were studied and found to coincide with properties of cell receptor site material. Inhibitory material did not directly affect host cells but was bound to virus. A possible role of receptor in elimination of virus during recovery from infection is discussed.

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Rate of Metabolism of 9,10-Dimethyl-1,2-Benzanthracene in Newborn and Adult Mice.* (28292)

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During investigations in this laboratory it was demonstrated that the newborn mouse is particularly susceptible to the action of carcinogenic chemicals(1,2). This has since been confirmed by others(3,4). In particular, the polycyclic aromatic hydrocarbon 9,10-dimethyl-1,2-benzanthracene (DMBA) has been found to induce a high incidence of lymphomas and other tumors in newborn mice at dosages, on a body weight basis, that would induce a few such tumors in the adult. In addition to this quantitative difference there is a qualitative difference in the neoplasms induced. The majority of tumors that have been recorded in animals treated at birth have been lymphomas, lung adenomas and a scattering of other interesting neoplasms(1,2,3,4). In the adult, injection of DMBA by the subcutaneous route results in induction of sarcomas, a tumor seen rarely in mice treated when newborn(5). A number of other carcinogens have been tested in the same system including the related polycyclic aromatic hydrocarbons 3,4-benzpyrene,1,2,5,6-dibenzanthracene and 20-methylcholanthrene, in addition to the unrelated compound urethan(2,6,7). All of these compounds manifested an enhanced response in the newborn with the same characteristics, but in none was the difference so marked as with DMBA.

A variety of reasons for the susceptibility of the newborn mouse to carcinogens has been advanced, but none have been tested experimentally. The experiments referred to were originally prompted by the demonstration of the usefulness of the newborn mouse in studies of viral carcinogenesis. The particular susceptibility of the newborn animal to various oncogenic viruses has been the foundation of the recent upsurge of work in this field. It is still unclear why the newborn responds in this manner, although the ill-informed nature

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of the antibody response at this stage of life appears likely to be a major factor. In the instance of chemical carcinogenesis it seemed to us that another explanation of the differences encountered might be due to differences in metabolic or detoxification rates between newborn and adult mice. This proposition has been examined in the studies to be reported.

Materials and methods. Swiss mice from our own colony were used. "Newborns" refers to mice 17 to 24 hours old at time of treatment. Adult mice were 10 to 12 weeks old at time of treatment.

Groups of 30 to 40 animals were injected intraperitoneally with a measured dose of DMBA in olive oil; newborn mice received 0.05 ml of a solution containing 3.2 mg/ml, adult mice received 0.10 ml of a solution containing 30 mg/ml (the exact dose was determined spectroscopically as the average of several blanks; subsequent results were expressed as the proportion of the injected dose recovered). Immediately after injection ('zero time'), and at 1 day intervals thereafter, 3 to 5 animals were sacrificed by breaking the neck and each carcass was homogenized in a Virtis homogenizer, with acetone as solvent. The homogenate was transferred to an extraction thimble and extracted for one hour in a Soxhlet apparatus. The acetone and water were removed from the extract by evaporation on a steam bath under nitrogen. The residue from the newborn animals was dissolved in 2,2,4-trimethylpentane (isooctane) and the DMBA in the solution estimated spectroscopically at 297 mμ. In the case of the adults the background absorption of the lipid was too great to permit direct measurement of DMBA and the polycyclic hydrocarbon was extracted by partition between cyclohexane and nitromethane(8). In the later stages of the experiments the nitromethane retained sufficient background absorbing material to make difficult the determination of the very small amounts of DMBA remaining. The DMBA was, in these cases, separated from the interfering material in the residue from evaporation of nitromethane either by paper chromatography (N, N-dimethylformamide/isooctane(9)) or by thin layer chromatography (silica gel/benzene-isooctane 1:19). The blue fluorescent DMBA band was eluted by extraction with benzene-ethanol 3:1, the solution evaporated to dryness and the absorption spectrum of the residue taken in a known volume of isooctane.

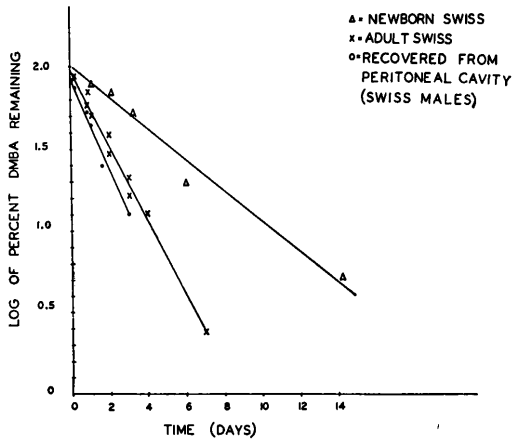

FIG. 1. Log of avg percentage of injected DMBA recovered at different times after injection.

In one series of experiments the DMBA remaining in the peritoneal cavity was measured by washing out the cavity with acetone, evaporating the solution and estimating the hydrocarbon in isooctane solution. The DMBA in the carcass was also determined in each case by the method described above.

All of the experimental procedures were carried out in diffused light to minimize photooxidation of DMBA and all evaporations and distillations were under nitrogen. Overall recovery of DMBA in control experiments was above 80%.

Results. The results given in Fig. 1 are expressed as percentages of the injected dose recovered; this eliminates small differences in quantity of DMBA injected from one experiment to another. In all cases the recovered DMBA falls exponentially with time until the quantity can no longer be measured accurately. A semilogarithmic plot of recovered DMBA against time (Fig. 1) shows that the slope of the graph is considerably steeper for the adults (including the data on the peritoneal cavity alone) than for the newborn animals. No sex difference was noted in the results with adults; sex was not de-

termined in newborns. The DMBA recovered from the carcass alone, on the other hand, remained almost constant until virtually all of the DMBA had been absorbed from the peritoneal cavity.

Discussion. The experimental studies reported demonstrate that the carcinogen 9,10-dimethyl-1,2-benzanthracene persists for long periods in the newborn mouse; it is cleared much more quickly from the adult mouse. Only 10% of the dose injected is present in the adult mouse after 4 days, whereas, in the newborn, 10% of the injected dose is still present 10 days after injection. Physical factors appeared to play some part in the clearance of the hydrocarbon, since the quantity of hydrocarbon in the carcass other than the peritoneal cavity remained fairly constant, while that in the peritoneal cavity declined exponentially. An experiment in which determinations were made in peritoneal cavity and carcass in the few hours immediately following DMBA injection, showed that the quantity of DMBA in the carcass rose to 200-250 μg within one hour, this quantity remaining virtually constant thereafter. In addition the rate of disappearance of the DMBA, expressed as a half-life, was almost the same whether the dose injected was 350 μg or 10 times as much (performed in another strain of mice).

Although further studies must be carried out to determine the distribution and persistence of DMBA in the body, as well as those of the metabolites of this carcinogen, certain inferences seem permissible from the information so far available. It appears that a correlation exists between the persistence of unchanged carcinogen and the neoplastic response, as seen in the adult and newborn mouse. In addition it is suggested that the carcinogen 9,10-dimethyl-1,2-benzanthracene is most likely a biologically active molecule in unchanged form. There does not appear to be a need, as has been widely assumed, for this compound to be metabolized into an active carcinogen; in fact, no metabolite of a polycyclic aromatic hydrocarbon has yet been isolated which has any but negligible tumorigenic activity.

The results given here do not, of course, resolve the qualitative differences in the effect of DMBA on adult and newborn mice; neither do they begin to settle many other outstanding problems in DMBA carcinogenesis. They do, however, provide the basis for some inferences on which further studies can be based.

Summary. Determination of the proportion of free 9,10-dimethyl-1,2-benzanthracene remaining in newborn and in adult mice following intraperitoneal injection, revealed that this compound disappeared more quickly from the adult than from the newborn animals. This supports the view that the greater susceptibility of the newborn to tumor induction by this carcinogen is due to longer persistence of the compound in the newborn than in the adult.

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