AN INCREASED SUSCEPTIBILITY to infections in leukemia and in primary malignant disorders of the reticuloendothelial system, especially in some forms, is generally admitted. The nature of the defects in host resistance to bacterial infections in these diseases has not been clearly defined. One possible cause which has been considered is a decrease in the ability to form circulating antibodies. In patients with malignant blood disorders, this power has usually been investigated by determining the circulating antibodies arising as a response to an artificially induced antigenic stimulus. Acute leukemia, chronic lymphatic leukemia and Hodgkin’s disease in particular were investigated in this way. The results, however, show certain discrepancies. Various authors used different antigens, and sufficient attention was not always paid to the stage of the neoplastic process, or to complications and other factors different for each patient—e.g., the manner of treatment.

The study of the ability to form circulating antibodies in leukemia is important from still another aspect. In recent years, immunologic patterns of defense of the animal organism have already been demonstrated in some experimental leukemias. It cannot be ruled out, though evidence is still lacking, that a similar mechanism may also be operative in cases of human hemoblastosis. Certain spontaneous remissions might well be interpreted as the result of successful immunologic defense. In defense of this nature, circulating antibodies may also play a role. If the chemo- and immunotherapy of leukemia become a practical possibility at some time in the future, it will certainly be important to know whether organisms affected by hemoblastosis (which frequently invades the RES and lymphoid system—known to be closely related to antibody formation), are still capable of immunologic response. With experimental tumors the question has already been raised in contemporary literature of whether the increased susceptibility of certain mouse strains to tumors of viral origin is not due to an inborn-deficient capacity to form circulating antibodies.

We have studied several factors of immunologic reactivity in patients suffering from malignant blood disorders, including the level of properdin, complement, isohemagglutinins, bactericidal activity of the serum against gram positive microorganisms, and the cellular composition of inflammatory exudates. In this study we were interested in the power to form circulating antibodies to the Brucella endotoxin and whether there was any difference in
this respect between various diagnostic groups of malignant blood disorders and a control group of healthy, normal subjects. We further studied the effect of the activity of the leukemic process, therapy and white cell count variations on antibody formation and differences between patients who were not susceptible to frequent infections.

**MATERIAL AND METHODS**

The formation of circulating antibodies to the Brucella endotoxin was investigated in 113 adults suffering from various kinds of malignant blood disorders. These comprised 25 patients with acute leukemia, 16 with chronic myelosis, 17 with chronic lymphatic leukemia, 23 with Hodgkin's disease, 14 with reticulosarcoma and lymphosarcoma, 10 with myeloma and 8 patients with myelofibrosis. Most of the patients investigated were in the active stage of the hemoblastic process.

**Brucella endotoxin.** Endotoxin of the *Brucella suis* strain was prepared by trichloracetic acid extraction; it represents an antigen of the Boivin type whose preparation was described in detail in "Experimental Immunochemistry" by Kabat and Mayer, 1961, p. 830. Its toxicity was determined, and the patients received doses well below the level of toxicity.* The testing of antibodies was performed in the usual way by the agglutination reaction with a suspension of the corresponding *Brucella suis* strain, heated for 1 hour at 70 °C. The mixture of serum and Brucella suspension was stored in a refrigerator; the final readings were made at the end of 7 days.

The Brucella endotoxin was given subcutaneously in three consecutive injections at 7-day intervals; each dose contained 0.048 mg. endotoxin dissolved in 0.3 ml. saline. Blood samples were taken for antibody determinations and examined shortly before the first injection of the vaccine (initial value) and then 2 weeks (marked as sample I), 4 weeks (sample II), and 8 weeks after the injection. The last values, recorded 8 weeks after the beginning of vaccination, were not included in the statistical analysis since the antibody titers at that time did not usually exceed the values recorded at previous examinations.

Several patients in an advanced stage of the disease died during the investigation so that the second sample could not be collected. On other, though exceptional, occasions, the patient escaped the first sampling, and only the second sample could be taken. In some patients vaccination and the examination of the Brucella circulating antibodies were repeated during a subsequent admission of the patient to the department.

The initial titers of circulating antibodies against Brucella endotoxin, examined prior to vaccination were not, as a rule, equal to zero; in most patients and in all healthy subjects low titers were already present. We were thus actually testing an anamnestic response and not a pure primary immune response. For this reason we did not evaluate the antibody titers themselves, but differences between the initial values and those obtained 2 and 4 weeks later. The differences were not expressed in terms of dilution but in degrees of titration. Since the titrations were carried out in geometric series with a coefficient of two, the dilution rate 1:4 could be considered to be equal to 1, 1:8 to 2, etc. These degrees of titration actually represent logs of dilution, to the base 2. All other calculations and conclusions are based on degrees of titration thus defined (DT). As it has been demonstrated that antibody formation in adult humans bears no relation to age,1,16 this factor was not taken into consideration in our analyses.

**RESULTS**

Statistical evaluation was made of the results in healthy subjects (control group) and in the 7 above-mentioned diagnostic groups of malignant blood disorders (table 1).

*The Brucella endotoxin was kindly supplied by J. Sterzl, M.D.
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Table 1.—Antibody Titers to Brucella Andotoxin (Expressed as Differences in Titration Degrees)

<table>
<thead>
<tr>
<th></th>
<th>Acute Leukemia</th>
<th>Chronic Lymphatic Leukemia</th>
<th>Chronic Myelosis</th>
<th>M. Hodgkin</th>
<th>Reticulosarcoma</th>
<th>Lymphosarcoma</th>
<th>Myeloma</th>
<th>Myelofibrosis</th>
<th>Controls</th>
</tr>
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<tr>
<td><strong>Results at 14 days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>25</td>
<td>17</td>
<td>15</td>
<td>23</td>
<td>14</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>2.97</td>
<td>0.059</td>
<td>3.333</td>
<td>0.913</td>
<td>0.643</td>
<td>0.778</td>
<td>2.875</td>
<td>1.882</td>
<td></td>
</tr>
<tr>
<td>s</td>
<td>2.70</td>
<td>1.37</td>
<td>3.11</td>
<td>2.04</td>
<td>1.76</td>
<td>1.53</td>
<td>3.25</td>
<td>1.78</td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>P &lt; 0.01</td>
<td>-</td>
<td>P &lt; 0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>P &lt; 0.01</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Comparison with controls</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

| **Results at 28 days** |                |                           |                 |            |                |               |         |              |          |
| n                    | 23             | 16                        | 16              | 22         | 14             | 10            | 8       | 17           |          |
| M                    | 1.739          | 1.000                     | 3.062           | 1.591      | 1.071          | 2.200         | 2.500   | 2.118         |          |
| s                    | 2.35           | 2.45                      | 3.13            | 1.90       | 1.67           | 2.14          | 2.96    | 1.67         |          |
| Change                | P < 0.01       | P < 0.01                   | P < 0.05        | P < 0.05   | P < 0.05       | P < 0.05      | P < 0.05| -            |          |
| Comparison with controls | P < 0.01 | P < 0.01                   | P < 0.01        | P < 0.05   | P < 0.05       | P < 0.05      | P < 0.05| -            |          |

- n = number of investigations; M = mean rise in titration degrees (expressed in titration degrees); s = the standard deviation of the means; Change = statistical significance of the rise in antibody titers during immunization in test groups and control group. The P values computed for M and s give the statistical significance of the M and s values as compared with the respective values of the control group. Wherever P is omitted, the difference from normal controls was not statistically significant.

It was first necessary to find out whether there was any statistically significant increase in the antibody titers in the test groups and in the control group during immunization. The mean differences in titration degrees (DT) were analyzed by means of the t-test for the mean difference. For changes taking place over the period of 14 days, a rise of 2.0 DT in acute leukemia and 3.333 in chronic myelosis, and also 1.88 for the control group was shown to be statistically highly significant (P < 0.01); a rise of 0.913 in Hodgkin's disease is also significant, but at the 5 per cent level only. In the remaining 4 diagnostic groups, i.e., in chronic lymphatic leukemia, reticulosarcoma and lymphosarcoma, in myeloma and myelofibrosis, there was also a rise in the titration degree, this increase, however, was not statistically significant.

The changes in DT at the end of 28 days, again showed a statistically significant rise (P < 0.01) in acute leukemia, chronic myelosis and Hodgkin's disease as well as in the control group. In reticulosarcoma and lymphosarcoma, and in myeloma the rise was significant at the 5 per cent level only. In chronic lymphatic leukemia and myelofibrosis the increase was not statistically significant. In myelofibrosis the mean increase in DT was notably high (higher rise was observed only in chronic myelosis), the lack of significance is, however, due to the relatively small number of patients.

The main object in our study was to compare the changes (differences in DT) in each of the above test groups with those found in healthy controls. The standard deviation of the differences of DT (s) (with the aid of the F-test) and the magnitude of the mean changes (M) (using the t-test) were compared. For the results at 14 days, greater standard deviation was found in
acute leukemia, chronic myelosis and myelofibrosis than in the controls (in all instances the difference was significant at the 5 per cent level only). At 28 days, the standard deviation of the differences in DT was significantly higher in chronic myelosis (P < 0.01) and in myelofibrosis (P < 0.05) than in controls.

The mean rise in DT (M) did not differ significantly in six test groups from the mean rise seen in controls at 14 and 28 days. In chronic lymphatic leukemia only, the average value recorded at 14 days (the mean rise of 0.06 DT only) differed significantly from the mean rise in a control group. This difference is highly significant (P < 0.01). At 28 days, however, even in chronic lymphatic leukemia a significant difference could no longer be seen in the rise in the degree of titration as compared with the control group of healthy subjects.

In acute leukemia the capacity for antibody formation to the Brucella endotoxin was usually well preserved. In some patients in an advanced stage or in the preterminal stage of the disease it exhibited a tendency to decrease; this does not hold true in general, however. Therapy with 6-mercaptopurine, buthiopurine (derivative of 6 MP) and prednisone, in the usual clinical doses, does not depress the capacity for antibody formation; nor was it inhibited by neutropenia.

In patients with chronic myeloid leukemia irrespective of the degree of activity of the leukemic process, the capacity for antibody formation against Brucella endotoxin was well preserved. This was the case even in patients who received x-ray treatment or myleran, as well as in those with infectious complications.

In chronic lymphadenosis, especially in the advanced stages of the disease, antibody formation to Brucella endotoxin was clearly deficient.

In Hodgkin's disease antibody formation to the Brucella endotoxin tended to be decreased especially in patients in a very advanced stage of the disease; the difference in the mean degree of titration of the whole group as compared with the control group of healthy subjects, however, was not statistically significant.

In reticulosarcoma and lymphosarcoma impaired antibody formation was found especially in cases with a very advanced stage of the disease. This impairment, however, judged by the mean value of the entire group, was not statistically significant as compared with the control group.

In cases of myeloma there was a tendency for deficient antibody formation tested 14 days after vaccination. If, however, the values obtained at 28 days were taken into consideration, it could be seen that in some patients no impairment of antibody formation to Brucella endotoxin could be demonstrated even in a very advanced stage of the disease.

Most patients with myelofibrosis showed a well preserved capacity for antibody formation.

Since in some malignant blood disorders, especially in acute leukemia and in Hodgkin's disease, neutropenia or pancytopenia not infrequently occur especially in the advanced stages of the disease, we were interested to see whether these alone, without hemoblastoses, were capable of influencing anti-
body formation. We therefore tested 2 patients suffering from chronic idiopathic leukopenia and 2 patients with chronic idiopathic agranulocytosis for Brucella endotoxin antibodies. In the 2 patients with chronic leukopenia the ability to form antibodies Brucella endotoxin was well preserved; in 2 patients with chronic agranulocytosis this ability was very high (titer 1:16,384, DT = 10, resp. 1:2048, DT = 7). These were the highest values that we ever measured in the whole group of our patients, healthy controls included.

It looks as if in these cases an increased capacity for forming antibodies compensated the long-term deficiency in neutrophil leukocytes. It was interesting to note that the above-mentioned patients with severe neutropenia were, with one exception, not susceptible to infectious complications.

DISCUSSION

The relationship between the mode of treatment and antibody formation was investigated. In none of the test groups was x-ray treatment, chemotherapy (6-mercaptopurine, buthiopurine, Myleran, Leukeran, endoxan, chloralkylamine) or corticoid therapy in clinical doses found to inhibit antibody formation to Brucella endotoxin. In animal experiments, on the contrary, x-ray treatment,8'9'32 many chemotherapeutic agents (e.g. 6-mercaptopurine,27'32 chloralkylamines,24'30'32 and also corticoids7 are known to inhibit antibody formation if administered during or prior to the administration of the antigen. The inhibitory action of these agents, however, can only be demonstrated during the induction phase of antibody formation.27'32

The Brucella endotoxin was given to some of our patients when treatment was started or in the course of treatment, and in spite of this we did not observe that 6-mercaptopurine, buthiopurine, endoxan, Myleran, Leukeran, melphalan, prednisone, or x-irradiation, inhibited antibody formation in response to stimulation by the antigen. We explain this by the fact that in clinical therapy we usually employ much smaller doses than those used in animal experiments.

Data are also encountered in the literature offering evidence that clinical doses of corticoids do not inhibit antibody formation;22'24 similar data are available concerning chloralkylamines.11'28 Other authors likewise confirm in general that treatment neither influenced antibody formation in hemoblastoses25'26 nor increased susceptibility to infectious as claimed by Miller and Karnofsky21 for x-ray treatment and chloralkylamines in chronic lymphadenosis. Data on the effect of corticoids on antibody formation show discrepancies. Most investigators presume that in experiments, large doses of corticoids inhibit antibody formation when given at the beginning of the induction phase; however, opposed views are found, too. According to Winter et al., the administration of corticoids to rats before antigenic sensitization tended to stimulate antibody formation.33 In humans, antibody formation to the polysaccharides of pneumococcus capsules was investigated during ACTH and cortisone therapy and was not found to be suppressed. However, antibody formation in response to typhoid-paratyphoid vaccination was found to be impaired in some of these patients.22

We failed to demonstrate any relation between the white cell count in the
peripheral blood and antibody formation in chronic lymphatic or myeloid leukemia, where this count was usually increased, or in acute leukemia, where it was often reduced. In chronic idiopathic agranulocytosis antibody formation to Brucella endotoxin was surprisingly elevated. Here, the deficiency of neutrophil polymorphonuclears, which play an important role in defense against infection, seems to be compensated by an increase in antibody formation. We have so far observed this in 2 patients; therefore, it would obviously be necessary to confirm it in a larger series. The fact that in these patients we did not see so pronounced a tendency to frequent infectious complications as in acute agranulocytosis, (e.g., drug-induced)—where infectious complications occur practically in every case, with a sudden onset and, as a rule, a severe course—seems to argue in favor of the possibility of certain adaptive processes of an immunologic nature in chronic idiopathic agranulocytoses.

The role, which the RES and the lymphoid tissue play in antibody formation is very well known. It is therefore not surprising that in diseases connected with an alteration in these antibody-forming tissues, as in patients with advanced chronic lymphadenosis, malignant lymphogranuloma, reticulosarcoma and lymphosarcoma, a tendency to a decrease in antibody formation occurs.

Some of our patients in advanced stages of the disease presented various grades of cachexia so that the question arises as to whether undernutrition alone can cause impairment in antibody formation. Balch, who investigated this problem, showed as good an antibody formation in cachectic patients as in healthy subjects and proved that antibody formation continues until death. Similar findings were also reported by Saslaw et al., Silver et al., and by Bieler et al., who also do not consider anemia as a possible cause of decrease in antibody formation. Balch has further shown that in cachectic subjects antibody formation was related neither to total serum proteins nor to albumin or globulin levels. Nor does antibody formation in adult humans depend on age.

Some data in the literature, though based mostly on a few isolated observations, claim a relation between agammaglobulinemia and the inability to form antibodies to various antigenic stimuli as noted in patients with chronic lymphadenosis, and report simultaneous loss of resistance to infectious diseases. We have also made a similar observation in 3 patients. This relationship seems mainly to exist in chronic lymphatic leukemia, for it is known that hypogammaglobulinemia, when occurring in other diseases, need not necessarily be associated with an increased susceptibility to infections.

According to Hamilton Fairley, who investigated the antibody response to tetanus toxoid, patients suffering from chronic lymphadenosis form antibodies inadequately when encountering an antigen for the first time, but they do so more satisfactorily if immunized previously; in other words, they show a better response to reimmunization than to primary immunization. We were also testing an anamnestic response rather than a pure primary immune response.

Whereas, according to our results and those of other investigators, antibody formation is impaired in chronic lymphadenosis, in acute leukemia, as ob-
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served in our patients, it was preserved. It tended to decrease only in a few patients in an advanced stage of the disease. However, in this whole group of patients there was a high incidence of infectious complications (in 19 of 22 patients). Therefore, it follows that a factor other than impaired antibody formation probably accounts for the increased susceptibility to infection. A well preserved capacity to form antibodies was also described in acute leukemias by Silver et al.29 who used five different antigens for the immunization of their patients (the parotitis virus, mixed typhoid vaccines, tetanus and diphtheria toxoids, and influenza vaccines); furthermore, it was described in a few cases by Larson and Romlinson who used the pneumococcus polysaccharides as antigen.15

We have observed well preserved antibody formation to Brucella endotoxin in patients with chronic myeloid leukaemia. In contrast to this, Saslaw et al.25 using tularaemia antigen observed a weaker response.

In our patients with Hodgkin's disease the antibody response to Brucella endotoxin tended to decrease, especially in advanced stages of the disease; however, the difference in mean values between this group of patients and healthy controls was not statistically significant. Schier et al.,28 who used the parotitis virus as antigen, report an adequate antibody response similar to that in controls, but unsatisfactory delayed type of immunologic response. In agreement with this, Kelly et al.13 observed prolonged homograft survival in Hodgkin's disease. Hoffman and Rottino,12 who used mixed typhoid and paratyphoid vaccines, likewise describe preserved capacity for agglutinin formation in 13 patients. Larson and Tomlinson,15 employing pneumococcus polysaccharides as antigen, described a poor antibody response in 3 of 6 patients with Hodgkin's disease. Geller11 likewise investigated the quantitative antibody response in 11 patients with Hodgkin's disease and in 13 patients suffering from lymphosarcoma. Saslaw et al.,25 who used tularaemia vaccines, also noted impaired antibody response in Hodgkin's disease. Dubin (quoted by Schier) studied patients with Hodgkin's disease who contracted brucellosis; only in some of his patients did he find specific antibodies against brucellae. Wise (quoted by Schier) had a similar experience. This clearly shows the discrepancies of the data in the literature. This may be due to the use of various antigens, different methods of investigation, and also to different selection of patients. For example Schier studied only outpatients, obviously with milder disease. Our survey, on the contrary, comprises a number of patients in very advanced stages.

We also recorded impaired antibody response to Brucella endotoxin in patients with reticulosarcoma; the difference in the mean values between this group and the control groups of healthy subjects, however, were not statistically significant.

Barr et al.4 observed a significantly impaired antitoxin formation to primary immunization with tetanus toxid in patients with chronic lymphadenosis, lymphosarcoma, Hodgkin's disease, and the myeloproliferative syndrome. Impaired response to reimmunization was found only in disorders affecting the lymphoid tissue; in others it was not seen.
We have not observed any increased susceptibility to infectious complications in patients with Hodgkin's disease, reticulosarcoma, lymphosarcoma, and leukemia in stages I and II of the disease; it was not until advanced stages of generalization of the disease that increased susceptibility was observed. Schier also failed to demonstrate susceptibility to infections in his outpatients with Hodgkin's disease.28

Our patients with plasmocytoma showed a tendency to decreased antibody response to Brucella endotoxin, especially 14 days after vaccination; the difference from the control group, however, was not statistically significant. There are few data available concerning the capacity for antibody formation in patients with myeloma. Larson and Tomlinson14 investigated 4 patients; 2 of them were not capable of forming antibodies against pneumococcus polysaccharides. Balch1 observed normal antibody formation to the stimulus of diphtheria antitoxin in 1 patient with myelomatosis. Lawson et al. investigated for agglutinins and lysins to the typhoid fever salmonella, Escherichia coli and some other microorganisms in 9 patients with myeloma.16 The patients were not immunized and the results were compared with normal controls. The author found a striking decrease in antibody titers in these patients with myeloma. Marks19 also found decreased staphylococcus alpha-antitoxin and O-anti-streptolysin values in most patients with myeloma. Recently, the failure to form agglutinins in response to mixed typhoid and paratyphoid antigens has been described in macroglobulinemia.23

In patients with myelofibrosis, we did not find a decrease in the mean values of antibodies against Brucella endotoxin. However, there was a large standard deviation of the values, and the number of patients was not representative enough to permit a definite conclusion. We have not found any data in the literature on antibody formation in myelofibrosis.

**SUMMARY**

If we summarize our observations, we see that the capacity for antibody formation as a response to Brucella endotoxin stimulation is well preserved especially in acute leukemia, chronic myelosis, and in myelofibrosis. It is significantly decreased only in chronic lymphadenosis. A decrease, but lacking statistical significance, was further observed in reticulosarcoma and lymphosarcoma, Hodgkin's disease, and myeloma, i.e., in affections in which the RES is mainly involved.

The very frequent infectious complications affecting patients with acute leukemia cannot in most cases be ascribed to deficient capacity to form antibodies, as often erroneously claimed, but rather to other factors which will be dealt with elsewhere. In contrast to this, in chronic lymphadenosis the frequent infectious complications are very probably due to impaired antibody formation together with relatively frequent hypogammaglobulinemia.

Chemotherapy, corticoid and x-ray treatment in the usual clinical doses were not found to inhibit antibody formation as a response to an antigenic stimulus.

Even changes in the leukocyte count—leukocytosis, neutropenia or lympho-
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penia—did not seem to inhibit the capacity for antibody formation to Brucella endotoxin.

In 2 patients with idiopathic chronic agranulocytosis, who were examined for comparison, we found a strikingly high titer of circulating antibodies to the Brucella endotoxin. It is suggested that, in chronic agranulocytosis, the capacity for circulating antibody formation may be enhanced to compensate for the decrease in the number of polymorphonuclears.

SUMMARIO IN INTERLINGUA

Le formation de anticorpore circulante anti endotoxina brucellic esseva investigate in 113 patientes adulte, omnes afficite de varie typos de hemoblastosis.

Le activitate e le stadio del morbo, le therapia, le presentia de associate complicationes infectiose, e le numeration leucocytic omnes prendite in consideration in le evaluation final del resultatos, le quales esseva compare con le resultatos obtenite in un grupo de controlo de subjectos normal.

ACKNOWLEDGMENTS

I am much indebted to J. Sterzl, M.D., D.Sc., for valuable advice, for kindly supplying Brucella endotoxin, and for the help of his laboratory in reading the agglutinations; I express my thanks to Mr. V. Malý for statistical analysis of the data and to Mrs. V. Horáčková for technical assistance.

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Study of Immunologic Reactivity in Hemoblastosis. Circulating Antibody Formation as a Response to Antigenic Stimulus in Leukemia, Malignant Lymphoma, Myeloma and Myelofibrosis

J. LIBÁNSKY