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# Blastogenic Responses of Lymphocytes from Patients with Untreated and Treated Lymphomas [1, 2]

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## *Introduction*

Small lymphocytes play a central role in mammalian immune responses (1). These cells proliferate in lymphoid tissues, a proportion are long lived (2) and recirculate from lymphoid tissues through lymphatic channels to peripheral blood, and back through the lymphoid tissues (3). These cells participate in delayed hypersensitivity, homograft and graft versus host responses and may be the effector cells of these reactions (1). They are the immunological memory cells and both specific immunological reactions and tolerance can be transferred with them from one animal to another (1).

An effective method for study of human peripheral blood lymphocytes is to culture them with mitogenic agents such as the red kidney bean extract phytohemagglutinin (PHA) (4). This substance induces the majority of peripheral blood lymphocytes from normal subjects to enlarge into easily recognizable lymphoblastoid cells. This response has been used as a measure of the functional normality of lymphocytes although it does not necessarily reflect their ability to respond immunologically (5).

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[2] Presented in part at the Annual Meeting of the American Association of Cancer Research, June, 1966 in Denver, Colorado and the Second International Congress of Lymphology, March 20, 1968 in Miami Beach, Florida.

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Patients with Hodgkin's disease have an immunological deficiency characterized by development of impaired delayed hypersensitivity (possibly early in the course of their disease) and a variably associated inability to mount a primary antibody response (6). This immunological deficiency has recently been correlated with a failure of untreated patients' lymphocytes to respond normally to PHA and other stimulation (7, 8, 9). The current study was designed to further evaluate *in vitro* lymphocyte responses in Hodgkin's disease and other lymphomas. The cells of treated patients were compared with those of untreated patients and normal controls. It was hypothesized that differences in responsiveness between treated and untreated patients' lymphocytes might provide a clue to understanding the nature of the immunological deficiency in lymphoma.

*Material and methods*

Forty-four patients were studied. Nine had untreated Hodgkin's disease, two had untreated reticulum cell sarcoma (RCSA), 26 had treated Hodgkin's disease and seven treated RCSA. Evaluation for staging the extent of disease include lymphangiography in every patient. The staging criteria were: stage 0, no evident disease activity; stages I–IV were those of the committee on the staging of Hodgkin's disease (10). The subclassifications A or B to indicate the absence or presence of systemic symptoms were also used.

The only treatment was radiotherapy. The radiation dose varied from 1500 to 4000 r to involved and adjacent node bearing areas, the dose being determined by a protocol for the study of intensive radiotherapy of lymphoma (11). Average time between the end of radiotherapy and *in vitro* testing was 9.5 months with a range of 1–36 months. There were 20 normal control subjects.

Lymphocyte cultures were set up, processed and evaluated in the following manner. Twenty-five ml of venous blood were mixed with 300 units of heparin and 2.5 ml 3% dextran (m.w. 240.000). The red blood cells were gravity sedimented for one hour at room temperature in an inclined, screw cap, 20 x 150 mm tube. The resultant white cell rich plasma was centrifuged, the cells and plasma separated and the white cells washed twice in 10 ml. Hank's balanced salt solution with 10% fetal bovine serum. The cells were finally resuspended in Eagles basal medium and cultures were set up with $2 \times 10^6$ lymphocytes, 1 ml of plasma and 2 ml media in 13 x 170 mm screw cap tubes.

Simultaneous cultures of patients' and normal controls' cells were set up, each in both patients' and controls' plasma, each with and without stimulation with PHA-M (0.1 ml per culture). The cultures were incubated for five days at 37 °C in an atmosphere of 5% $CO_2$ in air. The cells were harvested by centrifugation, fixed in 10% glacial acetic acid in methanol, mounted on slides and stained with Giemsa stain.

Five hundred cells were counted on each slide. Cell types identified included small lymphocytes, large lymphoblastoid cells, dead lymphoid cells, macrophages and cell in mitosis. In addition to slide counts, hemocytometer chamber counts were done on unstimulated cultures at the end of the culture period. This approach gave an accurate estimate of lymphocyte survival and allowed comparison with estimates made by slide

counts. The ratio of large lymphoblastoid cells (blasts) to small lymphocytes in the PHA cultures was calculated. The result gave an estimate of the proportion of cells which did not respond to PHA.

*Results*

The overall results of the lymphocyte cultures in the patient and control groups are shown in table 1. It can be seen that both lymphocyte survival and blastogenesis were poor in the patients' cultures compared to the controls. The blastogenesis was poor in terms of the percentage of blasts, percentage of mitoses and the increased proportion of non-responding lymphocytes remaining in the patients' PHA stimulated cultures. These results were similar to those obtained in previous studies. In these over-all results which included studies of treated and untreated patients, lymphocyte survival was in the normal range in 52% of the patients' cultures, blastogenesis was in the normal range in 53%, mitosis in 62% but the blast/lymphocyte ratio was the most sensitive indicator of impaired lymphocyte function in these patients.

Table 1 Lymphocyte Cultures in Lymphoma Compared to Normal Controls.

| | Unstimulated Cultures | | PHA Stimulated Cultures | | | | | |
|---|---|---|---|---|---|---|---|---|
| | % Surviving Lymphocytes | | % Lymphoblastoid Cells | | % Mitoses | | Blast/Lymphocyte Ratio | |
| | P | N | P | N | P | N | P | N |
| Median | 37.5 | 75.0 | 56.5 | 75.5 | 0.20 | 0.80 | 5.4 | 23.5 |
| Mean | 30.7 | 71.8 | 51.2 | 74.2 | 0.38 | 0.84 | 6.7 | 28.0 |
| Range | 1.5–76.0 | 23.0–90.0 | 1–89 | 53–91 | 0–1.6 | 0.2–1.6 | 2–35.5 | 12.5–90 |
| P | 0.01 | | 0.01 | | 0.05 | | 0.01 | |

PHA = phytohemagglutinin P = patients N = normal controls

A careful search was made for factors which might be present in the patients' plasma to account for their abnormal lymphocyte function. The subject's cells were washed two times in Hank's solution with 10% fetal bovine serum and then resuspended in both allogeneic normal and autochthonous patients' plasma. This was done simultaneously for both the patients and their matching normal control. The overall results of these comparative studies are shown in table 2. As can be seen from the data there were no significant differences between cells cultured in autochthonous or allogeneic plasma. This finding was true for both patients' cells and controls' cells.

These results suggested that plasma factors were not involved in the deficient lymphocyte function in Hodgkin's disease. However, although there were many minor variations in the matched patient-control studies, in five instances patients' plasma was clearly toxic. Both patients' and controls' cells survived better and had better blastogenic

Table 2 Effect of Autochthonous and Allogenic Plasma on *In Vitro* Lymphocyte Function.

| | % Surviving Lymphocytes | | | | % Lymphoblastoid Cells | | | |
|---|---|---|---|---|---|---|---|---|
| | Patient | | Normal | | Patient | | Normal | |
| Source of Serum | I | A | I | A | I | A | I | A |
| Median | 37.5 | 35.5 | 75.0 | 76.5 | 56.5 | 56.5 | 75.5 | 71.5 |
| Mean | 30.7 | 31.2 | 71.8 | 70.0 | 51.2 | 47.4 | 74.2 | 71.6 |
| Range | 1.5–76 | 0–58 | 23–90 | 55–90 | 1–89 | 0–81 | 53–91 | 42–91 |
| | % Mitoses | | | | Blast/Lymphocyte Ratio | | | |
| | Patient | | Normal | | Patient | | Normal | |
| Source of Serum | I | A | I | A | I | A | I | A |
| Median | 0.20 | 0.20 | 0.80 | 0.80 | 5.4 | 4.4 | 23.5 | 26.5 |
| Mean | 0.38 | 0.35 | 0.84 | 0.99 | 6.7 | 6.1 | 28.0 | 28.0 |
| Range | 0–1.6 | 0–2.0 | 0.2–1.6 | 0.4–2.0 | 0.2–35 | 0.3–23 | 12.5–90 | 13.5–65 |

I = autochthonous A = allogeneic.

responses in normal plasma. The patients' plasma was clearly toxic to normal cells. The difference in cellular responses between the normal and patients' plasma were at least 10%. Three of these patients had untreated stage III or IV Hodgkin's disease. The other two who had Hodgkin's disease and reticulum cell sarcoma, were treated and had no clinical evidence of active disease. A lymphotoxic factor may be a hallmark of untreated Hodgkin's disease since 33% (3/9) untreated patients exhibited it while it was found in only 3.9% (1/26) of the other patients. The clinical data on these five patients is given in table 3.

The results of the studies in the treated and untreated patients were compared and are given in table 4. These data were compared without regard to the stage of the patients' disease. This decision was necessary since most patients (28 out of 33) changed stage after treatment, and if stage were considered, the effects of treatment would be obscured. The relationship with stage will be considered later.

The lymphocyte survival and blastogenic responses of both the treated and untreated patients were consistently lower than normal. Also survival and blastogenesis of untreated patients' cells were consistently lower than those of treated patients cells. Mean values for untreated and treated patients respectively were: 23% and 34% lymphocyte survival, 42% and 56% lymphoblastoid cells, 0.29% and 0.43% mitoses and blast lymphocyte ratios of five and 7.8 respectively. These differences were at the borderline of significance. These observations were interpreted as suggesting that the lymphocyte responses of patients with lymphoma may improve after therapy and when states of remission are achieved.

This point is further illustrated by a comparison of the lymphocyte responses of patients with different stages of disease, especially patients who had been treated and

Table 3 Clinical Data on Patients with Lymphotoxic Plasma Factor (s).

| No. | Diagnosis | Age | Duration of Disease[3] | Stage at Onset | Stage at Testing | Therapy | Interval Between Therapy and Test | Results of DNCB Testing |
|---|---|---|---|---|---|---|---|---|
| 1. | H. D.[1] | 50 | 2 months | III B | III B | None | | Not Done |
| 2. | H. D. | 25 | 2 weeks | III B | III B | None | | Negative |
| 3. | H. D. | 15 | 7 weeks | IV B | IV B | None | | Negative |
| 4. | H. D. | 24 | 6 months | II A | 0 A | X-ray[4] | 3 months | Negative |
| 5. | RCSA[2] | 35 | 7 months | III B | 0 A | X-ray[5] | 5 months | Negative |

[1] Hodgkin's Disease
[2] Reticulum Cell Sarcoma
[3] Time from Tissue Diagnosis to Test
[4] 3500–4000 Rads to Involved & Adjacent Node Bearing Areas
[5] 1500 Rads to Involved Areas Only

achieved stage 0. Two approaches to the evaluation of stage were used. In the first, an attempt was made to correlate lymphocyte function with the stage at the time of diagnosis, while in the second lymphocyte function was correlated with the stage at the time of testing. The results are shown in tables 5, 6, and 7. Only Hodgkin's disease data was evaluated in this way. Table 5 shows that patients with no evident disease or localized disease had better lymphocyte function than patients with widespread disease. This effect of the stage of the disease was seen in both treated and untreated patients. Of course no patient had stage 0 disease until after they were treated. Table 6 illustrates that patients with stage 0 disease who originally had stage III disease (before treatment) also showed significantly better lymphocyte function than did patients with active disease. The corollary of this observation was also true; that a correlation between function and stage could only be made for current stage and not for original stage. In other words lymphocyte function was approximately the same in patients with stage 0 disease, whether they had been stage I, II or III originally.

There was no correlation between the interval from treatment to testing and the degree of *in vitro* responsiveness. Likewise there was no correlation between the extent or intensity of treatment and the subsequent degree of *in vitro* survival, blastogenesis or mitosis. Table 7 shows the interrelationship of stage, DNCB skin test results and lymphocyte function. Thirty-seven of the studied patients had been stimulated with DNCB and were challenged repeatedly through their subsequent followup. Of the 11 patients DNCB positive at the time of *in vitro* testing nine had stage 0 disease and two were stage II. It is important to note that all patients were DNCB negative at the time of initial diagnosis and the onset of therapy. Of greatest interest in table 7 is that the patients who were treated, had no evidence of active disease and were DNCB positive had almost completely normal *in vitro* lymphocyte function!

Table 8 shows the results of the cultures of lymphocytes from the nine patients with reticulum cell sarcoma. These patients had significantly diminished lymphocyte

Table 4 Lymphocyte Cultures in Treated and Untreated Patients with Lymphomas.

| | % Surviving Lymphocytes | | | % Lymphoblastoid Cells | | | % Mitoses | | | Blast/Lymphocyte Ratio | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | U | T | N | U | T | N | U | T | N | U | T | N |
| Median | 8.0 | 39 | 75.0 | 40.5 | 57.5 | 75.5 | 0.20 | 0.30 | 0.80 | 2.5 | 6.0 | 23.5 |
| Mean | 22.8 | 34 | 71.8 | 41.7 | 55.9 | 74.2 | 0.29 | 0.43 | 0.84 | 5.0 | 7.8 | 28.0 |
| Range | 1.5–62 | 2.5–58 | 23.0–90 | 2.5–86 | 2–89 | 53–91 | 0–1.0 | 0–1.6 | 0.2–1.6 | 0.2–21 | 0.6–36 | 12.5–90 |
| Significance | $0.05 < P < 0.10$ | | | $0.1 < P < 0.2$ | | | $0.05 < P < 0.10$ | | | $0.05 < P < 0.10$ | | |

U = untreated T = treated N = normal control

Table 5 Lymphocyte Function in Patients With Limited and Widespread Hodgkin's Disease.

| Stage at Time of Test | % Surviving Lymphocytes | | % Lymphoblastoid Cells | | % Mitoses | | Blast/Lymphocyte Ratio | |
|---|---|---|---|---|---|---|---|---|
| | 0–II A | III B–IV | 0–II A | III B–IV | 0–II A | III B–IV | 0–II A | III B–IV |
| Median | 41.5 | 3.0 | 57.0 | 27.5 | 0.3 | 0.00 | 6.0 | 1.6 |
| Mean | 37.5 | 14.3 | 57.3 | 29.4 | 0.4 | 0.12 | 8.9 | 1.9 |
| Range | 6–67 | 1–41 | 5–89 | 2–70 | 0–1.6 | 0–1.0 | 1–36 | 2–6.5 |
| Significance | 0.01 | | 0.01 | | 0.05 | | $0.02 < P < 0.05$ | |

Table 6 Lymphocyte Function in Patients with No Evidence of Disease and With Widespread Hodgkin's Disease.

| | % Surviving Lymphocytes | | % Lymphoblastoid Cells | | % Mitoses | | Blast/Lymphocyte Ratio | |
|---|---|---|---|---|---|---|---|---|
| Stage | 0 | III B–IV | 0 | III B–IV | 0 | III B–IV | 0 | III B–IV |
| Median | 40.0 | 3.0 | 64.0 | 27.5 | 0.60 | 0.00 | 8.4 | 1.6 |
| Mean | 44.4 | 14.3 | 63.4 | 29.4 | 0.51 | 0.12 | 9.1 | 1.9 |
| Range | 6.8–62 | 1–41 | 22–85 | 2–70 | 0–1.0 | 0–1.0 | 2.6–21.4 | 0.2–6.5 |
| Significance | 0.01 | | 0.01 | | 0.02 | | 0.01 | |

0 = Stage when tested; had stage III disease at diagnosis
III B–IV = stage when tested

Table 7 Relationship Between Stage, DNCB Skin Sensitivity and *In Vitro* Lymphocyte Function.

| Stage | | III B–IV | 0 | 0 | Control |
|---|---|---|---|---|---|
| DNCB reaction | | negative | negative | positive | |
| parameter | result | | | | |
| | mean | 14.30 | 33.80 | 47.00 | 71.80 |
| percent | median | 3.00 | 37.50 | 49.00 | 75.00 |
| lymphocytes | range | 1.0–41.5 | 6.8–49.0 | 21.0–76.0 | 23.0–90.0 |
| | mean | 29.40 | 55.60 | 67.30 | 74.20 |
| percent | median | 27.50 | 55.00 | 67.00 | 75.50 |
| lymphoblasts | range | 2.0–70.0 | 11.0–78.5 | 49.3–76.0 | 53.5–91.4 |
| | mean | 0.12 | 0.23 | 0.42 | 0.84 |
| percent | median | 0.00 | 0.20 | 0.40 | 0.80 |
| mitoses | range | 0.0–1.0 | 0–0.6 | 0–0.8 | 0.2–1.6 |
| | mean | 1.90 | 3.31 | 11.40 | 28.00 |
| blast/lymph | median | 1.60 | 1.42 | 11.30 | 23.50 |
| ratio | range | 0.20–6.50 | 0.31–12.8 | 1.6–23.1 | 12.5–90.0 |

responses in all parameters tested. One patient had a lymphtoxic plasma factor. Approximately 3/4 of the nine patients had abnormalities in at least one of the lymphocyte function parameters measured.

To further evaluate lymphcyte survival, unstimulated cultures of 21 patients and 10 controls were evaluated by hemocytometer chamber as well as slide counts at the end of the culture period. This method gave a precise quantitative estimate of the degree of lymphocyte survival. Only intact viable cells were counted. The results of the chamber and slide counts correlated well with a correlation coefficient of 0.87 (Fig. 1). The median percent surviving lymphocytes were 48% in the patients' cultures and 80% in the controls cultures. Fifty-six percent of the patients' cultures were completely outside the normal range (Fig. 2).

Table 8 Culture of Lymphocytes from Patients with Reticulum Cell Sarcoma.

| | Unstimulated Control | | PHA Stimulated Cultures | | | | | |
|---|---|---|---|---|---|---|---|---|
| | % Surviving Cells | | % Blasts | | % Mitoses | | Blast/Lymph Ratio | |
| | P | N | P | N | P | N | P | N |
| Median | 36.5 | 75.0 | 56.5 | 75.5 | 0.2 | 0.80 | 4.0 | 23.5 |
| Mean | 35.3 | 71.8 | 56.1 | 74.2 | 0.3 | 0.84 | 6.3 | 28.0 |
| Range | 5.0–76.0 | 23.0–90 | 22.5–85.5 | 53–91 | 0–0.8 | 0.2–1.6 | 0.5–21.5 | 12.5–90.0 |
| p | 0.02 | | 0.02 | | 0.05 | | 0.01 | |

*Discussion*

Immunological deficiency in Hodgkin's disease is a phenomenon that has fascinated the medical community for over 20 years and which has excited many investigations and speculations. These need not be reviewed in detail here since they have been the subject of several recent reviews (6, 12, 13). Although etiology and pathogenesis of immunological deficiency are not known there are certain immunological phenomena that are well documented. These include normal secondary antibody responses (14), normal (15) or depressed (16) primary antibody responses, and anergy to delayed hypersensitivity testing (17). Recently, attention has focused on lymphocyte function in Hodgkin's disease. Lymphocyte depletion is characteristic of advanced disease (18). One cannot transfer delayed hypersensitivity with normal lymphocytes to anergic patients with Hodgkin's disease; although this can be done to anergic patients with sarcoidosis (6). Lymphocytes from patients with Hodgkin's disease are deficient in the lymphocyte transfer test (19). Finally the blastogenic responses of lymphocytes from patients with this disorder are deficient in their *in vitro* responses to PHA, allogeneic leukocytes and antigens (7, 8, 9).

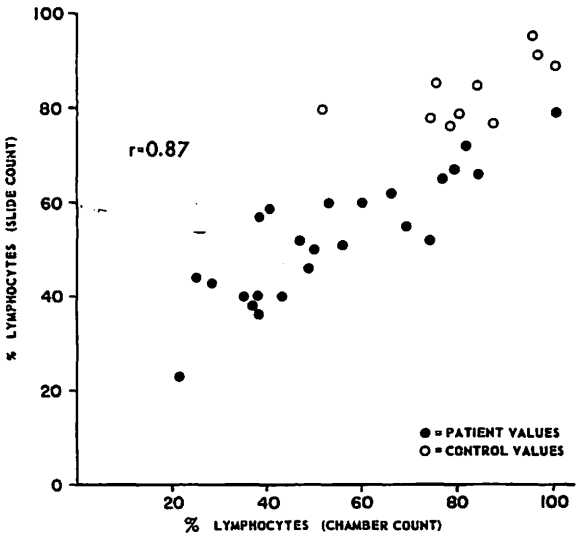


Fig. 1

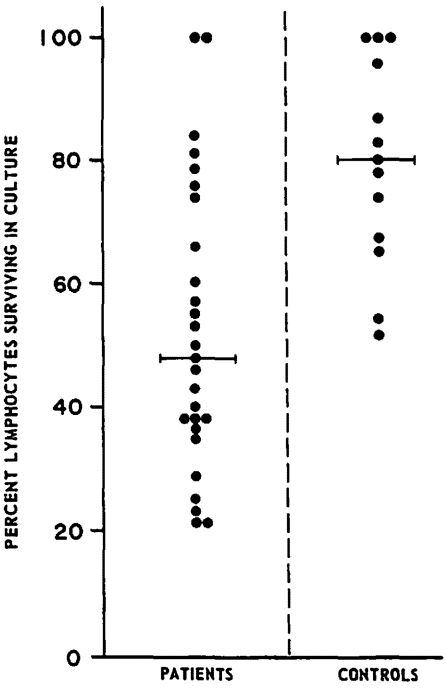


Fig. 2

The data presented in the current study are important in two regards. First they indicate that the immunological deficiency, at least measured by *in vitro* lymphocyte function reverts completely to normal during states of remission and at times when *in vivo* delayed hypersensitivity is normal. This observation suggests that failure of delayed hypersensitivity and blastogenesis is not primary but rather is related to other factors such as depletion of a responsive population or lymphotoxic factors. The latter is suggested by the poor *in vitro* lymphocyte survival although a lymphotoxic serum factor is not found in the majority of the patients.

The second area of interest in the current study is that in a related but distinct disorder (reticulum cell sarcoma) a lymphocyte functional abnormality similar to Hodgkin's disease has now been observed. Thus, not only are these patients as anergic as the patient with Hodgkin's disease (20) but they have decreased lymphocyte survival and diminished blastogenesis and mitosis *in vitro*. This finding suggests that diminished lymphocyte function is characteristic of many if not all of the lymphoproliferative disorders.

Several recent studies related to lymphocyte function and Hodgkin's disease are worthy of further comment. *Crowther* et al. (21) studied cell morphology and $H^3$-thymidine incorporation of lymphocytes freshly harvested from patients with Hodgkin's disease. They found an increased percentage of large lymphocytes which had increased thymidine incorporation compared to normal controls. A similar phenomenon was noted in patients with autoimmune diseases, post infection states and in subjects who had recently undergone immunization. This finding suggested that the patients with Hodgkin's disease have an ongoing active immune response to something, perhaps a tumor specific immune response. This does not explain the diminished response of the patients' lymphocytes *in vitro* since only a few percent of the patients' cells are affected in this way.

Of great interest are the observations that *in vivo* and *in vitro* alterations of the lymphocytes' environment can profoundly influence their function. For example, tuberculin skin reactivity is depressed during measles (22) and lymphocytes from such subjects manifest diminished blastogenic responses to tuberculin *in vitro* (23). The direct addition of measles virus to lymphocyte cultures has similar effects (24). *In vitro* lymphocyte blastogenesis has also been found deficient in association with acute viral hepatitis and the effect has been related to a plasma factor active at 1 : 1000 dilution (25). Finally the addition of non-viable mycoplasma can reversibly inhibit mitosis in human peripheral blood leukocyte cultures (26). These studies indicate that lymphocyte function can be markedly depressed during certain infections and when certain infectious agents are added to leukocyte cultures *in vitro*. One wonders whether these same infections might also result in the increased number of thymidine incorporating large lymphocytes observed by *Crowther* et al. (21). This may in turn bear on the etiology of Hodgkin's disease.

*Brown* et al. (27) have recently reported a careful simultaneous study of a battery of immunological tests done in patients with untreated, newly diagnosed Hodgkin's disease. Parameters studied included: primary antibody response to tularemia vaccine, delayed skin allergy to natural antigens, the induction of delayed skin allergy with DNCB, peripheral blood/lymphocyte counts, and peripheral blood lymphocyte cultures.

They found that 84% of the patients responded either to at least one natural skin test antigen or could be sensitized to DNCB. This represented more reactivity than had been observed in previous studies of delayed hypersensitivity in Hodgkin's disease. The authors suggested that the poor responsiveness in other studies related to the longer duration of active disease or to prior therapy. We would favor the former explanation since recovery has been noted in several studies after therapy (6). Deficiency in the parameters of *in vivo* delayed hypersensitivity *in vitro* lyphocyte blastogenesis and circulating lymphocyte levels correlated with the stage of disease (parameters most deficient in stage IV disease). There was, of course, also a strong correlation between lymphopenia and the other parameters. The authors felt that lymphopenia, related to the extensive lymph node replacement of advanced Hodgkin's disease might be enough to explain the immunological deficiency.

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*Summary*

*In vitro* lymphocyte function was studied in 44 patients with treated or untreated malignant lymphoma. The categories were: untreated Hodgkin's disease (H. D.), 9; untreated reticulum cell sarcoma (RCSA), 2; treated H. D., 26; treated (RCSA), 7. The % surviving lymphocytes, % blastoid cells and % mitosis in response to phytohemagglutinin were 37.5, 56.5 and 0.2 for the patients and 75.0, 75.5 and 0.8 for the controls. A lymphocytotoxic plasma factor was found in the serum of five of the patients and none of the 20 controls. For all parameters studied in H. D., lymphocyte function was most deficient in the untreated patients and in the patients with widespread disease. The responses of the treated H. D. patients were better and the responses of the treated patients who had achieved stage 0 and who had regained delayed skin allergy were almost normal. Lymphocyte function was also abnormal in RCSA. For the above mentioned parameters the values in RCSA were 35.0, 55.0 and 0.3 compared to their controls of 72.0, 74.0 and 0.8. The data suggest: A) that there may be a return to normal lymphocyte function after radiotherapy induced remission of H. D. and B) that a defect of lymphocyte function similar to that of H. D. may be present in RCSA.

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# Problems of Radiation Dosimetry in Endolymphatic Therapy with Radioactive Isotopes

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To date we have applied endolymphatic therapy using $^{131}I$ labelled oily contrast medium to 35 patients and using $^{32}P$- $^{131}I$ labelled Lipiodol to 8 patients suffering from Hodgkin's disease and metastases involving the retroperitoneal lymphnodes (11). In 30 of these patients we investigated the course of the radioactive material in order to calculate the radiation doses absorbed by the lymphnodes and other tissues. Some of these results have been recently published (4, 5).

For daily external measurements of the $^{131}I$-distribution following the administration of $^{131}I$ to the lymph channels of the dorsum of both feet the following method was applied:

The patient lies on a couch over which a 2 inch sodium-iodide scintillation – counter is moved horizontally at a distance of about 35 cm. The counter is shielded by a slit collimator so that it responds to the $^{131}I$ in the whole width of the body, but lengthwise to only the short section opposite the counter. A strip chart recorder draws a „profile" curve, indicating the $^{131}I$ distribution along the length of the body.

The topographic positions corresponding with the peaks on the profile curves were identified by rectilinear area scanning methods (Picker Magna Scanner III with photorecording system) as well as by radiological studies of the distribution of the contrast medium.