Induced Hypersensitivity to Cold

Abstract. *Extreme hypersensitivity to cold can be induced in the rat by a single subcutaneous injection of carrageenin. In animals so treated, exposure to cold induces necrotizing thrombohemorrhagic lesions in the nose, paws, and tail, accompanied by renal lesions resembling those of the generalized Sanarelli-Shwartzman phenomenon. The same treatment elicits hemorrhagic necroses of transplants of Murphy lymphosarcomas in rats.*

In man, extreme hypersensitivity to cold has often been attributed to cold agglutinins, because at low temperatures these cause conglutination of erythrocytes with thrombus formation in the microcirculation. Such changes appear to play an important role in paroxysmal cold hemoglobinuria and in certain forms of Raynaud's phenomenon (*1*). These two conditions frequently develop in association, and they may lead to symmetrical cyanosis, hemorrhage, or even necrosis, especially in the acral regions (hands, feet, nose) when the patient is exposed to cold (*2*). Hence it may be of interest to describe an experimental model of chemically induced extreme sensitivity to cold in the rat; this sensitivity is associated with similar acral thrombohemorrhagic lesions and can be reliably reproduced by a single subcutaneous injection of carrageenin.

Twenty female Sprague-Dawley rats of the Holtzman strain, with an average body weight of 102 g (range 98 to 108 g) were subdivided into two equal groups, one of which was given 200 mg of carrageenin (Marine Colloids Inc.) in 5 ml of distilled water by subcutaneous infiltration on the entire back to permit easy absorption. Three days later, both groups were exposed to minus 2°C in a refrigerated room (five rats per cage) for a period of 2½ hours. The controls showed no obvious morbid lesions, whereas among the carrageenin-treated animals four died, and these, as well as the survivors, developed first an intense cyanosis and then thrombohemorrhagic necrosis of the nose, paws, and tail (Fig. 1). Upon autopsy next day, these animals (unlike the controls) exhibited bilateral renal cortical necrosis with pronounced reddish-purple discoloration of the renal medulla.

Histologically, the acral cutaneous lesions were characterized by the formation of erythrocyte agglutination and fibrin thrombi in the microcirculation (especially the veins and capillaries) with hemorrhages. In the renal cortex, necrosis of the tubules was associated with formation of fibrin thrombi and agglutination of erythrocytes in the glomerular capillaries. The medulla showed extreme dilatation of the vasa recta with perivascular hemorrhages. There was also hematuria and hemoglobinuria. The lesions showed individual variations of degree, but all carrageenin-treated animals gave markedly positive responses.

Thus, microscopically, the cutaneous and renal lesions were virtually indistinguishable from those characteristic respectively of the local and generalized reactions of the Sanarelli-Shwartzman phenomenon elicited by bacterial endotoxins.

A second experiment was designed to establish the optimal conditions for the production of this form of cold sensitivity. It was found that the minimum amount of carrageenin necessary for this purpose is between 25 and 50 mg and that thrombohemorrhagic lesions limited to the paws and tail can be obtained if, instead of placing the animals in a refrigerated room, they are merely forced to walk on a plate of ice for 5 hours at room temperature. Under these conditions, renal lesions do not develop and there is no mortality. When animals are thus tested the cold sensitivity begins to be evident on the 2nd or 3rd day after the injection and remains demonstrable for several weeks.

Since Shwartzman-active substances are known to produce hemorrhagic necrosis in tumors, a third experiment was performed under identical conditions on rats which had been inoculated with a suspension of Murphy rat-lymphosarcoma tissue into the thigh, 7

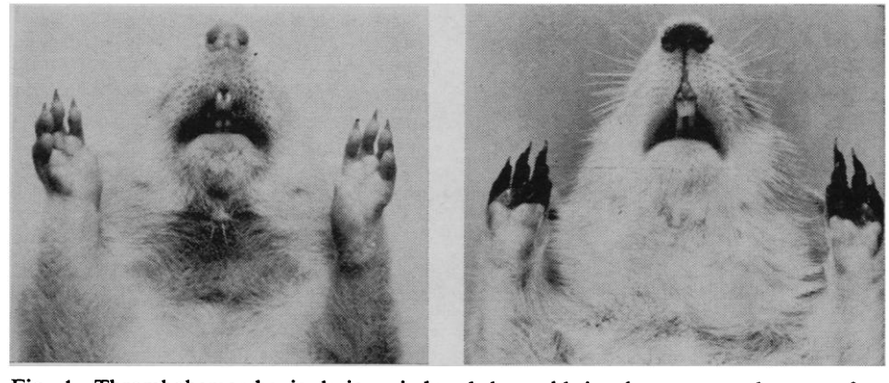

Fig. 1. Thrombohemorrhagic lesions induced by cold in the paws and nose of a rat sensitized by carrageenin. Left: before exposure to cold; right: after exposure.

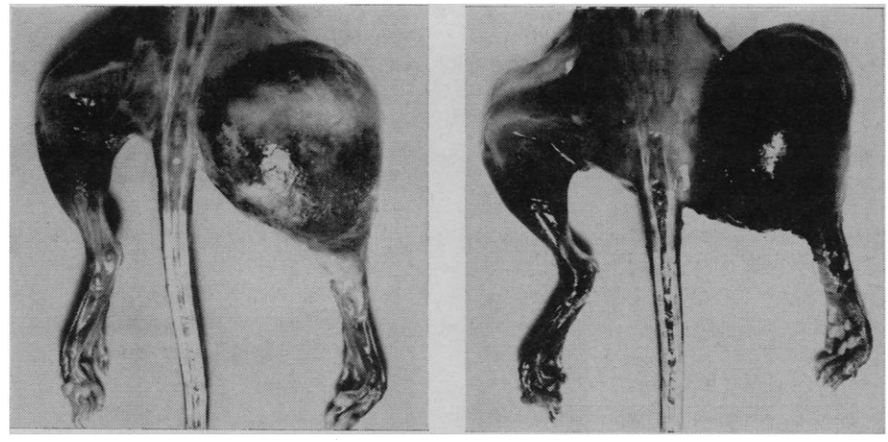

Fig. 2. Thrombohemorrhagic necrosis of a tumor in a rat sensitized to cold by carrageenin. Left: control; right: after exposure to cold.

days before the carrageenin injection. Exposure to cold surrounding temperature (as in the first experiment) 3 days later elicited the usual cutaneous and renal lesions but, in addition, the tumor transplants underwent severe hemorrhagic necrosis (Fig. 2). Controls receiving the same carrageenin treatment without exposure to cold exhibited no hemorrhagic lesions in the tumor transplants.

This model may be of use in the study of the so-called diseases *a frigore*; it also furnishes us with a new technique for the production of hemorrhagic necrosis in tumors.

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References and Notes

1. D. G. McKay, *Disseminated Intravascular Coagulation: An Intermediary Mechanism of Disease* (Harper and Row, New York, 1965), p. 493; A. Linke, *Arch. Dermatol. Syphilol. (Berlin)* **191**, 123 (1949); P. E. Hansen and M. Faber, *Acta Med. Scand.* **129**, 81 (1947).
2. D. Stats and J. G. M. Bullowa, *Arch. Internal. Med.* **72**, 506 (1943); W. Baumgartner, *Helv. Med. Acta* **15**, 411 (1948).
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Phytotoxin Isolated from Liquid Cultures of Ceratocystis ulmi

Abstract. *Phytotoxic material has been isolated from liquid cultures of* Ceratocystis ulmi. *One component of the material has been obtained in pure form and has proved to be a rather thermostable glycoprotein. This compound induced disease symptoms, similar to those produced by the fungus itself, in elm sprouts and trees.*

Phytotoxic material present in the filtrate of a nutrient medium in which *Ceratocystis ulmi* (Buisman) C. Moreau had been grown was discovered by Zentmyer (*1*) and the production of toxic substances has been confirmed (*2–5*). To Zentmyer and Horsfall (*6*) this thermostable toxic material appeared to consist of more than one component, one of which Dimond (*2*) characterized as a polysaccharide. Feldman *et al.* (*3*) and Lafayette and Howard (*7*), however, considered this component as rather unimportant in relation to the pathogenicity of the fungus. Beckman (*5*) showed a pectinase and cellulase to be present in the culture filtrate.

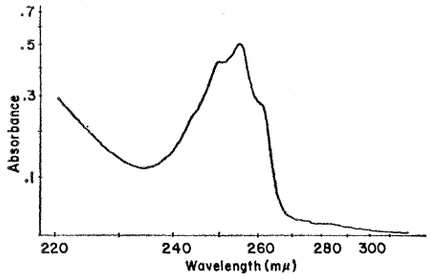

Fig. 1. Ultraviolet absorption spectrum of compound A.

In our experiments *Ceratocystis ulmi* was cultured at 24°C in a nutrient medium of the following composition (per liter of tap water): glucose, 20 g; L-asparagine-monohydrate, 2 g; KH_2PO_4, 1.5 g; $MgSO_4 \cdot 7H_2O$, 1 g; $ZnSO_4 \cdot 7H_2O$, 20 mg; $FeCl_3$, 10 mg; pyridoxin, 1 mg; and thiamin, 1 mg. After 2 to 3 weeks the mycelium was separated from the shake culture by centrifugation and filtration.

The culture filtrate, all fractions, and all substances derived from it during the process of isolation and purification were tested for their toxicity to elm sprouts. Sprouts were raised from root callus according to the method of Tchernoff (*8*); these had the advantage of being genetically homogeneous. All sprouts originated from the susceptible elm *Ulmus hollandica* Mill, clone *belgica*. Each sprout was placed with its base in an aqueous solution of an isolated fraction or substance in concentrations that were present in the original filtrate. The control consisted of similar shoots placed in water, in nutrient medium, and in culture filtrate. If the isolated fractions were toxic, the sprouts showed some of the typical symptoms of elm disease, that is, young leaves wilted and died, older leaves curled upward or developed necrotic spots between the veins. Saplings of the *belgica* clone, aged 1 to 2 years, were used for testing the toxicity of the substances isolated from the culture filtrate. Solutions were taken up by wounds cut into the stems (*9*). For control, spore suspensions, culture filtrate, or water was administered to the trees.

Concentration of the culture filtrate 15 times (in a vacuum) and addition of an equal volume of ethanol to the concentrate caused the precipitation of a polysaccharide. After filtration of the solution and evaporation of the ethanol, the concentrate (*p*H 3.5 to 4) was dialyzed against running tap water until the *p*H of the solution became 5; above *p*H 6.5 loss of phytotoxicity might occur (*3, 10*). Therefore, after 2 days the process was continued with 0.05*M* pyridine–acetic acid buffer (*p*H 4.8). The buffer was changed once a day for 1 week. Concentration and subsequent freeze-drying of the remaining colorless and viscous solution yielded 50 to 150 mg of a white powder (per liter of culture filtrate) which was highly soluble in water and rather thermostable with regard to toxicity. Ultracentrifugation of this substance in 0.1*M* NaCl revealed inhomogeneity. A main compound (A) comprising approximately 95 percent of the total amount, with a molecular weight of about 25,000, and a polydisperse minor constituent (B), with a molecular weight of about 10^6, were present. So far it has not been possible to separate the components from each other by gel filtration on Sephadex G-100 or on Biogel P-100. However, through preparative ultracentrifugation of crude toxin (A + B) isolated from 10 liters of culture filtrate we obtained 300 mg of the pure compound A.

The compound B has not yet been isolated in pure form. Its phytotoxic properties are therefore still unknown. The toxin (A + B) induced in the shoots phytotoxic phenomena similar to those induced by the culture filtrate of *Ceratocystis ulmi*. It appeared to be rather thermostable with regard to toxicity. This material did not possess the activities of pectinase and cellulase as reported by Beckman (*5*). To each tree of a group of 25, a dose of 25 to 200 mg of crude toxin (A + B), dissolved in 5 ml of water, was administered. The quantity used depended on the size of the treated elm. The discoloration of the leaves, the vascular browning, and the plugging of the vascular system with gums and tyloses were similar to those induced by the culture filtrate and also similar to the initial symptoms shown by susceptible trees after inoculation with a spore suspension. Compound A, when applied to shoots, appeared to be as effective as the crude toxin, even in concentrations as small as 10^{-5} mole/liter.

Compound A proved to be a glycoprotein. Figure 1 shows the typical ultraviolet absorption spectrum of this substance. The protein moiety probably consists of one peptide chain, as shown by oxidation of the glycoprotein with sodium periodate and isolation of the product by paper electrophoresis. Total