constantly exposed to strong light, my copy, which I have mounted on strong cardboard and keep folded when not in use, has not changed perceptibly in nearly a year. The chart is known as the "Fischer Color Chart" and may be obtained from the Secretary of the New England Gladiolus Society, Mr. C. W. Brown, 13 Park Road, Ashland, Massachusetts.

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# SPECIAL ARTICLES

## A GROUP OF CHEMICALS ACTIVE IN INCREASING TISSUE PERMEABILITY AND ENHANCING CERTAIN INFECTIOUS PROCESSES

ATTEMPTS have been made to isolate and identify the factor demonstrated in testicle and certain other normal tissues, which has the property of increasing tissue permeability and enhancing the lesions resulting from infectious agents. Recently it has been shown (Goodner, Duran-Reynals) that certain invasive micro-organisms possess an extractable substance with similar properties.

In the course of this study, many chemicals and various tissue components as widely related as histamine, tyrosine or nucleic acid have been repeatedly tested in different ways with the hope of tracing the specific properties to some known physiological agents. However, the substances so far investigated have failed to show any power in increasing tissue permeability or augmenting infectious lesions.[1]

When working on the fractionation of testicle extract and the isolation of the soluble factor from staphylococcus, it was found that the activity in solutions would always correspond to a strong diazo reaction, while inactive fractions discarded during the purification would regularly give negative chemical tests. This observation indirectly led to the trial of pure diazo-compounds on dermal permeability. These proved to possess the property of reproducing the effect so far obtained only with extracts from certain tissues and virulent bacteria. The present paper deals with the activity of the diazide of sulfanilic acid under various conditions.

The p-sulfanilic acid itself from which the reagent is prepared is entirely inert, and the response is never different from that of the control. On the other hand, when a certain quantity of the diazonium derivative, namely p-diazo-benzol-sulfonic acid, is dissolved in water by means of sodium hydroxide and intradermal injections are made with India ink as indicator, this is followed by sudden and complete diffusion of the material injected. However, contrasting with the speed with which diffusion takes place, one observes a limitation as regards the area involved, and the surface of diffusion can not be appreciably increased even by the use of much more concentrated solutions.

The explanation of this fact was that we were dealing with a substance rather soluble in water which would first spread rapidly through the tissue spaces but would soon be taken up by the larger lymphatic vessels and carried away into the circulation. Consequently, it was thought that more favorable conditions for the production of the phenomenon could be obtained if the active reagent were linked to a larger molecule. Coupling of the diazide with gelatin, casein, egg albumin and horse serum was attempted. The preparations obtained from gelatin and casein were not very satisfactory. On the other hand, the diazo compound reacts readily with egg albumin and serum. These proteins can absorb large amounts of the reagent if adequate alkaline reaction is provided, and they yielded very active preparations.

The intradermal injection in rabbits of 0.5 cc of such azo-proteins with 0.25 cc of India ink suspension as indicator results in a gradual spreading of the material and the diffusion may continue to progress during several hours. The ink particles are almost completely carried away from the site of inoculation and in certain instances preparations can be made so that the diffusion area easily covers more than 120 sq. cm of the skin. The ink diluted with Ringer's solution and injected intradermally in the same amount remains for some time in a distinct bleb and its eventual diffusion is limited to a small area immediately around the site of injection and rarely greater than 4 sq. cm.

Moreover, the diazo-compound so combined with albumin or serum also possesses the property of enhancing infectious processes. Tested with progressive dilutions of vaccine virus it not only has the power markedly to increase the size of the local lesions (45 sq. cm as against 6.0 sq. cm for the control in a typical experiment), but exhibits a definite enhancing effect. In the higher dilutions, while the control injections remained completely negative, the same material with the azo-protein was able to develop typical and well-defined lesions.

[1] McClean has reported that certain protamines possessed the property of increasing dermal permeability, but inhibited infectious agents through their antiseptic power. However, these protamines were extracted from fish sperm and, in a recent private communication, McClean stated that by means of fractionations carried out with Morgan, he was able to refer their action on tissue permeability to the testicle factor itself contaminating the sperm proteins.

Experiments in view of determining which group in the molecule is actually responsible for this specific activity are under way, and other diazo- and azo-compounds are being tested in this respect.

The study has not progressed to a point where it is possible to discuss the chemical relationship between the synthetic spreading factor and the biological one obtained from certain tissues and from invasive bacteria. As far as can be judged, their activity in increasing tissue permeability and enhancing infections is identical, and no other chemicals have been found which bring about these results. Recently a crystalline product has been obtained from testicle extract and from *Staphylococcus aureus* extract. In the latter this product is comparatively easily isolated, and even after five consecutive recrystallizations it retained the typical spreading and enhancing properties. The chemical constitution of this material is being investigated.

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## A PHYTOPHTHORA DISEASE OF SNAPDRAGONS

DURING March, 1932, a wilt disease of snapdragons appeared in several greenhouses near San Leandro, California. Approximately 50 per cent. of the plants were either killed or so badly injured that they were of no commercial value. As a result of the damage done by this disease several thousand dollars' worth of flowers were destroyed. The grower was not familiar with the trouble and had no conception of what was doing the damage. He thought it might be due to improper soil or water conditions. The disease was found in all parts of the affected houses. Plants were observed in all stages of infection ranging from those on which a few stem branches were wilted to those which were browned and drying up. A careful examination showed that the outer tissues at the bases of the stems and on the larger roots were decayed. On a good many of the plants the cortical tissue was so badly decayed that it had become separated from the plant, resulting in the wilted condition. On some of the plants only a very narrow band of living tissue on one side of the stem was keeping the plant alive. The lesions were found at the ground line or beneath it. They ranged in color from a very light yellow to a deep brown. Affected tissue appeared at first to be water-soaked. As the disease progressed the lesions became sunken, followed by sloughing off of the outer tissues, thus eliminating all the growing part of the plant stem. On a few of the plants the fungus was observed attacking branches several inches above the surface of the soil, but this occurred only when the plant stems were very wet.

Isolations were made from tissues and three organisms were found; a bacterium which produced a yellow culture, a fungus *Cephalosporium acremonium* Corda, and a Phytophthora which has been tentatively identified as *Phytophthora cactorum* (Leb. and Cohn) Schrt. These three organisms were repeatedly isolated from diseased tissue both separately and together in mixed cultures. A pure culture of each organism was used to inoculate plants under greenhouse conditions. Snapdragon plants were grown from seed in sterilized soil until they were near the blooming stage. They were inoculated with cultures of the fungi which were grown on corn-meal and sand and with a spore suspension of the bacterium. Inoculations were so made that each organism was placed on plants alone and in combination with the other two. As a result of these inoculations it was found that only the Phytophthora was pathogenic on the snapdragon plants. Neither the Cephalosporium fungus nor the bacterium gave any indication of being pathogenic. All the plants exposed to the Phytophthora fungus wilted down within two weeks of exposure and showed symptoms identical with those noted in the greenhouse at San Leandro. The Phytophthora fungus was reisolated from all the inoculated plants which became diseased. Tissues from the diseased plants were fixed, sectioned and stained. A study of these slides shows that the fungus belongs to the *Phytophthora cactorum* group of Phytophthora. Studies are now in progress covering the growth of the fungus on various kinds of media. An effort was made to trace the source of the infestation in the greenhouse. It was found that one of the sources of the water in the greenhouse was contaminated and also the compost soil used by the grower was infested. The disease was entirely eliminated during the past winter season by using clean water and sterilizing all soil from the compost pile. Work on this problem is continuing and a more complete account of the study will be given in a later paper.

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