The Persistence of Growth Inhibition in Young Rats Induced by 1,2,5,6-Dibenzanthracene

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The highly active carcinogenic hydrocarbons have been shown to retard or even inhibit the bodily growth of young rats. The subsequent confirmation by several investigators corresponds so closely to the results of the pioneer work done by Haddow, Scott, and Scott (6) that this observation may be accepted as an established fact.1

Perhaps the most remarkable feature of these experiments is the persistence of growth inhibition by the carcinogenic hydrocarbons. The retardation of growth subsequent to a single injection of 10 to 20 mgm. of a highly potent carcinogenic hydrocarbon is described as being extremely prolonged, if not permanent. This is in marked contrast to the action of other growth-retarding agents, such as colchicine and lead, which in one application provoke only a transient interference with growth, followed by resumption of the normal rate of growth.

In view of the earlier prevalent opinion that carcinogenic hydrocarbons are eliminated from the body in a relatively short time following their administration, their prolonged inhibitory effect on growth was surprising. This opinion was based on the studies of Chalmers (4), Chalmers and Peacock (5), Berenblum and Kendall (3), and Hieger (7) which indicated that the period of elimination of administered carcinogenic hydrocarbons was relatively short, the time depending upon the nature of the solvent and the mode of application. In 1937, however, Hieger (8) cautioned against drawing final conclusions, pointing out that the sensitivity of the methods of assay was still under investigation. More recent work, with improved methods, by Peacock and Beck (10) has shown that the period of time required for the elimination of administered carcinogenic hydrocarbons from the body of the experimental animal is significantly longer than formerly assumed.

In view of recent studies of elimination of these compounds the possibility exists that the extremely prolonged growth-inhibitory action of carcinogenic hydrocarbons may be due to the protracted presence of the substances at the site of injection, whence a gradual but uninterrupted resorption takes place. It would be of interest, therefore, to ascertain whether the growth-retarding effect of a compound would continue if the deposit were removed by surgical intervention at a time when unmistakable inhibition of growth had become manifest, or whether the removal of the hydrocarbon would be followed promptly by an increase in the growth rate of the animal. The experiments reported in this paper were made to investigate this problem.

Technical Considerations

The surgical removal of the hydrocarbon several days or weeks after its injection presents serious difficulties. In the experiments of Haddow (6), Lees (9), and others the highly potent carcinogenic compound, emulsified or dissolved in oil or lard, was injected either into the peritoneal cavity or into the subcutaneous tissue of the animal's back or groin. For the purposes of the present study it would have been necessary to remove the oil or lard 15 to 30 days after the injection. It is obviously impossible to remove such material surgically from the peritoneal cavity. Nor would a dissection of the material from the subcutaneous tissue seem feasible because this procedure would introduce a large and indefinite experimental error.

Andervont's (2) experiments on immunization suggested that the injection of the hydrocarbon into the subcutaneous tissue of the rat's tail, with subsequent amputation of the tail cephalad to the site of the deposit, at a suitable time, would be a satisfactory procedure. It seemed to the author that this method would provide a unique opportunity for the study of the resulting changes in the growth rate of the animal.

Much time and work were wasted in a first series

1 An earlier series of the writer's experiments (1) undertaken for a different purpose, appeared to show that the body growth of young rats had been retarded by exceedingly small doses of carcinogenic hydrocarbons. It is to be admitted freely that this was an erroneous observation due to the omission of certain precautions which at the time did not appear important to the investigator since those studies were conducted with no more than a passing interest in the determination of body growth. In the course of subsequent experiments, undertaken as a check on these results, the author was unable to obtain any distinct reproducible effect. On the contrary, this work confirmed once more the fact established by Haddow (6), by Morelli and Guastalla and by Lees (9) that to induce a striking inhibitory effect a minimum of about 6 to 7 mgm. of a powerful carcinogenic hydrocarbon is needed.—Author.
of 14 experiments on 255 rats with caudal injections of 1,2,5,6-dibenzanthracene in oil. No satisfactory conclusions could be reached because of the high morbidity and mortality of the experimental animals. The chief cause of death was fat embolism resulting, in spite of all possible precautions, in sudden death, or more frequently in chronic infarction of the lung. Infarction of the lung, verified at autopsy, was difficult to diagnose while the animal was alive. This caused embarrassing uncertainties as to whether or not such animals should be included in the calculations.

The prime factor in this condition of embolism and infarction appears to be some peculiarity of the structure of the rat’s tail, which favors the direct entrance into the blood stream of fluid media injected into the caudal subcutaneous tissue under pressure greater than that of the venous pressure. In view of the frequency of these complications, the first series of experiments was discarded and the method replaced by one more free from uncertainties and accidents.

**Experimental**

The following procedure was carried out in 6 experiments with 109 test rats and 58 controls:

A first caudal amputation was performed about 6 to 8 cm. from the tip of the tail. By pushing back the skin a cup 5 to 6 cm. deep was left after dividing and removing the bony portion. This skin cuff was filled to half of its capacity with 10 to 20 mgm. of a thick aqueous paste of crystals of 1,2,5,6-dibenzanthracene. A running suture of two or three stitches closed the cuff. A dressing fixed with mastisol was kept over the wound during the first 24 hours after the operation.

In the control rats, operated upon in the same manner and on the same day as the test animals, the skin cuff was half filled with dextrose.

Evidence of the fact that this seemingly more extensive operation is less drastic than caudal injection under pressure is the observation that a decrease in weight, which occurred frequently after injection, was the exception rather than the rule after caudal amputation.

Some uncertainty as to the precise amount of dibenzanthracene applied is, of course, inevitably associated with this method. The knowledge of the exact amount, however, was not considered essential.

After the lapse of a certain time, when impaired growth of the test rats had become unmistakable, a second caudal amputation was performed on both the test and control animals. At this operation a segment 20 to 30 mm. long was removed from the stump of the tail.

**Results**

Experiment 3, Series II, involving 19 test and 10 control rats, was the first which could be carried to a successful finish. The data, summarized in Table I, are shown graphically in Fig. 1. In spite of the poorly vascularized cicatrix encapsulating the paste of crystals and in spite of the absence of fat which would have aided solution of the compound the results indicated clearly that the dibenzanthracene was absorbed slowly and regularly. The growth of the test animals was definitely retarded.

By the 7th day following the caudal application of 10 mgm. of 1,2,5,6-dibenzanthracene the curve of percentage increase in weight of the test rats was falling steadily below that of the controls. This retardation of growth rate was considerable by the 30th day, when the second caudal amputation was performed to remove the deposit of dibenzanthracene. After the second caudal amputation the curve of percentage increase in weight of the test animals was accelerated. The curve of percentage increase became parallel to that of the controls, but at a lower level, on which it remained until the 110th day, when the experiment was terminated.

Experiments 4, 5, and 6, Series II, involving a total of 90 test rats with 50 controls were carried out to verify this observation.

On account of the highly irritating property of crystals of dibenzanthracene placed in the skin cuff of the rat’s tail the slightest slip in asepsis leads to infection and suppuration. This occurred in Experiments 4 and 5. While the stumps of the tails of all the controls healed by primary intention, the majority of the...
40 test animals used in these two experiments showed signs of localized inflammation and suppuration. In Experiments 4 and 5 dibenzanthracene failed to induce the slightest retardation of growth. On this account Experiment 4 was not pursued beyond the 29th day. In Experiment 5, on the 13th day, when the growth rate of the test animals was the same as that of the controls, a second caudal amputation 20 to 30 mm. nearer the body, was performed and 20 mgm. of dibenzanthracene were placed in the new skin cuff. Although the stumps healed aseptically, no inhibition of growth occurred during the next 30 days, at which time the experiment was terminated.

In Experiment 6, Series II, 20 mgm. of 1,2,5,6-dibenzanthracene was placed in the skin cuff after the first caudal amputation. The results, presented in Table II and Fig. 2, were practically the same as those of Experiment 3. The only noteworthy difference between the two was that in Experiment 6 the retardation of growth rate following the application of dibenzanthracene occurred earlier than it did in Experiment 3. This effect appeared to be attributable to the larger amount of the compound applied in Experiment 6. The stunting of the animals persisted after the removal of the deposit of dibenzanthracene on the 24th day.

**DISCUSSION**

In the experiments of Series II, described in this communication, there was no significant mortality among the test and control animals, in contrast to the losses which occurred in Series I. In Series II the experiments were started and terminated with a sufficiently large number of animals. The consistent results of Experiments 3 and 6 are therefore regarded as significant evidence that 1,2,5,6-dibenzanthracene applied in the manner described produces retardation of growth of young adult rats and that an effect on growth of the animals persists after removal of the compound. Although the results are strictly applicable only to the substance used, namely, 1,2,5,6-dibenzanthracene, they are regarded as evidence confirming the original observation of Haddow (6) concerning
the persistence of inhibition of growth exerted by highly active carcinogenic hydrocarbons.

Two effects were noted in these experiments; (a) permanent dwarfing and (b) progressive retardation of growth rate. These will be discussed briefly.

The dwarfing of rats by dibenzanthracene was irreversible during periods of 204, 180, 142, 110, and 30 days. Apparently this retardation of growth was permanent. On the other hand, the progressive character of growth retardation is probably not an inherent property of the carcinogen. It is the result of a combination of two factors. One factor is the permanent dwarfing effect of the absorbed carcinogen. The second factor, responsible for the progressive course of growth retardation, is the continuous and protracted absorption of the compound from a deposit in the tissues. The moment the deposit is removed by surgical intervention the progressive character of the inhibition of growth ceases.

Although the mode of action of the carcinogenic compounds is obscure, it appears probable that a clearer understanding of their effects may be gained through the knowledge that the general retardation of growth produced by absorbed dibenzanthracene is permanent.

The avoidance of infection is essential for the success of experiments of this type. It seems difficult to escape the deduction from Experiments 4 and 5 that not only the suppuration of the stump but also the induration which follows in the wake of subsiding cellulitis higher in the tail prevented absorption of dibenzanthracene from its deposit in the skin cuff. This deduction is in accord with the opinion of Halsted concerning the genesis of swelling of the arm following operations for cancer of the breast.

**SUMMARY AND CONCLUSIONS**

Two series of experiments were performed to investigate the effect of 1,2,5,6-dibenzanthracene upon the growth of young adult white rats.

In Series I, comprising 14 experiments, the hydrocarbon dissolved in oil was injected under pressure into the subcutaneous tissue of the rat's tail. Most of the animals treated in this manner died of fat embolism or of infarction of the lung, as the result of passage of the oily material into the caudal veins. Because of these complications which introduced great uncertainties, this method was discarded.

In Series II, a watery paste of crystals of 1,2,5,6-dibenzanthracene was placed in a skin cuff formed by amputation of a portion of the tail. When inhibition of growth had become unmistakable, 24 to 30 days after the first application of the carcinogen, the deposit was removed by a second caudal amputation. This procedure was entirely satisfactory.

The results of two experiments, in which 50 test animals and 40 controls were used, showed that 1,2,5,6-dibenzanthracene was absorbed gradually and regularly from the skin cuff and produced a persistent inhibition of growth. After removal of the deposit of the compound a rate of growth similar to that of the controls was resumed.

On the basis of these observations, it is concluded that:

1. 1,2,5,6-dibenzanthracene applied locally in the tail of the young adult rat causes a permanent dwarfing of the animal.
2. Progressive inhibition of growth rate is not an inherent property of this carcinogen, but is dependent upon continued absorption of the compound from the local deposit.

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