Aminopterin and sulfasuxidine have also been reported to cause morphological changes in the intestinal wall(17,18); and they may act, then, by interfering with some reaction taking place there, a reaction which is vital in the chain of reactions ultimately producing ascorbic acid.

Summary. Possible mechanisms by which dietary aminopterin and sulfasuxidine decrease levels of liver ascorbic acid in the rat have been investigated. In previous studies an increased rate of ascorbic acid excretion after feeding aminopterin had been found not to occur. In the present studies, dietary aminopterin has been found not to induce an increased destruction of ascorbic acid by liver homogenate or a translocation of liver ascorbic acid to other organs. Also, aminopterin and sulfasuxidine do not depress liver ascorbic acid in guinea pigs receiving an adequate daily supply of ascorbic acid. Therefore, all of these findings are considered as evidence that aminopterin and sulfasuxidine decrease liver ascorbic acid of the rat by inhibiting the biosynthesis of the vitamin. Possible mechanisms of this inhibition are discussed.

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Influence of Photodynamic Effects on Diffusion in Rabbit Dermis.* (21257)

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Castellani(1) stressed the importance of an unknown photodynamic effect on mucopolysaccharides of connective tissues, namely hyaluronic acid. *In vitro* he observed a steady reduction of viscosity of hyaluronic acid mixed with hematoporphyrin and exposed to visible light, as compared with hyaluronic acid mixed with hematoporphyrin and not exposed to light. According to the author, such reduction of viscosity is due to a depolymerization of this mucopolysaccharide.

In view of the importance which this phenomenon might have for interpretation of the pathogenesis of cutaneous diseases caused by

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TABLE I. Diffusion Areas after Intradermal Injection of 0.1 ml of a Mixture of Equal Amounts of Haematoporphyrin and India Ink. Six animals.

	Diffusion surfaces in mm²		
	Exposed to light	Unexposed	△
	209	45	164
	118	52	66
	388	71	317
	220	108	112
	98	26	72
	77	25	52
Mean	185	54	131

△ Difference between surfaces in mm².

photodynamic effects, it seemed useful to investigate it *in vivo*. The following materials have been used: A) hematoporphyrin (prepared by the Nordmarck Werke, Hamburg) at 5% dilution in distilled water. B) India ink (Pelikan trade-mark of the Günther Wagner firm). C) diphtheria toxin (prepared by the Istituto Sieroterapico e Vaccinogeno Sclavo of Siena).

Methods. Experiments were carried out on albino rabbits of the same breed, weighing approximately 2 kg each. The above mentioned substances were intradermally injected in the rabbits' back after careful shaving. The animals were inoculated in the left side and subsequently exposed to light; control injections were symmetrically given on the right side. Six rabbits received, both in the right and left sides, two 0.1 ml injections (one side each) of a suspension containing equal parts of A (hematoporphyrin) and B (India ink). The right side was covered with a wet cloth, and a black sheet of paper was fixed along the spine. The left side was exposed to the light of 3 electric bulbs for a total of 1600 W at 15 cm distance. The exposed area was then cooled by means of ventilator draught and moistened every 3 minutes so that the cutaneous temperature should never exceed 28-30'. The exposure to light went on for 2 hours, then the areas where the inoculated material had diffused were outlined in ink and traced on cellophane. Measurements were recorded on coordinate paper in order to have the surface value in mm². Data of this series are shown in Table I. Two rabbits were given 2 injections a side of 0.2 ml of a solution made with equal amounts of hematoporphyrin and diphtheria toxin: the dry toxin contained in an ampoule was dissolved in 0.5 ml of saline, which yielded a concentration 6 times higher than is normally used for the Schick reaction. The left side was exposed to light as the other series, the right side being protected against light. After 2 hours' exposure to light, the diffusion area (which was clearly visible) of hematoporphyrin was measured. After 24 hours the area of initial central necrosis and surrounding inflammatory edema was recorded. After 48 hours the area of both the edema and the necrotic hemorrhagic lesion was again recorded. Figures for this series of experiments are shown in Table II. To check whether a variation of diffusion might be due to any cause other than exposure to light, 2 rabbits were inoculated in both flanks with 0.2 ml of India ink, the left side only being exposed to light, protection against light being equally provided for the right side. To the same purpose another rabbit was inoculated in both sides with 0.2 ml of diphtheria toxin, its concentration being half that

TABLE II. Inflammation Areas after Intradermal Injection of 0.2 ml of a Mixture of Equal Amounts of Haematoporphyrin and Diphtheria Toxin.

Animal No.	2 hr*			24 hr*			48 hr*		
	A	B	△	A	B	△	A	B	△
1	130	28	102	655	128	527	1280	433	847
	100	25	75	550	64	486	570	350	220
2	99	40	59	1492	133	1359	942	263	679
	96	21	75	1370	162	1208	1283	360	923
Mean	106	28	78	1016	121	895	1018	351	667

* Time elapsed between inoculation and measurement of inflammation area.
A—Inflammation area in skin exposed to light.
B— " " " normal skin.
△—Difference between surfaces in mm².

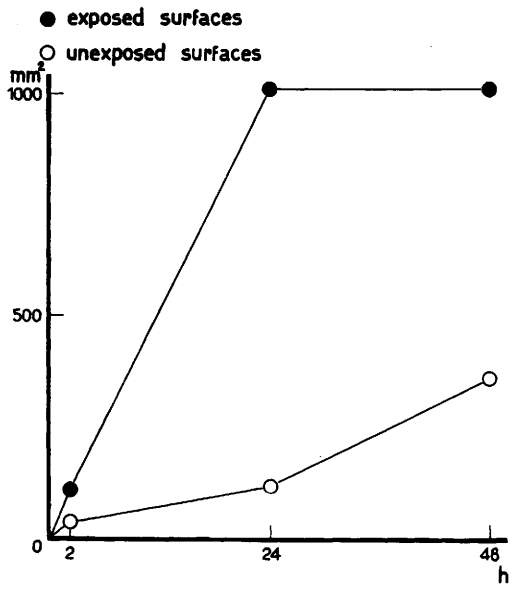

FIG. 1. Mean surface value of inflamed areas inoculated with haematoporphyrin and diphtheria toxin.

used for the previous group treated with hematoporphyrin and toxin: the final toxin concentration being therefore the same. The left side was exposed to light while the right side was protected.

Results. Besides the data shown in the tables, it should be pointed out that in the group treated with India ink and hematoporphyrin the diffusion areas of unexposed skin showed clear-cut margins, whereas the exposed skin areas were more irregular and less sharply defined. On the controls of the group treated with diphtheria toxin and hematoporphyrin the typical necrotic lesion surrounded by slight edema was clearly seen within 24-48 hours. On the contrary, the exposed skin lesions showed less necrosis, whereas the surrounding edematous area was wider and highly hyperemic. At 72 hours the necrotic lesion of the unexposed side was quite evident and remarkably large whereas that of the exposed side was much smaller with more irregular edges; the edematous zone had practically disappeared. The 2 control rabbits inoculated with India ink only, showed no appreciable difference between the skin spots that had been exposed to light and those that had been protected. No difference was observed between the exposed and the unexposed skin spots of the control rabbit inoculated only with diphtheria toxin.

For the interpretation of the phenomena observed, some reference seems indicated to the many recent observations which emphasize the relationship between the spreading power of some substances (bacteria, viruses, dyes, toxins, etc.) inoculated into the skin, and the state of the connective tissue ground substance. The spreading effect after destruction of the ground substance by either hyaluronidase or the action of various chemical substances (ascorbic acid, azoproteins) is well known. It has also been demonstrated that the degree of polymerization of the ground substance is in relation to the effect of various hormones (Opsahl, White, and Duran-Reynals(2); Lurie and Zappasodi(3); Catchpole, Gersh, and Pan(4) which thus gain more importance also for what concerns the diffusion phenomena on the ground substance. On the other hand, the above mentioned possibility of hyaluronic acid being depolymerized *in vitro* by a photodynamic action has been recently demonstrated. Since it is well known that luminous radiations easily find their way through the epidermis, it seems reasonable that the increased spreading power observed in the experiments here reported is the result

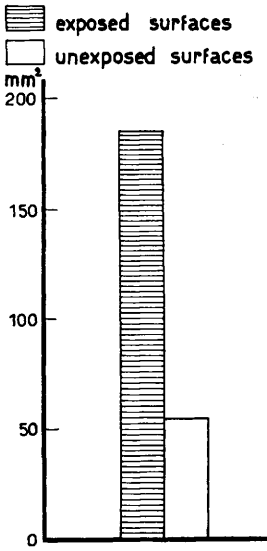

FIG. 2. Mean surface value of inflamed areas inoculated with haematoporphyrin and India ink.

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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