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HOW TO INFLUENCE THE METASTASIS OF HEPATIC TUMOURS INDUCED BY BUTTER-YELLOW

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(Received August 18, 1955)

The formation of metastases is considered one of the practically most important and biologically most significant and interesting problems of both clinical and experimental oncology.

Most authors hold that the metastasis of tumours, i. e. the transfer of tumour cells from the primary focus to distant parts of the body, and their proliferation on the new site, has much in common with transplantation. Therefore, they expected a solution of the pertaining biological problems from the study of transplanted tumours, especially as regards their capacity to form metastases (6). Useful and practicable as investigations of this kind have proved to be, this way of approaching the said problems is far from being suited for observing and elucidating all the obscure questions arising in the pathology of metastases.

There are essential differences between spontaneous and transplanted tumours ; we cannot refer here but to the most significant of these differences, such as, for instance, the ontogeny of spontaneous tumours, including those of man, which is entirely different from that of transplants, or the fact that spontaneous tumours are formed from the own tissues of the organism, that their type of growth differs from that of transplanted tumours and that immunological reactions in animals with spontaneous tumours have been observed to be different from those in animals with transplanted tumours (Hackmann-5).

Since induced tumours show a greater resemblance to spontaneous growths than do transplants (6), investigations into the metastases of the former may be expected to throw light on such rules governing the metastatic processes of human cancers as are inaccessible to experiments with transplants. Investigations of this kind are, however, greatly hindered by the fact that metastases of induced tumours are a comparatively rare phenomenon.

In order to study the process of metastasis in connection with induced tumours it is, first of all, necessary to achieve such an increase in, and such a standardization, of the metastases as to make their occurrence reliably certain and, therefore, suitable for systematic pathological investigations. The present experiments have been started with this end in view. As it was the dietary

treatment and a prolongation of the life of tumourous animals which has seemed to offer the most appropriate physiological method among the numerous treatments used with a view to promoting the metastasis of transplanted tumours, these have been adopted to approach the problem in question.

Material and methods

In the experiments, 150 young albino rats, mostly male, of approximately the same age and weight (130 g, on the average), taken from a strain reared at the Institute of Pathological Anatomy of the University in Debrecen, were used. Dividing them into 3 groups, each of the groups was placed on a different diet to which an identical amount of butter-yellow (para-dimethylamino-azobenzene, p—DAB) was added. The food of group G consisted of a mixture of equal quantities of cereals such as wheat, maize and oatmeal, supplemented by carrot every other day. The animals of group K were kept on a semisynthetic diet consisting of casein (12%), wheat starch (78%), Sós' [11] salt mixture [4], edible oil (5%) and rice bran (1%). The animals of group E received the normal diet of Sós [12]. However, at the onset of the experiment the members of this group had been fed for two weeks a diet deficient in methionine and poor in tryptophane [11]; and also later whenever their weight was observed to have increased, these rats were fed that inadequate diet for a fortnight; this occurred three times in the course of the experiment. Each of the animals received a daily ration of 10 g, irrespective of the composition of the diet. Vitamin D_2 was administered regularly to all groups.

The animals were weighed every two weeks; the state of their livers was closely observed not only by palpation but also by laparotomy and biopsy. Many of the animals, on being found tumourous and marasmic were placed on a dye-free diet in order to prolong their life.

While some of the animals died spontaneously (especially during the first weeks of treatment), others — in the first place those with tumours — were sacrificed in ether or chloroform anaesthesia. The organs were fixed in 4 percent formaldehyd, the lymph nodes in Susa's or Carnoy's fluid or in ice-cooled 80 percent, alcohol solution. Liver, lungs and lymph nodes were subjected to systematical, other organs to occasional, histological studies.

Results

Hepatic tumour developed in 65 rats out of the total of 150 animals of the three groups (43,3 percent). While the incidence was found to be approximately equal in the different groups, the average length of time needed for the growth of the tumour varied according to the diet (428 days in group G, 280 in group K, and 306 in group E).

The proportion of the various histological types of tumour was also more or less identical in all groups.

The average weight of the tumourous livers was about 26 g in all groups, with a minimum of 7,75 g and a maximum of 63 g.

Autopsy revealed 59 cases of malignant and only 6 cases of benign tumours. Metastasis was found in 25 (42,3 percent) out of the 59 malignant cases. The distribution of metastasizing tumours was more or less equal in the various groups (Table I).

Table I

Group	Number of malign. tum.	Number of metastases
E	14	5
G	21	10
K	24	10

The proportion of metastasis among tumours of different histological structure was likewise equal.

Metastases were encountered both in the lymph nodes and the lungs. The

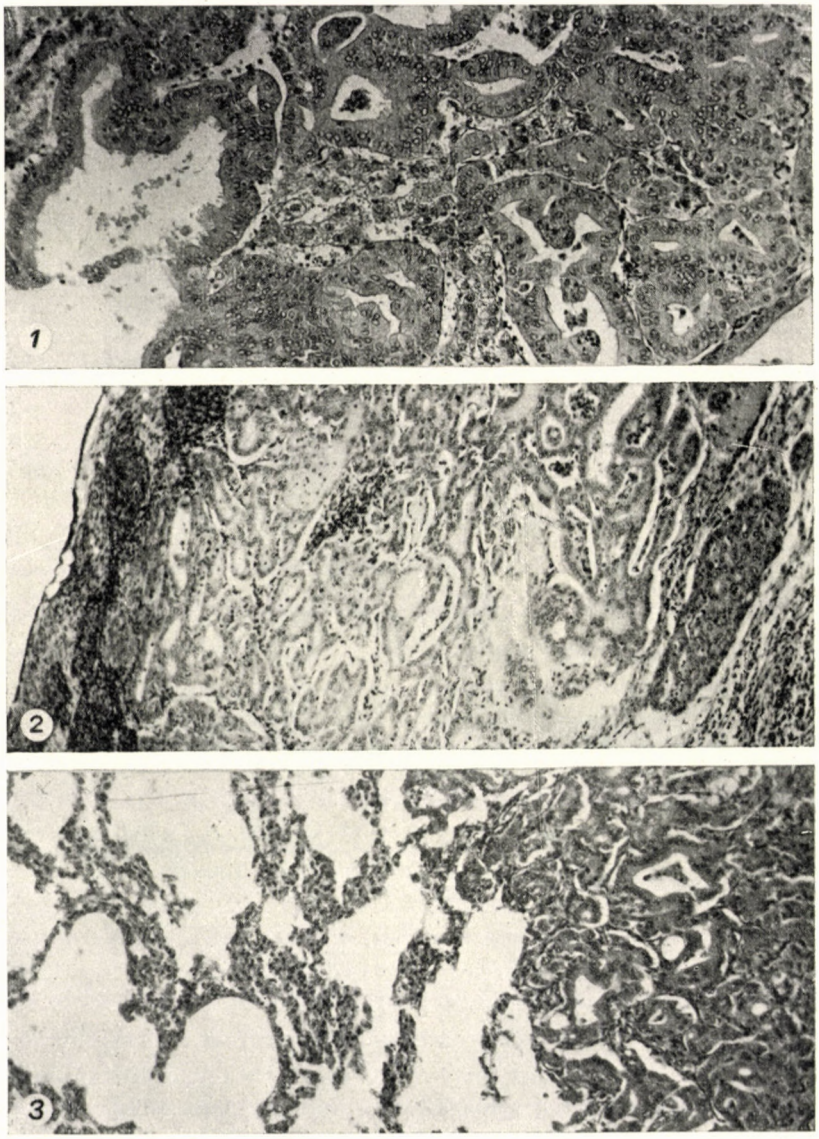

Fig. 1. Cholangiocellular adenocarcinoma. The original architecture is preserved in the metastases, as is usually the case with all tumours induced by butter-yellow. (G. 47, dye-free diet during the last 31 days. ×110)

Fig. 2. Lymph-node metastasis of the same tumour, with a similar architecture and expansive growth ×110

Fig. 3. Pulmonary metastasis of the same tumour, with a similar architecture ×110

metastases generally retained the histological structure of the primary growth (Figs. 1, 2, 3). The most frequent, almost typical site of lymph-node metastases were the so called mediastinal, subclavicular or paratracheal lymph nodes in the thorax, situated on both sides of the trachea at the height of the apices of the lungs.

Having encountered so many metastases in these lymph nodes, it was deemed important that the anatomical relationship between the liver and these nodes should be clarified, especially the eventual existence of a direct communi-

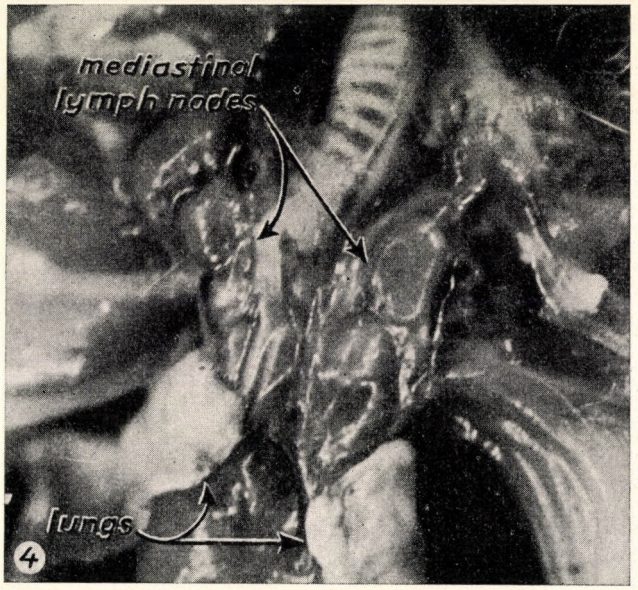

Fig. 4. Mediastinal lymph nodes situated on both sides of the trachea above the level of the apices as the most frequent, almost exclusive, site of the lymphatically transferred metastases of butter-yellow induced hepatic tumours. The lymph nodes in the picture contain metastases. (Group K ; dye-free diet during the last 50 days)

cation between the liver and the said nodes, in other words to ascertain whether the metastases encountered in these nodes owed their origin to a transfer through lymphatics or blood vessels (Fig. 4).

The fact itself that metastases were sometimes found in the lymph nodes when it was impossible to detect tumorous tissue in the lungs however minutiously they had been worked up, seemed to point to the probability of a transfer through the lymphatic system. In order to investigate the problem more closely, a small amount of India ink was injected beneath the liver capsule of healthy animals ; on killing them 24 hours later, the lymph nodes in question were found to be blackened, while no such coloration was seen in any other organ, including the lungs, a proof that no India ink could have entered into the blood

circulation, and further of the existence of a lymphatic communication between the said group of nodes and the liver, with the consequent possibility for hepatic tumours to form metastases in the said lymph nodes through the lymphatics.

The majority of the pulmonary metastases was of the multiplex type. They were observable on gross examination as small yellowish-grey nodules, mostly protruding from the surrounding surface. Any gross observation of metastases in the lung is, however, made difficult by the bronchiectases, a change frequently observed in the form of multiplex cystic nodules in rats subjected to prolonged treatment.

It has already been mentioned that, to keep them alive as long as possible, the greater part of the tumourous animals was placed on a dye-free diet as soon as a tumour was diagnosed either by palpation or by laparotomy. The tumourous animals may, therefore, be divided into two categories: those which were treated with butter-yellow until, or were placed on a diet free from dye not more than 5 days before, their death; and those which, after the tumour had been diagnosed, were kept on a normal diet until their death, always for more than 5 days (28 days on the average). After administration of butter-yellow had been discontinued there was always some improvement in the condition but the tumour began to grow rapidly. While the average weight of the liver of animals kept on butter-yellow until the end of the experiment was 18,31 g, the liver of animals fed on a dyeless diet after the appearance of the tumour reached an average weight of 34,2 g. A comparison of the number of metastases in these two categories revealed conspicuous differences (Table II).

Table II

Treatment	Number of animals with tumour	Number of cases with metastasis	Average survival from beginning of experiment
Butter-yellow given until death or until five days prior to death	32	6 (18,7%)	315 days
Placed on diet free from butter-yellow after diagnosis of tumour, and continued on such diet for an average length of 28 days	27	19 (70,3%)	373 days

Discussion

There are but few data in the literature concerning metastasis of hepatic tumours induced by butter-yellow or its derivatives, and the few available reports describe metastasis as a rare occurrence. SUGIURA and RHOADS [13] observed it in only one fifth of their tumourous animals, while GELSTEIN [4] encountered pulmonary metastasis in 14 per cent of mice with liver tumour.

OPIE [7], too, saw metastasis only occasionallly in animals surviving 12 months, although metastases were carefully sought after, if only because he refused to regard tumours as malignant unless they had metastasized. PRICE, HARMAN and MILLER—MILLER [9], encountered no metastases at all, supposedly because the animals had been killed too early, and because microscopic metastases had not been sought for. Nor did the present author, in the course of experiments made in collaboration with TÓTH and SÓS [15], see metastases being formed by hepatic tumours induced in animals fed deficient diets, although the lungs had invariably, and the lymph nodes in most cases, been subjected to histological examination. This, too, may have been due to the fact that the animals had been sacrificed too soon after the development of the tumours. Only RICHARDSON and BORSOS-NACHTNEBEL [10] reported on metastasis as a surprisingly frequent occurrence (peritoneal implantation in 87 per cent, metastases in the lungs in 74 per cent, in the lymph nodes in 46 per cent, in the heart in 6 per cent of the cases). It was demonstrated by TANNENBAUM [14] that a reduction of the caloric value of the food consumed by the animals decreases the frequency of tumours and also delays the development of tumorous growths. RUTH CORTELL [1] observed that when in 9 animals the administration of the tumour-inducing agent had been discontinued after a certain time and the animals were fed fox chow (Purina), tumours appeared earlier and became more malignant, as proved — among others — by the increased frequency of metastases in the lungs.

The metastasis of hepatic tumours induced by p — DAB did not appear to be influenced by differences in the diet (G, K, E) as applied in the present experiments.

On the other hand, the incidence of metastases in animals whose life had been — so to say — prolonged by placing them on a dye-free normal diet at a given moment was found to be three times that of the incidence in the rest (70,3 per cent vs. 18,7 per cent, Table II). According to statistical analyses made with the method of the four-compartment table, the result in question appears to be significant in respect of a 5 percent probability level, as seen from Table III.*

Table III

x^2	$f^2 = \frac{x^2}{n}$	f	Degree of freedom	5% level	Conclusion
15,983	0,271	0,521	1	3,841	Significant

In the literature concerning metastasis of transplanted tumours there are many references to the fact that parallel with the prolongation the life of the

* The statistical computations were performed by the Debrecen Section of the Institute for Applied Mathematics (Director: B. Gyires) to whom we are indebted for their assistance.

tests animals there is corresponding increase in the number of metastases. ZEIDMAN [16] attributed this phenomenon chiefly to the successive release and transfer of tumourcell emboli. OSTENFELD [8] shared ZEIDMAN's view emphasizing that time was too short for the development of metastases and that this was the reason why usually no spread was observable. Also, clinical experience and post-mortem findings seem to corroborate that a considerable part of human tumours — about 20 to 30 percent according to various statistical data (FISCHER, 3) — does not reach the stage of metastasis-formation during the life of the host organism. DRUCKREY et al. (2) observed wide-spread metastases after the surgical removal of Flexner—Jobling's carcinoma, and ascribed them partly to the prolongation of the life of the test animals and partly to the fact that operations are unavoidably followed by an additional intensive spread of cells. According to the authors, tumours would create in the organism their own conditions of growth and dissemination by the spread of the disintegrating tumour cells, and therefore, tumours would have to go through a so called "lag phase" prior to metastasis.

The increase in the intensity of metastasis-formation in the present experiments was likewise made possible by a prolongation of the life of the test animals. On the other hand, degenerative regressive processes in the tumour, greatly intensified along with the rapid growth, also seem to be factors of significance. The prolonged survival and the said processes led to a gradually increasing dissemination of the decomposing tumourous elements, so that, as a result, an adequate readjustment of the organism was brought about and a medium favourable for the formation of metastases was prepared.

With the method described in this paper the incidence of metastasizing can be so raised as to allow systematical investigations. The results obtained in the present experiments with tumours more similar to spontaneous neoplastic growth than are transplants, seem to prove that metastasis, far from being a fortuitous occurrence or some particular *a priori*-property of certain tumours, is a regular process characterising a given phase in the development of every tumour. Metastases are a manifestation of the change in the relationship between tumour and organism, a phenomenon the tumour sooner or later is bound to provoke.

Summary

(i) Induced tumours are held more suitable for the study of certain problems in metastasis than transplants; this their advantage is, however, very much impaired by the fact that induced tumours metastasize but rarely.

(ii) Experiments have been made with a view to increasing the number of and standardizing the metastases of hepatic tumours induced by butter-yellow. Differences in the diet did not influence the incidence of metastasis.

(iii) By discontinuing the administration of carcinogen at a given moment it has been possible to raise the incidence of metastasis of hepatic tumours to more than three times (70 per cent, vs. 20 per cent) of the incidence observed without this measure, and thus to obtain test objects suitable for studying certain problems connected with metastasis.

(iv) The increased formation of metastases observed has been attributed to the prolonged survival of the animals, as also to the gradually increasing dissemination of the tumorous elements conditioned by both the said prolongation of life and the rapid growth of the tumours.

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ВЛИЯНИЕ НА МЕТАСТАЗИРОВАНИЕ ВЫЗВАННЫХ «БУТТЕРГЕЛЬБ»-ОМ ОПУХОЛЕЙ ПЕЧЕНИ

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1. По мнению автора индуцированные опухоли принципиально более подходят к исследованию определенных вопросов патологии метастазов, чем трансплантированные опухоли. Однако, их применению для исследований такого характера препятствует то обстоятельство, что индуцированные опухоли весьма редко метастазируют.

2. Настоящие исследования автора были направлены на повышение метастазирования и на стандартизацию индуцированных «Буттергельб»-ом (p-di metilam no-azobenzol) опухолей печени. Примененные автором три различных режима питания не оказывали влияния на число метастазирования опухолей.

3. Прекратив подачу «Буттергельб»-а в определенный момент автору удалось в значительной степени повысить метастазирование опухолей печени (на троекратное = 70% метастазирования, наблюдаемого в ходе прочих опытов), причем он получил для определенных исследований патологии метастазов подходящие подопытные объекты.

4. Автор объясняет повышенное метастазирование прежде всего значительным повышением продолжительности жизни животных, и связанным с этим, как и с бурным разрастанием опухолей постепенным повышением заноса элементов опухолей.

BEEINFLUSSUNG DER METASTASENBILDUNG VON MIT BUTTERGELB HERVORGERUFENEN LEBERGESCHWÜLSTEN

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1. Induzierte Geschwülste erscheinen zur Untersuchung gewisser Fragen der Metastasen-Pathologie prinzipiell geeigneter als transplantierte Geschwülste. Ihre Verwendung bei Untersuchungen dieser Art wird jedoch durch den Umstand verhindert, dass induzierte Geschwülste äusserst selten metastasieren.

2. Die gegenwärtigen Untersuchungen richten sich auf die Steigerung der Metastasenbildung und auf die Standardisierung der durch Buttergelb hervorgerufenen Lebergeschwülste. Die Häufigkeit der Metastasen wurde durch die angewandten drei verschiedenen Diätarten nicht beeinflusst.

3. Durch Einstellen der Dosierung von Buttergelb in gegebenen Zeitpunkt gelang es die Metastasenbildung der Lebergeschwülste in bedeutenden Masse zu erhöhen auf das dreifache (70%) der in den übrigen Experimenten beobachteten Häufigkeit, und auf diese Weise geeignete Versuchsobjekte zur Untersuchung der Metastasen-Pathologie zu gewinnen.

4. Die gesteigerte Metastasenbildung wird in erster Linie mit der bedeutenden Erhöhung der Lebensdauer der Tiere, und der damit, sowohl als auch mit dem stürmischen Wachstum der Geschwülste zusammenhängenden sukzessiven Steigerung der Verschleppung von Geschwulstelementen erklärt.

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