progressive rise. For 20 to 40 seconds the heart struggles along with slow beats. As the coronary circulation and vigor of contractions gradually improve, the pressures reach supernormal levels which gradually fade back to normal ranges.

If fibrillation lasts longer—roughly a minute or more—even such a slow recovery may fail. After 2 minutes of fibrillation, regular beats are started; but under the conditions of our experiments they never become strong enough to give the myocardium sufficient nourishment for revival. Blood pressures remain low and death supervenes.

Conclusions. The dog's ventricles did not generally survive interruption of coronary circulation during fibrillation for more than about 1 minute, in the sense that useful coördinated beats sufficient for recovery are reëstablished without massage. These results caution us not to entertain too high hopes of resuscitating human hearts by the countershock method. Countershock can stop fibrillation; but complete anoxia beyond one minute generally prevents a resumption of beats sufficiently vigorous to reëstablish viable blood pressures.

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Antihemorrhagic Compounds as Growth Factors for the Johne's Bacillus.*

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One of the first bacterial growth factors to be studied in any detail was a substance necessary for the growth of the Johne's bacillus *(Mycobacterium paratuberculosis)*. Twort and Ingram[1] showed that certain other acid-fast bacteria including the tubercle bacillus contained a necessary growth factor for the original cultivation of the Johne's bacillus. Crude concentrates of the active substance were prepared from *Mycobacterium phlei.* We have long been interested in the growth of the Johne's bacillus, and for some time have attempted to isolate the active growth factor. In this paper

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1 Twort, F. W., and Ingram, G. L. Y., *Johne's Disease,* London, 1913.

concentration of the active factor will be described and it will be shown that it can be replaced by the antihemorrhagic vitamins.

The strain of the Johne's bacillus grows as a dull, brittle, granular film on the surface of liquid media. It always grows better in the presence of phlei cells or an extract of them, and we have not been able to subculture it more than 2 or 3 times on a purely synthetic medium although it has been artificially cultivated about 20 years. In our experience most strains of the Johne's bacillus become continuously cultivable on a synthetic medium, although the phlei factor is needed for primary cultivation; but all strains always grow faster and give a better yield when the factor is present.

The basal medium was composed of the following substances per liter of solution: glycerol 70 cc, asparagine 5 g, sodium citrate 0.5 g, K_2HPO_4 1 g, $MgSO_4.7\ H_2O$ 1 g, iron citrate 0.063 g. Twenty cc portions of this basal medium were sterilized by autoclaving in 50 cc Erlenmeyer flasks. Substances to be tested for growth factor activity were incorporated in the basal medium at suitable dilutions. Triplicate flasks of each dilution of a substance to be tested were employed. An inoculum about 2 to 3 mm in diameter was floated on the surface of the media. The inocula could not be strictly uniform. If the cells were suspended in order to obtain uniform inocula, the lag phase of growth of the submerged cells was considerably lengthened. The bacteria for the inocula were grown on the basal medium to which had been added 2% of dried cells of *M. phlei.*

Three months were required for all the cultures to attain maximum growth; at the end of the growth period the cells were filtered from the medium, washed, dried, and weighed.

The source of the growth factor was the dried cells of *M. phlei* which had been grown on a synthetic medium.[2] One percent of this material when added to the synthetic basal medium gave excellent growth. It was found that the active substance could be extracted from the cells by boiling water, or by boiling acetone. The best concentrate obtained in this work was prepared as follows: *M. phlei* cells were extracted 3 times with boiling acetone. The extracts were concentrated under reduced pressure to an oil which was dissolved in ether. The ether solution was extracted 4 times with water. While the ether phase still contained some activity, the aqueous extracts showed maximum effect when added at a level equivalent to 1% of *M. phlei* and this amount of material supplied only 10 γ of solids per cc of medium.

[2] Ingraham, M. A., and Steenbock, H., *Biochem. J.*, 1935, **29**, 2553.

At this point in our studies, the note of Almquist and Klose[3] appeared, in which it was reported that phthiocol possessed vitamin K activity. Since this substance had been isolated from acid-fast bacteria[4] and since the Johne's bacillus growth factor was soluble in fat solvents as well as in water, it seemed possible that phthiocol was the active substance present in our concentrates. Dr. R. J. Anderson kindly supplied us with a sample of synthetic phthiocol, and assay showed this material to be effective in promoting growth of the Johne's bacillus. It then became of interest to test other antihemorrhagic compounds. A highly potent concentrate of vitamin K was effective, as was 2-methyl naphthoquinone (kindly supplied by Dr. A. Black of E. R. Squibb Company). Representative data are shown in Table I.

It soon became evident that while the antihemorrhagic vitamins were growth factors for the Johne's bacillus, something else in addition was supplied by the dried cells or metabolites of *M. phlei.* The growth on the phlei was more rapid as shown by the accompanying photograph (Fig. 1) taken of cultures 2 months old. Furthermore, growth was not always obtained in subcultures on the basal medium supplemented with the vitamin.

TABLE I.

Growth of the Johne's Bacillus in Media Containing Antihemorrhagic Compounds.

Culture medium	Dry wt of Johne's bacilli produced per 20 cc of medium after 3 mo growth, mg
Assay 1	
Synthetic Medium	120
" " + dried phlei cells (40 mg per cc)	260
" " + phthiocol (10 γ per cc)	207
" " + " (1.0 " ")	250
" " + " (0.1 " ")	227
" " + " (0.01 " ")	147
Assay 2	
Synthetic Medium	139
" " + phlei filtrate* (0.05 cc per cc)	195
" " + 2-methyl naphthoquinone (1.0 γ per cc)	167
" " + " " (0.15 " ")	206
" " + " " (0.1 " ")	212
" " + " " (0.01 " ")	152

* The filtrate of a culture of *M. phlei* on the synthetic medium; it had been concentrated by heat to about 1/30 the volume of the original medium. The filtrate was used instead of phlei cells here to obviate the difficulty of separating the Johne's bacilli from the phlei cells to obtain the weight of the former.

[3] Almquist, H. J., and Klose, A. A., *J. Am. Chem. Soc.*, 1939, **61**, 1611.

[4] Newman, M. S., Crowder, J. A., and Anderson, R. J., *J. Biol. Chem.*, 1934, **105**, 279.

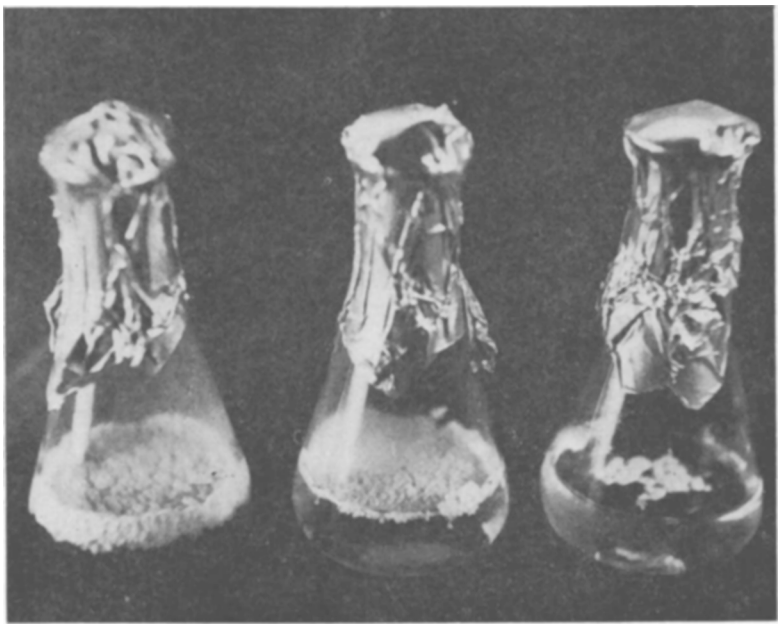

FIG. 1.
Relative stimulation of the Johne's bacillus by phlei filtrate and by 2-methyl naphthoquinone (2 months' growth).
Left—Synthetic medium + phlei filtrate.
Center—Synthetic medium + 2-methyl naphthoquinone.
Right—Synthetic medium.

Almost without exception bacterial growth factors have been found to be water-soluble compounds. Results of the present work show that the fat-soluble vitamins also play a rôle in the metabolism of certain bacteria. The effectiveness of antihemorrhagic compounds in promoting the growth of the Johne's bacillus demonstrates for the first time the part played by such compounds in bacterial nutrition. It is of interest to consider whether the utilization of a fat-soluble growth factor is in any way concerned with the very slow growth rate, or with the high fat content of these organisms. It is possible that further study with bacteria may be useful in the elucidation of the rôle of vitamin K in cell physiology.