inhibited in the presence of a sufficiently high concentration of tyrothricin. On the contrary, there was little or no correlation between Gram staining ability and the bacteriostatic potency of the pyocyanines, hemipyocyanine and pyocyanase.

The inhibitory action of the antibiotic substances studied is not confined to bacteria but extends to other groups of microorganisms such as yeasts and fungi. High inhibitory activity towards one type of microorganism does not necessarily signify similar potency towards other types: hemipyocyanine was much more inhibitory for yeasts and fungi than for bacteria.

Summary. The inhibition of growth of bacteria, yeasts and pathogenic fungi by natural and synthetic pyocyanine, synthetic hemipyocyanine, and natural pyocyanase and tyrothricin has been investigated. Natural and synthetic pyocyanine have equal activities which surpass those of pyocyanase. Tyrothricin is particularly inhibitory for Gram positive bacteria, especially streptococci. The considerable fungistatic potency of tyrothricin and hemipyocyanine suggests that these substances may be of value in the treatment of fungus infections.

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Protein Content of Rabbits' Aqueous Humor Following Intravenous Injection of *E. coli* toxin.*

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It has been shown[1,2] that culture filtrates of Gram-negative microörganisms capable of producing the Shwartzman phenomenon[3] (such as meningococcus, coli, typhoid, Shiga, proteus, and choleræ toxins) produce in rabbits a primary reaction characterized by iridoconjunctival hyperemia, and, upon removal, coagulation of the aqueous humor. This reaction appears several minutes after intravenous administration of toxin and reaches a maximum intensity in a few hours. The ocular symptoms are bilateral, usually of equal intensity, and disappear in the ensuing 24 hr.

In view of the possible bearing of this reaction on the problem of uveitis and other intraocular conditions, such as epidemic dropsy, the influence of intravenous injection of *E. coli* toxin on the protein content of the aqueous humor of rabbits has been investigated.

Methods and Material. A strain of *E. coli communior,* freshly isolated from a case of purulent cholecystitis, was inoculated in nutrient agar slants, of pH 7.0. After 24 hr at 37°C, the growth of each slant was washed into 100 ml of nutrient broth, of pH 7.2. The 24 hr broth cultures, tested aerobically and anaerobically for contaminants, were centrifuged, and the supernatants filtered through Mandler candles No. 9. Filtrates, controlled for sterility, were stored at 4°C. Male albino rabbits, weighing about 2 kg, received *E. coli* toxin in saline dilutions ranging from 1:5 to 1:500 in volume of 1 ml per kg of body weight.

Rabbits' aqueous humor, placed into small stoppered tubes, was weighed. The presence or absence of coagulation was noted

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1 Ayo (Ajo), C., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 500.

2 Ayo, C., accepted for publication by the *Journal of Immunology.*

3 Shwartzman, G., *Phenomenon of Local Tissue Reactivity,* Paul B. Hoeber, N.Y., 1937.

after several hours standing. To each sample, brought to a volume of 1 ml with distilled water, 0.2 ml of 2% gum ghatti and 1 ml of 5% sulphosalicylic acid were added. Tubes were shaken lightly and allowed to stand for several hours. Protein was then determined by either of two methods. When no precipitate was present in the solution, a modification of the Looney-Walsh nephelometric method[4,5] was used: The turbidity was compared with a blank in a photoelectric colorimeter[6] and the galvanometer values were read from a standard curve, calibrated with known serum protein dilutions. When precipitates were present, protein was determined by the Kjeldahl method. After standing overnight in the icebox, the tubes were centrifuged and carefully drained. The precipitate, washed with 2.5% sulphosalicylic acid, was transferred with sulphuric acid into Kjeldahl flasks. A conversion factor of 6.25 was used.

Experiment 1. In the first experiment a comparative study was made of the protein content of the aqueous humor and the previously established ocular symptoms, iridoconjunctival hyperemia and aqueous humor coagulation. Sixty-one rabbits were used. Observation varied from 30 min to 3 hr after toxin injection. In many cases, the protein content did not parallel the dose of toxin; this confirmed the finding[2] that the ocular reaction is considerably influenced by variations in the response of the animals.

Ocular hyperemia was evident when the protein content was above 150 mg %. It was usually proportional to the protein content of the aqueous humor but did not always show a close parallel. Coagulation of the aqueous humor consistently occurred with a protein content of 500 mg % or more, while fibrin threads could be observed with protein concentrations of 200-500 mg %. Red blood cells were never found microscopically even with the highest protein concentrations. Results similar to those obtained in this experiment were recently observed[7] using other Shwartzman toxins.

Experiment 2. In a study of the optimal time of the ocular reaction, 2 groups of 12 rabbits were injected with coli toxin in dilutions 1:5 and 1:50 respectively. Aqueous humor was removed after 30, 60, 90, and 120 min and every hour thereafter up to 12 hr after injection. At every interval one eye each of 2 new rabbits per group was used. In the first group, which received the stronger dose of toxin, protein concentrations were higher, reached a maximum in 2 hr, and started to drop in 6 hr. In the second group the protein content reached a maximum in 3 hr and dropped after 5. Twelve hr after injection the protein content was still elevated in both groups, being 466 and 440 mg % and 190 and 90 mg % respectively.

It was observed in these experiments that the aqueous humor removed more than 3 hr after toxin injection may show fibrin threads or complete coagulation even with protein concentrations of as low as 50-100 mg %. This seems to indicate an earlier decrease of albumin and globulin than of fibrinogen as the reaction subsides.

Experiment 3. The protein content of the secondary aqueous humor was determined in 35 rabbits treated with coli toxin in varying doses. 0.2 ml of aqueous was removed 90 min and 4 hr after toxin injection. As shown in Table I, the animals were divided into 3 groups: Group I those without toxin treatment; Groups II and III including animals injected intravenously with coli toxin, and having a protein concentration in the primary aqueous in the normal limits and above normal respectively. The abnormally high protein content of the secondary aqueous of Group II indicates a toxic effect on the capillaries of the ocular vessels although no primary reaction had been evident. Damage to extraocular structures (paralysis) in the absence of ocular manifestations had been observed previously.[7]

Experiment 4. Nine animals received twice, at 4 weeks interval, the same dose of a filtrate of *E. coli* which proved to be of

[4] Looney, J. M., and Walsh, A. I., *J. Biol. Chem.*, 1939, **127**, 117.

[5] Kronfeld, P. C., *Am. J. Ophth.*, 1941, **24**, 1121.

[6] Weech, A. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 858.

[7] Ayo, C., unpublished experiments.

TABLE I.
Influence of *E. coli* Toxin on Protein Content of Secondary Aqueous Humor.

Group	No. of rabbits	Treatment	Protein content (mg%) Primary Avg	Primary Low	Primary High	Secondary Avg	Secondary Low	Secondary High
I	6	None	36	19	51	565	281	993
II	18	*Coli* toxin	41	16	68	2160	869	2590
III	17	" "	435	77	1025	2211	1297	3800

unchanged potency in control rabbits. Results as shown in Table II confirm the observation[2] that a previous administration of toxin confers a considerable degree of protection against a subsequent one. The nature of this protection is being investigated.

TABLE II.
Protein Content of Aqueous Humor Following Injection and Reinjection (4 Weeks Later) of *E. coli* Toxin.

Toxin dilution	Primary aqueous humor protein (mg%) 90′ after 1st inj.	2nd inj.
1:100	547	431
1:200	1108	97
1:200	1025	55
1:235	77	79
1:240	130	68
1:240*	38	55
1:240	26	70
1:300*	1582	45
1:400	312	48

*Aqueous humor removed 4 hours after 1st and 2nd injections.

Experiment 5. The nature of the proteins in the aqueous humor after injection of coli toxin was studied electrophoretically (Dr. D. H. Moore) by the Tiselius method in a microcell of about 2 ml. Two hr after toxin injection, fluid was removed from the anterior chambers of 8 rabbits. Coagulation of the pooled fluid was prevented by sodium citrate, 6 mg per ml of fluid. The sample contained 353 mg % of protein. It was dialysed at +4°C for 3 days against 0.05 molar barbiturate buffer and 0.1 ionic strength (pH 7.83). The relative concentrations of the proteins after one hr were: albumin 77.5%, beta globulin 11.5%, gamma globulin 6.1%, fibrinogen 4.9%. The A:G ratio was 3.44. A fast component[8] corresponding to hyaluronic acid[9] was not visible, since its concentration was probably too low. The elevated A:G ration seems to indicate a faster diffusion into the anterior chamber of the proteins of low molecular weight.

Summary. Intravenous injection of coli toxin produced in rabbits a rise in protein content of the aqueous humor. Above 500 mg % protein the aqueous coagulated. The protein content of the secondary aqueous of toxin-treated animals was higher than in normal controls, even when that of the primary aqueous had been normal.

A previous dose of coli toxin partially or completely prevented a rise in the protein content after a second injection.

Electrophoresis of the aqueous humor of toxin treated rabbits indicated albumin, beta and gamma globulin, and fibrinogen, with an A:G ratio of 3.44.

[8] Meyer, K., and Chaffee, E., *J. Biol. Chem.*, 1940, **133**, 83.

[9] Meyer, K., Smyth, E. M., and Gallardo, E., *Am. J. Ophth.*, 1938, **21**, 1083.