animals and a marked reduction in the fifth as demonstrated in the pH of the gastric mucosa of seven different regions of the stomach. These values appear in Table I, reference to which indicates that while in the average figures for five animals the fasting pH shows little change, the level after histamine is considerably elevated following the implantation of the jejunal segment.

The effect on the gastric analysis is also consistent and definite. Here the average fasting free and combined acidities are diminished somewhat after the jejunal transplantation has been performed and a further decrease occurs following histamine (Table II). Particularly noteworthy is the fact that the combined acidity is lower in each instance following operation, indicating that the reduction in free acidity is not to be explained on the basis of neutralization of the gastric juice by the alkaline secretion of the jejunal mucosa.

TABLE II

GASTRIC ANALYSES BEFORE AND AFTER JEJUNAL TRANSPLANTS

	Before transplant			After transplant		
	Fasting	10 min. after histamine	20 min. after histamine	Fasting	10 min. after histamine	20 min. after histamine
Dog No. 1839						
Free	60	92	70	24	12	5
Combined ...	38	44	65	26	36	30
Total	98	136	135	50	48	35
Dog No. 1841						
Free	60	78	90	40	20	10
Combined ...	56	60	44	36	22	20
Total	116	138	134	76	42	30
Dog No. 1842						
Free	50	70	87	44	30	8
Combined ...	45	60	58	40	36	32
Total	95	130	145	84	66	40
Dog No. 1887						
Free	40	48	60	36	36	22
Combined ...	35	38	34	30	20	12
Total	75	86	94	66	56	34
Dog No. 1888						
Free	42	50	63	50	42	30
Combined ...	50	46	40	30	28	20
Total	92	96	103	80	70	50
Averages						
Free	50	68	74	39	30	15
Combined ...	45	50	48	32	28	23
Total	95	118	122	71	58	38

These changes have been shown to occur within 45 minutes of the transplantation and in the completely studied animals to persist for at least four months.

In two animals when the effects of the transplant were established, it was then resected and further pH measurements carried out. These indicated that within one hour one animal, number 1723, showed the normal reaction to histamine, although the fasting pH remained slightly higher than before the graft was implanted. In the other, number 1339, the pH of the mucosa and its reaction to histamine corresponded to the normal findings in all respects.

Further studies of the effect of such transplants on other aspects of gastric secretion are in progress.

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THE EFFECTS OF ANTI-MICROBIAL SUBSTANCES OF BIOLOGICAL ORIGIN UPON BACTERIAL TOXINS[1]

DURING recent years, the anti-microbial properties of various substances produced by different bacteria and fungi have been studied extensively and some of them have been utilized as chemotherapeutic agents in the treatment of infections of animals and man. Among the more important of these substances are the following: Pyocyanase, isolated from *Pseudomonas aeruginosa* by Loeb; penicillin, obtained by Fleming from *Penicillium notatum;* tyrothricin (gramicidin and tyrocidin) isolated by Dubos and Hotchkiss from *Bacillus brevis;* and actinomycin A and B isolated by Waksman from *A. antibioticus.* Although the action of these substances upon growth and survival of many bacteria and fungi is now well known, only scant information is available in regard to possible effects upon bacterial toxins. It has been stated that pyocyanase inactivates diphtheria toxin.[2] Tyrothricin and actinomycin A inhibit fibrinolysis by *beta hemolytic streptococcus* and plasma coagulation by pathogenic staphylococcus.[3] However, it is not known as yet whether these substances act directly upon fibrinolysin and coagulase themselves. In the following communication, the results of experiments on the effects upon bacterial toxins of various anti-microbial substances of biological origin are presented.

Tyrothricin, actinomycin A, pyocyanase and dimethyl-benzylammonium chloride (Zephiran) were tested for possible antitoxic activity. The latter substance was included because it is a mixture of alkyl radicals from C_8H_{17} to $C_{18}H_{37}$ as contained in the corresponding fatty acids of cocoanut oil. Tetanus toxin was diluted either in buffer solution (pH 7.2) or in infusion broth and then mixed with appropriate amounts of the respective substances. The mixtures were injected subcutaneously into the leg of white mice (18 to 24 g) either immediately or following incubation of 37° C for various periods of time. The animals were observed daily for the development of

[1] The author wishes to express his appreciation to Dr. D. F. Robertson, associate medical director, Merck and Company, for tyrothricin and pyocyanase; to Dr. Edwin F. Voigt, Director, Human Biological Division, Lederle Laboratories, for tetanus and diphtheria toxin; and to Dr. Selman A. Waksman, State of New Jersey Agricultural Experiment Station, New Brunswick, for actinomycin A.

[2] R. Emmerich, O. Loew and A. Korschun, *Zentr. Bakt. Parasitenk.*, 31: 1–25, 1902; Okhubo, *Z. Immunitaesf.*, 5: 428, 1910.

[3] E. Neter, *Proc. Soc. Exp. Biol. Med.*, 49: 163–167, 1942.

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.

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[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.