

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glycosides with the introduced chemicals serving as aglucons can take place quite generally among the higher plants.³ Unpublished results with carrot roots (*Daucus carota* L. var.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, decolored 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 $[\alpha]_D^{25} = -49.4^\circ$ (CHCl₃, 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 derivatives⁵ 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 C₃₂H₃₈O₁₈Cl: 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 chloral 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.

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 Burekhard Helferich und Ernst Schmitz-Hillebrecht, *Ber. d. Chem. Ges.*, 66: 378, 1933.

⁸ Microanalyses by H. Jeanne Thompson.

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.

It is quite likely, of course, that treatments with these chemicals stimulate the production of gentiobiose.

Details of these experiments will be published elsewhere.

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CHEMICAL EXAMINATION OF THE LIPID FRACTION OF ROYAL JELLY

HEYL¹ has reported that dilute NaOH extracts or aqueous pyridine extracts of royal jelly, when injected subcutaneously for five days into 21-day-old female rats, caused an increase in follicular activity of the ovaries. Melampy and Stanley² have recently failed to confirm this finding. However, it is important to note that the latter workers used *acetone-dried* royal jelly. The use of such a solvent to remove water completely would also remove sterols, phenols, acids, esters, glycerides, etc., some of which are the very types of compounds most likely to possess gonadotropic activity.

For nearly two years the chemical fractionation of royal jelly has been progressing in this laboratory and biological testing of the fractions has been commenced. In a paper at present in press, the authors describe the preliminary results of the chemical examination. Royal jelly contains an ether-soluble fraction of some complexity from which the authors have been able to isolate six substances, and there are indications of several others. Royal jelly, dried to constant weight over P₂O₅ and then powdered, when ex-

haustively extracted with ether in a Soxhlet apparatus, yields 10 to 15 per cent. of its weight as a cream-colored, semi-crystalline material of waxy consistency. This fraction has a characteristic flowery to spicy odor. The ether-soluble material contains about 0.1 per cent. phosphorus and 0.3 per cent. nitrogen. About 85 per cent. of this fraction can be extracted from ethereal solution by dilute sodium hydroxide. The separation of phenols and acids from this alkali-soluble material, and of wax, phospholipid, sterols and glycerides from the alkali-insoluble portion, has been accomplished. The difficulty of obtaining sufficient quantities of these individual substances delayed commencement of the biological experiments.

However, some feeding experiments performed over a year and a half ago, using the fruit fly, *Drosophila melanogaster*, revealed that the ether-soluble fraction possessed a remarkable influence on the number of eggs laid and the rate of reaching sexual maturity. These results have not been reported because we had hoped to be able to identify the compound responsible for this effect before publishing. The failure of Melampy and Stanley, using acetone-dried royal jelly, to confirm Heyl's results, appears to support our finding that the active material influencing the reproductive system is in the ether-soluble fraction. The detailed results of the *Drosophila* experiments will be published shortly. Our chemical and biological studies are being continued.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

ACCURACY IN ANATOMICAL DRAWING

To encourage greater accuracy in anatomy students' drawings, this year one class was provided with 8 × 10 inch sheets of cellulose acetate (du Pont Plastacele, 1/50 inch thick) ruled in centimeter squares with black auto lacquer. The students themselves prepared 8 × 10 inch heavy bristol board sheets with India ink rulings in centimeter squares (one side), in 1½ cm squares (opposite side), in ½ cm squares (another sheet, one side), and in 2 cm squares (opposite side). Both cellulose acetate and bristol board sheets were numbered vertically and horizontally so that the coordinates of points could be read off with ease.

For drawing, the clear cellulose acetate sheet is laid over the specimen and the appropriate surface of the proper bristol board inserted under the drawing paper. To draw natural size, the surface of the bristol board ruled in centimeter squares goes under the drawing sheet, its rulings clearly showing through the page.

¹ H. H. Heyl, *SCIENCE*, 89: 540, 1939.

² R. M. Melampy and A. J. Stanley, *SCIENCE*, 91: 457, 1940.

To enlarge to 1.5 × the 1½ cm ruling is used, for 2 × the 2 cm ruling, for reductions to 0.5 × the ½ cm ruling. By quickly plotting the chief features of the structures to be drawn on their appropriate coordinates on the drawing paper, the outlines are easily obtained.

Increased facility for making accurate drawings is especially apparent in dissection of nervous or circulatory systems, where simultaneous vision of all related parts is impossible without extensive excision of other organ systems. If the tip of the snout, the sternum or other landmark be fixed upon for repeated reference, the details visible at any one time may be plotted in on the coordinates, then intervening structures moved aside, and the deeper ones exposed. Replacing the cellulose acetate sheet with reference to the chosen point allows the drawing of deeper structures to continue with no distortion. This has proven especially valuable where superficial structures are exposed days before deeper ones. The drawing begun with reference to well-chosen points can be continued after deeper portions are exposed even though the superficial struc-