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Effect of Subcutaneous Injury on Tumor Growth in the Mouse.*

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Several devices were explored for inducing inflammation under the skin of the mouse, to be followed by tumor implantation. Among these were the injection of acid, alkaline, or distilled water; the implantation of a small wad of glass wool; the injection of an air bleb, alcohol, turpentine, or histamine; and mechanical separation of the skin from the abdominal muscle wall. None but the last mentioned of these irritants produced consistent alterations in the growth of implanted tumor fragments. The type of inflammation induced by mechanical injury was, therefore, studied in detail in respect to its effect on tumor growth, and this effect comprises the main subject of this paper.

Procedure. A sharp-pointed 3-inch surgical scissors was inserted under the skin in the inguino-abdominal region and directed anteriad to a point about one-fourth inch from the axilla. With the position of the scissors' fulcrum lying at the point of original entry, the scissors was opened broadly so that the outer surfaces of its shears radically cleaved the skin from the underlying muscle tissue, without however puncturing the skin. Local bacterial infection was not evident in mice so treated, even when rigid surgical asepsis was not observed.

Groups of mice were so prepared for tumor implantation. Into some of these mice, fragments (approx. 2 mm^2) of sarcoma 180 were implanted immediately after the cleavage operation by inserting a trocar through the incision made by the scissors; the seeding fragment was deposited as close as possible to the center of the cleaved area. Fragments of comparable size and from the same donor tumor were implanted in the corresponding region of mice which had not been given the cleavage treatment; these served as controls.

Additional experiments were set up in which groups of cleavage-treated mice received tumor implants at various intervals after cleavage: at 24 hours after cleavage, at 48 hours, 72 hours, 6 days, and 12 days. In each instance tumors were implanted into parallel control groups of non-cleaved animals. The implanted fragment was allowed to grow for the usual 6 to 7 day period, at the end of which the tumorous growth was dissected out and wet-weighed. Before weights were taken, a puncture was made of the dissected tissue mass and exudative fluid was drained off. Rockland white male mice of 18-22 g were used throughout.

Results. For the first 4 days following fragment implantation into the cleaved subcutaneous area, tumor growth appeared to progress normally and was indistinguishable topographically from that of the controls. Between the 4th and 6th day after implantation a very marked swelling would become apparent in the area where the implant was deposited. Within 24 to 48 hours after the onset of this swelling, the experimental tumor appeared to attain a mass of from 5 to 10 times that of the control tumor (Plate I, Fig. 1 & 2). Upon dissection it was found that an extensive vesicle had developed throughout the cleavage area; and that a bleb of exudate had formed in close association with the growing tumor fragment (Plate I, Fig. 3). The vesicle fluid accounted for about half the total volume of the swelling. By the 9th day a hardening of the soft mass became evident. By the 12th day the edematous fluid was considerably replaced by the ingrowing tissue; the mouse in all such cases died before this process was complete, death usually occurring between the 12th and 15th day after implantation.

Animals implanted with tumor fragments immediately after cleavage exhibited the

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TABLE I.

Weights of tumors (sarcoma 180) dissected from mice during the 6-7 day period after implantation. Control mice had received no previous treatment; others were subjected to skin-muscle cleavage at various intervals before implantation (see text).

		Interval between cleavage operation and time of tumor fragment implantation			
Exp. No.	Control g	0-hr g	24-hr g	48-hr g	72-hr g
1	.15	.72	.47		.58
	.28	.39	.21		.42
	.15	.59	.47		.27
	.30	.22	.73		.12
	.20	.63	.12		.41
	.06				
	.25				
2	.20			1.00	
	.18			.45	
	.33			.54	
	.33			.41	
	.24			.67	
				.66	
				.16	
3	.25	.77	.48		.32
	.18	.89	.53		.47
	.18	.55	.63		.54
	.33	.47	.13		.24
	.20	.47	.86		.41
	.42	.19			.44
	.23				.20
	.18				
4	.46	.69	.46	.62	
	.51	.45	.31	.84	
	.26	.36	.33	.39	
	.29	.25	.81	.51	
	.27	.45	1.17	.61	
	.31	.71	.70	.70	
		.45	.18	.21	
		.87			
5	.39	1.10			
	.26	.86			
	.24	.31			
	.20	.96			
	.16	.28			
	.22	.74			
		.90			
Mean tumor wt	.25	.59	.50	.54	.36

above-described effect to an extent not distinguishable from that in animals in which the implantation was made 24 or 48 hours after cleavage. The effect showed signs of lessening in animals implanted 72 hours after cleavage; was barely apparent in those implanted 6 days after cleavage; and was completely absent in animals implanted 12 days after cleavage, at which time, apparently, complete healing of the original injury had occurred.

The data of Table I indicate that the mean weight of tumor tissue from cleaved animals sacrificed 6 to 7 days after implantation was in the order of twice that of the non-cleaved controls. It was noted, on the other hand, that had weighing been done at a later date, say during the 8-10 day period, the difference between cleaved and control groups would probably have been recorded as considerably greater. In Table I it will be noted, too, that the growth-enhancement effect occurred in

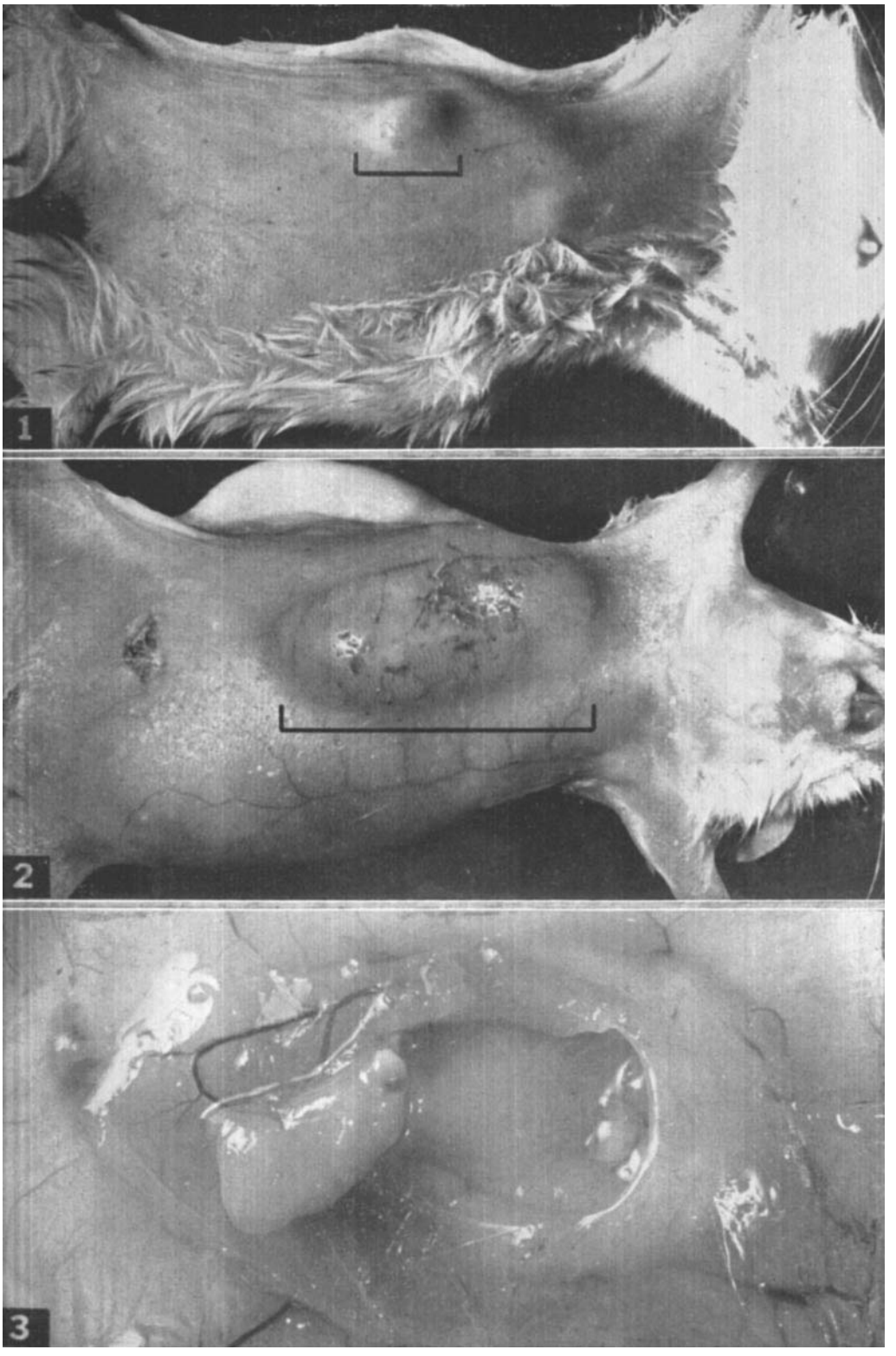
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PLATE I.

Abdominal aspect of epilated mice to show relative growth of sarcoma 180 implant.
FIG. 1. Control tumor at 6-7 day stage (approx. ×2).
FIG. 2. Cleavage tumor at 6-7 day stage (approx. ×2).
FIG. 3. Cleavage tumor with skin removed and edematous bleb cut open (approx. ×4).

about 80% of the cleavage-treated mice, the remainder showing normal tumor growth. Occasionally an animal would lose its newly implanted fragment; such animals were rejected from the experiment.

Exudate withdrawn by syringe from the edematous bleb 6 days after tumor implantation was a pale pink serum, of pH 7.6, which when examined fresh or as a Wright's stain smear showed a sparse distribution of erythrocytes, some cells resembling lymphocytes and monocytes, and some polymorphonuclear leucocytes—a picture confirmed in Bouin-fixed hematoxylin-and-eosin sections of the tissue and bleb mass. At the interface of the serum and its encasing tissue (which appeared to be an intermixture of proliferating sarcoma cells and connective tissue elements) loose strands and masses of tumor cells extended inward, containing many cells in active mitosis (Plate II, Fig. 3, 4, & 5). The occurrence of such an inward infiltration was confirmed by examination of tissue fixed at 6, 8, 10, and 12 days after implantation. Very clearly, the induration of the mass palpably observed during the 8-12 day period was due to this ingrowth. Interspersed among the tumor cells at the growing fringe at the aforementioned interface were polymorphs, although their number was not such as to suggest suppuration, except in later stages when necrosis in the center of the vesicle often became more marked. Concomitant with tumor growth and the thickening of the vesicle wall, the primary tumor mass became undemarcably involved.

In further experiments a number of mice were subjected to the cleavage operation, but were not implanted with tumor tissue. Mice so prepared were killed at 1-day intervals following cleavage; the abdominal skin was incised and retracted so that observation could be made of the gross damage resulting from the cleavage. In most cases the inner surface of the cleaved skin showed some extravasation of blood, derived, obviously, from rupture of the larger vascular channels of the connective tissue during the scissors-cleavage procedure. The visible remains of such extravasation diminished after the 3rd day, and was no longer observable after the 6th. Restoration of fusion of the skin with the abdominal wall was not clearly complete until about the 5-6 day stage, as could be determined by the relative ease with which the skin could be withdrawn from the abdominal wall during dissection. Suppuration was never observed. The inner skin surface in the region cleaved showed some hyperemia, but never in conspicuous degree, and this hyperemia disappeared during the 3-6 day period. None of some 30 cleavage-control animals examined showed any discernible swelling of the area or any external evidence of edema.

The pronounced exudation observed in cleavage animals receiving tumor implants appeared to result from a specific interaction between the actively proliferating tumor tissue and the injured connective tissue substrate. Evidence of such a specificity was derived from an experiment in which fragments of muscle, tongue, intestine, spleen, and liver, instead of tumor tissue, were implanted into the cleaved area. In no instance did a palpable edema develop. The implanted non-tumorous tissue fragments were eventually resorbed without incident.

Comment. To interpret the interaction between tumor growth and the type of subcutaneous injury herein described, it may be of advantage to picture the events occurring under the mouse skin following cleavage injury. Moreover, it is necessary to consider what occurs when a tumor fragment is implanted in the usual fashion into non-injured subcutaneous tissue. In the latter case, the insertion of the trocar probably does not tear but merely distends the connective tissue. Thus the little damage that may occur is almost mechanically repaired at withdrawal of the trocar. Similarly, when the fragment is deposited, the connective tissue is stretched somewhat, but not

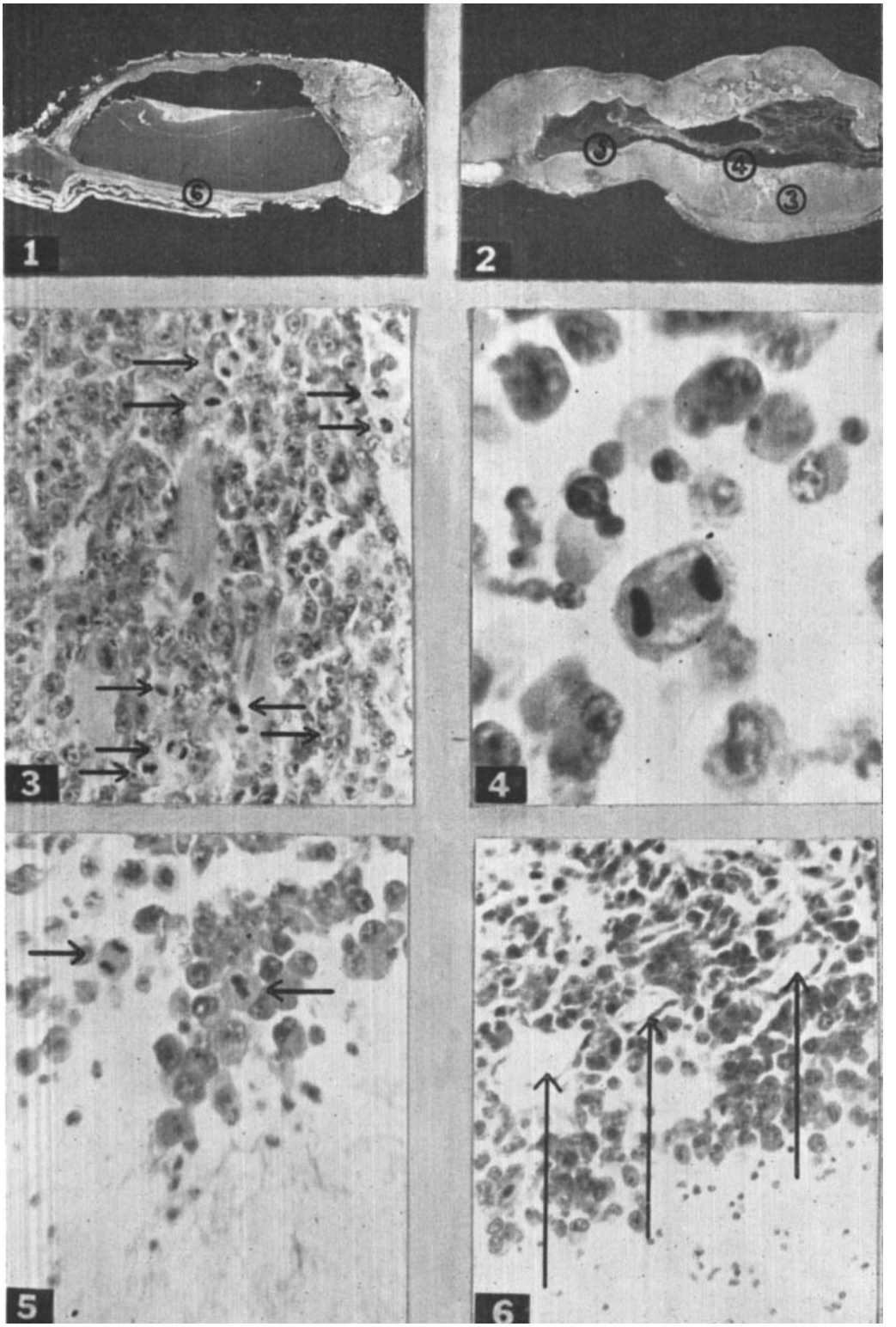
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PLATE II.

FIG. 1. Shadowgraph of median sagittal section of 6-7 day cleavage tumor (approx. ×2.5).
FIG. 2. Shadowgraph of median sagittal section of 9-10 day cleavage tumor (approx. ×2.5).
FIG. 3. Area from Fig. 2, as indicated. Note large number of mitoses (high dry).
FIG. 4. Area from Fig. 2, as indicated, to show mitotic sarcoma cell (oil immersion).
FIG. 5. Interface between vesicle fluid and growing tumor surface, from Fig. 2, as indicated. Note mitoses (high dry).
FIG. 6. Interface between vesicle fluid and growing tumor surface, from Fig. 1, as indicated. Note new capillary channels (high dry).

radically torn. During such normal implantation procedure there is little or no vascular rupture nor extravasation of blood.

The initial obvious reaction of the connective tissue to a tumor implant is one of classical imflammation, involving, hyperemia, appearance of polymorphs, and subsequently of mononuclear leucocytes.[1-3] The connective tissue area surrounding the new implant almost immediately becomes hyaline, and within a few days becomes infiltrated as a firm and expanding tumor mass. The original fragment does not change appreciably in size or shape (although its cells start undergoing degenerative changes almost at once after implantation) until about the 9th day when a fusion with surrounding necrotizing tissues renders its outlines and structure somewhat amorphous.

Following implantation of a tumor fragment, it is probable that phagocytic cells—as they do in a localized bacterial infection—attack the tumor cells at the periphery of the fragment. The tumor cells obviously win in the ensuing competition, for they proliferate vigorously and at the same time stimulate the growth of capillaries and, later, of larger vascular channels; in the meantime attracting large numbers of reticulo-endothelial elements. One of the fundamental differences between malignant and non-malignant tissue lies in the former's capacity to survive phagocytic or lytic onslaughts and to excite vascular proliferation in the substrate tissue.

Is the explosive edema described in this paper a tissue shock reaction due to the super-permeability of cleavage-inflamed capillary walls? If so, why is it elicited specifically by a tumor fragment and not at all by a normal tissue implant? It seems probable that cleavage injury, being vastly greater than that produced during simple trocar implantation, excites an excessive inflammatory and reparative response on the part of subcutaneous tissues involved. When into this dynamically unstable substrate a fragment of tumor tissue is implanted, the proliferative and stimulatory factors presumably released by the metabolizing tumor fragment find a richly vascularized and nutritious substrate in which to act or re-act.

The type or irritation produced by turpentine has been reported as not affecting carcinogenesis by benzpyrene,[4,5] which is consistent with our failure to note tumor growth enhancement by turpentine as well as by the variety of other irritating agents tested. Croton oil, on the other hand, has been reported as markedly augmenting chemical carcinogenesis, presumably by the exertion of a precarcinogenic action on skin tissues so treated.[6] Perhaps more cogent to the present discussion is the report that deep incisions made in the skin of mice previously treated by carcinogenic compounds showed localization to some extent of subsequent tumor growths.[6] It has been suggested, too, that cancer cells find a favorable environment in the region of healing wounds.[7] Whether such actions as these are related or analogous to the type of injury effect described in this paper is difficult to determine, due mainly to the variety of technics and the diversity of tumor types em-

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ployed.

Summary. When a subcutaneous inflammation is induced in the mouse through mechanical injury, and when into such a traumatized site a fragment of experimental sarcoma is deposited, a striking edematous reaction ensues, accompanied by a conspicuous acceleration of tumor growth; these associated phenomena being well defined by the 4th to 6th day after implantation.

This effect is construed as a specific interaction between the neoplastic characteristics of the tumor fragment and the cellular and/or vascular sequences of subcutaneous inflammation. The effect does not occur when nonmalignant tissues are similarly implanted.

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Veratrinic Effects of Pentamethylenetetrazol (Metrazol) and 2,2-Bis (P-Chlorophenyl) 1,1,1 Trichloroethane (DDT) on Mammalian Neuromuscular Function.*

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An extraordinarily diverse group of agents produces in excitable tissues a stereotyped, veratrinic effect: a single brief stimulus evokes a burst of repetitive responses.[1,2] Incomplete evidence indicates that the appearance of the veratrinic response in nerve fiber always is accompanied by a similar alteration in muscle fiber, and suggests that repetitive response is a general phenomenon which occurs in all excitable tissues exposed to veratrinic agents.

To explore further this general phenomenon, the study reported here was made of two agents which produce altered states of excitability. *Pentamethylenetetrazol* (metrazol) was selected because of its predominant effect on central neural activity and investigated for its effect on the peripheral neuromuscular unit. *2,2-bis (p-chlorophenyl) 1,1,1 trichloroethane* (DDT) was studied in the mammalian preparation because recent extensive investigations have demonstrated its potent veratrinic effect on the peripheral nerve of both Hexapoda and Crustacea.[3,4]

Methods. Details of the preparation and recording technics have been described.[2] Briefly, adult rats under pentobarbital anesthesia were used throughout. Isometric myograms were made from the triceps surae by means of resistance-wire strain gauges and recorded by means of a cathode ray oscillograph. Electromyograms were recorded through needles placed in the belly of the muscle. Electroneurograms were recorded from the sciatic-tibial nerve which had been isolated centrally and peripherally, Square-wave supramaximal stimuli were delivered to the sciatic nerve or directly to the curarised muscle. Metrazol was injected intraperitoneally (i-p) in doses of 25 to 100 mg/kg. DDT† was suspended in peanut oil-saline homogenate by means of lecithin (Asolectin)‡[5] and injected i-p in doses

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† A twice-crystallized sample was kindly provided by the Medical Division, Army Chemical Center, Edgewood, Md.

‡ Generously provided by Associated Concentrates, Inc., Woodside, Long Island, N. Y.