carotene, is not only needless, but it actually inhibits the formation of the blue color with carotene and of the blue color with oils rich in vitamin A at room temperature and of the rose or violet-red after heating. Aged solutions of pyrocatechol exert even greater inhibitory powers than fresh solutions.

7840 P

Cultivation of the Johne's Bacillus in a Synthetic Medium.*

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Twort and Ingram[1] succeeded in isolating the causative organism from the intestine of a cow which had died of Johne's disease, by using a special medium containing the dead cells of tubercle bacilli. They later found that other acid-fast organisms would give the same stimulation, the timothy grass bacillus, *Mycobacterium phlei,* giving especially good results. Since then the cells, various extracts of the cells, or the products of growth of *M. phlei* have been incorporated in the media used to cultivate the Johne's bacillus. The nature of the "essential substance", which may be chemical or physico-chemical has, however, remained obscure.

Johne's disease (paratuberculosis) is a chronic enteritis affecting the cow and the sheep and caused by the multiplication of the Johne's bacillus in the intestinal mucosa, the impairment of the function of which results in the emaciation of the animal. The disease is usually fatal. The disease occurs in isolated herds in the United States but is not yet of serious economic importance. In France, however, the disease has spread within the last 20 years from 2 to 41 departments of the country according to Rinjard.[2] It would seem, therefore, that the United States would profit by combating the disease before it becomes more widely distributed. In the eradication we are hampered by the lack of a sufficiently good diagnostic agent. A "Johnin" prepared like old tuberculin neces-

* Published with the consent of the Director of the Agricultural Experiment Station. Aided by a grant from the special research funds of the University.

[1] Twort, F. W., and Ingram, G. L. Y., A Monograph on Johne's Disease. Baillière, Tindall and Cox, London, 1913.

[2] Rinjard, M. P., *La paratuberculose bovine en France.* Office International des Epizooties R 51, 1934.

sarily contains fractions of *M. phlei,* which Dunkin[3] reports may lead to a false positive reaction, and the potency of different batches varies widely and cannot be easily standardized. For these reasons avian tuberculin has been used to diagnose Johne's disease by Hagan and Zeissig[4] and others. It would seem, however, that a specific product should yield better results than the avian tuberculin.

We have found that under certain conditions the Johne's bacillus can be induced to grow in Dorset's[5] synthetic medium without the addition of any material of unknown composition such as the cells of *M. phlei.* We have successfully carried one of our strains of the Johne's bacillus through 10 subcultures on the synthetic medium during the last 2 years. The growth of the Johne's bacillus on media not containing the "essential substance" in one subculture has been reported by Dunkin.[3] Such evidence is not conclusive of growth without the "essential substance", since some of it might have been carried over with the inoculum to the first subculture.

The conditions necessary for the growth of the Johne's bacillus on the synthetic medium are that the thin, even-surfaced rather than the thick, granular type of pellicle growth shall be used as the inoculum, and that a mass of growth at least 7 or 8 mm. in diameter per 50 cc. of medium shall be used. The inocula are floated on the surface of the medium.

The type of growth on the synthetic medium differs from that on the same synthetic medium containing in addition the dead cells of *M. phlei.* On the latter medium the growth spreads in a thin, coherent film over the surface in 3 to 4 weeks and then thickens and wrinkles, attaining the maximum in 2 months; whereas on the synthetic medium the growth occurs in discrete particles, thin in some places and thick in others. The total amount of growth on the synthetic medium is never as great as that on the phlei medium. At the end of 5 months the growth on the phlei medium has retained the same appearance as it had after 2 months, while the growth on the synthetic medium is wet and sunken. As good growth occurred in the tenth subculture as in the first subculture on the synthetic medium. If transfers are made from any subculture back to the phlei medium, the type of growth characteristic of the phlei medium at once appears.

The cultivation of the Johne's bacillus on a synthetic medium

[3] Dunkin, G. W., *J. Comp. Path. and Ther.*, 1933, **46**, 159.

[4] Hagan, W. A., and Zeissig, A., *Ann. Rpt. Cornell State Veterinary College,* 1927-28, 172.

[5] Henley, R. R., *Am. Rev. Tuberc.*, 1929, **19**, 660.

has enabled us to prepare a solution of the Johne's bacillus protein for diagnostic purposes according to the ultrafiltration method of Seibert[6] for preparing tuberculin. This "Johnin" eliminates certain disadvantages of previous products, since it contains none of the foreign proteins which are introduced by the use of the phlei medium, and is practically free of the crystalloids of the culture medium. It can be easily standardized by measuring the amount of protein precipitated from a sample of the solution by trichloracetic acid.

Conclusions. The dead cells of *M. phlei* contain an *accessory* rather than an *essential* growth substance for the Johne's bacillus.

A purified "Johnin" containing no proteins other than the Johne's bacillus proteins has been manufactured for use as a diagnostic agent.

7841 P

Effect of Adrenalectomy and Hypophysectomy upon Experimental Diabetes in the Cat.

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We have previously reported some preliminary observations upon 5 adrenalectomized-depancreatized cats[1] and upon 2 hypophysectomized-depancreatized cats.[2] In the first series of adrenalectomized-depancreatized animals the operations were performed in 3 stages, but in the present group of 5 animals we have found it possible to remove the remaining adrenal and all the pancreas at one operation. These animals have survived 8, 9, 9, 16 and 28 days respectively. By this procedure we have avoided any loss of weight prior to total adrenalectomy and pancreatectomy, and since the results obtained are substantially identical with the previous ones the criticism of Ring[3] that previous inanition was responsible for the effects observed would appear to be unfounded. In addition, depancreatized

[6] Seibert, F. B., *J. Biol. Chem.*, 1928, **78**, 345.

[1] Long, C. N. H., and Lukens, F. D. W. *Science*, 1934, **79**, 569.

[2] Long, C. N. H., and Lukens, F. D. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 326.

[3] Ring, G. C., *Science*, 1934, **80**, 97.