been kept frozen for a few days at –22°C. The specimen was used in a final dilution of 1:4. C) Normal guinea pig serum (pooled specimens from at least 12 animals; kept frozen at –22°C for periods up to 4 weeks; known from subsidary tests to be active against 6C3HED lymphoma cells *in vivo*). This was likewise regularly used in a final dilution of 1:4; alone it did not harm the cells in any of the present experiments, as will be mentioned again further on, while in the previously reported experiments whole guinea pig serum did not harm 6C3HED lymphoma cells when held in contact with them for 6 hours at 37°C(1). Control mixtures were included to test the viability of the cells kept under the actual conditions of each experiment, and for any effects the guinea pig serum itself might have on the lymphoma cells.

The mixtures were incubated 4 hours at 37°C, then gently centrifuged with removal of virtually all the supernatant liquid and resuspension of the lymphoma cells in the Ringer's solution buffered at pH 7.4 to which glucose 2 mg per cc had been added. Approximately 2 million of the resuspended lymphoma cells were then implanted into each flank of 3 susceptible C3H mice, with careful observation of the test animals afterwards. To insure that the *in vitro* conditions were such that any antibodies present in the test serums would have a favorable chance to act in conjunction with the guinea pig serum, 2 additional control mixtures were included in 4 experiments. These mixtures both contained, in addition to the known number of 6C3HED lymphoma cells, an antilymphoma immune serum made by injecting the 6C3HED lymphoma cells together with Freund's adjuvants into 6 rabbits and pooling the serum, which was harvested 3-6 weeks after the immunizing injections. In one of these mixtures, the immune serum was used alone, in a final dilution of 1:64; in this concentration, the immune serum was regularly devoid of effect on the lymphoma cells. In the other mixture the immune serum was used in a final dilution of 1:64, together with guinea pig serum in a final dilution of 1:4; this mixture regularly killed all the lymphoma cells with which it was held in contact in the 4 experiments.

Findings. Nine experiments were done as just described. In these, tests were made with

7 specimens of pooled serum from normal C3H mice, with 5 specimens of pooled serum from C3H mice with enlarging 6C3HED lymphomas (resulting from implantations made 5 to 14 days before bleeding), with one specimen from 10 C3H mice that had once overcome 6C3HED lymphomas and resisted challenge re-implantations made 11 days before bleeding, with 5 specimens from normal A mice, and with 5 specimens from A mice that had overcome 6C3HED lymphomas and proved resistant to re-implantation with 6C3HED cells 8 days before bleeding. In each experiment the lymphoma cells that had first been incubated in mixture with the buffered-glucose-Ringer's solution, then centrifuged, resuspended, and implanted as described above, regularly formed palpable tumors within 7 to 10 days, and these grew progressively in the implanted test mice, usually bringing about death between the 20th and 30th days. The same was true of the cells that had been exposed to the effects of either guinea pig serum alone or immune rabbit serum alone. The lymphoma cells that had been exposed to the immune rabbit serum in mixture with guinea pig serum, however, regularly failed to grow, as has already been stated. The lymphoma cells that had been exposed to the effects of guinea pig serum in mixture with the specimens of pooled mouse serum mentioned above, all grew quite as readily as did the cells from the control mixtures, the findings providing strong evidence that none of the test specimens contained isoantibodies capable of killing the lymphoma cells *in vitro* in mixture with the guinea pig serum.

To pursue the matter further, an experiment was made to compare the effectiveness of guinea pig serum against the 6C3HED lymphoma cells *in vivo* in C3H mice of a "relatively susceptible" and a "resistant" strain, respectively. This was done to learn whether the guinea pig serum might have enhanced effectiveness in the resistant mice, on the assumption—in spite of the negative indication provided by the experiments already given—that the resistant animals might conceivably have isoantibodies, or be capable of developing these rapidly, in titer sufficient to prove effective against the 6C3HED lymphoma cells *in vivo*. C3H/JAX mice (procured from the Jackson Memorial Laboratory, Bar Harbor), were used as "resistant" mice. Recent experiments made in this laboratory have shown that these animals, when implanted with 2-4 million 6C3HED lymphoma cells in each groin, regularly develop palpable tumors within 7-10 days which enlarge notably during the ensuing week and are quite comparable in size and initial course to those developing in the "relatively susceptible" strain of C3H mice customarily used in this laboratory. These latter were originally procured from Dr. L. C. Strong and have been random-bred in this laboratory during the past several years; they are here referred to as C3H/CMC mice. More than 90% of the mice of this C3H/CMC strain that have been implanted with 2 million or more 6C3HED cells during the past year have developed progressively enlarging subcutaneous tumors and died with widespread lymphomatosis some 18 to 30 days following the implantations. In the C3H/JAX mice, however, the subcutaneous 6C3HED lymphomas have regularly regressed completely during the period 12 to 22 days following implantation, and the animals have remained healthy thereafter. In the experiment made to see whether guinea pig serum is more effective against the 6C3HED lymphoma cells in resistant mice, 4 groups of the C3H/JAX mice and 4 groups of the relatively susceptible C3H/CMC strain of mice (four animals in each group) were implanted with 2 million of the lymphoma cells in the subcutaneous tissue of each groin. One group of mice of each sort was then kept under observation without treatment as controls. One hour after implantation the mice of another group of each sort was given 2.0 cc of guinea pig serum intraperitoneally, and additional groups of each sort were given 1.0 and 0.5 cc, respectively. In the control mice of the relatively susceptible C3H/CMC strain, tumors almost 1.0 cm across were palpable on the 7th day following implantation; these growths enlarged progressively, killing 2 of the 4 mice on the 20th day and a third on the 22nd day, while the fourth animal was sacrificed when moribund on the 27th day. The growths of the control untreated mice of the

resistant C3H/JAX strain were quite comparable in size to those of the untreated C3H/CMC mice during the period 7 to 12 days following implantation. On the 11th day, for example, subcutaneous tumors measuring 13 to 21 mm across were present at each implantation site in the four hosts, and these growths were approximately the same size on the 12th day. On the 14th and 15th days, however, the growths were all much smaller, and by the 18th day they had disappeared entirely from all four mice. The guinea pig serum in general proved equally inhibitory in mice of the two sorts. Thus growths failed to appear in 3 of the 4 C3H/CMC mice given 2 cc of guinea pig serum, while small bilateral growths made a much delayed appearance in the fourth animal of this group; the same was true of the group of C3H/JAX mice given 2 cc of guinea pig serum, there being complete inhibition of the growths in 3 of the 4 animals, with delayed appearance of bilateral growths in the remaining one. 1 cc of guinea pig serum gave complete inhibition of the growths in 1 of the 4 C3H/CMC mice, with delayed appearance of growths in the other 3, while this amount of serum completely inhibited growth in two of the C3H/JAX mice and delayed the appearance of growths in the remaining two. The 0.5 cc of guinea pig serum likewise inhibited the growths markedly but incompletely in the relatively susceptible and resistant mice respectively, the results being quite comparable in the 2 groups. The findings as a whole made it plain that the inhibitory effects of the guinea pig serum was not notably enhanced in the resistant mice.

Summary. In the light of a previous observation—that normal guinea pig serum is highly effective against 6C3HED lymphoma cells *in vivo* but not *in vitro*, the host animal obviously participating in the reaction *in vivo*—a number of experiments were done to learn whether isoantibodies, capable of acting in conjunction with guinea pig serum against the lymphoma cells, might be present in the serum of normal mice of susceptible and resistant strains, in that of susceptible hosts carrying 6C3HED lymphomas, or in that of mice that had overcome implanted 6C3HED lymphoma cells and proved immune to re-implantation with them. The findings are given in some detail. They provide strong but not conclusive evidence that such isoantibodies are absent from the blood of mice in all the categories mentioned above. It follows that host factors or conditions of some other sort might profitably be sought to account for the participation by the host in the reaction whereby normal guinea pig serum, injected into animals carrying 6C3HED lymphomas, brings about death of the lymphoma cells.

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Relationship of Vitamin E to Embryonic Development of Avian Eye. (21259)

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The relationship of eye disorders to vit. E has been reported in a study of retrolental fibroplasia in human infants(1), and in an eye disorder suggestive of retrolental fibroplasia in rats(2). Jensen(3) stressed the importance of vit. E in practical turkey rations in relation to embryonic mortality and hatchability. The effect of adding alpha-tocopheryl acetate to a practical type diet, with combina-

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