(2) About sixty-six per cent. of the eggs are in early prophase (two new asters have arisen; chromosomes are becoming organized; and the nuclear membrane is still intact). In normal, untreated, control eggs, under comparable conditions, only about twenty-five per cent. are in prophase.

There is also some evidence that in these DNC treated eggs, darkly staining chromatin may be recognized somewhat earlier than in normal eggs, that is during the late resting phase instead of early prophase.

These experiments, as well as others on unfertilized eggs and the fertilization process, indicate that so far as suppression of division is concerned, DNC acts on the nucleus rather than on the cytoplasm or plasma membrane, and that this effect may be due to some modification of or interference with an oxidative process occurring in the late resting phase or early prophase. Further experiments are being conducted to determine the mode of action of the dinitrophenols and their reduction products.

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POSITIVE INFECTION TRIALS WITH ELM "WILT" FUNGI

FOLLOWING the isolation of a variety of fungi from diseased American elms in Illinois, as reported by Harris[1] in 1932, a considerable series of inoculation tests has been carried out and success in reproducing infection has been obtained with 3 of the fungi considered by Harris as most important, namely, two strains of Coniothyrium, designated as "A" and "B," and Phoma "B." It now appears advisable to announce the results of some of these tests.

Each of four three-year-old elm seedlings were inoculated with Coniothyrium "A" in the laboratory on April 6, 1934. A sliver of elm wood, previously sterilized and then well inoculated with a pure culture of the fungus, was inserted through a T-shaped slit in the bark so as to lie in contact with the xylem of the seedling. A glass tube, containing moist cotton at the base, was fitted on the inoculated branch and held in place by perforated corks, in order to surround the inoculation with a moist atmosphere, and the cotton was kept moist during the first few weeks. Pycnidia of the Coniothyrium soon formed on the exposed xylem in the T-shaped slits of all four of the seedlings as well as on the wood slivers used as carriers of inoculum. By the middle of July the inoculated lateral branch of one seedling had shown a slowly progressing but definite "wilt" and, three weeks later, was dead from the tip down to 9 cm below the point of inoculation. Cultural platings from this seedling yielded the Coniothyrium "A," with which it had been inoculated, as far as 20 cm below the point of inoculation and 11 cm below any external evidence of infection.

In another test a positive result was obtained with Coniothyrium "B." Two three-year-old seedlings were inoculated on January 4, 1934, in the following manner. The bark was scraped off one side of the stem for a vertical distance of 1 cm, exposing the xylem. Corn-meal agar containing actively growing mycelium of the fungus was placed in contact with the xylem and covered by a layer of moist cotton. This was enclosed by Cellophane held in place by adhesive plaster. Of these seedlings, one showed symptoms of a general infection by the end of March, and cultural isolations were made from it on April 10. Coniothyrium "B" was obtained from points as far as 15 cm above and 12 cm below the point of inoculation.

Phoma "B," the third agent used, was found to be capable of infecting elm leaves within a very short time. Drops of a spore suspension were placed upon living, detached leaves suspended in a petri dish with the open end of the petiole immersed in water, in modification of the method described by Clinton and McCormick.[2] Development of mature pycnidia took place in the mesophyll within five days. A more abundant infection took place when spores were planted on the upper leaf surface, in spite of the fact that stomates are much more numerous on the lower surface. While these experiments with the Phoma do not furnish conclusive proof of the ability of that fungus to infect a healthy elm tree, they suggest the means by which it gains entrance to the trees from which it is isolated in culture.

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AN ANTIRACHITIC DERIVATIVE OF CHOLESTEROL[1]

RECENTLY Koch, Koch and Ragins[2] reported that provitamin D is not limited to ergosterol but can be formed from cholesterol. In this laboratory an antirachitic substance produced from cholesterol but different from vitamin D has now been isolated in pure form and known constitution through an investigation of the chemistry of the Bills[3] method for the

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[1] Journal paper No. J182 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 103.

[2] F. C. Koch, E. M. Koch and I. K. Ragins, *Jour. Biol. Chem.*, 85: 141, 1929.

[3] C. E. Bills, *Jour. Biol. Chem.*, 67: 753, 1926; C. E.

antirachitic activation of cholesterol with fuller's earth. Bills,[3] and Kon, Daniels and Steenbock[4] concluded several years ago that this active substance was different from vitamin D. However, in working with purer preparations we found that the effect on intestinal reaction, iron reduction and even bone calcification[5] was similar to that of irradiated ergosterol products.

Data with experimental procedures to be reported elsewhere have led to the clarification of the chemistry of the fuller's earth activation of cholesterol. The cholesterol activating constituent of the reactive earth was found to be sulfuric acid or its anhydride. The initial reaction was a dehydration of the cholesterol not only to the dicholesteryl ether, as Bills found, but to the ultimate dehydration product, cholesterilene, of Mauthner and Suida.[6] The final reaction then was found to be sulfonation at the site of the double bond created by the removal of a molecule of water. Actually the concentration of the antirachitic substance was much increased by the treatment of the cholesterilene in carbon tetrachloride with a small amount of sulfur trioxide. This is a well-known method for the sulfonation of aromatic hydrocarbons. Still better yields of the antirachitic substance were obtained when the Friese[7] method for the sulfonation of hydroaromatic hydrocarbons was applied with some modification to the sulfonation of cholesterilene. Using the Shipley technique for the "line" test, protocols were obtained showing degrees of calcification induced by the substance made by various modifications of the Friese method.

If the active substance is a sulfonic acid it should be soluble in water and precipitated by barium. In fact, addition of barium hydroxide or acetate solution to the water soluble acid residue remaining after the evaporation of the acetic acid solvent precipitated a crystalline barium salt. This was filtered off, dried, digested in alcohol, dissolved in carbon tetrachloride and reprecipitated in alcohol. The precipitate was dried at 100° C. The percentage of barium in three such preparations was 13.38, 13.21 and 13.18 and of sulfur, 5.94, 6.16 and 6.28, respectively. The calculated percentages for barium cholesterilenesulfonate $(C_{27}H_{43}O_3S)_2Ba$ are: barium 13.31 and sulfur 6.21. This salt does not melt below 330° C.

Two of these analyzed preparations, 104.4 and 107.2, were converted into the free sulfonic acid by digestion with an equivalent of sulfuric acid and "line" tested in a qualitative way for antirachitic potency. A continuous ++ line of calcification was produced by 3 mg of the cholesterilene sulfonic acid.

Since the insolubility of the barium salt in dilute acid or alkalis indicated a possible formation of the corresponding calcium salt from the free sulfonic acid and the calcium-containing ration in the digestive tract, a preparation 110 of the calcium salt was also "line" tested. Preparation 110 contained 4.37 per cent. calcium and 6.68 per cent. sulfur. The calculated values for $(C_{27}H_{43}O_3S)_2Ca$ are: 4.27 per cent. calcium and 6.86 per cent. sulfur. This salt melts at 320 to 325° C. Its antirachitic potency compared well with its molecular equivalent of the free sulfonic acid.

The potassium salt was prepared from the barium cholesterilenesulfonate by double decomposition with a molecular equivalent of potassium sulfate. This salt melted at 277° C.

A more direct method for the preparation of cholesterilene sulfonic acid and its isolation as the alkaline earth salt is through the sulfonation of cholesterol by modifications of the Friese method. In a preliminary run a 66 per cent. yield of the crude calcium salt was readily obtained.

The cholesterilene sulfonic acid is almost tasteless, soluble in water and also in oils when dehydrated. The monovalent metal salts are generally soluble. The bivalent metal salts are tasteless, insoluble to slightly soluble in water and soluble under certain conditions in organic solvents and oils. Due to their relatively low antirachitic potency overdosage is difficult. However, marked purgative effects were noted with rats, receiving during a four-day period 125 mg of calcium cholesterilenesulfonate.

It was not an objective of this station project to develop another antirachitic substance and it is proposed through letters patent to protect the interests of the public from a promiscuous substitution of such an antirachitic for vitamin D before its pharmacological action is further investigated.

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