thus provides a sensitive, specific assay method. The results obtained for normal peripheral white blood cells, lymphocytes, and peripheral white cells from our patients are presented (Fig. 1).

Normal white blood cells and the thoracic-duct lymphocytes (nonleukemic cells) had measurable amounts of the methyl donor (each about 2 μg per gram of wet tissue). Leukemic cells had much more SAMe (8.6 $\pm$ 1.6 μg/g), the difference being statistically significant. The increased amounts of white-cell SAMe coincide with increased serum B_{12} and white-cell folic acid (*9*). All of these substances are important in the chemical transfer of methyl groups, but the physiological significance of these findings is not evident.

The increase in the adenosylmethionine is associated with a high proportion of immature cells in leukemic blood smears. Thus the question arises whether the elevations are unique to leukemic white blood cells, or merely correlate with metabolic immaturity in general. Minor elevations (about twice the adult concentrations) occur in the liver and brain of the newborn rat (*10*). On the other hand, there is a striking lack of direct correlation between SAMe content and the degree of immaturity of cells in the blood smears of our patients (Table 1).

The measurement of adenosylmethionine in normal marrow, while theoretically important, would not be comparable to preparations of peripheral blood cells from leukemic patients, since marrow contains immature red as well as white cells. In addition, the amounts of normal human marrow needed for assay could neither be obtained readily, nor without significant dilution with peripheral blood cells. A study of this compound in animal marrow and blood cells might provide adequate amounts of material, but would be of questionable comparability with human leukemic cells. The study of white blood cell preparations from patients with acute leukemia would be useful, since they contain high proportions of immature cells.

While there is no known direct relation of adenosylmethionine to nucleic acid metabolism, methyl transfer is important in the synthesis of the methylated pyrimidine, thymine (*2, 3*). Therefore, it would be well to search for methylation antimetabolites useful in therapy. There is information indicating that certain methyl acceptors such as pyrogallol, and certain potent monoamine oxidase inhibitors such as pargyline, can lower the amounts of SAMe in rat tissues strikingly (*10*). That such a lowering agent might inhibit tumor growth is suggested.

To one fasted patient with chronic myelocytic leukemia, 50 mg of pargyline was given orally during a 24-hour period, before and after which blood samples were taken, white cell preparations were made, and assays for the adenosylmethionine were performed. The patient was clinically unchanged and ate normally throughout the experimental period. The amount of SAMe in the cells before treatment was 6.2 μg/g, and after treatment it fell to 1.8 μg/g; however, this single finding cannot be interpreted until further data are available.

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11. We thank Dr. C. Roth of Georgetown University Hospital, Washington, D.C., for performing the B_{12} assays.

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Abnormal Immune Mechanism in Allogeneic Radiation Chimeras

Abstract. *Mice were subjected to x-rays (950 roentgens) and injected with isogeneic (isologous) or allogeneic (homologous) bone marrow. Six to 8 months later these chimeras were injected with* Salmonella typhi *flagellar antigen, and the formation of antibodies resistant and sensitive to destruction by treatment in vitro with 2-mercaptoethanol was determined. The allogeneic chimeras showed almost normal amounts of serum antibody after a third injection of antigen but a relative defect in their ability to synthesize antibody resistant to 2-mercaptoethanol. Apparently control of antibody formation becomes abnormal in the presence of the immunologic tolerance existing between the host and the foreign hematopoietic graft.*

The immunologic recovery of lethally irradiated mice treated with isogeneic (*1*) bone marrow was nearly normal when tested two or more months later with particulate antigens (*2*). In contrast, the immune status of irradiated mice treated with allogeneic (homologous) or xenogeneic (heterologous) marrow remained subnormal well beyond the critical period of secondary disease that these mice experience 20 to 40 days after irradiation. This reduced capacity in chimeras injected with foreign bone marrow is difficult to reconcile with the current opinion that a state of immunologic tolerance exists between the transplanted graft and foreign host. In contrast to the preceding findings, Garver *et al.* (*3*) reported a normal agglutinin response in allogeneic chimeras stimulated with multiple injections of human red-cell antigens 8 months after irradiation and marrow treatment. Vos and Weyzen (*4*) reported normal reactivity of allogeneic chimeric lymph-node cells against transplantation antigens not present in the chimeric host or grafted tissues. Thus, chimeric lymph-node cells injected into an irradiated mouse of a strain unrelated to either host or chimeric donor showed a killing effect comparable to lymph-node cells obtained from normal mice.

The immune response following antigen injection in many species of animals consists of the sequential formation of two major molecular forms of antibody: the 19*S* macroglobulins and the 7*S* γ-globulins (*5*). Depending upon the species of animal and the type and dosage of antigen, the antibody formed during the first and second weeks after antigenic stimulation is usually the 19*S* variety, and it

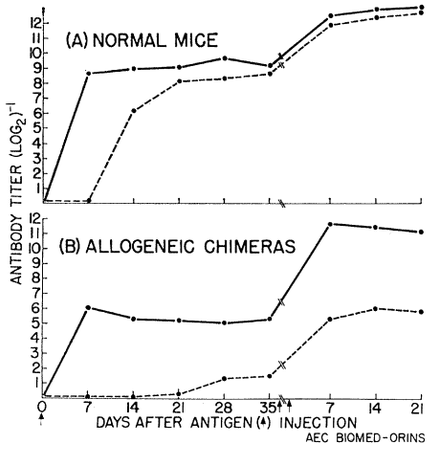

Fig. 1. Agglutinin response to *Salmonella typhi* flagellar antigen in (*A*) normal unirradiated mice and (*B*) allogeneic radiation chimeras after a primary (first) and tertiary (third) antigen injection. Titration before (solid lines) and after (broken lines) 2-mercaptoethanol treatment of the serum samples.

is then replaced during the subsequent weeks by 7*S* molecules. Furthermore, treatment in vitro of antibody with 2-mercaptoethanol (2-ME) has been shown to result in dissociation and inactivation of 19*S* molecules without affecting 7*S* antibody proteins (*6*). This provides another means of classification of antibody, that is, 2-ME resistant and 2-ME sensitive. Since this change in the type of antibody produced is a key qualitative feature of the "normal" immune response, it was possible that any impairment of immune competence in allogeneic chimeras would be reflected in impaired conversion from "large-antibody" to "small-antibody" synthesis. Therefore, the ability of allogeneic chimeras to show the sequential development in 2-ME–sensitive (large) and 2-ME–resistant (small) antibodies was determined and is reported here.

Mice of the $1C3F_1$, that is, (101/Cum × C3H/AnCum)F_1, strain were exposed to x-rays (950 roentgens, 250 kv-peak, 15 ma; inherent filtration, 1 mm Al; added filtration, 1 mm Al; half-value layer, 0.5 mm Cu; target-object distance, 60 cm; dose rate in air, 150 r/min). Within 2 hours after irradiation, groups of mice were injected with 10 to 50 × 10^6 cells of allogeneic, that is, (C57BL/6Cum × DBA/2Cum)F_1, or isogeneic bone marrow. Six to 8 months later, tests on the blood of the mice treated with allogeneic cells showed 100 percent donor-type red cells. At this time, groups of isogeneic and allogeneic radiation chimeras and normal unirradiated control mice (six to ten mice per group) were injected intraperitoneally and subcutaneously with *Salmonella typhi* flagellar antigen (*7*). The animals were bled from the retro-orbital sinus weekly for 5 weeks after injection, reinjected with the antigen 6 and 10 weeks after the first injection, and bled at weekly intervals for 3 weeks after the third antigen injection. All serum samples were frozen at −20°C until titrated. The individual serums were titrated for *S. typhi* flagellar (H) agglutinins before and after treatment in vitro with 2-ME (*7*). After the titrations, the remaining portions of serum for each day on which bleedings were made were pooled for ultracentrifugation in sucrose density gradients. Sucrose gradients from 10 to 40 percent, 4.6-ml volume, were made with an automatic mixing device and 0.4 ml of diluted serum (0.2 ml serum plus 0.2 ml saline) was layered on the surface of each gradient. These were then centrifuged for 15 hours (35,000 rev/min, 5°C) in a Spinco L-2 preparative ultracentrifuge with a swinging bucket rotor (SW39). Gradient fractions were obtained by piercing the bottom of the celluloid tubes, each fraction consisting of 25 drops. The fractions were then titrated for antibody. Since sedimentation coefficients have not yet been determined on the serum antibodies discussed here, they will be referred to as either 2-ME sensitive or resistant depending on treatment of the serum with 2-ME and as "large" or "small" antibodies as determined by sedimentation in sucrose density gradients.

Figure 1, *A* and *B*, shows the results of the first and third antigenic stimuli on antibody formation in normal mice and allogeneic chimeras. Since the results with the isogeneic chimeras resembled the normal they are not presented. In the normal group, the antibody formed in the first 7 days after the first injection of antigen (primary response) is inactivated by 2-ME, but from the 14th day on through the 35th day the presence of 2-ME–resistant antibody is evident. The third injection of antigen (tertiary response) resulted in the appearance of predominantly 2-ME–resistant antibody. The allogeneic chimeras, in contrast, showed a lower primary response with a relatively small amount of 2-ME–resistant antibody during the 35-day interval, but an excellent tertiary response. Figure 1*B*, however, shows that this response consists predominantly of 2-ME–sensitive antibody.

The serum samples obtained after the primary and tertiary injections of antigen were studied in sucrose density gradients (Fig. 2). The presence of almost entirely "large" antibody, that is, rapidly sedimentating type, in the 7-day sample of the primary response in normal mice is evident. The synthesis of "small" antibody, that is, more slowly sedimentating type, is well under way 14 days after the initial antigen injection, and predominates throughout the 35-day period. The booster response to the third antigen injection continues to show this quantitatively greater production of "small" antibody, although an increase of "large" antibody above that in the 35-day primary serum is also apparent. In the mice treated with allogeneic cells, the primary response, although showing the initiation of "small"-antibody production 14 days after antigen injection, shows a significant difference from the normal control in the proportions of "small" and "large" antibody formed throughout the 35-day period. An impaired conversion to "small"-antibody synthesis among the allogeneic mice, however, is evident mainly after the third antigen injection, where, although high titers of antibody comparable to the normal

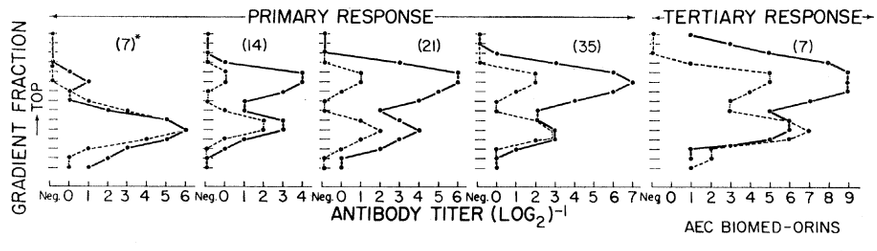

Fig. 2. Titration for antibody in sucrose gradient fractions of immune serum obtained from normal, unirradiated mice (solid lines) and allogeneic radiation chimeras (broken lines) according to the immunization scheme shown in Fig. 1. Numbers in parentheses indicate days after immunization.

group were obtained and an increase in "small" antibody was found, the response consisted predominately of the rapidly sedimentating "large" antibody (Fig. 2). The deficiency in converting to "small"-antibody synthesis in the tertiary response of the allogeneic chimeras might possibly be related to the small amount of this antibody produced in the primary response. To test this, groups of normal mice were injected with an antigen dose designed to yield a weak primary response with an amount of "small"-antibody production comparable to that obtained with the allogeneic mice. The normal mice were then given a third course of *S. typhi* antigen, and the titrations for antibody and sucrose gradient studies were performed. This response (data not shown) to the third antigen injection also consisted predominately of "small" antibody, similar to that shown for the normal control group in Figs. 1*A* and 2. Thus, the low amount of "small" antibody in the allogeneic chimeras after the primary injection was not the factor that prevented maximum conversion to "small"-antibody synthesis after the third antigenic stimulus.

Finally, the absence of 2-ME–resistant antibody in the primary response of chimeras (Fig. 1*B*) does not agree completely with the sucrose gradient results (Fig. 2), which show the presence of "small" antibody. Therefore, some caution should be applied in the current usage of "small" or "large" antibody terminology to denote 7*S* or 19*S* antibody, respectively, when the analysis is based on 2-ME–inactivation studies alone. In this regard, Rocky and Kunkel (*8*) have reported the presence in human serum of antibodies having intermediate sedimentation coefficients of 9*S* to 15*S* which are also sensitive to 2-ME treatment. The antibodies discussed in this report may thus represent a broad range of molecular size, as the sucrose density-gradient studies alone do not permit distinction between 7*S* and 9*S* molecules, and similarly, in the more rapidly sedimentating zone, distinction between 15*S* and 19*S* antibody molecules would not be possible.

Thus, allogeneic chimeras possess an abnormal mechanism for the production of humoral antibody. Also, additional studies have revealed that this defect is present in varying degrees among such mice; great latitude exists from truly abnormal to almost normal ability to convert to small-antibody formation. Whether this variability is related to the status of the immunologic tolerance between host and graft tissues in these animals is not known. Such chimeras may be used as models for the study of the sequential conversion of synthesis of large antibody to that of the smaller type; the physiology of this process in normal animals is not yet clearly understood (*9*).

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References and Notes

1. Recommended by G. Snell [*Transplant.* **2**, 655 (1964)], the new terminology for tissue transplantation has been used in this report. Homologous, previously used to denote genetic differences within a species, has been replaced by allogeneic; heterologous, previously used to denote genetic differences between species, is replaced by xenogeneic; and isologous, once used to denote the same genetic origin, is replaced by isogeneic.
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Polyribosomes from Escherichia coli: Enzymatic Method for Isolation

Abstract. *Polyribosomes can be rapidly extracted from* Escherichia coli *by sequential passage of the cells through solutions of a chelating agent and lysozyme in a centrifugal field. Biosynthesis of protein in whole cells and in a cell-free system occurs almost exclusively in the polyribosomes.*

Although techniques for the isolation of polyribosomes from animal tissues have been developed by Wettstein *et al.* (*1*) for rat liver, Penman *et al.* (*2*) for HeLa cells, and Gierer (*3*) and Marks *et al.* (*4*) for rabbit reticulocytes, it has proved more difficult to obtain them from *Escherichia coli* cells.

Staehelin *et al.* (*5*) demonstrated the existence of polyribosomal aggregates in *E. coli*, and Schaechter (*6*) has extracted polyribosomes from a number of bacterial species, including *E. coli*, by gentle lysis of the whole organisms in a French pressure cell. Kiho and Rich (*7*) have also been able to obtain polyribosome-rich extracts of *E. coli* by treatment of the cells with penicillin and subsequent lysis of the protoplasts by detergent.

We now have isolated polyribosomes from *E. coli* by a technique (*8*) for producing spheroplasts of this organism. The spheroplasts produced by the original method will not yield significant amounts of polyribosomes, apparently because during the formation of spheroplasts, EDTA (disodium ethylenediaminetetraacetate) can effect chelation of intracellular divalent cations and thus break down the polyribosomes. If the spheroplasts are incubated in a growth medium, polyribosomes, presumably produced from newly synthesized messenger RNA (*9*), can be isolated.

If the cells could be briefly exposed to EDTA and lysozyme in succession, rather than together, the enzyme might be able to act on the cell wall without the undesired accompanying depletion of intracellular cations (*10*). Such treatment was achieved by sedimenting the bacterial cells through sucrose solutions of different densities with EDTA in an upper and lysozyme in a lower layer. The resulting cells are not true spheroplasts, but their cell walls are weakened sufficiently to be readily opened by detergents. Removal of unbroken cells and cellular debris then yields an extract rich in polyribosomes.

The technique is simple, rapid, and reproducible; the protein synthesizing activity of crude extracts of *E. coli* is concentrated in these polyribosomes both in vivo and in vitro.

Escherichia coli was grown in Medium A of Davis and Mingioli (*11*) (the disodium citrate being omitted) with 0.2 percent glucose added; the cultures (100 ml) were incubated at 37°C on a rotary shaker. During exponential growth the cells were harvested by centrifugation and suspended in magnesium-tris buffer (15 m*M* magnesium acetate, 5 m*M* tris buffer, *p*H 7.6 at 23°C). One milliliter of the cell extract [at 20 to 40 mg (wet weight)/ml] was layered over four layers of sucrose solutions in a 10-ml cellulose tube. These layers were (from the bottom up): (i) 2 ml of 25 ercent sucrose in the magnesium-tris buf-