system may be involved, for essentially the same results can be secured by post-irradiation exposure to KCN before the incubation period at optimal temperatures.

4) Since anaerobic recovery is greatest when the deoxygenated eggs are held at optimal temperatures, it appears that anaerobiosis, as well as KCN treatment, functions in some other way than merely by preventing the cytochromes from carrying out oxidative processes that are necessary for the expression of the latent damage in the irradiated cell. While the cytochromes are being held in abeyance, a positive function, probably the anaerobic synthesis of proteins and other substances essential for recovery of the damaged cell, is taking place. The exact nature of these anaerobic recovery processes must await further experimentation.

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22 August 1955

## Effect of DDD and Some of Its Derivatives on Plasma 17-OH-Corticosteroids in the Dog

In 1949, Nelson and Woodard (*1*) reported that 2,2-bis-(para-chlorophenyl)-1,1-dichloroethane (DDD) causes atrophy of the adrenal cortex in the dog. Other workers have confirmed this finding and have obtained indirect evidence of decreased adrenal function following DDD administration (*2–4*). Recently, Larson *et al.* (*5*) studied various DDD derivatives in order to determine the relationship of chemical structure to the production of adrenal cortical atrophy or hypertrophy. In the present investigation (*6*) the effect of DDD, of 2,2-bis-(para-ethylphenyl)-1,1-dichloroethane (Perthane), and of 2-hydroxy, 2,2-bis-(para-chlorophenyl)-1,1-dichloroethane (FW-152) on adrenal function in dogs has been followed as indicated by changes in the plasma 17-hydroxycorticosteroids after injection of ACTH, this response being determined before and after the administration of each of the three compounds. Compound FW-152 differs from DDD and Perthane in that it has been found to produce the histological appearance of adrenal cortical hypertrophy rather than atrophy (*5*).

Plasma 17-hydroxycorticosteroids were determined by the method of Nelson and Samuels (*7*). Preliminary experiments revealed that the normal level of circulating 17-hydroxycorticosteroids in dogs is much lower than that found in human beings. In 32 determinations on 9 dogs, the mean level with S.D. was 2.6 ± 1.5 μg/100 ml of plasma. Since these values are at the lower limit of accuracy for the method, it was necessary to work with dogs whose adrenals had been stimulated with an intravenous injection of ACTH. Initially, time-response curves were run on several animals, and it was found that the plasma 17-hydroxycorticosteroids reached a maximum level between 1.5 and 2 hours after 20 I.U. of ACTH (*8*) had been given intravenously. In all subsequent experiments, a control blood sample was drawn, ACTH was given, and a second blood sample was taken at the end of 2 hours. In this way, a 2.5- to 10-fold increase in plasma 17-hydroxycorticosteroids over control levels was obtained; this permitted the measurement of a significant change after the administration of an atrophy-producing compound. Although the range of ACTH response varied among dogs, the response in the individual animal was relatively constant.

In Figs. 1 and 2, the control 17-hydroxycorticosteroid levels after injection of ACTH are represented by a vertical bar that gives the extremes and mean values for three to five tests on each animal. Fig. 1 shows the marked diminution of adrenal responsiveness that occurred after administration of various doses of DDD. Fig. 1*A* illustrates a fall in plasma levels from 20 to 0 μg/100 ml. This animal showed no signs of distress, and after discontinuance of the compound, normal adrenal response to ACTH gradually returned. On subsequent treatment with a

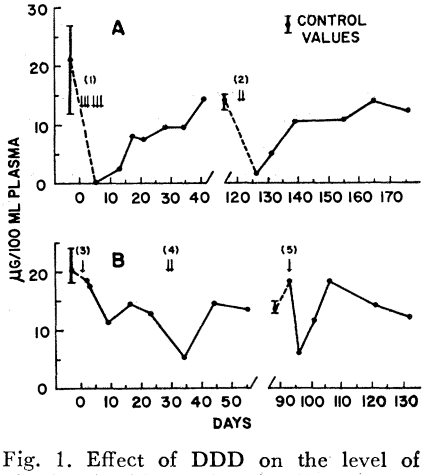


Fig. 1. Effect of DDD on the level of plasma 17-hydroxycorticosteroids 2 hours after injection of ACTH; ↓ indicates oral administration of DDD as a 20-percent solution in corn oil as follows: *1, 2, 3,* and *4*—100 mg/kg day; *5*—200 mg/kg day.

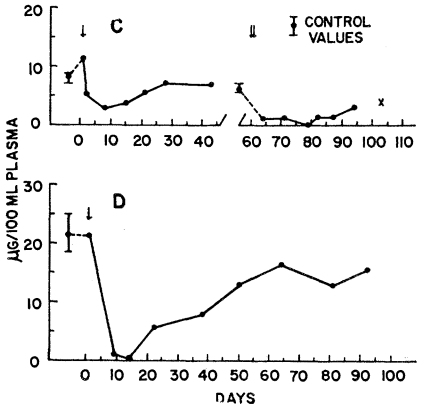


Fig. 2. Effect of Perthane on the level of plasma 17-hydroxycorticosteroids 2 hours after injection of ACTH; ↓ indicates oral administration of Perthane as a 20-percent solution in corn oil at 200 mg/kg day; *X,* animal sacrificed.

smaller dose, a similar fall and recovery were noted. A fall in plasma 17-hydroxycorticosteroids to zero was also obtained with several animals that were treated with Perthane, data on two of which are shown in Fig. 2. One animal (Fig. 2*C*) recovered so slowly after the second treatment with this compound that it was sacrificed at the end of 4 weeks for histopathological study of the adrenals, which showed the typical adrenal cortical atrophy that is produced by Perthane (*5*). The second dog (Fig. 2*D*) evidenced a marked sensitivity to Perthane—a single dose produced a fall in plasma 17-hydroxycorticosteroids to zero. Another dog, not shown, was given six doses of Perthane of 200 mg/kg day. On the fifth day, the plasma level was zero, and death occurred on the eighth day.

As is apparent in Figs. 1*B* and 2*C*, levels somewhat higher than normal were occasionally obtained 6 hours after the compounds were given. This effect was fleeting and was not consistently found.

Two dogs were treated daily with FW-152 at 50 mg/kg day. At the end of 1 week, one died suddenly of an unknown cause, having shown no significant change in the 17-hydroxycorticosteroid level. The other dog was sacrificed after 26 days, when it was in a moribund state. Because of poor fixation, histological examination was unsatisfactory. During the experimental period, the plasma 17-hydroxycorticosteroids fell slowly from 14 to < 2 μg/100 ml, a finding that is of interest in view of the microscopic adrenal changes indicative of hypertrophy that have been previously reported following administration of this compound (*5*).

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6. This investigation was supported in part by a grant from the Lasdon Foundation and in part by a grant from the Rohm and Haas Company.


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## Increased Production of Carotene by Mixed + and – Cultures of Choanephora cucurbitarum

The production of carotenoid pigments by filamentous fungi is apparently quite common, but knowledge of their functions in fungus metabolism is meager. There is evidence that β-carotene is concerned with phototropic responses of certain fungi, and in many fungi, visible amounts of carotenoids are produced only in the reproductive structures. Goodwin (*1*) has reviewed the literature on this subject. *Phycomyces blakesleeanus* has been the principal test fungus, and the mycelium of either the + or the – sex (*2*) has been used. *Choanephora cucurbitarum* is not mentioned as a carotene producer.

*Choanephora cucurbitarum* is heterothallic (*3*) and zygospores have been observed and described (*4, 5*). No mention has been made of carotenoids in the mycelium, and only Wolf (*5*) reports observing "numerous yellowish oil globules" in immature zygospores.

During routine culturing of *C. cucurbitarum,* it was observed that the mycelium of combined + and – cultures in liquid medium became bright yellow within a few days, while the mycelium of either the + or – sex cultured alone was only slightly yellowish. This paper (*6*) reports results of subsequent experiments that show that the production of β-carotene by *C. cucurbitarum* is greatly increased in mixed + and – culture.

The + and – cultures of *C. cucurbitarum* used in this study were isolated from the same diseased pumpkin flower at Morgantown, W. Va., in 1954. A liquid medium (glucose, 25 g; acid-hydrolyzed casein, 2 g/lit, essential salts, and thiamine) at an initial *p*H of 6.0 was used.

The fungus was cultured at 25°C in 250-ml flasks containing 25 ml of medium without agitation and at 28°C in 9-lit bottles containing 6 lit of medium through which sterile air bubbled continuously. The experiments have been repeated a number of times with similar results, although quantitative carotene determinations were made on only some of the cultures.

Under both sets of cultural conditions, the mycelium in the mixed + and – cultures began to show yellow pigmentation after about 3 days, reaching a maximum intensity about the fifth or sixth day. The + and – mycelia produced little yellow pigment when they were grown separately.

Under all conditions of growth, the mycelium contained much oil after a few days, but only the mycelium in the mixed cultures showed conspicuous yellow pigments in the oil. Later, during sexual reproduction, these pigments usually became concentrated in the suspensor cells that subtend the immature zygospores. Some yellow pigment is evident in the oil droplets in immature zygospores.

The yellow mycelium was squeezed between layers of cloth to remove excess water, and drying was completed with absolute methanol. The pigments were extracted with petroleum ether, saponified and reextracted with petroleum ether. The β-carotene content of each extract was determined by measuring the optical density of 460 mμ in a Beckman photoelectric spectrophotometer according to the procedures of Garton *et al.* (*7*).

By using chromatographic adsorption techniques (*7*) and repeated recrystallizations from a 1-to-1 solution of ethanol and petroleum ether (bp 40° to 60°C), it was possible to isolate the primary pigment. This material was characterized by the use of chromatographic adsorption techniques (*7*), molecular extinction curves and extinction values ($E_{1\ cm}^{1\ percent} = 2400$ at 455 mμ in cyclohexane) (*8*), and identified as β-carotene. The yields of β-carotene in one typical experiment are presented in Table 1.

Under these conditions, the mycelium grown in mixed + and – cultures produced 15 to 20 times as much β-carotene per gram of mycelium as did that of either sex grown alone. The evidence suggested that the stimulatory substances responsible for the enhanced carotene production were secreted by the mycelium of the opposite sex.

When the + and – mycelia are grown on opposite sides of a cellophane membrane, both mycelia usually show increased pigment production, indicating that the stimulating substances are diffusible through cellophane and that the effect is reciprocal. The failure of these cultures to form zygospores after further incubation is evidence that the mycelium did not penetrate the cellophane.

Increased carotene production by the mycelium in mixed + and – cultures of a fungus does not seem to have been previously reported. Since there is normally no anastomosis between the + and – vegetative hyphae of the Mucorales, which would result in heterocaryotic mycelium, the stimulation in *C. cucurbitarum* must originate as secretions from the mycelium of the opposite sex. These secretions are possibly of the nature of hormones. It seems probable that some relationship exists between the production of carotene and sexual reproduction in *C. cucurbitarum.*

Table 1. Weight of dry mycelium and β-carotene after 6 days in 6-lit aerated cultures when + and – sexes were grown separately and together.

| Culture | Dry mycelium (g) | β-carotene (μg) | Amt. β-carotene in dry mycelium (μg/g) |
|---|---|---|---|
| + | 25 | 1,140 | 45.6 |
| – | 22 | 1,377 | 62.6 |
| ± | 18 | 16,560 | 920.0 |

These investigations, as well as others designed to give more information on the role of carotenoids in the fungi and studies particularly concerning the relationship of carotene to sexual reproduction of *C. cucuribitarum,* are being continued.


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12 August 1955

## Fate of Radiostrontium Fed to Habrobracon Females

Strontium-89 has been of interest to us, not only because it is a biologically important product of nuclear fission, but more specifically because it is the only pure beta-emitting radioisotope with which we have been able to produce per-