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## Experimental Studies of Factors Influencing Hepatic Metastases: XI. Effect of Hepatic Trauma in Hypophysectomized Animals.* (27104)

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We have reported(1) that hepatic trauma greatly augmented the take and growth of experimentally induced hepatic metastases. The same results were obtained in adrenalectomized animals(2), indicating to us that adrenocortical "stress" was probably not responsible for this phenomenon. Recently we noted that the incidence of such tumors was markedly reduced in hypophysectomized animals and that suppression of metastases appeared independent of the general somatic response to hypophysectomy or to deficiency of ACTH(3). This provoked the question as to whether or not the augmentory tumor response to trauma was eliminated in pituitary ablated animals or whether or not the suppressive tumor effect of hypophysectomy could be overcome by liver damage. The following presents the effect of hepatic trauma upon artificially induced hepatic metastases in hypophysectomized animals.

*Materials and methods.* Adult female normal and hypophysectomized Sprague-Dawley rats‡ weighing 175-200 g were used in all experiments. They were maintained in individual cages and permitted water *ad libitum*. Hypophysectomized animals were received in the laboratory 2 days after operation. Upon arrival both hypophysectomized and control animals were placed on a diet of one-half ground Chow§ and one-half dog food‖ with a daily supplement of fresh orange and raw carrot. Following 8 days of *ad libitum* feeding, paired-feeding of normal controls to non-traumatized hypophysectomized animals was begun and maintained until sacrifice. The latter continued to eat *ad libitum*. After a week of such feeding, groups of normal control animals, hypophysectomized control rats and pituitary ablated animals to be subjected to hepatic trauma were injected intraportally with Walker carcinoma cells. This tumor has been propagated in our laboratory for 4 years. The method of tumor cell preparation and inoculation has been described(4).

All animals in these experiments were lightly anesthetized with ether, subjected to celiotomy, and injected intraportally *via* a large mesenteric vein with ½ ml of a saline-plasma solution containing 5,000 tumor cells. Experiments were devised so that control and experimental animals received injections of the same tumor cell preparation. Hepatic trauma, as previously reported(1), was produced by massage of the liver either 5 minutes, 2, 7, or 14 days following injection of tumor cells, and animals were sacrificed 14 days following the manipulation. Careful gross examination of livers for tumor was

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§ Purina.

‖ Ken-L-Ration.

TABLE I. Incidence of Hepatic Metastases in Non-traumatized and Traumatized Hypophysectomized Animals.

| Group | Condition of pituitary | Liver trauma and time after inj. | Time of sacrifice, days after inj. of tumor cells | No. of rats | | | |
|---|---|---|---|---|---|---|---|
| | | | | Total | + | — | % + |
| I | Hypox | None | 14 | 205 | 3 | 202 | 1 |
| II | " | " | 21 | 56 | 2 | 54 | 4 |
| III | " | " | 28 | 50 | 7 | 42 | 14 |
| IV | Intact | " | 14 | 30 | 14 | 16 | 47 |
| V | " | " | 21 | 38 | 21 | 17 | 55 |
| VI | " | " | 28 | 40 | 25 | 15 | 63 |
| VII | Hypox | Immed. | 14 | 48 | 22 | 26 | 46 |
| VIII | " | 48 hr | 16 | 27 | 5 | 22 | 19 |
| IX | " | 7 days | 21 | 24 | 1 | 23 | 4 |
| X | " | 14 " | 28 | 30 | 3 | 27 | 10 |

carried out and questionable lesions were microscopically examined. Effectiveness of hypophysectomy was assessed by measurements of the width of tibial cartilages(5).

*Results.* Hypophysectomized rats inoculated intraportally with 5,000 tumor cells and sacrificed either 14, 21, or 28 days following injection (Groups I, II, III respectively; Table I) demonstrated a markedly decreased incidence of liver metastases when compared with similarly treated animals having intact pituitaries (Groups IV, V, VI). When hypophysectomized animals were similarly injected with tumor but were subjected to hepatic trauma by liver massage immediately following inoculation (Group VII), 22 of 48 rats (46%) had liver tumor at sacrifice. If such animals were subjected to hepatic trauma 48 hours after tumor cell injection (Group VIII), 19% were "positive" for tumor. When such damage was produced 7 or 14 days after introduction of tumor cells (Groups IX and X) and animals were sacrificed 14 days later, no effects were elicited. Only 4% and 10% of such animals demonstrated tumor, an incidence quite similar to that observed in hypophysectomized untraumatized animals injected with the same number of tumor cells and permitted to survive for the same length of time (Groups II and III; Table I).

*Discussion.* These experiments indicate that the profound inhibition of hepatic metastases observed in hypophysectomized animals is overcome when livers of such animals are traumatized immediately following tumor cell inoculation. At sacrifice 14 days later the number of hypophysectomized animals bearing tumor was similar to that in a group of normal control rats. This effect was still evident when hepatic manipulation was performed 48 hours after tumor injection but was not observed when longer time intervals (*i.e.*, 7 or 14 days) between injection and trauma were investigated. This differed from findings in animals with intact pituitaries. It has been reported that, in the latter, manipulation of the liver 14 days after tumor injection resulted in a 4-fold increase in animals exhibiting such lesions after another week. It was suggested that tumor cells could remain viable but dormant in the liver for a protracted period of time before being "triggered" into activity by the liver trauma. That such results were not observed in the hypophysectomized animal when trauma was instituted at a similar length of time after injection (*i.e.*, 14 days) suggests that in such animals either tumor cells do not remain viable in a dormant state or, less likely, that under such conditions this stimulus of trauma is inadequate for tumor growth.

It is conjectural as to what local changes might occur in the livers of traumatized hypophysectomized rats which could account for the findings. At present, changes in liver catalase are being evaluated. It has been reported that hypophysectomy results in a 2- to 3-fold increase in activity of this enzyme and that with growth of tumor there is a marked reduction. There is a possibility that the growth of metastases in traumatized hypophysectomized animals may not be entirely due to "soil" alteration but may be medi-

ated through a humoral factor. This is suggested by the observation of similar results (6) in hypophysectomized animals subjected to dorsal celiotomy and subcutaneous implantation of tumor cells.

*Summary.* These findings reveal that the profound inhibition of metastases observed in pituitary ablated animals is overcome by hepatic damage. The effect was no longer evident in animals subjected to hepatic manipulation 7 and 14 days after injection of tumor cells.

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## Elucidation of a Factor Essential in Oxidative Phosphorylation of a Mammalian System.* (27105)

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Whole homogenates of rat liver preincubated with RNAase lose the capacity to convert mevalonic acid (MVA) into non-saponifiable material (NSF) or cholesterol (CHL) (1). Such treated liver homogenates, in contrast to controls, are devoid of ATP(2). RNAase inactivation of MVA utilization may be prevented or reversed by an autoclaved extract of fresh liver previously treated with a Dowex-1 anion exchange resin to remove any residual ATP that it might contain, and conversion of MVA to NSF and CHL in this system is proportional to the ATP level maintained(3,4). The conclusion has been drawn that there exists in autoclaved liver extract a factor concerned with maintenance of ATP levels in liver homogenates incubated aerobically(3,4). Subsequent study has shown that this activity is not confined to autoclaved liver extract, however, since the effect of autoclaved liver extract may be duplicated with DNA, large amounts of RNA, or other polyanions such as polyethylene sulfonate or heparin(5). The properties of the factor from autoclaved liver extract including, for example, failure to be retained by a strong anion exchange resin, lability to both weak acid and alkali, loss of activity on dialysis, and resistance to both RNAase and DNAase (4) all suggested that the factor present in autoclaved liver extract, required by RNAase-treated homogenates to maintain a level of ATP is not simply RNA or DNA. This paper is concerned with fractionation studies that have led to some understanding of the nature of the factor of autoclaved liver extract. A preliminary report of the findings has been presented(6).

*Materials and methods.* The experimental procedures involved in biosynthesis of NSF and CHL from MVA by rat liver homogenates have been described(1-7). In brief, cooled liver (1 volume) from young rats was homogenized quickly in a "loose" Potter-Elvehjem homogenizer (1 mm clearance) with pH 7.0 phosphate (0.1 M)-magnesium (0.006 M)-nicotinamide (0.03 M) buffer (2 volumes) and centrifuged (200 × g, 3 min.) to remove connective tissue. This essentially whole homogenate was dispensed (5 ml aliquots) into flasks (125 ml Erlenmeyer) containing ATP (1 mg), DPN (10 mg), sodium succinate (100 mg), and RNAase where in-

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