Officers for the coming year are:

President: V. O. Graham, 4028 Grace Street, Chicago.
First Vice-president: T. H. Frison, State Natural History Survey, Urbana.
Second Vice-president: C. R. Moulton, Museum of Science and Industry, Chicago.
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Editor: Grace Needham Oliver, State Geological Survey, Urbana.
Junior Academy Representative: Miss Audry Hill, Chester High School, Chester.

Winners of the American Association for the Advancement of Science academy grants in aid for research for 1940–41 were:

Dr. F. O. Green, Department of Chemistry, Greenville College.
Dr. C. L. Furrow, Department of Zoology, Knox College.
Dr. F. R. Cagle, Southern Illinois State Normal University.

R. F. PATON,
Secretary

SPECIAL ARTICLES

DIMINISHING RESPONSE OF THE SKIN TO FREQUENTLY REPEATED REINFECTION WITH INVASIVE BACTERIA

IN experiments concerned with the mode of action of sulfanilamide we wished to make observations on the difference in the response to the drug by a primary infection as compared to the response found once an infection had been established. In devising the experiments we felt that injecting four or five small doses of the bacteria in different parts of the skin, at intervals of a few hours, would give more information than injecting a single large dose. As a preliminary step leading to this study, rabbits were injected intradermally, following this procedure but without sulfanilamide being administered. The main purpose of this preliminary note is to describe a phenomenon which was encountered constantly in the course of these studies. Details of the experiments will be published in the *Yale Journal of Biology and Medicine.*

Two groups of bacteria were studied, (*a*) two strains of *Streptococcus hemolyticus* and two strains of *Staphylococcus aureus,* which were invasive bacteria and produced large amounts of spreading factor, and (*b*) two strains of *E. coli* and one of *S. anolium,* which were not invasive and did not produce spreading factor. Broth cultures of exactly the same age were employed for the injections in each rabbit. In general, the amount of bacterial culture administered in each injection was that which when injected for the first time into rabbits would induce lesions measuring from 10 to 20 sq cm after 24 hours. For instance, when streptococcus was employed 0.1 cc of 18-hour broth culture was usually injected; when *E. coli* was employed 0.5 cc of culture were injected.

It was found that when the invasive bacteria were injected in four or five identical doses within a period of from one to twenty-four hours, using a different part of the skin for each injection, the resulting lesions, measured twenty-four hours after injection, showed marked differences both in severity and in the area through which the infection had spread; the second lesion being smaller and less severe than the first; the third smaller than the second, and the fourth and fifth, when present, smaller and much less severe than all the lesions resulting from earlier injections. These results were not affected by the site injected. The phenomenon may distinctly manifest inself as early as one hour after the first injection. Frequently the area of the lesion resulting from the last injection was as much as fifteen times smaller than that resulting from the first; indeed, oftentimes such lesion was no more than a pimple.

This phenomenon of the diminishing skin response was completely absent in infections caused by *E. coli* and *S. anolium.* However, when larger doses of these non-invasive bacteria were given so as to induce a more severe first lesion, a slight decrease in the response to the following injections was sometimes observed; and if the same amounts of bacteria were injected together with spreading factor either from streptococcus or from testicle extracts so that very large first lesions were induced, then the phenomenon was clearly present.

Antibodies were not found in the serum by the usual serological tests, and blood counts made at different intervals showed no unusual or marked variations.

These findings led us to make identical experiments with heat-killed bacteria and with bacterial filtrates. The results were as follows:

The phenomenon was practically never present when the bacteria, either invasive or non-invasive, were killed by heat. In testing the filtrates India ink was used as an indicator of the area through which each inoculum spread. Filtrates of non-invasive organisms failed to spread at all, and the area of spread of all inocula was the same. Streptococcus filtrates, endowed with a pronounced spreading power, seemed to elicit the phenomenon in some instances, and not in a very marked degree.

In identical experiments in which dilution of rattlesnake venom, a secretion containing much spreading

factor, was substituted for the streptococcus filtrate, but without using India ink, the same inconclusive results were obtained as when the bacterial filtrate was employed.

As regarding the effect of sulfanilamide on the phenomenon, it is difficult to make a conclusive statement in a preliminary note. The individual results will be analyzed separately when the work is fully reported. At present the point with which we are mainly concerned is the phenomenon which occurs with invasive bacteria without the aid of sulfanilamide.

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INDUCED FORMATION OF β-GENTIOBIOSIDES IN GLADIOLUS CORMS AND TOMATO PLANTS TREATED WITH CHEMICALS

When potato tubers (*Solanum tuberosum* L.) or *Gladiolus* corms are treated with ethylene chlorohydrin in order to break the rest period,[1] the ethylene chlorohydrin absorbed by the tissues is converted into β-2-chloroethyl-*d*-glucoside.[2] Further experiments with other plant tissues and with additional chemicals have shown that the formation of glycosides with the introduced chemicals serving as aglucons can take place quite generally among the higher plants.[3] Unpublished results with carrot roots (*Daucus carota* L. var. *sativa* D.C.) and wheat tops (*Triticum aestivum* L.) have shown that these plants also form β-2-chloroethyl-*d*-glucoside from absorbed ethylene chlorohydrin. However, when gladiolus corms are treated with *o*-chlorophenol, the glycoside formed is not β-*o*-chlorophenol-*d*-glucoside even though corms of the same variety form a β-glucoside when ethylene chlorohydrin is absorbed.[4] The acetyl derivative of this *o*-chlorophenol glycoside from gladiolus corms was prepared by acetylating the material obtained by continuous extraction with ethyl acetate of an aqueous extract of treated corms which had been precipitated with lead acetate, deleaded with hydrogen sulfide and concentrated with reduced pressure. After several recrystallizations from absolute ethanol, it melted at 207.5° to 208.5° (Corr.) and had a specific rotation $[\alpha]_D^{25°} = -49.4°$ ($CHCl_3$, Concn. 2.66 g in 100 cc). Tests with partially purified preparations of the glycoside from aqueous extracts of the corms had shown that on emulsin hydrolysis two moles of reducing sugar, calculated as glucose, are liberated for each mole of *o*-chlorophenol set free, and preliminary studies with the benzimidazole derivatives[5] indicated that both sugars comprising the disaccharide were *d*-glucose. This suggested that the glycoside might be a gentiobioside and accordingly β-*o*-chlorophenol-gentiobioside heptaacetate was synthesized.[6,7] The melting point and specific rotation of this synthetic gentiobioside were identical with the corresponding values for the isolated acetyl glycoside. Theory for $C_{32}H_{39}O_{18}Cl$: C, 51.44; H, 5.26; Cl, 4.75. Found:[8] C, 51.45; H, 4.97; Cl, 4.60. The propionyl derivatives of both the synthetic and gladiolus glycoside were also prepared and melted at 178.5° to 179° and a mixed melting point determination showed no depression. The glycoside formed in gladiolus corms is thus shown to be β-*o*-chlorophenol-gentiobioside. The quantity of β-*o*-chlorophenol-gentiobioside formed in the treated corms averaged about 0.5 g per 100 cc of expressed juice.

When tomato (*Lycopersicon esculentum* Mill.) plants were grown in sand culture supplied with a complete nutrient solution, and 0.1 to 0.2 millimole of *o*-chlorophenol added daily for about 15 days and then sampled, the roots were found to contain about one millimole of an *o*-chlorophenol glycoside per 100 cc of expressed juice. This glycoside was also β-*o*-chlorophenol-gentiobioside, since the acetyl and propionyl derivatives had the same melting point and showed no depression in mixed melting point determinations with the corresponding synthetic gentiobiosides. A β-glycoside was also formed when tomato plants were grown in the presence of chloral hydrate. The acetyl derivative of the glycoside, melting at 184° to 185°, was obtained from both tops and roots by a procedure similar to that previously used for the preparation of β-2-chloroethyl-*d*-glucoside tetraacetate from gladiolus corms.[2] When trichloroethyl alcohol was added to the nutrient medium instead of choral hydrate, the same glycoside was formed. Synthetic heptaacetyl β-trichloroethyl-gentiobioside, prepared by the reaction between trichloroethyl alcohol and heptaacetyl-bromogentiobiose in the presence of silver carbonate, had the same melting point, and it thus appears that the tomato plant forms β-trichloroethyl-gentiobioside from both chloral and trichloroethyl alcohol. It is of interest that in the detoxication of chloral in the tomato plant, as in animals, a reduction to the corresponding alcohol takes place.

These results indicate that gentiobiose is more widely distributed in plants than was previously supposed.

[1] F. E. Denny, *Am. Jour. Bot.*, 13: 118, 1926; *Contrib. Boyce Thompson Inst.*, 8: 473, 1937.
[2] *Contrib. Boyce Thompson Inst.*, 9: 425, 1938; 10: 139, 1939.
[3] *Am. Jour. Bot.*, 25: 15s, 1938.
[4] *Contrib. Boyce Thompson Inst.*, 11: 25, 1939.
[5] Stanford Moore and Karl Paul Link, *Jour. Biol. Chem.*, 133: 293, 1940. I am indebted to these authors for providing me with a copy of this paper prior to its publication.
[6] The β-octaacetyl gentiobiose used in this synthesis was kindly supplied by Professor William Lloyd Evans, of Ohio State University.
[7] Burckhard Helferich und Ernst Schmitz-Hillebrecht, *Ber. d. Chem. Ges.*, 66: 378, 1933.
[8] Microanalyses by H. Jeanne Thompson.