# CHANGES IN THE ELECTROPHORETIC MOBILITY OF MOUSE LYMPHOCYTES, THYMOCYTES, MACROPHAGES AND TUMOUR CELLS FOLLOWING IMMUNISATION

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THERE is evidence of excessive cell death in many tumours. This has prompted the use of immunological methods to detect the presence of specific antigens on tumour cells and a study of the activity of antibodies and/or lymphocytes against tumour cells.

Myer (1966) using mixed-antiglobulin and immune-adherence tests found anti-tumour antibodies in C3H mice immunised against Ehrlich ascites tumour, but no strong histocompatability antigens were detected on the tumour. Hartveit (1965*a*, *b*) used complement-induced lysis to show that Ehrlich ascites tumour cells became progressively coated with antibody when grown in mice of a closed colony and that this also happened when Ehrlich cells were grown in C3H mice (Hartveit, 1966). After the removal of tail-transplants of Ehrlich tumour, Thunold (1967) found that peritoneal cells formed characteristic rosettes round the tumour cells.

The above studies were all carried out in systems in which the tumour cells grew as an allograft. However, antibody coating also occurs in isogeneic systems (Hartveit, 1964, 1965*c*). Cater and Waldmann (1967) found that lymphocytes from C57Bl mice immunised with BP8 tumour plus Freund's complete adjuvant, would protect C3H mice against fatal challenge with the isogeneic BP8 tumour, when the lymphocytes were given i.p. Rook and Cater (1968) found these " immune " lymphocytes would also protect when given i.v., but not if the C3H mice were given sublethal total-body irradiation. They also showed the adherence of these " immune " lymphocytes to BP8 cells in tissue culture.

The electrokinetic behaviour of many types of biological cells—erythrocytes, tumour cells, cells in tissue culture etc.—arises because of the ionising groups on the cell surface. Information on the adsorbtion of antibody on the cell surface has also been obtained by the technique of cell electrophoresis (Sachtleben, 1965).

In the present paper we demonstrate by means of changes in the electrophoretic mobility the appearance of a new population of lymphocytes, thymocytes and macrophages in C57Bl mice after immunisation with BP8 cells plus Freund's complete adjuvant. We have also found changes in the electrophoretic mobility of Ehrlich ascites tumour cells and of BP8 ascites tumour cells at various times after transplantation, and compared these *in vivo* changes with *in vitro* studies in which the electrophoretic mobility of BP8 cells was found to fall after treating the cells with anti-BP8 serum.

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## MATERIALS AND METHODS

*Apparatus.*—The electrophoresis chamber used was of circular cross-section (Seaman and Heard, 1961) as modified by Mehrishi (1962). (See also Leaman, 1965).

*Solutions.*—Hanks' solution, containing 5 units of heparin per ml., was used as suspending medium for the cells in the majority of the experiments. In some the cells were suspended in physiological saline, pH 7·2 ± 0·2 (Seaman and Heard, 1960). Studies on tumour cells and lymphocytes processed in saline and parallel experiments in Hanks' solution have shown no damage to the membrane of the cells—as judged by phase contrast microscopy and/or permeability of the cell membrane to potassium ions (Mehrishi and Butterworth, 1968).

### *Experimental procedure*

*Immunisation.*—The C57Bl mice were given weekly injections of BP8 cells in Freund's complete adjuvant (0·1 ml/mouse). The time intervals and number of mice are shown in Fig. 2.

### *Tumour transplantation*

*BP8 tumour.*—Ten adult C3H mice were injected i.p. with $5 \times 10^4$ cells. One mouse received $10^3$ tumour cells and 3 mice $10^4$. The mice were killed at intervals after injection as shown in Figs. 3 and 4.

*Ehrlich tumour.*—Twelve male and 12 female Tuck No. 1 mice were injected i.p. with 0·2 ml. of whole tumour ascites from a 7 day transplant (approx. $1{\cdot}6 \times 10^7$ tumour cells). The mice were killed at intervals after injection (Fig. 6). Three untreated mice served as controls.

*Preparation of cell-suspensions.*—The tumour cells were washed 3 times by suspending in 50 volumes of Hanks' solution, or saline, and centrifuging gently ($160 \times g$ for 3 min.). The lymph-nodes and thymuses were removed and minced with scissors in Hanks' solution. The resulting suspensions were filtered through 4 layers of gauze. The cells were then spun down ($160 \times g$) and resuspended in Hanks' solution for a further wash; while 3 min. sufficed for the thymocytes, 6 min. were needed for the lymphocytes.

In non-tumour bearing mice the peritoneal macrophages were also removed following the injection of 1–2 ml. of Hanks' solution into the peritoneal cavity. The cells were then washed 3 times as for the tumour cells.

*Serum-treated cells.*—Pooled antiserum was obtained from C57Bl mice immunised with 4 injections of BP8 tumour cells in Freund's adjuvant as described above. The agglutinating titre was 1/64 when the suspension contained $2{\cdot}5 \times 10^5$ BP8 cells.

Sera from untreated C3H mice, untreated Tuck No. 1 mice and Tuck No. 1 mice carrying 8 day transplants of Ehrlich ascites carcinoma were also used. Details of the concentrations used are given in Table III.

The washed tumour cells were incubated in Hanks' solution with added serum for 30 min. at 37°. The suspensions were agitated gently at approx. 5 min. intervals. After incubation the tumour cells were spun down and then washed twice in Hanks' solution as described above. Control suspensions were treated similarly.

### *Cell electrophoresis*

The electrophoretic mobility determinations were carried out at 25 ± 0·1° and the mobility expressed in $\mu$ per sec. per V. per cm. Approximately 30 cells were examined from each suspension and studies were completed within 3 hr. of the death of the mouse. The reliability of the apparatus was checked repeatedly with saline-washed erythrocytes (human).

## RESULTS

### *Section I. Electrophoretic mobility of lymphocytes, thymocytes and macrophages from C57Bl mice injected with BP8 cells plus Freund's complete adjuvant, compared with cells from normal C57Bl mice*

After two injections the lymph-nodes were hypertrophic and they continued to increase in size with the repeated weekly injections. No changes in size of the thymus were noted.

*Lymphocytes.*—A comparison was first made between the electrophoretic mobility of lymphocytes from normal mice and those that had received 4 injections of BP8 cells in Freund's adjuvant. These results are shown in Fig. 1. Note that while the majority of cells from the normal mice clustered round 1 mean, the cells from the immunised mice fall into 2 populations. These data were analysed further (see appendix, and Fig. 7). From this analysis it was possible to define a dividing line between the 2 populations of cells in the immunised mice. Cells with a mobility

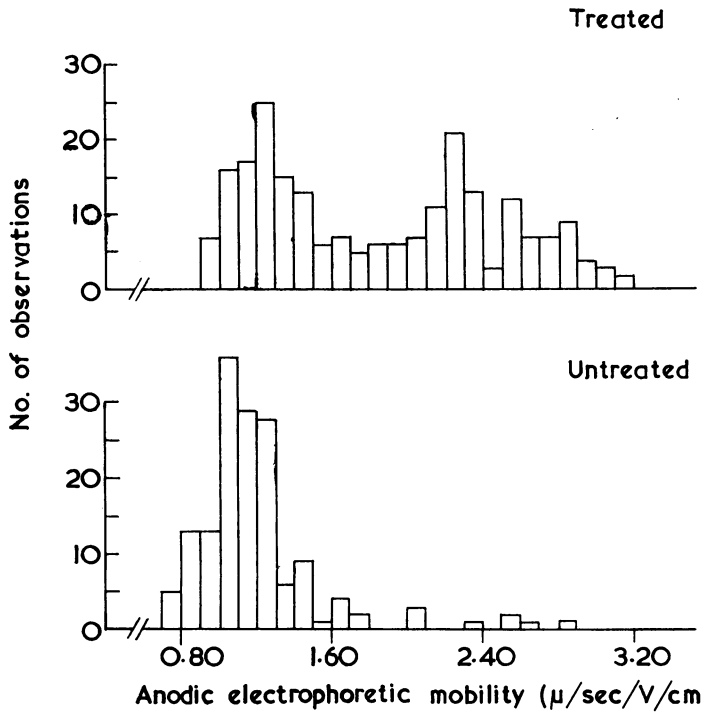


FIG. 1.—Histogram showing the distribution of lymphocytes with different electrophoretic mobilities. A new fast population appears in the C57Bl mice immunised with BP8 cells plus Freund's complete adjuvant.

greater than — 1·73 $\mu$/sec./V/cm. were classified as " fast " and those with a lesser mobility as " slow ". This dividing line was used in further analysis of the data on lymphocytes.

Table I shows the mobility of lymphocytes related to the number of weeks of immunisation. The bimodal pattern emerges clearly. The mean for the fast lymphocytes increased gradually following repeated immunisation. The mean for the slow cells showed less variation.

The percentage of cells in each class also changed (Fig. 2). Very few fast lymphocytes were present in the normal C57Bl mice, but after 2 weeks of immunisation their number had risen to 40 per cent. The increase continued to just over 50 per cent after 4, and after 5 injections.

*Thymocytes.*—Table I shows that the electrophoretic mobilities of thymocytes also followed a bimodal pattern. As the mean values were comparable to those found for the lymphocytes, the same dividing line between fast and slow was used. The percentage of fast cells was however lower (Fig. 2). In the normal mice only

TABLE I.—*Electrophoretic Mobility* (μ/sec./V/cm.) of Lymphocytes, Thymocytes, and Macrophages from C57Bl mice related to Time after Repeated Immunisation (weeks) with BP8 Cells plus Freund's Complete Adjuvant; Compared with Cells from Normal C57Bl Mice (day 0)*

| Cell type | Duration of immunisation (weeks) 0 | 2 | 4 | 10 |
|---|---|---|---|---|
| Lymphocyte | | | | |
| Fast | −2·27±0·13 (10) | −2·33±0·06 (42) | −2·38±0·03 (116) | −2·52±0·05 (58) |
| Slow | −1·12±0·01 (144) | −1·15±0·03 (66) | −1·26±0·02 (106) | −1·26±0·04 (43) |
| Thymocyte | | | | |
| Fast | −1·79±0·01 (3) | −2·45±0·08 (16) | −2·10±0·06 (41) | −2·60±0·07 (42) |
| Slow | −1·16±0·01 (169) | −1·04±0·02 (68) | −1·37±0·01 (175) | −1·27±0·03 (59) |
| Macrophage | −1·11±0·02 (95) | −1·03±0·03 (58) | −1·04±0·02 (80) | −1·04±0·04 (53) |

* Number of cells examined in parentheses.

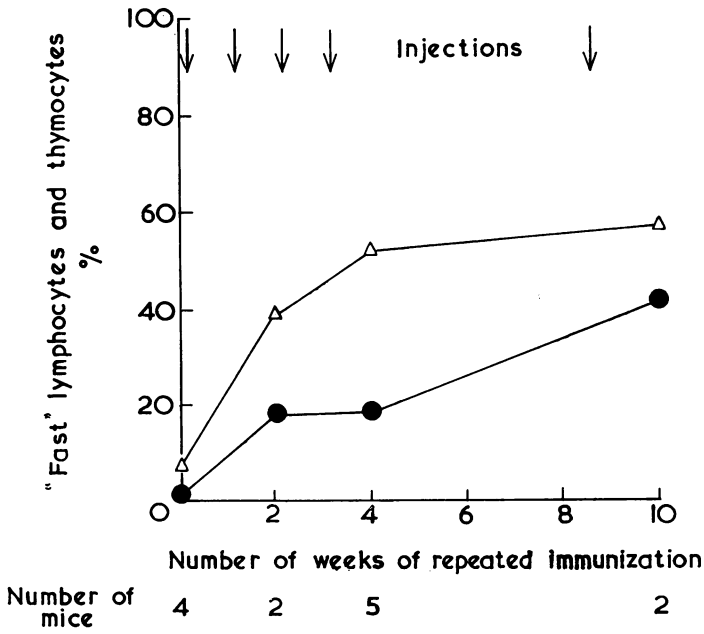


FIG. 2.—The percentage of "fast" lymphocytes — △ — △ and "fast" thymocytes — ● — ●, plotted against the overall time of immunisation. C57Bl mice immunised with BP8 cells + Freund's complete adjuvant.

3 cells were classed as fast, and as these gave a mobility marginally above the dividing line it is likely that they represent the " tail " of the slow curve and not a population of fast cells. On the other hand after 2 weeks of immunisation a fast population was established and this increased from 20–40 per cent following repeated immunisation.

*Macrophages.*—The mobility of these cells remained remarkably constant despite immunisation (Table I).

*Section II. The electrophoretic mobility of lymphocytes, and thymocytes in C3H mice with BP8 ascites tumour*

Following tumour transplantation the lymph-nodes and thymus were as a rule hypertrophic in mice with early transplants. Eight days or more after transplantation they were atrophic, and in some cases it was difficult to obtain enough cells from them, for example the 10 day sample (Table II).

*Lymphocytes.*—The electrophoretic mobility of C3H lymphocytes (Table II) showed a bimodal distribution as found in the immunised C57Bl mice. The electrophoretic mobility of fast cells rose with the time after tumour transplantation until day 7. The number of fast cells rose to a peak (32 per cent) on day 7, after which they were no longer found (Fig. 3).

The electrophoretic mobility of the slow population decreased by 8 per cent on day 4, returned to control level at 7 days but fell on the 10th, and again on the 14th day (Table II).

TABLE II.—*Electrophoretic Mobility (μ/sec./V/cm.) of Lymphocytes and Thymocytes, Related to the Time after Transplantation (Days) of 5 × 10⁴ BP8 Ascites Tumour Cells in C3H Mice, Compared with Control Mice (day 0)*

| | Time after transplantation (days) | | | | |
|---|---|---|---|---|---|
| Cell type | 0 | 4 | 7 | 10 | 14 |
| Lymphocyte | | | | | |
| Fast | −1·82±0·02 (10) | −2·09±0·15 (4) | −2·18±0·07 (26) | — | — |
| Slow | −1·31±0·03 (80) | −1·21±0·02 (102) | −1·38±0·02 (56) | −1·12±0·03 (27) | −1·04±0·04 (28) |
| Thymocyte | | | | | |
| Fast | — | −2·38±0·07 (22) | −2·15±0·05 (24) | — | — |
| Slow | −1·14±0·03 (76) | −1·14±0·03 (101) | −1·11±0·04 (59) | −1·27±0·06 (9) | −1·18±0·03 (29) |
| Macrophage | −1·09±0·04 (42) | — | — | — | — |

*Thymocytes.*—Here the fast population was present at 4 and 7 days only (Table II), the percentage rising from 18–28 per cent (Fig. 3). The slow population showed a reasonably constant mobility throughout (Table II).

When the inoculum of BP8 cells was reduced from $5 \times 10^4$–$10^3$, fast lymphocytes (10 per cent) and fast thymocytes (13 per cent) were present on days 10–14.

*Macrophages.*—In the control C3H mice the macrophages gave a mean mobility of − 1·09 ± 0·04 μ/sec./V/cm. (Table II).

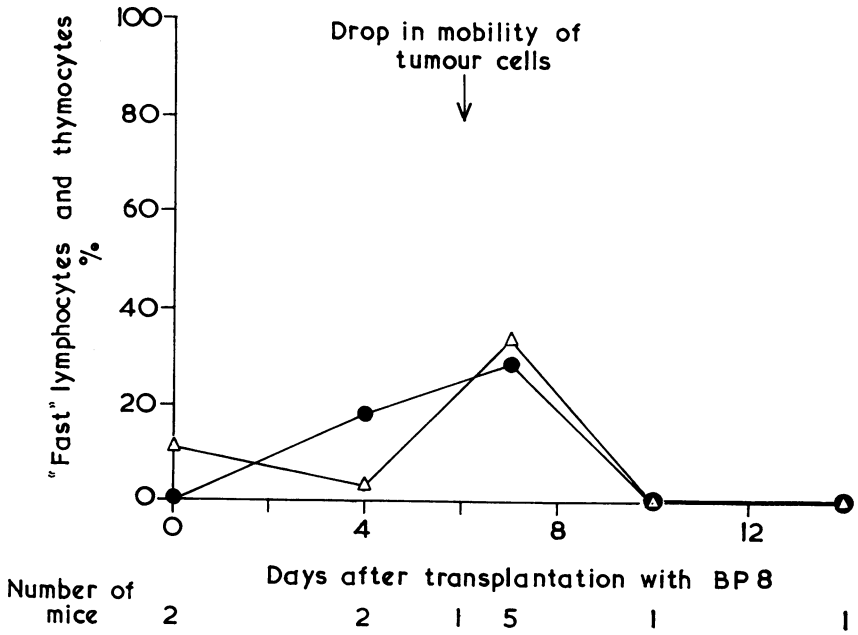


FIG. 3.—The percentage of " fast " lymphocytes — △ — △ and " fast " thymocytes ● — ●, in C3H mice, plotted against days after transplantation of BP8 ascites tumour.

*Section III. The electrophoretic mobility of BP8 ascites tumour cells related to time after transplantation* in vivo *and the effect of antiserum* in vitro

*BP8 tumour cells* in vivo.—After inoculation with $5 \times 10^4$ cells the mobility of the cells in the growing transplant fell with time, and the mobility of the cells in late transplants was related to the number of cells in the original inoculum (Fig. 4).

*BP8 tumour cells treated with antiserum* in vitro.—Treatment of tumour cells with antiserum gave a consistent decrease in the electrophoretic mobility, 15–20 per cent (Table III). These reductions in mobilities were not related to the amount of antiserum used when the reaction time was 30 min. Control experiments in which the tumour cells were treated with normal C3H serum showed a reduction of only 5 per cent which is near the the limit of experimental error. Similar results were obtained with the second batch of antiserum of comparable titre.

*Section IV. The electrophoretic mobility of lymphocytes and thymocytes in Tuck No. 1 mice with Ehrlich ascites tumour*

*Lymphocytes.*—A very fast population of lymphocytes was found in the control mice (Table IV). Three and 4 days after transplantation the mobility of the fast population had fallen by about 15 per cent. The number of fast cells present is shown in Fig. 5. It was high (39 per cent) in the controls but fell to 10 per cent 3 days after transplantation. By the 4th day there was a sharp rise to near 50 per cent, but by 8 days the fast population had virtually disappeared.

In the control mice the mobility of the cells in the slow population was $-1{\cdot}42 \pm 0{\cdot}03$ $\mu$/sec./V/cm. when they constituted 61 per cent of the total

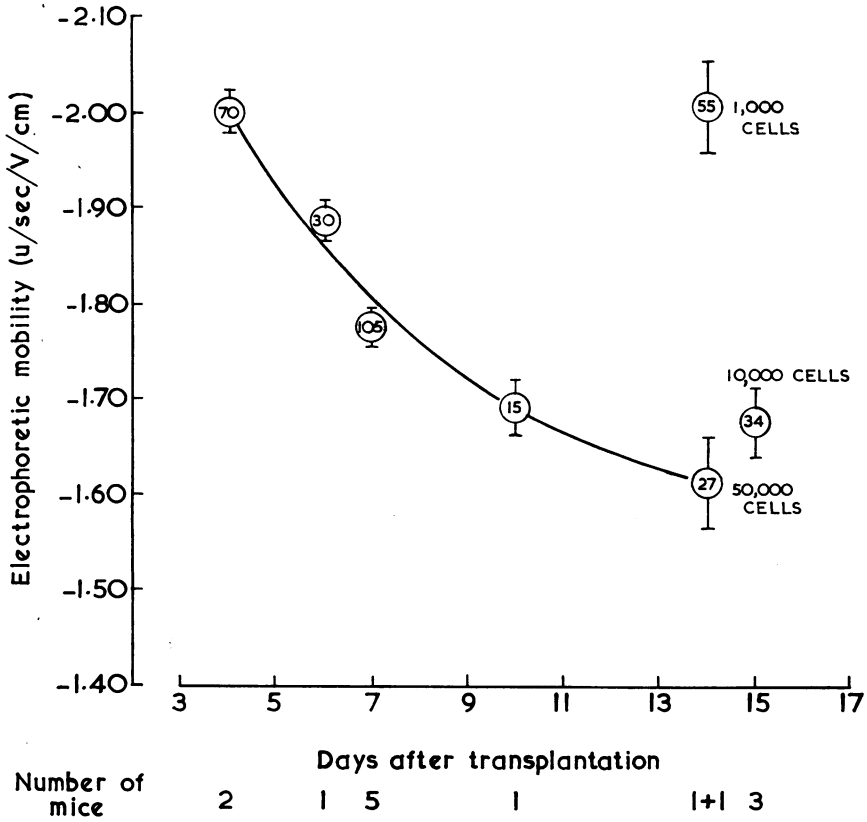


FIG. 4.—The anodic electrophoretic mobility of BP8 ascites tumour cells, removed from C3H mice, falls with the number of days after tumour transplantation, unless the innoculum is very small (1000 cells).

TABLE III.—*The Reduction in Electrophoretic Mobility (μ/sec./V/cm.) of Washed BP8 Ascites Tumour Cells after Treatment with Normal C3H Serum, Normal C57Bl Serum and Serum from C57Bl Mice Immunised with BP8 Cells plus Freund's Complete Adjuvant*

| Serum type | Serum dilution | Electrophoretic mobility (μ/sec/V/cm) Control | Electrophoretic mobility (μ/sec/V/cm) Treated | Reduction in mobility (per cent) |
|---|---|---|---|---|
| Normal C3H | 1/16 | −2·13±0·02 (126) | −2·02±0·03 (80) | 5% |
| Normal C57Bl | 1/20 | −1·68±0·04 (34) | −1·46±0·03 (25) | 13% |
| Immunised C57Bl | 1/5 | −2·01±0·04 (70) | −1·70±0·04 (36) | 15% |
| ,, | 1/16 | −2·13±0·03 (126) | −1·75±0·05 (56) | 18% |
| ,, | 1/20 | −1·69±0·04 (34) | −1·36±0·04 (20) | 20% |

Cell concentration $10^6$ cells/ml.
Number of cells examined in parentheses.

TABLE IV.—*Electrophoretic Mobility* (μ/sec./V/cm.) of Lymphocytes and Thymocytes Related to the Time after Transplantation (Days) of Ehrlich Ascites Tumour in Tuck No. 1 Mice Compared with Control Mice (day 0)*

| Cell type | Time after transplantation (days) 0 | 3 | 4 | 8 |
|---|---|---|---|---|
| Lymphocyte | | | | |
| Fast | −2·47±0·05 (36) | −2·19±0·10 (9) | −2·14±0·04 (53) | † |
| Slow | −1·42±0·03 (56) | −1·16±0·02 (81) | −1·40±0·04 (54) | −1·14±0·02 (70) |
| Thymocyte | | | | |
| Fast | −2·20±0·04 (89) | −2·33±0·09 (12) | −2·15±0·06 (24) | ‡ |
| Slow | −1·47±0·03 (36) | −1·03±0·03 (78) | −1·20±0·03 (49) | −1·08±0·02 (74) |
| Macrophage | −1·19±0·03 (188) | — | — | — |

* Number of cells examined in parentheses.
† 3 fast in one mouse (−2·16) (μ/sec./V/cm.).
‡ 1 fast in same mouse (−2·32) (μ/sec./V/cm.).

population. Three days after transplantation the mobility dropped markedly from this high value but rose again at 4 days. By the 8th day the mobility had fallen once more (Table IV).

*Thymocytes.*—Fast thymocytes were present in the control mice and at 3 and 4 days after transplantation (Table IV). The percentage of these cells was extremely high (69 per cent) in the control mice but fell to 13 per cent, 3 days after

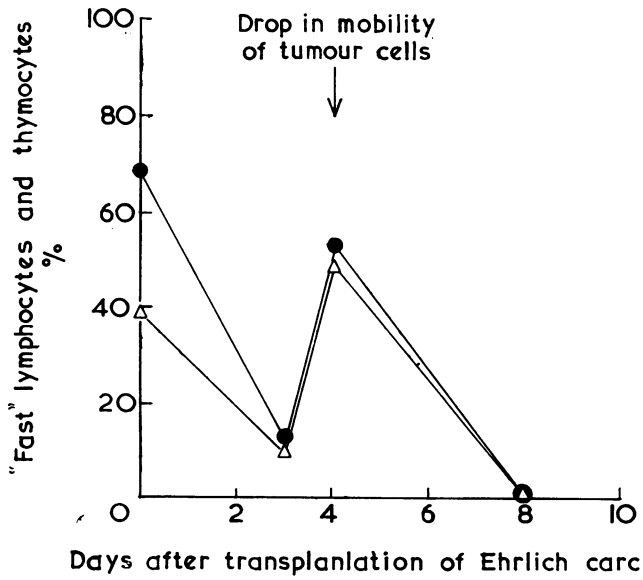


FIG. 5.—The percentage of " fast " lymphocytes △ — △ and " fast " thymocytes ● — ●, in Tuck No. 1 mice, plotted against the days after transplantation of Ehrlich ascites carcinoma. The number of mice were: 3 on day 0, 2 on day 3, 2 on day 4 and 2 on day 8.

transplantation. This fall was followed by a sharp rise to just over 50 per cent at 4 days. At 8 days the fast population had virtually disappeared (Fig. 5).

The mobility of the slow cells followed a pattern similar to that of the slow lymphocytes (Table IV).

*Macrophages.*—The electrophoretic mobility of the macrophages in the control mice was $-1{\cdot}19 \pm 0{\cdot}03$ $\mu$/sec./V/cm. (Table IV).

*Section V. The electrophoretic mobility of Ehrlich ascites tumour cells related to the time after transplantation* in vivo *and the effect of antiserum* in vitro

*Ehrlich tumour cells* in vivo.—The mobility of the tumour cells fell with time after transplantation and doubling the innoculum produced a greater fall (Fig. 6).

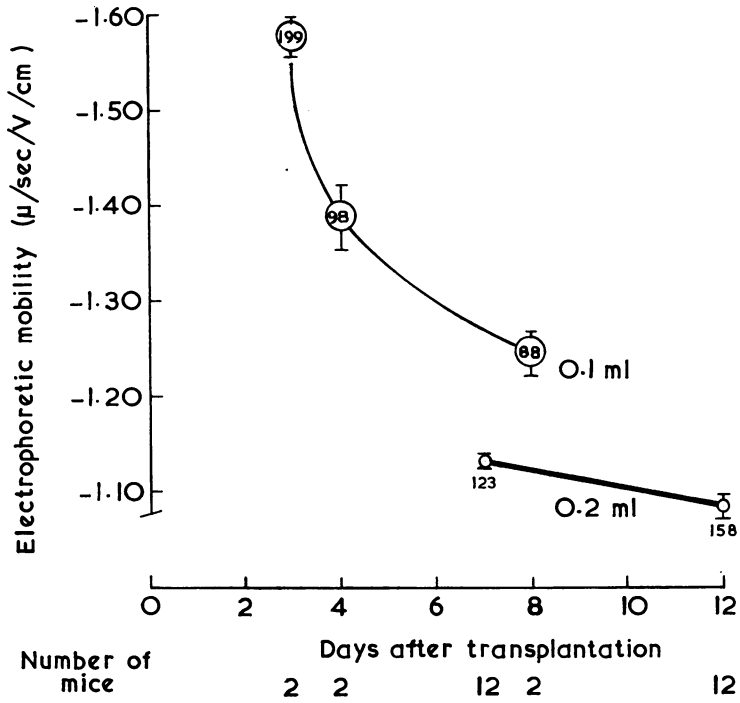


FIG. 6.—The anodic electrophoretic mobility of Ehrlich ascites tumour cells, removed from Tuck No. 1 mice, falls with the number of days after transplantation of the tumour and the size of the innoculum.

*Ehrlich tumour cells treated with antiserum* in vitro.—Treatment of cells with serum from mice with 8 day transplants led to a drop in mobility of 22 per cent. This serum did not agglutinate the red blood cells from the mouse that supplied the tumour cells. Treatment with normal Tuck No. 1 serum gave a 7 per cent reduction in mobility (Table V).

No consistent differences in mobility were found between cell suspension made up in Hanks' solution and physiological saline, and no significant differences related to the sex of the tumour bearing mouse were recorded.

TABLE V.—*The Reduction in Electrophoretic Mobility* (μ/sec./V/cm.) of Washed Ehrlich Ascites Tumour Cells Following Treatment* in vitro *with Serum from Tuck No.* 1 *Mice Compared with the Reduction in Mobility after Treatment with Serum from Tuck No.* 1 *Mice Bearing* 8 *day Ehrlich Ascites Tumours*

| Serum | | Electrophoretic mobility (μ/sec./V/cm.) | | Per cent reduction in mobility |
|---|---|---|---|---|
| Type | Dilution | Control | Treated | |
| Tuck No. 1 (Pooled normal) | 1/5 | $-1\cdot58\pm0\cdot02$ (199) | $-1\cdot47\pm0\cdot04$ (61) | 7 |
| Tuck No. 1 with 8 day Ehrlich | 1/5 | $-1\cdot58\pm0\cdot02$ (199) | $-1\cdot24\pm0\cdot03$ (81) | 22 |

* Number of cells examined in parentheses.

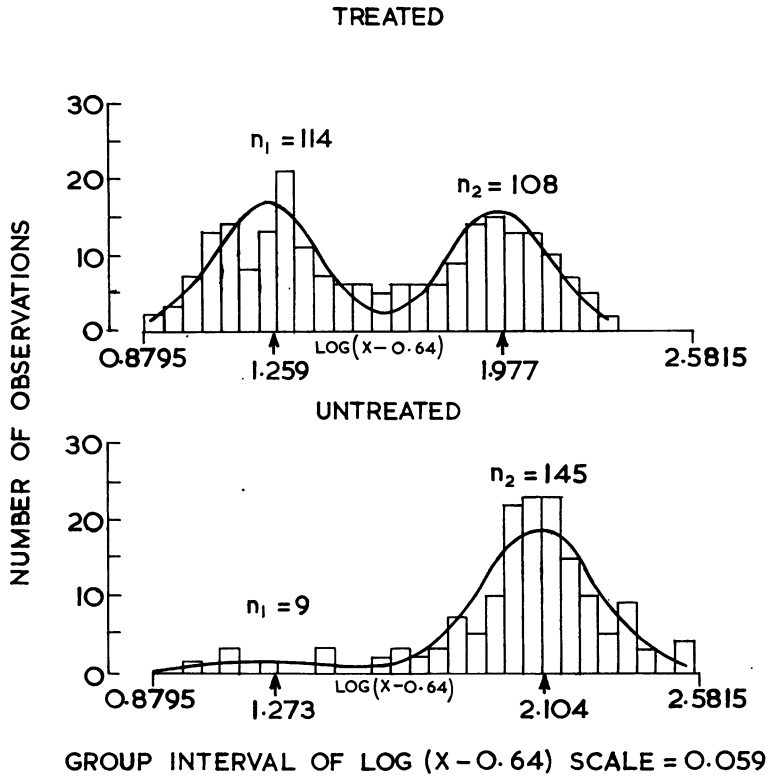


FIG. 7.—Histogram showing the distribution of lymphocytes plotted against log (time in seconds − 0·64). The lymphocytes were taken from C57Bl mice " untreated " or " treated " (immunised with BP8 cells plus Freund's complete adjuvant).

## DISCUSSION

Our experiments show that following antigenic stimulation a new population of lymphocytes and thymocytes appears with a mean mobility increased by 80 per cent. On the other hand cells harvested from the peritoneal cavity appeared to be unaffected by the immunisation procedures. This perhaps helps to justify their classification as macrophages.

The surface changes on the lymphocytes and thymocytes (cells that could be expected to be immunologically competent) were remarkably constant irrespective of mouse strain or type of antigenic stimulus. Thus, with C57Bl mice given BP8 and Freund's adjuvant, C3H mice given BP8 cells or Tuck No. 1 mice given Ehrlich cells, two cell populations were swiftly established. These do not appear to be related to the two populations of lymphocytes in normal rats reported by Ruhenstroth-Bauer and Lücke Huhle (1968) in which the mobilities differed by only 12 per cent and both were of the same order as our slow population.

Following immunisation of C57Bl mice the fast population of lymphocytes appeared before the fast thymocytes. This difference was not seen in the tumour transplantation experiments using C3H or Tuck mice. In the Tuck mice there were numerous fast lymphocytes and thymocytes in the control animals. After injection of the tumour these fast cells were no longer present in the lymph-nodes and thymus but they reappeared later. In the C3H control mice only a few fast lymphocytes were present and these behaved in the same way. We think the fast lymphocytes and thymocytes present in the control Tuck mice may have been due to chronic infection, because the percentage present and their mobility were similar to those in the C57Bl immunised mice. (Pathogen-free mice were not available to resolve this). Following the tumour injection, *i.e.* a new antigenic stimulation, the fast cell population disappeared from the lymph-nodes but on the 4th day more fast cells appeared (Fig. 6). It is tempting to postulate that the fast cells represent 'primed cells ", *i.e.* cells able to react specifically against this stimulating antigen.

Turning now to the experiments on the mobility of tumour cells, these cells represent a living antigenic target for specific antibody. The BP8 cells are antigenic in C3H mice by virtue of their tumour specific antigen; the Ehrlich tumour is antigenic in Tuck mice by virtue of its ability to grow as an allograft.

We interpret our results as follows: the fast electrophoretic mobility shown by the tumour cells 3 or 4 days after transplantation (Figs. 4 and 6) represents the primitive state of the cell surface unsullied by antibody. Then there is a slow reduction in mobility over several days with BP8 cells (Fig. 4) and a rapid reduction in mobility with Ehrlich tumour cells (Fig. 6). This is probably due to the coating of the surface with antibody (*c.f.* erythrocytes, Sachtleben, 1965). Hartveit (1963) reported that the morphology of Ehrlich cells at 3 days is markedly different from that in later transplants. In our experiments large inocula, providing a greater antigenic stimulus, were found to accelerate the loss of mobility of both BP8 and Ehrlich cells.

Our *in vitro* experiments support the interpretation that the change of mobility is due to antibody coating the tumour cells. Normal C3H serum in the case of BP8 cells and normal Tuck No. 1 mouse serum in the case of Ehrlich cells produced only a 5–7 per cent reduction in mobility. The significantly greater reduction in mobility produced after *in vitro* addition of anti-BP8 serum (15–20 per cent) and serum from mice with 8 day Ehrlich transplants (22 per cent) indicated that specific binding had occurred on to the cell surface. It is of note that the latter serum had this effect, as this implies that it contained circulating antibody, although an effect of toxic substances such as that reported by Holmberg (1962) cannot be excluded. Holmberg (1968) reports that his polypeptide has no effect on the electrophoretic mobility of erythrocytes and Chang liver cells.

The time of appearance of the new population of fast lymphocytes and thymocytes coincided with the drop in the mobility of the living tumour cells carried by

the same mouse. Thus, mice with Ehrlich showed a peak incidence of fast lymphocytes and thymocytes 4 days after transplantation, while the mobility of the tumour cells dropped sharply between the 3–4th day. The mice with BP8 tumour showed a peak incidence of fast lymphocytes and fast thymocytes at 7 days (this could have been earlier as the previous reading was taken on day 4) while the drop in mobility of the BP8 cells had started on day 6.

This relationship between the occurrence of fast cells in the lymph nodes and thymus and the decrease in mobility of the tumour cells adds support to the hypothesis that the two may be causally related—the primed cells in the nodes supplying the antibody that coats the antigenic tumour cells. This idea of *in vivo* sensitisation of transplanted tumour cells has been discussed in detail previously (Hartveit, 1965*a*, *b* and *c*).

There was a marked drop in the number of fast lymphocytes and thymocytes present in the mice with late transplants of both BP8 and Ehrlich, in contrast to the continued increase in number of fast cells following repeated immunisation of C57Bl with BP8 and Freund's adjuvant. Fast cells disappeared between 7–10 days after the transplantation of BP8 and between 4–8 days after the transplantation of Ehrlich cells. The lymph-nodes in these mice with late transplants were small and atrophic, while those from mice with early transplants and from the immunised C57Bl mice were hypertrophic. This suggests that the antigenic stimulation following tumour transplantation had been great enough to exhaust the lymph-nodes of immunologically competent cells. This finding may correlate with reported defects in the immunological response of cancer patients (see Alexander and Fairley, 1967, for review). It is possible that the drop in the mobility of rat lymph-node cells following immunisation with bacterial antigens (TAB and *Vibrio cholerae*) reported by Sundaram, Phondke and Ambrose (1967) might be explained on this basis. However, these authors inferred that the slow cells were probably plasma cells. Our lymphocyte and thymocyte preparations appeared to be free from plasma cells as checked by phase-contrast microscopy.

While the present experiments stress the changes in the cells in the lymph-nodes and thymus following antigenic stimulation, they also indicate that the fast cells seen following immunisation may be the immunologically active ones. These cells appear in response to stimulation, and arise in all probability from the more stable slow population. The finding that the glands become depleted of fast cells in mice with late tumour transplants raises the question of the fate of these cells. They might be the cells responsible for the local cellular immune response, but more data are needed before this possibility can be fully evaluated.

## SUMMARY

In C57Bl mice, immunised with BP8 ascites tumour cells plus Freund's complete adjuvant, a new population of lymphocytes and thymocytes appeared with a high anodic electrophoretic mobility ($-2{\cdot}52 \pm 0{\cdot}05$ $\mu$/sec./V/cm.), double that of the normal slow population.

" Fast " lymphocytes and thymocytes were present on the 4–7th day in C3H mice with BP8 ascites tumour and in Tuck No. 1 mice with Ehrlich ascites tumour.

The electrophoretic mobility of BP8, and Ehrlich tumour cells is high on the 4th and 3rd day after transplantation; respectively, but then falls with time after transplantation.

BP8, or Ehrlich tumour cells incubated with antisera and washed, showed significant reduction (15–22 per cent) in electrophoretic mobility compared with controls.

The significance of these findings is discussed in relation to the tumour/host relationship.

We wish to thank Mr. E. King for providing us with Tuck No. 1 mice, Miss J. Wright for skilled technical assistance, Mr. R. G. Carpenter and Mrs. C. R. G. Saunders for the statistical report, the mathematical laboratory for the use of computer facilities, and Professor J. S. Mitchell for facilities and encouragement. Financial support from the British Empire Cancer Campaign for Research is gratefully acknowledged and one of us (J.N.M.) also received help from Professor Mitchell's Research Fund.

## APPENDIX

The data were submitted to the Medical School Section, Department of Human Ecology· Mr. R. G. Carpenter and Mrs. C. R. G. Saunders reported as follows:—

The distribution of the mobilities observed in treated and untreated groups is shown in Fig. 1. Fig. 1 suggests that there are at least two populations of cells in the treated group and that the distribution of mobilities in both populations is approximately normal but with different standard deviations.

Rao (1952) describes a method for estimating the parameters of data comprising a mixture of 2 normal distributions with equal standard deviations.

In the present case the raw data corresponding to the times in seconds (t) taken by each cell to move a fixed distance were analysed. It was found that if

$$x = \log (t - c)$$
$$= \log \left(\frac{1}{m} c\right)^{-}$$

Then, when the observations x were grouped into 25 equal intervals and c was chosen to be 0·64 sec., that the data was accurately represented by a mixture of 2 normal distributions with equal standard deviations, Fig. 7. The constant c was chosen as follows. Rao's method was programmed for a digital computer (Titan). The fitting was repeated for a range of values of c. The value of c chosen gave a minimum value to the $\chi^2$ measuring the goodness of fit of the theoretical distribution to the data. The minimum goodness of fit $\chi^2$ (20 degrees of freedom) = 19·33, $P = 0{\cdot}05$. The proportion of cells in the two populations and the other parameters of the distributions are shown in Fig. 7.

If the mid-point between the means, $\chi = 1{\cdot}618$, m = 173, t = 5·68 sec., is chosen as the dividing line between " fast " and " slow " moving cells, the probability of cells of either population being misclassified will be equal. (Note the number of either type misclassified will depend on the ratio of " fast " and " slow " moving cells in the mixture.)

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