local or generalized tetanus and death. A few experiments were carried out with diphtheria toxin; guinea-pigs weighing from 150 to 250 g were used in these studies.

The experiments revealed that tyrothricin (0.05 mg and less) has no immediate effect upon the toxicity of tetanus toxin. The mixture of tetanus toxin and tyrothricin causes tetanus just as tetanus toxin alone. However, tyrothricin has a marked effect upon tetanus toxin which has been diluted in physiological salt solution or in buffer solution and kept either at 37° C or 4° C for 24 hours or more. Such a diluted toxin loses rather rapidly in toxicity. Tyrothricin in amounts of 0.05 mg to 0.000005 mg partially or completely inhibits this loss of toxicity of tetanus toxin. In one particular experiment, for instance, diluted tetanus toxin, which had been incubated together with tyrothricin at 37° C, caused tetanus and death, whereas the tetanus toxin control had become completely devoid of toxicity. It is interesting to note that tyrothricin also inhibits the loss of toxicity of tetanus toxin which has been exposed to heat (55° C). In regard to the mode of action, it may be pointed out that tyrothricin is a mixture of two polypeptides, namely, gramicidin and tyrocidin, and that peptones likewise inhibit the loss of toxicity of diluted tetanus toxin.[4] No evidence was obtained that tyrothricin increases the toxicity of tetanus toxin *per se*. It does not prevent the neutralization of tetanus toxin by the homologous antitoxin. Tyrothricin also inhibits the loss of toxicity of diphtheria toxin which has been diluted in physiological salt solution or buffer solution and kept at 37° C or 4° C.

Actinomycin A, an orange-colored pigment with marked bacterio-static activities, has no effect upon the toxicity of either diphtheria or tetanus toxins: in amounts of 0.005 mg and less, it neither prevents the loss of toxicity of these toxins which have been diluted in physiological salt solution, nor does it inhibit or enhance their toxicity.

Pyocyanase exerts a definite effect upon tetanus toxin. A preparation of pyocyanase was obtained from Merck and Company through the kindness of Dr. D. F. Robertson. It is a brownish, black slave-like material, soluble in ether and alcohol, but mainly insoluble in water. Following incubation for 24 to 48 hours, this pyocyanase preparation in amounts of 1 mg inhibits the toxic and lethal effects of tetanus toxin. This effect takes place in the presence of broth. Injection of tetanus toxin immediately after the addition of pyocyanase resulted only in a slight delay of the appearance of signs of tetanus.

Zephiran, too, exerts a definite effect upon tetanus toxin. In dilution of 1: 10,000, it completely prevents the toxic effects of tetanus toxin in mice, even when the toxin is injected immediately following the addition of this substance. It is important to note that the effects of zephiran upon tetanus toxin are somewhat inhibited in the presence of infusion broth and even more so in the presence of human serum.

The foregoing experiments revealed that certain substances of biological origin with marked anti-microbial properties, such as pyocyanase and zephiran, inhibit the *in vivo* effects of tetanus toxin. Whether or not they irreversibly inactivate the toxin and change its antigenic pattern, remains to be determined. Certain others, such as tyrothricin, inhibit the loss of toxicity of tetanus and diphtheria toxins which have been diluted in physiological salt or buffer solution. The effects upon other bacterial toxins need further investigation, and it remains to be seen whether the antitoxic properties of antimicrobial substances of biological origin can be utilized with efficacy and safety in the treatment of localized and generalized infections in which bacterial toxins play an important role.

ERWIN NETER
DEPARTMENT OF BACTERIOLOGY OF CHILDREN'S HOSPITAL AND UNIVERSITY OF BUFFALO SCHOOL OF MEDICINE

[4] K. Halter, *Zeitschr. Hyg. Infektionskr.*, 118: 245–262, 1936.

CAROTENOIDS OF TELIAL GALLS OF GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE LK.[1]

THE rust fungus *Gymnosporangium juniperi-virginianae* Lk. infecting the common juniper (*Juniperus virginiana* L.) forms caulicolous galls, globoid or reniform in shape, varying in diameter from 5 to 30 mm or more. The aeciospores produced during the summer on the cultivated apple are transferred to the juniper and cause infection. The mycelium remains dormant until the following spring when the telial galls become visible. These galls grow throughout the summer, mature in the fall and give rise to the teliospores the next spring.[2]

The mature galls used in this work were gathered when the telia were 1 to 2 mm in diameter by 5 to 10 mm long. The galls ranged in size from 10 to 50 mm in diameter and were of a cedar-brown color, while the telia were of a deeper reddish brown.

The leaves of the juniper contained 50 per cent. water at the time of gathering the galls, while the galls contained 68 per cent. water. The color of the interior of the galls when opened was pale green near the rind, while the body was light yellow. On exposure to the air, however, this color deepened to

[1] Contribution No. 274 from the Department of Chemistry.

[2] F. L. Stevens, "The Fungi Which Cause Plant Disease." Macmillan, 1921.

orange-yellow. Microscopic examination of the crushed galls showed that they consisted largely of teliospores and mycelium with the color confined to the teliospores.

Entire galls weighing 10 to 15 g were diced, weighed and placed in Erlenmeyer flasks. One hundred ml of saturated alcoholic potash was added and the whole refluxed for one-half hour. The gall residue was separated by suction filtration and the residue mixed in a Waring blender for 2 minutes with 50 ml of alcohol. The mixture was again refluxed for 15 minutes and the alcoholic extracts combined.

Complete removal of all the carotenoids present was accomplished with three extractions, using small amounts of petroleum ether (b.p. 30–60°). The pigments were epiphasic against 90 per cent. methanol, indicating the absence of free or esterified xanthophylls.

The combined petroleum extracts were dried over anhydrous Na_2SO_4 and brought to a small volume by distillation *in vacuo*. They were then chromatographed by the Strain[3] technic, using as adsorbent a mixture of MgO and Hyflo Super Cel (1:1). Only two zones separated. The lower zone was subsequently shown by spectrum analysis to be β-carotene. The upper, more strongly adsorbed red-orange zone had absorption maxima in petroleum ether at 4600Å and 4900Å with a minimum at 4800Å. The latter pigment, by its behavior on the adsorbent and its absorption spectrum, appears to be identical with γ-carotene.

The total carotene concentration of the gall was 3.31 mg per 100 g, of which 36 per cent. was β-carotene and 64 per cent. the γ-isomer. By comparison, a similar chromatographic study of the leaves gave 4.03 mg per 100 g of total petroleum-phasic carotenoids distributed as follows: 7 per cent. α, 83 per cent. β and 10 per cent. γ-carotene. Small amounts of xanthophylls were present in the leaves, but these were not investigated.[4]

The remarkably high content of the γ-isomer in the gall is of particular interest. This isomer is quite rare in plants, constituting only 0.1 per cent. of the total carotene prepared from ordinary sources.[5] Small amounts have been found in apricots (*Prunus armeniaca*).[6] Mackinney[7] has reported the marsh dodder (*Cuscuta salina*) to be a relatively rich source. Emerson and Fox[8] found a remarkably high concentration of γ-carotene in the male gametangia of the aquatic Phycomycete *Allomyces*. The latter workers point out the probability that "carotenoids may play important biological roles in sexuality and the process involved in the metabolism of reproduction."

The gall described is the richest source of γ-carotene which has come to the attention of the authors.

SUMMARY

In an investigation of the pigments of the telial galls of the common rust fungus *Gymnosporangium juniperi-virginianae* Lk. β- and γ-carotenes were shown to be the only carotenoids present, with the γ-isomer predominating. The identification of γ-carotene was based on its more characteristic properties, behavior on an adsorbent, and its absorption spectra. Neither free nor esterified xanthophylls were present, and only traces of chlorophyll.

The leaves of the juniper, besides containing chlorophyll, showed the presence of α-, β- and γ-carotene.

B. L. SMITS
W. J. PETERSON

DEPARTMENT OF CHEMISTRY,
KANSAS STATE COLLEGE

SCIENTIFIC APPARATUS AND LABORATORY METHODS

CHROMATOGRAPHIC ANALYSIS IN REVERSE

THE adsorption of substances from solution has generally been accomplished in one of two ways: by shaking the solution with the adsorbent and then filtering or by means of a chromatographic adsorption column. In the latter, the solution is allowed to percolate through a column packed with an adsorbent and the various adsorbable substances in the solution form bands in the adsorbent column, which can later be removed and eluted separately.

Because of the recognized value of chromatographic analysis, a modification of this technique found in this laboratory appears to have interesting possibilities as a research method. This modification consists in reversing the usual Zwett technique. Instead of passing the solution through the adsorbent column and then separating the bands by washing, the solution is placed in a tube and the adsorbent allowed to settle slowly through it, a small portion at a time. The powdered adsorbent falling through the solution sets up eddy currents which mix the solution sufficiently.

[3] H. Strain, *Jour. Biol. Chem.*, 105: 523, 1934; *ibid.*, 111: 85, 1935.

[4] M. Tswett has reported the presence of the xanthophyll, rhodoxanthin, in *Juniperus virginiana. Compt. rend.*, 152: 788, 1911.

[5] R. Kuhn and H. Brockmann, *Naturwiss.*, 21: 44, 1933; *Ber.*, 66: 407, 1933.

[6] H. Brockmann, *Z. physiol. Chem.*, 216: 45, 1933.

[7] G. Mackinney, *Jour. Biol. Chem.*, 112: 421, 1935.

[8] R. Emerson and D. L. Fox, *Proc. Royal Soc. London*, 128: 275, 1940.