TUMOURS PRODUCED IN HAMSTERS BY BENZPYRENE¹

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Experiments on rabbits, rats, mice, and guinea-pigs have shown that rodents of different species do not react uniformly to the application of carcinogenic substances. We therefore undertook to establish whether tumours produced by carcinogenic substances in hamsters show any peculiarities as regards development, course, and spread. Apart from a short communication published meantime by Gye and Foulds (1) no observations on this animal have been recorded. We used the Syrian, or golden, hamster (*Cricetus auratus*), of which one strain is bred in our laboratory.

Animals weighing about 80 gm. were injected subcutaneously, in the abdominal region, with benzpyrene Meurice, 0.25 c.c. of a solution in lard, every three or four weeks, the injections being interrupted as soon as a tumour became apparent. Forty animals were treated between Nov. 6, 1935, and April 28, 1938. Nine died within the first three months of treatment. Of the 31 remaining, 29, or 93 per cent, developed tumours.

The earliest beginning of a tumour can not usually be established with certainty, for infiltrations forming after injection may be misleading. Definite tumours may appear after two months, but the average period is three months.

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So far none of our hamsters with primary benzpyrene tumours has shown metastases post mortem, though Gye and Foulds observed metastases in the lung in one of 2 hamsters in which they were able to produce benzpyrene tumours.

Histologically the benzpyrene tumours are polymorphous-cell sarcomata showing a few individual differences which are of no appreciable significance (Fig. 1). Some of the primary tumours were inoculated into other hamsters. The results varied, as is the case with our primary benzpyrene tumours in rats. As in the latter, some tumour strains gave 100 per cent takes in successive passages.

A comparison of the results of our benzpyrene experiments in hamsters and in rats brought out certain interesting differences.

(1) The latent period of the primary benzpyrene tumours is shorter in hamsters than in rats, an average of three months as compared with four and a half to five months.

(2) In passages carried out by the same technique—subcutaneous transplantation with a cannula—the resulting tumours in hamsters show a greater tendency to be adherent to skin and muscle, that is a greater tendency towards infiltrative growth than did the rat tumours (Fig. 2).

One strain, H. M., which we have carried through successive passages since Feb. 22, 1938, is of special interest because of its peculiar tendency to lymph node metastasis. Much has been written on the spread of spontaneous tumours and experimentally induced tumours in animals. Some strains never show metastases, whereas they are produced by others with a certain degree of
regularity. An incidence of 50 per cent for any tumour strain must be considered high (for the literature up to 1913, see Woglom, 2). Metastases usually appear mainly in the lungs. Ashburn (3), for example, in autopsies on 480 female mice with spontaneous mammary carcinoma found metastases in 217 or 45.2 per cent, and all were localized in the lungs, only 5 animals showing deposits in other organs as well. Flaks (4, 5) inoculated Jensen sarcoma into the thigh of suckling rats and regularly succeeded in obtaining metastases in the lumbar lymph nodes. Recently Koch (6) has reported a gradual increase in the percentage of metastases in a strain of Ehrlich mouse carcinoma from 5 per cent to 80-90 per cent. He believes that this has been brought about by using metastatic tumours for inoculation. A marked tendency to metastasis is seen, also, in ascites carcinoma of mice (W. Schmidt, 7).

Cook and Kennaway (8) cite the literature on metastasis of tumours induced by carcinogenic substances. Metastases are rarer in the lymph nodes than in the lungs. In a more recent work Vannfält (10) reports the development of node metastases in 36 per cent of mice having tar tumours. Gye and Foulds (1) found lung metastases in one of their original hamsters with a benzpyrene tumour and in another hamster in which this had been successfully transplanted. In rabbits Burrows and Boyland (9) observed metastases from adenocarcinoma of the uterus following the administration of 1:2:5:6-dibenzanthracene in all cases. Reference will be made later to the node metastases observed by Oberling and M. and P. Guérin (11) in rats treated with large doses of benzpyrene, as well as to the changes in the lymph nodes occurring in Parsons' (12) strain.
In 4 benzpyrene tumour strains which we have carried through as many as 70 passages in rats, we have never found metastases. Repeated transplantation of nodes which appeared to be enlarged and histologic examination of these nodes have yielded negative results.

The behaviour of tumour strain H. M. was, therefore, all the more striking. The original animal was a hamster which developed a tumour after 4 subcutaneous injections of 2.5 mg. benzpyrene; the tumour was carried through 14 passages from Feb. 22, 1938, with 100 per cent takes. The original animal showed no metastases at autopsy. It was only in later generations that spread into the nodes was observed. Autopsies were carried out on 54 hamsters with tumours, and 39 of these, or 72 per cent, showed node involvement. The shortest time elapsing between transplantation and a positive finding in the nodes at autopsy was three weeks.

Practically all the transplanted tumours were in the abdominal region. Dissemination followed a definite course. The nodes affected were those of the axillary region, groin (Fig. 3) and mediastinum, and a chain in front of the spine beginning between the kidneys and stretching into the pelvis (Fig. 4). Those most frequently affected and those attaining the greatest size were the axillary nodes, sometimes on the side opposite to the tumour. It was frequently noted that dissemination was greatest in animals which survived transplantation the longest. In these cases small nodules were also observed in the diaphragm, peritoneum and omentum, and in one case there was infiltrative growth in the liver (Fig. 5).

Genuine metastases elsewhere than in the nodes have so far been observed
only three times with this tumour strain. In addition to a particularly wide-
spread dissemination in the nodes, there were sharply defined round nodules in
the kidneys (Fig. 6) in two cases and lung metastases in the third (Fig. 7).
There exist therefore a marked affinity for the lymphatic system and a practi-
cally strictly limited localisation of metastases. The foci in the thorax in
the neighbourhood of the thymus are occasionally strikingly large. These tumours
finally infiltrate the thoracic wall and penetrate the intercostal spaces.

Enlarged nodes were transplanted in 14 cases, with 13 positive results, the
tumours resembling the original.

Blood from a hamster which showed metastases was used for inoculation
on 31 occasions, about 0.25 to 0.5 c.c. being used. Blood was taken from the

heart and injected subcutaneously into normal hamsters; tumours resulted in
8 animals. In all these cases metastases in the animals from which passage
was made were limited to the lymph nodes.

Nodes which had not yet become appreciably enlarged were found on micro-
scopic examination to consist almost entirely of normal tissue, with here and
there scattered foci of tumour cells (Fig. 8). From this beginning every stage
may be observed up to the point where gland tissue can no longer be identified
and only tumour cells are seen (Fig. 9).

Professor Adler of the Department of Parasitology of the Hebrew Univer-
sity, using this strain in his work on leprosy, observed metastases in the earliest
passages before we could establish their presence in our animals. Since the
hamsters for the leprosy experiments were splenectomised, it seemed not im-
possible that the absence of the spleen favoured the formation of metastases.
Eighteen splenectomised hamsters were therefore inoculated with H. M. sar-
coma. Of these, 14 showed dissemination into the lymph nodes, while 4 remained negative. If reliance may be placed on such a comparatively small number of cases, the occurrence of metastases does not differ appreciably in splenectomised and in normal animals (77 per cent as against 72 per cent). Eight hamsters received general irradiation with x-rays (180 kv.; filter; 400 r on the surface) before tumour transplantation; 6 showed dissemination, 2 remained negative. Here, too, the incidence of metastases (75 per cent positive) deviates but little from that in untreated hamsters, but the cases are too few for a definite conclusion.

Our strain H. M. shows a certain resemblance with regard to lymph node metastases to a strain described by Parsons (12). In the latter case the original animal was a mouse treated with soluble dibenzanthracene. The resulting sarcoma produced no node metastases up to the 122d passage. Blood and tumour filtrate were then inoculated into mice previously treated with x-rays. These animals showed wide dissemination in the lymph nodes particularly of the axilla, groin, and lumbar region, and in the mesenteric and pancreatic nodes. After this the strain retained its metastatic properties on further passage. Large mediastinal tumours such as were observed in our hamsters do not seem to have occurred. Inoculation of blood gave a higher percentage of success than in our experiment (40.8 per cent). In spite of the fact, therefore, that the blood was rich in tumour-forming material, this strain produced metastases in no organ other than the lymph nodes.

It must be noted that Parsons' tumour could be transmitted by means of a filtrate which had passed through a collodion membrane. We have not used filtrates of our hamster tumours for inoculation. We have, however, inoculated material derived from tumours and gland metastases after intensive irradiation with x-rays. This procedure we based upon our experience with irradiation of Rous sarcoma cultures. Doljanski and Halberstaedter (14), by using suitable doses of radium rays, succeeded in freeing the agent of Rous sarcoma from active cells while retaining its infectivity. Were a similar tumour-forming agent present in the hamster tumour which could be effective without the presence of actively growing cells, it should be demonstrable with the help of irradiation. But attempts in this direction have so far failed.

Oberling and Guérin in a recent study on the occurrence of node metastases in rats treated with benzpyrene found that while animals which develop tumours after normal doses never show metastases, these appear when the dose is greatly increased. At the same time the number of takes on transplantation of the primary tumours increases and the latent period is shortened. With 75 mg. of benzpyrene metastases develop in 50 per cent of the animals. In view of the fact that metastases appear only after large doses of benzpyrene and increase with increasing dosage, Oberling and Guérin conclude that either the larger doses have a toxic effect which weakens the resistance of the organism, or that they produce a peculiarly aggressive and proliferating race of cell. In our tumour strain we can exclude the first possibility, for the metastases appeared on passage, that is in animals which had no contact with benzpyrene. We must, then, be dealing with a special property of the tumour cells. The question of an indirect effect does not arise, for metastases appeared in a large percentage of animals which were not subjected to other treatment (such as
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splenectomy or irradiation). Nor can it be said that we may be dealing with a peculiar property of the hamster, for not every hamster tumour strain produces metastases in the lymph nodes. So far out of a series of tumour strains only H. M. has shown any tendency towards metastasis formation, although all were produced by benzpyrene. The preference in this strain for certain organs also appears to be a specific property of the tumour cell. Here we are dealing, with few exceptions, with a dissemination typically affecting the nodes and giving the appearance of a systemic disease. In Parsons' strain dissemination took place only into the lymph nodes of the affected mouse.

There still remains the question whether the dissemination into the lymph nodes observed in Parsons' mouse tumours and in our hamster tumours represents true metastasis. Parsons is for various reasons inclined to a negative reply, mainly on the ground of the multiplicity of the affected nodes independent of the localisation of the primary tumour and because of the successful transmission with filtrate. She discusses the possibility that the changes in the lymphatic tissue "are due to (1) some chemical substance elaborated from metabolic processes in the tumour cells, (2) an agent existing and multiplying locally in the sarcoma cells which produces some substance acting systemically and selectively on the lymph glands, or (3) an ultramicroscopic agent introduced on grafting a sarcoma and itself subsequently involving the lymphoid tissue and causing neoplastic changes in the glands." Fränkel (13) was able to obtain tumour growth with organs of mice with Ehrlich carcinoma even when no tumour could be demonstrated in the organs used for inoculation. He was most successful with lung, spleen, and brain. He concluded that a chemical or fermentative agent possessing certain organ affinity was present.

In our experiments on hamsters the argument that it is the cells themselves which are disseminated into the nodes and form the metastases is upheld by the fact that the foci present in the nodes appear to be histologically identical with the primary tumours and that subcutaneous inoculation of nodes produces sarcoma which corresponds in structure and behaviour to the tumour strain. Further histologic investigation must, however, be undertaken. The specific affinity for certain organs may be a property of cells or of agent. Solution of this problem depends on the result of transplantation of cell-free material. These experiments must be continued either by the method of filtration or of irradiation. Furth, Tuggle and Breedis (15) proposed to utilise the difference in radioresistance between cells and virus to distinguish between virus-containing and virus-free neoplasms.

**Summary**

The hamster (*Cricetus auratus*) reacts to the subcutaneous injection of benzpyrene, like the rat and mouse, by the formation of sarcoma.

The percentage of tumour production is high (93 per cent); the average incubation period is three months.

A hamster tumour strain is described having a marked tendency to metastasise, the percentage of animals with metastases being 72 per cent.

The metastases were mainly localised in the lymph nodes, as in systemic disease.
The nodes affected were those in the axillae, groins, and mediastinum, as well as the lumbar and sacral nodes.

Roentgen irradiation or splenectomy before inoculation caused no appreciable increase in the percentage of animals with metastases.

The tendency to metastasis and the manner of dissemination are believed to be due, in part at least, to the specific properties of the tumour cells.

BIBLIOGRAPHY