Europ. J. Cancer Vol. 16, pp. 1399–1407

0014-2964/80/1101-1399 $02.00/0

Blood Coagulation Changes in JW Sarcoma, a New Metastasizing Tumour in Mice*

J. CHMIELEWSKA,† A. POGGI,‡ L. MUSSONI, M. B. DONATI and S. GARATTINI

Laboratory for Haemostasis and Thrombosis Research, 'Mario Negri' Institute for Pharmacological Research, Via Eritrea, 62 20157 Milan, Italy

Abstract—*A new murine tumour, which arose spontaneously in the lung, was characterized as a possible model of blood clotting changes during tumour and metastasis growth. The JW sarcoma (JWS), when transplanted s.c. in BALB/c mice, grows locally and spontaneously metastasizes to the lungs. The transplanted animals survive for* 6 weeks. *Thrombocytopenia and increased leukocyte count were observed during the metastasis growth. Concomitantly, increased fibrinogen level and reduced fibrinolytic activity were observed. At* 4 weeks *after tumour implantation, survival of labelled fibrinogen was prolonged. This experimental model may offer useful information on the haematological and blood clotting changes in cancer patients.*

INTRODUCTION

FACTORS involved in haemostasis and thrombus formation have been suggested as playing a role in tumour growth and metastasis formation [1, 2]. Moreover, clinical observations indicate that neoplastic diseases are accompanied by haemorrhagic and/or thromboembolic complications [3, 4]. The study of blood clotting changes in tumour-bearing animals could provide some insight into the mechanisms of tumour cell–fibrin interaction; this approach, however, has not been followed extensively so far.

In only two models of solid tumours, (the Walker 256 carcinosarcoma and the Lewis lung carcinoma) and in two rat leukaemias (L5222 and BN-ML) have changes in the host's haemostatic system been investigated [5–9]. Interactions between cancer cells and fibrin may conceivably vary according to the type of tumour, the modalities of cancer cell inoculum and growth and the animal species considered. It is therefore important to extend such a study to different experimental models, which should reproduce spontaneous rather than artificial metastases because the former mimic more closely the dissemination pattern in patients bearing solid, metastasizing tumours [10].

A metastasizing murine tumour which arose spontaneously in the lung of a BALB/c mouse was described by Dr. Janik in Warsaw [11] and characterized as a sarcoma; it was thus named JW sarcoma (JWS). The tumour was maintained by s.c. passages; at the end of 1976, an ascitic form was obtained which was subsequently maintained by weekly passages in the same mouse strain.

In this paper, we report the growth characteristics and haematological changes occurring at various phases of JWS growth and metastasis.

MATERIALS AND METHODS

Animals

Five hundred BALB/c male mice, 6–8 weeks old, obtained from Charles River

Accepted 3 April 1980.

*This work was performed in the frame of a collaborative project on "The Role of Fibrin in Tumour Growth" between the Institute of Nuclear Research, Warsaw, Poland, and the Mario Negri Institute for Pharmacological Research, Milano, Italy.

The partial support of the Polish Ministry of Health (Grant No. PR-6 1F 9S6X, Polish Government Research and Development Programme on Control of Neoplastic Diseases), the National Institute of Health, National Cancer Institute, Bethesda, Maryland, U.S.A. (Grant NIH PHR B-1 R01, CA 12766-01) and the Italian Government (National Research Council Project No. 78-02792.96 on Control of Tumoral Growth) is gratefully acknowledged.

†On leave of absence from the Institute of Nuclear Research, Warsaw, Poland.

‡To whom requests for reprints should be addressed.

Breeding Laboratory (Calco, Italy) were used for this study. The animals, weighing 20–25 g at the start of the experiments, were housed in plastic cages in air-conditioned premises with a constant light–dark rhythm; they had free access to food pellets and water. Out of the total number of BALB/c mice, 170 were used for the biological characterization of the model, 240 for the haematological studies, and the remaining 90 for kinetic studies and for preparation of purified fibrinogen. For the latter purpose, also 50 C3H, 50 DBA-2 and 50 C57Bl 6J mice were employed.

Tumour

JWS was maintained *in vivo* by weekly intraperitoneal (i.p.) injections. For each passage cells were collected from the peritoneal cavity and washed once in phosphate-buffered saline pH 7.4, 0.15 M. Viable cells were counted by light microscopy employing the Trypan-blue dye exclusion technique, resuspended at a concentration of 5×10^6/ml and injected i.p. in a volume of 0.1 ml.

For the experiments reported in this paper, JWS cells prepared as described above and diluted at various concentrations were injected subcutaneously (s.c.) in 0.1 ml into the upper part of the mouse back. Cells from the 10th to the 50th serial i.p. passage were used. After s.c. injection, a solid tumour mass grew locally and spontaneous metastases were visible in the lungs.

Experimental design

Experimental work was done according to the following scheme:

(a) Biological characteristics of the model;
(b) Haematological studies;
(c) Fibrinogen kinetic studies.

Biological characteristics of the model. One hundred and fifty BALB/c mice were injected with three different inocula of JWS cells (10^4, 5×10^4, 10^5 respectively), prepared as described above. Different groups of animals were used to evaluate primary and lung metastases growth. Fifteen animals per cell dose were allowed to survive till spontaneous death and the number of tumour takes and the survival time were recorded. Caliper measurements of the two largest tumour diameters were taken twice a week, starting from day 10 till day 43, to plot the primary tumour growth curve. Caliper measurements were converted into weight using the formula:

$$W = \frac{a \times b^2}{2}$$

where a = length in mm, b = width in mm, and W = weight in mg. The assumption was made that the two short axes were of the same length and that specific gravity was approximately one [12].

Tumor weights, determined as described above, were plotted on a semilogarithmic scale and the growth curve fitted according to the Gompertz equation, which has been found to mathematically describe several types of biological growth, including the growth of transplantable animal tumours. It describes an exponential growth curve with a simultaneous exponential decrease of the specific growth rate [13, 14]. The Gompertz equation for tumour growth can be written as follows:

$$S'_{(t)} = S_o \cdot e^{\beta/\alpha}(1 - e^{-\alpha t})$$

where $S_{(t)}$ is the tumour size at time t, S_o is the initial tumour size, and α and β are constants. Tumour mass doubling times (t_d) can be calculated according to the equation:

$$t_d = \frac{-1}{\alpha}\left\{\ln - \frac{\ln 2}{\ln S_\infty / S}\right\} S < \tfrac{1}{2} S_\infty$$

where S_∞ represents the asymptotic value for tumour size. To evaluate lung metastasis growth, 5 mice for each cell dose were killed at 5-day intervals, starting from day 20 until day 45. A total of 30 animals per group was used. At death, both lungs were inflated with Indian ink, excised and fixed in formalin according to Wexler [15].

Lung metastases were counted and the numbers plotted on a semilogarithmic scale.

Histological studies. A group of 18 animals were injected with 5×10^4 cells and three of them killed at weekly intervals. For histological examination, some tissues (i.e., tumour, lung, kidney, spleen, liver and heart) were excised, fixed with 10% neutral buffered formalin, embedded in paraffin, cut at 4–6 μm, stained with haematoxylin–eosin and observed by light microscopy.

Haematological studies. For the whole series of experiments a group of 120 mice injected s.c. with (5×10^4) JWS cells and a group of 120 normal BALB/c mice taken as controls were followed simultaneously for 6 weeks. Five con-

trols and 5 tumour-bearing mice were examined at weekly intervals. In some cases, native blood was collected from the retro-orbital plexus by means of a 20 μl Konstriktionsipipette (H. Pedersen, Oslo, Norway). In others, blood was obtained by intracardiac puncture from open-chested animals under slight ether anesthesia. For anticoagulation, 9 parts of blood were mixed directly in a disposable plastic syringe with 1 part 0.126 M trisodium citrate.

Blood erythrocyte, leucocyte and platelet counts and differential counts on blood smears were made by standard haematological procedures [16]; blood fibrinogen level was measured by the Fibrin Polymerization Time Test (FPT) adapted to mouse blood [6, 17]. As a measure of the prothrombin complex activity, thrombotest was made on citrated blood using a commercially available reagent (Nygard, Immuno, Pisa, Italy). Blood fibrinolytic activity was studied using the Dilute Blood Clot Lysis Time (DBCLT) [18]; fibrin(ogen) degradation products(FDP) were determined in serum using the Staphylococcal Clumping Test [19] and the presence of soluble fibrin monomer complexes was detected by the Protamine sulphate test (PS) [20].

In the light of the results of the first series of experiments, some selected tests were made on mice bearing 4-week old JWS and on the corresponding controls. These tests were: plasminogen determination using a chromogenic substrate [21], DBCLT and plasma euglobulin lysis time (ELT) according to Gallimore [18], plasma urokinase inhibitors according to Paraskevas *et al.* [22] and FDP [19].

Fibrinogen kinetic studies. Mice fibrinogen was prepared from plasma pools of four different mouse strains (C3H, DBA-2, C57Bl, BALB/c) according to the method of Regoeczi [23]. Purity of the prepared protein was checked by SDS–polyacrylamide gel electrophoresis. Before reduction, only one protein band was observed, whereas after treatment with 2-mercaptoethanol, three bands were seen, corresponding to the resolved fibrinogen chains. Purified fibrinogen was labelled with ^{131}iodine (obtained from OPIDI, Institute of Nuclear Research, Swierk, Poland) according to McFarlane [24]. The clottability of the ^{131}I–fibrinogen was evaluated by standard methods [25].

Labelled fibrinogen (0.05 μCi in 0.1 ml sterile isotonic saline) was injected into the tail vein of control mice and of tumour-bearing animals 2, 4 and 6 weeks after implantation. Six animals per group were studied. Radioactivity was measured in a Packard model 578 gamma counter on 20 μl native blood samples, drawn from the retro-orbital plexus, 3, 6, 9, 12, 15 and 24 hr after injection. Linear regression analysis of ln radioactivity values vs time was performed for each animal, and the slope (b) of each individual curve was established. The half-times of labelled fibrinogen disappearance ($t_{\frac{1}{2}}$) were calculated as ln 2/b [25, 26] and the fibrinogen survival times as the reciprocal (1/b) of the slope of the regression curve [27]. Fibrinogen turnover rates were calculated as the ratios of fibrinogen concentration to survival time and expressed as mg/ml/day [27].

Statistical analysis

One and two way analysis of variance, Student's t-test [28], Dunnett's test [29] and Tukey's test for unconfounded means [30] were performed as specified in the captions to each figure or table.

RESULTS

Growth characteristics and histological findings

In a first series of experiments the effect of various inoculum sizes was studied on the animals' mean survival time. It was 55 ± 1 days for 10^4 JWS cells, 49 ± 2 days for 5×10^4 cells and 41 ± 1 days for 10^5 cells.

Tumour take was 100% for each inoculum size.

Primary tumour growth, determined by means of caliper measurements of the two largest diameters of the tumour, was well fitted by the Gompertz equation. Three representative parameters of this equation (α, β and S_0) for each curve are shown in Table 1. Both curves had similar values for constants α and β and only different initial sizes (S_0). The number of lung metastases in animals injected with the 3 doses of cells increased according to exponential curves.

Figure 1 shows the growth curve of primary tumour weight and metastasis number after

Table 1. Growth characteristics of JWS tumour after three different inoculum sizes: parameters of the Gompertz equation

Cell dose	S_0	α	β
10^4	1.9×10^{-8}	1.1×10^{-1}	2.3
5×10^4	3.3×10^{-5}	1.1×10^{-1}	1.4
10^5	1.1×10^{-4}	1.1×10^{-1}	1.3

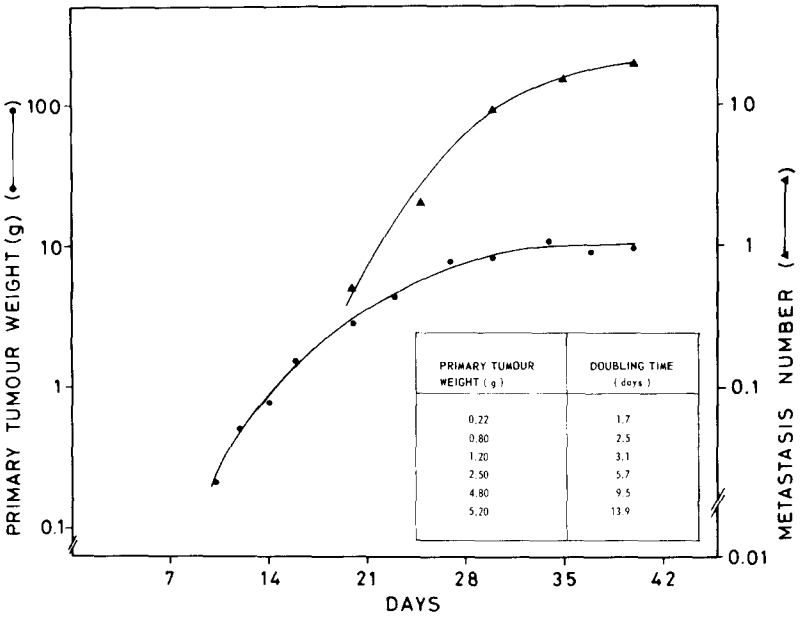

Fig. 1. Increase in primary tumour weight (●—●) and lung metastasis number (▲—▲) after s.c. injection of 5×10^4 JWS cells. Each point is the mean of results from 10–15 animals. The table gives doubling times (days) for different tumour weights (g). The curves were calculated using the Gompertz equation.

s.c. injection of 5×10^4 JWS cells, the inoculum size chosen for further experiments. The primary tumour reached a plateau of 8–9 g at the fourth week. The figure also shows the theoretical doubling times of the primary tumour as a function of its weight. This value increased from 1.7 days when the tumour weight was 0.22 g, to 13.9 when the weight was 5.2 g. Lung metastases could be measured from the fourth week after tumour transplantation. An average of 15–20 metastases was reached at death of the animals (approximately 6 weeks after tumour implantation). At that stage, lung weight was 410 ± 53 mg (mean ± S.E.) in a group of 10 JWS bearing mice and 163 ± 21 mg in a group of 10 control animals.

The s.c inoculation of JWS cells into BALB/c mice resulted in the growth of a nodular, non-encapsulated mass, involving on one side the subepithelial and on the other the muscular structures. Neoplastic tissue was polymorphous, with areas of mixed elongated and epitheliod cells and frequent pyknotic or clearly necrotic areas. The nuclei varied from round to oval, with either granular or dense chromatin structures. Cytoplasm was relatively poor because of the high compression of the tissue, which was almost completely without vasculature and stromal structures. The few visible vessels looked like lacunar spaces.

Lung inspection showed metastatic cells appearing to invade the lung tissue with infiltrative growth. Occasionally, cancer cells were observed in the spleen and heart.

Haematological studies

Figure 2 shows the blood cell counts during growth of JWS.

Erythrocyte count did not vary throughout the observation period. Leucocyte count rose significantly after the 3rd week, because of an increase in neutrophilic granulocytes, visible in the differential count on blood smears (data not shown). The number of platelets remained almost constant during the first 3 weeks but it fell progressively, thereafter, to approximately 25% of the initial values at death of the animals.

Figure 3 shows fibrinogen, DBCLT and PS in JWS bearing mice.

Blood fibrinogen concentration increased towards the end of the observation period and was significantly higher than in control mice 5 weeks after tumour implantation. No changes in Thrombotest clotting times were observed throughout this period (data not shown). Whole blood fibrinolytic activity, measured by the DBCLT, was particularly low at the fourth week after tumour implantation. Serum fibrin(ogen) degradation products were unchanged during the first 3 weeks and pro-

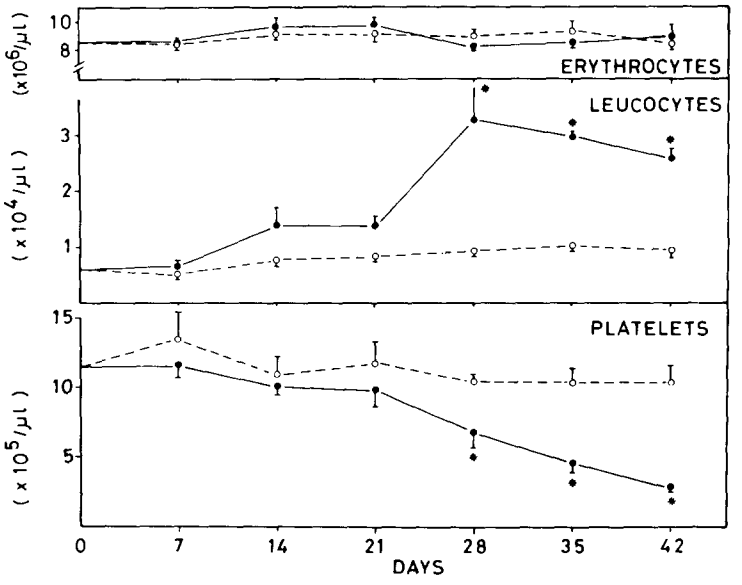

*Fig. 2. Course of erythrocyte, leucocyte and platelet counts during development of JWS. Each point is the mean ± S.E. of results from 5 animals. (○----○); control values; (●—●): JWS-bearing mice. *P<0.05 compared to the respective controls. Statistical analysis by Tukey's test (unconfounded means).*

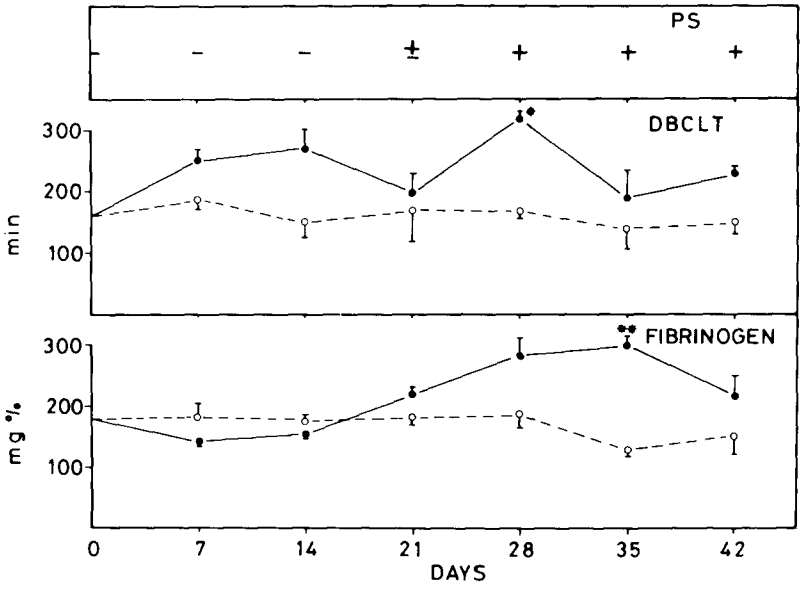

*Fig. 3. Course of blood fibrinogen and dilute blood clot lysis time (DBCLT) during development of JWS. Each point is the mean ± S.E. of results from 5 animals. Statistical analysis as in Fig. 2: *P<0.05; **P<0.01 (○----○): control values; (●—●); JWS-bearing mice. The results of the protamine sulphate test on samples from tumor-bearing mice are indicated at the top of the figure. This test gave negative results in control mice at the same intervals.*

gressively increased thereafter, being 0.25 ± 0.14 µl/ml (mean ± S.E.) in a group of normal mice and 4.56 ± 2.63 µg/ml in a group of mice bearing 6-week-old JWS. During the same period, the protamine sulphate test was positive, suggesting the presence of circulating fibrin monomer complexes.

A more detailed evaluation of the host's fibrinolytic system was performed in mice bearing 4-week-old JWS. Results are set out

in Table 2. DBCLT and ELT were significantly prolonged in tumour-bearing animals, and plasminogen was significantly higher than in controls animals. In contrast, plasma urokinase inhibitors did not differ in the two groups. A slight though not significant increase in serum fibrin(ogen) degradation products was observed.

Table 2. Fibrinolytic activity in mice 4 weeks after implantation of JWS cells. Mean ± S.E. of 6 animals per group

	Control	Tumour
DBCLT (min)	184.4 ± 12.8	227.1 ± 24.7†
ELT (min)	39.7 ± 2.9	62.4 ± 3.6†
Plasminogen (%)	87.3 ± 2.5	105.5 ± 2.5‡
Urokinase inhibitors (AU)	1.9 ± 0.2	1.7 ± 0.2
FDP (μg/ml)	0.25 ± 0.14	1.9 ± 0.4*

*$P < 0.10$.
†$P < 0.02$.
‡$P < 0.01$, Student's t-test for unpaired data.
DBCLT = Dilute blood clot lysis time; ELT = Euglobulin lysis time; FDP = Fibrin(ogen) degradation products.

Concomitantly with this fibrinolytic impairment, the half-life and survival time of radiolabelled fibrinogen were significantly prolonged in tumour-bearing animals (Table 3). Fibrinogen turnover was not significantly modified at any time, although it tended to rise at later stages of tumour growth.

DISCUSSION

This study aimed at characterizing the growth pattern of a new, syngeneic tumour in mice, as a possible model for evaluating tumour-associated haemostatic changes. The JW sarcoma, when implanted s.c., grows locally and gives spontaneous metastases mainly to the lungs. Cell-mediated immunological response is induced in JWS implanted mice as demonstrated by the lung colony assay in normal and whole-body irradiated mice [11, 31]. Both the primary tumour and the metastatic nodules lack encapsulation and heavily infiltrate host tissues. Similarly to other transplantable animal neoplasms [6, 13, 31], the primary tumour of mice implanted s.c. with JWS cells follows growth kinetics which can be described well by the Gompertz equation. This indicates tumour growth as being exponential at all times with a simultaneous exponential decrease of the specific growth rate [14]. Such a characteristic makes the model particularly suitable for evaluation of pharmacological modifications of tumour growth.

The lifespan of the animals injected with the cell numbers used in this study was long compared with that of other murine models of spontaneous metastasis such as the Lewis Lung carcinoma. It thus appeared particularly suitable for following the haematological changes induced in the host by tumour growth in conditions closer to those of human malignancies. In view of the relatively low growth rate of JWS it might also prove possible to assess the effects of multiserial treatments in this model.

Histological studies confirmed the macroscopic appearance of a non-encapsulated primary tumour, and the infiltrative metastatic growth invading the lungs. This resulted in the weight of the metastasis bearing lung at the 6th week after implantation being three times that of the lungs of mice 2 weeks after implantation. The infiltrative growth of lung metastases was such that it was impossible to enucleate the metastatic nodules from the remaining lung tissue as is done in other murine tumours (B16 melanoma, Lewis Lung carcinoma).

Table 3. Kinetics of labelled fibrinogen in JWS bearing mice at different times after tumour implantation. Mean ± S.E. of 6 animals per group

	Plasma fibrinogen (mg/100 ml)	Labelled fibrinogen		
		$t_{\frac{1}{2}}$ (hr)	Survival (hr)	Turnover (mg/ml/day)
Control	171.6 ± 22.1	14.6 ± 0.4	20.79 ± 0.6	1.98 ± 0.38
2 weeks	154.4 ± 5.0	16.0 ± 1.1	22.72 ± 1.6	1.63 ± 0.03
4 weeks	234.2 ± 23.2	21.3 ± 1.0*	30.03 ± 1.4	1.87 ± 0.16
6 weeks	216.2 ± 29.6	13.6 ± 1.2	19.04 ± 1.7	2.72 ± 0.17

*$P < 0.01$; Dunnett's test.

The main haematological changes observed during growth of the JWS were thrombocytopenia, increased leucocyte count, elevated fibrinogen level and depressed fibrinolysis. In contrast, the normal levels of Thrombotest indicated no relevant changes in the prothrombin complex activity and, therefore, in the liver function of the tumor-bearing animals. The observed thrombocytopenia is presumably to be ascribed to synthetic impairment, since no major signs of DIC or microangiopathy, which could favor intravascular consumption of platelets, were present in JWS-bearing mice. All the abnormalities occurred in the second half of the animals' life-span, when visible lung metastases were present. Similarly, in the 3LL, progressive thrombocytopenia and increase in fibrinogen level were observed during the period of metastasis growth [6]. However, in 3LL blood fibrinolytic activity was normal and fibrinogen survival time was shorter in tumour-bearing than in control mice, presumably because of fibrin deposition in the tumour [6].

In contrast, in JWS the depressed fibrinolysis was accompanied by a prolongation of labelled fibrinogen survival time. Reduced fibrinolytic activity has been reported in cancer patients [33–35] and in experimental leukemias such as rat L5222 and BNML [7, 8]. Depression of fibrinolysis could derive from production and release from cancer cells of inhibitors of blood fibrinolysis [36]. It may be worth noting that JWS cells taken from ascitic fluid show potent plasminogen activator activity [37, 38] but whether this property is modified in circulating cancer cells is at present not known. Leucocytes too are known to carry antiplasmins [39] and JWS bearing mice had very high leucocyte counts. Prolongation of the survival time of radiolabelled fibrinogen could be due to impairment in the catabolism of this protein; this might be the result of abnormal reticuloendothelial function or of decreased fibrinolysis, as is proabably the case in JWS bearing animals. Whatever their cause, both the depression of fibrinolysis and the prolongation of fibrinogen survival time were transient changes in JWS animals which were no longer detectable 6 weeks after implantation. The mechanism of these changes therefore merits further investigation.

At variance with 3LL-bearing mice, JWS-implanted animals did not show signs of microangiopathic haemolytic anaemia. This interesting difference could somehow be connected with the different vascularization pattern of the two tumours. The vascular net of JWS is very poor as compared with that of 3LL and micro-angiopathic haemolytic anaemia is a common event in the case of diseased vessels with fibrin deposits as in the 3LL. The different tissue where the tumours were implanted (s.c. in the case of JWS, i.m. in the 3LL) may also be relevant in this context.

In conclusion, the growth and metastases of JWS induced a number of haemostatic changes in the host, which could mimic laboratory changes in patients with some metastasizing tumours.

The possible connections between such changes and the intrinsic capacities of JWS cells to interfere with the clotting system are worth further investigation.

Acknowledgements—Dr. K. Berggren (Kabi Peptide Research, Stockholm, Sweden) and Dr. P. Taioli (Ortho-Cilag Chemie, Milan, Italy) kindly provided us with S-2251 substrate and Prof. L. Caprino (Serono Institute, Rome, Italy) provided the urokinase preparation used in the plasminogen assay. ^{131}Iodine was kindly supplied by OPIDI, Institute of Nuclear Research, Swierk, Poland.

Dr. Clelia Bertello, Mario Negri Institute, Milano, performed the mathematical analysis of the Gompertz growth curve by means of program BMDX 85 in a UNIVAC 1106 Computer.

Dr. Giovanna Balconi, Mario Negri Institute, Milano and Dr. Ewa Rackza, Institute of Nuclear Research, Warsaw, performed the histological studies.

The authors wish to thank Dr. Luciano Morasca for his assistance in interpreting histological results, Dr. Giovanni de Gaetano and Dr. Zbignew Latallo for fruitful discussions.

Judith Baggot, Vincenzo de Ceplie, Gigliola Brambilla, and Graziella Scalvini helped prepare this manuscript.

REFERENCES

1. P. Hilgard and R. D. Thornes, Anticoagulants in the treatment of cancer. *Europ. J. Cancer* **12,** 755 (1976).
2. M. B. Donati, A. Poggi, L. Mussoni, G. de Gaetano and S. Garattini, Hemostasis and experimental cancer dissemination. In *Cancer Invasion and Metastasis: Biologic Mechanisms and Therapy* (Edited by S. B. Day, W. P. L. Myers, P. Stansly, S. Garattini and M. G. Lewis) p. 151. Raven Press, New York (1977).
3. H. Al-Mondhiry, Disseminated intravascular coagulation. Experience in a major cancer center. *Thrombos. Diathes. haemorrh.* (*Stuttg.*) **34,** 181 (1975).

4. H. Rasche and M. Dietrich, Hemostatic abnormalities associated with malignant diseases. *Europ. J. Cancer* **13,** 1053 (1977).
5. P. Hilgard, R. Hohage, W. Schmitt and W. Köhle, Microangiopathic haemolytic anaemia associated with hypercalcaemia in an experimental rat tumour. *Brit. J. Haemat.* **24,** 245 (1973).
6. A. Poggi, N. Polentarutti, M. B. Donati, G. de Gaetano and S. Garattini, Blood coagulation changes in mice bearing Lewis lung carcinoma, a metastasizing tumor. *Cancer Res.* **37,** 272 (1977).
7. H. Rasche, D. Hoelzer, M. Dietrich and A. Keller, Hemostatic defects in experimental leukemia. *Haemostasis* **3,** 46 (1974).
8. P. Hilgard, Coagulation studies in the BNML rat leukemia. *Leukemia Res.* **1,** 175 (1977).
9. M. B. Donati, L. Mussoni, L. Kornblihtt and A. Poggi, Changes in the hemostatic system of rats bearing L5222 or BNML experimental leukemias. *Leukemia Res.* **1,** 177 (1977).
10. F. Spreafico and S. Garattini, Selective antimetastatic treatment: current status and future prospects. *Cancer Treat. Rev.* **1,** 239 (1974).
11. P. Janik, Lung colony assay in normal, irradiated and tumor bearing mice. *Neoplasma (Bratisl.)* **23,** 495 (1976).
12. L. Simpson-Herren and H. H. Lloyd, Kinetic parameters and growth curves for experimental tumor systems. *Cancer Chemother. Rep.* **54,** 143 (1970).
13. A. K. Laird, Dynamics of tumor growth. *Brit. J. Cancer* **18,** 490 (1964).
14. H. H. Lloyd, Estimation of tumor cell kill from Gompertz growth curves. *Cancer Chemother. Rep.* **59,** 267 (1975).
15. H. Wexler, Accurate identification of experimental pulmonary metastases. *J. nat. Cancer Inst.* **36,** 641 (1966).
16. J. V. Dacie and S. M. Lewis, *Practical Haematology*. Churchill, London (1970).
17. C. Vermylen, R. A. De Vreker and M. Verstraete, A rapid enzymatic method for assay of fibrinogen, fibrin polymerization time (FPT test). *Clin. chim. Acta* **8,** 418 (1963).
18. M. J. Gallimore, H. M. Tyler and J. T. B Shaw, The measurement of fibrinolysis in the rat. *Thrombos. Diathes. haemorrh. (Stuttg.)* **26,** 295 (1971).
19. M. B. Donati, J. Vermylen and M. Verstraete, The staphylococcal clumping test for detection of fibrinogen-like material. *Scand. J. Haematol. Suppl.* 13, 137 (1971).
20. Z. S. Latallo, Z. Wegrzynowicz, E. Teisseyre and M. Kopeć, Simple and rapid evaluation of the intravascular coagulation and fibrinolytic states by application of protamine sulphate and reptilase R. *Scand. J. Haematol.* Suppl. 13, 387 (1971).
21. L. Mussoni, E. Raczka, J. Chmielewska, M. B. Donati and Z. S. Latallo, Plasminogen assay in rabbit, rat and mouse plasma using the chromogenic substrate S-2251. *Thromb. Res.* **15,** 341 (1979).
22. M. Paraskevas, I. M. Nilsson and G. Martinsson, A method for determining serum inhibitors of plasminogen activation. *Scand. J. clin. Lab. Invest.* **14,** 138 (1962).
23. E. Regoeczi, Iodine-labelled fibrinogen: a review. *Brit. J. Haematol.* **20,** 649 (1971).
24. A. S. McFarlane, *In vivo* behavior of ^{131}I–fibrinogen. *J. clin. Invest.* **42,** 346 (1963).
25. M. J. Welch and K. A. Krohn, Critical review of the radiolabelled fibrinogen: its preparation and use. In *Radiopharmaceuticals* (Edited by G. Subramanian, B. Rhodes, J. Cooper and V. Sodd), p. 258. Society of Nuclear Medicine, New York (1975).
26. K. A. Krohn, S. J. De Nardo, D. W. Wheeler and G. L. De Nardo, I–fibrinogen as an oncophilic radiodiagnostic agent: distribution kinetics in tumour-bearing mice. *Brit. J. Cancer* **36,** 227 (1977).
27. L. A. Harker and S. J. Slichter, Studies on platelet and fibrinogen kinetics in patients with prosthetic heart valves. *New Engl. J. Med.* **283,** 1302 (1970).
28. G. W. Snedecor and W. G. Cochran, *Statistical Methods*, 6th edn. Iowa State University Press, Ames (1967).
29. C. W. Dunnet, Dunnett's test. *J. Amer. statist. Ass.* **50,** 1096 (1955).
30. R. E. Kirk, *Experimental Design: Procedures for the Behavioral Sciences*, p. 88. Brooks/Cole, Belmont (1968).

31. P. JANIK and B. SZANIAWSKA, Search for an influence of natural immunity on the lung colony assay of a syngeneic transplanted murine tumour. *Brit. J. Cancer* **37,** 1083 (1978).
32. F. M. SCHABEL, JR., Concepts for systemic treatment of micrometastases. *Cancer (Philad.)* **35,** 15 (1975).
33. A. BRUGAROLAS, E. G. ELIAS, H. TAKITA, I. B. MINK, A. MITTELMAN and J. L. AMBRUS, Blood coagulation and fibrinolysis in patients with carcinoma of the lung. *J. Med.* **4,** 96 (1973).
34. N. C. J. SUN, E. J. W. BOWIE, F. J. KAZMIER, L. R. ELVEBACK and C. A. OWEN, JR., Blood coagulation studies in patients with cancer. *Mayo Clinic Proc.* **49,** 636 (1974).
35. U. HEDNER and I. M. NILSSON, Pattern of the activator inhibitor in carcinoma and renal diseases. *Scand. J. Haematol.* **11,** 398 (1973).
36. P. M. FORNASARI, M. PANDOLFI and B. ÅSTEDT, On the proteolytic inhibitory effect of human foetal and neoplastic tissues. *Thromb. Res.* **8,** 829 (1976).
37. J. CHMIELEWSKA, E. RACZKA, E. TEISSEYRE and Z. S. LATALLO, Effect of neoplastic cells on plasma clotting and fibrinolytic system, *Thromb. Haemost.* **42,** 141 (1979).
38. Z. S. LATALLO, B. KOWALSKA-LOTH, J. CHMIELEWSKA, E. TEISSEYRE, E. RACZKA and M. KOPEĆ, A new approach to study factors from tumour cells which influence the clotting and fibrinolytic systems. In *Progress in Chemical Fibrinolysis and Thrombolysis* (Edited by J. F. Davidson, V. Cěpelak, M. M. Samama and P. C. Desnoyers) Vol. IV, p. 411. Churchill Livingstone, Edinburgh (1979).
39. N. WOLOSOWICZ and J. PROKOPOWICZ, Isolation and purification of anti-plasmins from human granulocytes. *Thrombos. Diathes. haemorrh. (Stuttg.)* **24,** 543 (1970).