RAT SARCOMA MODEL SUPPORTS BOTH "SOIL SEED" AND "MECHANICAL" THEORIES OF METASTATIC SPREAD

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Summary.—Following injection into the portal venous or vena caval systems, tumour cells are held up almost exclusively in the liver or lung respectively, and subsequent outgrowth of tumour only occurs in these organs.

Following systemic arterial injection, cells are distributed, and subsequently grow, in a variety of organs. However, the adrenal gland supports tumour growth from much fewer cells than the lung, and this is partly due to the fact the rate of tumour cell loss in the initial 48 h is very high in the latter compared to the former organ.

The factors determining the patterns of metastatic spread are complex and at present poorly understood. Two long-standing theories, "the soil seed hypothesis" of Paget (1889) and the "mechanical theory" of Ewing (1928) aroused considerable controversy for several decades and have been summarized by Willis (1952). Paget considered that the microenvironment of one organ might favour the seeding and growth of bloodborne tumour cells over another organ, while Ewing stated that "the mechanics of the circulation will doubtless explain most of these peculiarities; for there is no one parenchymatous organ more adapted than others to the growth of embolic tumour cells".

While published data have supported either Ewing's (Coman, Delong and McCutcheon, 1951; Coman, 1953) or Paget's (Sugarbaker, 1952; Kinsey, 1960) theories, we are not aware of a previous report which supports both theories with data from a single animal tumour model. The present study demonstrates a predominant influence of "mechanical" factors on the site of outgrowth of bloodborne tumour cells following injection into the vena caval or portal circulation and yet, on injection into the aorta, the outgrowth of the same tumour cells appears to be governed by other considerations, most probably involving variations in the local environment of different organs.

MATERIAL AND METHODS

Single-cell suspensions were prepared by enzymatic digestion from the 6–12th transfer generations of the MC1 sarcoma, maintained in the inbred Chester Beatty hooded rat strain of origin. Viable portions of tumour were incubated in 25 ml MEM (Microbiological Associates, U.S.A.) containing 0-13 g of trypsin, 0-15 g of collagenase and trace amounts of DNAase (all Sigma Type I), filtered through gauze and washed thoroughly. Radioactive label was incorporated by incubating 10⁶ single tumour cells in Falcon flasks containing 25 ml of MEM with 10% foetal calf serum (Microbiological Associates) and 5 μCi ¹²⁵IUr (N.E.N., Canada) for 48 h. The cells were then harvested by incubation for 10 min with 0-2% trypsin, and repeated washing to remove excess label. Such cells

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have a labelling index of > 97% on autoradiographic studies (personal observations) and following i.v. injection, produce a similar amount of lung tumour to the injection of an identical number of unlabelled tumour cells (Proctor et al., 1976). Volumes of 1 ml, containing between $2 \times 10^2$ and $10^4$ labelled or unlabelled single-cell suspensions, were injected intravascularly and the extent of tumour growth assessed by weighing infiltrated organs 3-4 weeks later. The initial distribution of tumour cells was assessed by counting radioactivity in organs on a conventional gamma counter 10 min after injection, and the loss of radiolabel from these organs was monitored by killing rats at various times thereafter.

RESULTS AND DISCUSSION

In an initial experiment, $2 \times 10^5$ MCl sarcoma cells, containing approximately $2 \times 10^4$ ct/min, were injected via the lateral tail vein, or following laparotomy under ether anaesthesia, via the superior mesenteric vein, and the distribution of the radioactivity and the subsequent outgrowth of tumour recorded. Tail vein injections led to an almost complete retention of cells and subsequent tumour growth in the lung, while following injection into the superior mesenteric vein, tumour cells were almost completely retained in the liver, and grew subsequently only in that organ (see Table I).

However, following injection of unlabelled tumour cells into the abdominal aorta, below the coeliac axis, macroscopic tumour was identified subsequently in a variety of organs and tissues (see Table II).

In subsequent experiments the distribution of radiolabelled tumour cells per organ was established 10 min after aortic injection of $2 \times 10^5$ radiolabelled tumour

TABLE I.—% of Injected Cells in Lung and Liver, 10 min after i.v. Injection, and their Subsequent Outgrowth

| Site of injection          | Number of animals/group | % (and range) of injected cells in Lung | Incidence of tumour growth in Lung | Incidence of tumour growth in Liver |
|----------------------------|-------------------------|----------------------------------------|-----------------------------------|-----------------------------------|
| Tail vein                  |                         |                                        |                                   |                                   |
| Experiment 1               | 5                       | 86·2 (82·4–93·1)                       | 5/5                               | 0/5                               |
| Experiment 2               | 5                       | 82·3 (77·4–92·4)                       | 2·0                               | 5·0                               |
| Superior mesenteric vein   |                         |                                        |                                   |                                   |
| Experiment 1               | 5                       | 1·5 (1·2–2·3)                          | 5/5                               | 0/5                               |
| Experiment 2               | 5                       | 1·2 (1·0–1·8)                          | 5/5                               | 0/5                               |

Some rats were exsanguinated and organs removed 10 min after injection, and the radioactivity within them counted on a conventional gamma counter (counts > 3 x background were considered significant). Others were killed 3-4 weeks after injection, and examined for macroscopic tumours in all organs and tissues. No tumour was identified in tissues or organs other than the lung and liver.

TABLE II.—Distribution of Macroscopic Tumour Nodules after Intra-arterial Injection of MCl Tumour Cells

| Incidence of tissue or organ involvement in 5 animals following injection with | 10^4 cells | 2 x 10^4 cells |
|-------------------------------|----------|---------------|
| Lungs                         | 5        | 5             |
| Prostate                      | 5        | 4             |
| Bone                          | 5        | 5             |
| Skeletal muscle               | 5        | 5             |
| Subcutaneous and peritoneal soft tissues | 5        | 5             |
| Adrenals                      | 5        | 4             |
| Lymph nodes                   | 5        | 2             |
| Seminal vesicles              | 3        | 1             |
| Liver                         | 2        | 0             |
| Kidney                        | 1        | 0             |
| Bladder                       | 1        | 0             |

No tumour was detected in spleen, pancreas, intestines, testes, brain, thyroid, thymus, heart or eyes.

Single-cell suspensions were prepared (see Table I) and $10^4$ or $2 \times 10^4$ tumour cells in 1 ml injected into the aorta above the renal arteries via a 27-gauge needle through a midline abdominal incision, under ether anaesthesia.

The animals were killed 3-4 weeks later, and organs and tissues examined for macroscopic tumour.
TABLE III.—Distribution Patterns of MC1 Tumour Cells and Subsequent Growth after Injection into the Abdominal Aorta

| Organ                      | Mean number of cells 10 min after injection | Incidence of tumour growth | Approximate mean weight of tumour (g) 21–28 days after injection |
|----------------------------|---------------------------------------------|----------------------------|---------------------------------------------------------------|
|                            | Per organ                                   | Per gram organ             | Per organ                                                   | Per gram organ                                             |
| Experiment 1               |                                             |                             |                                                             |                                                             |
| Lung                       | 168181                                      | 86663                      | 6/6                                                         | 2·18                                                       | 1·09                                                    |
| Adrenals                   | 372                                         | 3437                       | 6/6                                                         | 1·75                                                       | 17·50                                                   |
| Prostate and appendages    | 5662                                        | 9760                       | 6/6                                                         | 2·85                                                       | 4·31                                                    |
| Large intestine            | 10378                                       | 8392                       | 0/6                                                         | 0                                                          | 0                                                       |
| Experiment 2               |                                             |                             |                                                             |                                                             |
| Lung                       | 111290                                      | 68012                      | 5/5                                                         | 3·14                                                       | 1·89                                                    |
| Adrenal                    | 137                                         | 1245                       | 5/5                                                         | 1·96                                                       | 17·8                                                    |
| Prostate and appendages    | 5155                                        | 8372                       | 5/5                                                         | 2·21                                                       | 3·81                                                    |
| Large intestine            | 8129                                        | 7069                       | 0/5                                                         | 0                                                          | 0                                                       |

Single-cell suspensions of tumour were prepared and labelled with $^{125}$IUDR and injected into the abdominal aorta. Some animals were exsanguinated 10 min later and the organs weighed and counted for radioactivity. The number of tumour cells per organ was calculated as follows:

$$\text{Number of tumour cells/organ} = \frac{\text{ct/min/organ} \times \text{Total number of tumour cells injected}}{\text{Total ct/min injected}}$$

Other rats were killed 3–4 weeks later and the organs and tissues examined for tumour. The tumour in the lung, adrenal glands and prostate was weighed to the nearest 0·1 g, as follows:

$$\text{Approximate tumour wt/g of organ} = \frac{\text{Weight of organ with tumour} - \text{Weight of tumour-free organ}}{\text{Weight of tumour-free organ}}$$

cells containing approximately $2 \times 10^4$ ct/min, and the approximate amount of tumour resulting in each organ from these cells was determined by weighing infiltrated organs 3 weeks later.

Radiolabelled tumour cells dispersed widely following aortic injection (see Table III), but the proportion of cells in the adrenal glands was extremely low, considering the high incidence of tumour growth observed in these organs following injection with unlabelled cells (see Table II). Furthermore, each gram of tumour in the lung resulted from a much higher number of tumour cells than did each gram of tumour in the prostate, and more particularly in the adrenal gland (see Table III).

These findings support the hypothesis of Paget, and might simply imply different rates of tumour growth in these organs. However, previous experiments (Proctor et al., 1976), like those of Fidler (1970), have shown that, following retention of circulating tumour cells in the lung, the cells are rapidly destroyed there, in contrast to a much lower rate of cell loss from subcutaneous or intramuscular tissues (Peters and Hewitt, 1974). Therefore a further experiment was set up, to follow the initial fate of radiolabelled tumour cells in the above organs following injection into the aorta. The rate of tumour cell loss during the first 46 h after injection (see Fig.) in the adrenal gland, and to a lesser extent in the prostate gland, was very much slower than in the lung.

These findings explain partially the large amount of tumour resulting from a few tumour cells in the adrenal glands, compared to the similar amount of tumour resulting from a very large number of cells in the lung. They do not explain why no tumour grew in the large intestine, as there were still 2–3 times the number of cells in this organ compared to the number in the adrenal at 46 h. However, when expressed as a concentration of tumour cells/g of organ, the concentration in the adrenal is 20–30 times greater than in the small intestine, and this might explain the discrepancy in the
In summary, it is probable that, while the relative importance of "mechanical" and "soil" factors will vary from one form of cancer to another, "mechanical" factors may influence the pattern of secondary venous spread predominantly to the lung, while tertiary spread through the arterial circulation from such metastases may be determined to a much greater extent by "soil" factors.

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