

organisms capable of lysing Gram-positive bacteria, while Krasilnikov and Koreniako⁴ found actinomycetes producing bactericidal substances against acid-fast and Gram-positive bacteria. During the past five years, the senior writer has isolated a number of bacterial species which are antagonistic to phytopathogenic microorganisms in varying degrees. Special attention was given to two soil bacteria which showed a strong antagonism both to bacteria (Gram-positive and Gram-negative) and to certain fungi. One of these organisms was identified as *Bacillus vulgatus*, while the second one, a yellow spore-bearing bacillus, remains unidentified. The antagonism was tested on various solid and liquid media. On the solid media, a sterile zone was formed around the giant colony of the antagonist, while the liquid media into which a susceptible microorganism was introduced was usually completely cleared after 1 to 3 days, depending upon the organism used and the age of the culture. The two antagonists were active against the following phytopathogenic bacteria: *Erwinia amylovora*, *E. aroidae*, *E. carotovora*, *E. phytophthora*, *Phytomonas campestris*, *Ph. flaccumfaciens*, *Ph. insidiosa*, *Ph. juglandis*, *Ph. lachrymans*, *Ph. malvacearum*, *Ph. michiganensis*, *Ph. panici*, *Ph. pisi*, *Ph. sepedonica*, *Ph. stewarti* and *Ph. tumefaciens*. Of the fungi the following were affected by the antagonists: *Fusarium graminearum*, *F. lycopersici*, *Dematophora necatrix*, *Helminthosporium sativum*, *Verticillium albo-atrum* and *Phytophthora* sp. Other microorganisms tested with similar results were *Escherichia coli*, *Salmonella pulorum*, *S. typhi*, *Alkaligenes faecalis*, *Corynebacterium diphtheriae*, *Staphylococcus aureus*, *Streptococcus lactis*, *Mycobacterium phlei*, *M. sp.* (Grassberger's butter bacillus), 2 unidentified *Mycobacterium* sp. from the soil, *Leuconostoc mesenteroides* and *Lactobacillus acidophilus*.

The bactericides produced by the antagonists are water soluble and active in extremely small amounts. The bactericidal substance of *Bacillus vulgatus* was adsorbed by all Berkefeld filters but passed through a Chamberland L 3 filter. The bactericidal substance of the yellow antagonist was retained by both types of filters. Boiling for 60 minutes did not destroy the bactericide of either antagonist. However, when the bactericidal-containing medium of the yellow species was autoclaved, the bactericide was inactivated after

15 minutes at 10 pounds pressure, while that of *Bacillus vulgatus* was still active after 10 minutes sterilization at 20 pounds pressure. The strongest antagonism was observed in media containing dextrose and fructose, while no antagonism could be obtained either on a peptone sugar-free medium or in a nutrient medium plus maltose. Apparently the hydrogen-ion concentration did not affect the activity of the antagonists, since good sterile zones were obtained in media ranging from pH 4 to 10. All attempts to precipitate the active principle from culture solution with the aid of inorganic acids, ammonium sulfate, aluminum sulfate, aluminum nitrate, alcohols and ether, were unsuccessful. Bactericidal material of the culture media in which the antagonists grew can be concentrated by evaporating to dryness in a double boiler. Further studies on this phase of the problem are in progress. A more detailed report on the organisms discussed herewith and their relation to diseases will be published at a later date. Any one interested in these microorganisms may procure cultures gratis from the writers.

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HEMOGLOBIN REGENERATION IN ANEMIC TROUT FED LIVER FRACTIONS AND FLY MAGGOTS

In an earlier report the development of anemia in brook trout fed synthetic diets was described.¹ Trout recovered rapidly from this anemia when fed fresh beef liver. Inasmuch as a good test animal is needed for the study of the liver fractions used in treating human anemias, this phase of research with trout was extended during the past year. Through the generosity of four manufacturers, liver fractions used in the treatment of human anemia were provided.

About 400 trout averaging 9 grams in weight were made anemic by feeding them a diet of casein 20, starch 34, dextrin 34, yeast 5, cod liver oil 3 and Osborne and Mendel's salt mixture 4. When the red cell count had dropped to 850,000, the trout were divided into groups. The liver fractions were then weighed into capsules and fed to the trout individually. In addition to the groups fed the liver fractions, three others were used, one with no supplement, one fed fresh beef liver and one fed maggots of the common house fly. These larvae were reared upon the usual mixture of alfalfa leaf meal, bran, malt and yeast.

¹ A. V. Tunison, A. M. Phillips, C. M. McCay, C. R. Mitchell and E. O. Rodgers. Cortland Hatchery Report No. 8, for the Year 1939. New York State Conservation Department, Albany, N. Y.

² Rene J. Dubos and Carlo Cattaneo, *Jour. Exp. Med.*, 70: 249-256, 1939.

³ Grace M. Sickles and Myrtle Shaw, *Jour. Bact.*, 28: 415-431, 1934.

⁴ N. A. Krasilnikov and A. I. Koreniako, *Microbiology*, 8: 673-685, 1939.

Three trout were killed from each group at regular intervals and erythrocyte counts made in duplicate. The results are summarized in Table 1.

TABLE 1

Diet no.	Extract fed	Average of 6 counts in thousands at end of:			
		4 weeks	6 weeks	8 weeks	13 weeks
466	Fresh beef liver	973	1,051	1,130	1,120
467	Chappel's secondary	866	960	1,014*
468	Chappel's P.A. fraction..	861	851	1,025	1,010
469	Chappel's whole extract .	871	875	930	1,040
470	Armour whole dried liver	840	926	978	1,040
471	Lilly	820	901	993	1,040
472	Squibb	823	830	933	978
473	No supplement	671	651	656	660
474	Fly maggots	886	1,056	1,020	1,290

* Diet discontinued because of insufficient fish.

All liver fractions permitted the regeneration of erythrocytes, but none were as potent as the larvae. These must be very rich in the anti-anemic factor. In 1937 Trager² reported that both house fly maggots and liver were rich in a substance essential for the growth of mosquito larvae. Furthermore, his data indicated that one factor essential for the growth of mosquito larvae seemed to be involved in pernicious anemia, inasmuch as the urine of those suffering from this disease lacked the essential while normal urine contained it.

The factor needed for the regeneration of trout blood may also be similar to that found essential for chickens³ and pigeons⁴ by Hogan and his associates.

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A NOTE ON SERUM CHOLINESTERASE VARIABILITY IN MALE AND FEMALE RATS

DURING the course of an experiment involving the determination of cholinesterase in rat sera, it was observed that a large number of them displayed little or no activity. It was noted that the sex of the mature animals was the factor which determined whether the cholinesterase was high or low. In a group of 74 mature rats, all the male sera (41) showed little or no activity. All the female sera, with the exception of four, were relatively very active.

These exceptions suggested the possibility that the degree of sexual maturity of the females influenced the activity of serum cholinesterase, a point which was

² W. Trager, *Jour. Exp. Biol.*, 14: 240, 1937.

³ A. G. Hogan and E. M. Parrett, *Jour. Biol. Chem.*, 132: 507, 1940.

⁴ A. G. Hogan, L. R. Richardson, P. E. Johnson and R. N. Nesbit, *Jour. Nutrition*, 20: 203, 1940.

next investigated. Sera from thirteen immature female rats (50–80 g) were examined and found to possess a very low cholinesterase activity, similar to that of the normal males. Of five old and supposedly senile female rats, three had a low serum cholinesterase activity (in the normal male range) and two displayed the high activity characteristic of the normal mature female. Despite the fact that histological examination of the ovaries of all five of these animals revealed the presence of developing ova, the cholinesterase determinations suggested that these five animals did not constitute a representative sample from the same population as the normal mature female rats.

The sera of male and female mice, examined by the Warburg manometric method,¹ show that in this species also the serum cholinesterase of the female is markedly higher than that of the male.

Although a large number of additional experiments are indicated, due to the pressure of other duties the lead can not be further investigated at this time.

EXPERIMENTAL PART

The cholinesterase determinations were carried out at pH 7.2 by the continuous titration method.² The titration figures were so low in the case of males and immature females that there was some doubt as to the

TABLE I
VARIATIONS IN SERUM CHOLINESTERASE ACTIVITY WITH
SEX DEVELOPMENT

	No. of animals	Range		Average†	
		Warburg	Titrimetric	Warburg	Titrimetric
		cu. mm. CO ₂	cc	cu. mm. CO ₂	cc
Mature female rats ..	33	255– 617	0.68–2.77	415 ± 78	1.57 ± 0.51
Immature female rats ..	13	62– 160	x	107 ± 27	x
Senile (?) female rats ..	5	49– 447	x	217 ± 122	x
Mature male rats ..	41	77– 157	x	113 ± 9	x
Mature female mice .	5	1850–3000		2393 ± 325	
Mature male mice .	5	1300–2017		1545 ± 218	

x Practically all these were so low that they could not be distinguished with certainty from the blank.

† With average deviations from the mean shown.

actual presence of cholinesterase activity in these sera. By the more sensitive Warburg procedure³ it was

¹ All Warburg determinations were done by H. Rudney, to whom the authors extend thanks for technical assistance.

² Knaff-Lenz, *Arch. exp. Path. Pharmacol.*, 97: 242, 1923. Hall and Lucas, *Jour. Pharmacol. and Exp. Therap.*, 59: 1, 1937.

³ Ammon, *Arch. ges. Physiol.*, 233: 486, 1933.