of Fe^{55} for Richland residents, we analyzed some of their common foods (Table 2). Meat, grain products, and some eggs are probably the main sources. Cooling water from the nuclear reactors upstream from Richland empties into the Columbia River from which the inhabitants derive their drinking water, but analysis of this water showed only 0.0005 nc of Fe^{55} per liter —an insignificant contribution to the total body burden.

Since whole-wheat flour from various sources contains ten times as much Fe^{55} as does bleached white flour, the Fe^{55} is probably associated with the outer shell of the wheat. We have found similar differences with cesium-137 and manganese-54 in whole-wheat and white flour. This difference, plus the fact that vegetables contain very little Fe^{55}, indicates that the radionuclides probably reach the grain directly from the air rather than through the root system. Menzel *et al.* (*7*) and Rivera (*8*) found that most of the strontium-90 in wheat came from deposition on above-ground parts of the plants.

Relatively high amounts of Fe^{55} were found in smelt, salmon, and tuna caught in the Pacific Ocean. The content in tuna is about ten times the content reported by Seymour (*9*) in tuna from the Japanese fish markets in 1962. We do not know the exact location at which the fish we examined were caught; it is possible that some of the Fe^{55} in them may have derived from the Columbia River. Oysters, which tend to concentrate zinc-65 from ocean water (*10*), have low concentrations of Fe^{55} even though they have a higher iron content than any other food measured, except for caribou liver.

Caribou meat and liver samples have a higher content of Fe^{55} than do beef meat and liver from near Richland, as would be expected from the blood measurements. It is interesting that the content of stable iron in the meat and liver of Alaskan caribou and elk from Washington is also much higher than that in beef meat and liver.

Since the rate of fallout is now decreasing (*11*), the difference between amounts of Fe^{55} in Eskimos and residents of Richland will probably become greater. Because of the long retention of fallout nuclides by the lichens, the amounts in the caribou and Eskimos will increase until the rate of fallout is less than the rate of decay of the already deposited Fe^{55}, whose half-life is 2.7 years. Because of the short period of availability of the isotope on vegetation near Richland, we expect its concentrations in cattle, humans, and foods to decrease much sooner and more rapidly than in Eskimos and caribou.

HARVEY E. PALMER
THOMAS M. BEASLEY
Battelle Memorial Institute,
Pacific Northwest Laboratory,
Richland, Washington

References and Notes

1. E. P. Hardy, J. Rivera, W. R. Collins, *Fallout Program Quart. Summary Rep., HASL-146,* AEC, Health and Safety Lab., New York (1964).
2. C. J. Maletskos and J. W. Irvine, Jr., *Nucleonics* **14,** 84 (1956).
3. E. J. Underwood, *Trace Elements in Human and Animal Nutrition* (Academic Press, New York, 1962), p. 14.
4. H. E. Palmer, W. C. Hanson, B. I. Griffin, W. C. Roesch, *Science* **142,** 64 (1963).
5. Intern. Comm. Radiological Protect., *Recommendations: Report 2, Permissible Dose for Internal Radiation* (Pergamon, New York, 1960).
6. W. C. Hanson, H. E. Palmer, B. I. Griffin, *Health Physics* **10,** 421 (1964).
7. R. G. Menzel, D. L. Myhre, H. L. Roberts, Jr., *Science* **134,** 559 (1961).
8. J. Rivera, *ibid.* **133,** 755 (1961).
9. A. H. Seymour, "Gamma-emitting radionuclides in tuna samples from the Tokyo central fish market, 1962," Univ. of Washington, Lab. of Radiation Biol. Doc. UWFL-90.
10. W. A. Chipman, T. R. Rice, T. J. Price, *U.S. Fish Wildlife Serv. Fishery Bull. 135* (1958), pp. 279–292.
11. "Revised fallout estimates for 1964-1965 and verification of the 1963 predictions," Federal Radiation Council Rept. 6, October 1964.
12. We thank Dr. Lorentzen of the Alaska Native Health Hospital, Tanana, Alaska, for providing the Eskimo blood samples.

19 April 1965

Adult Thymectomy: Effect on Recovery from Immunologic Depression in Mice

Abstract. *A/Jax mice treated for 1 week with rabbit antiserum to A/Jax lymphocytes showed peripheral lymphopenia and tissue lymphocyte depletion which persisted for 4 weeks after initiation of serum treatment. During this time, such animals showed depression of the humoral antibody response and inability to reject skin allografts. After 4 weeks, mice recovered from effects of serum treatment. Normal blood and tissue lymphocyte levels returned, and immunologic competence was restored. Thymectomy of A/Jax mice (8 to 10 weeks old) prior to serum treatment resulted in peripheral and tissue lymphopenia and depression of antibody formation, which persisted twice as long as that shown by animals given serum alone. Similarly, skin allografts survive two to four times longer in serum-treated thymectomized mice, and, in some cases, persisted over 100 days in perfect condition.*

Thymectomy in mice performed within 24 hours of birth usually results in a syndrome characterized by progressive weight loss, cachexia, lethargy, ruffled fur, diarrhea, and, in a large proportion of the animals, subsequent death (*1*). Characteristically in such thymectomized animals there is a depletion of small lymphocytes in the blood and lymphoid tissues (*2*). These mice show immunological incompetence as determined by (i) impaired ability to reject skin allografts and xenografts (*3*) as well as grafts of normal and neoplastic allogeneic cells (*4*), and (ii) by decreased ability to form antibody to a number of antigens (*5*). In contrast, mice thymectomized at 8 weeks of age also show decreased numbers of small lymphocytes in the peripheral blood and lymphoid tissues, but immunological competence is unimpaired (*6*). Similarly, thymectomy of adult rabbits (*7*), rats (*8*), and guinea pigs (*9*) has no effect on immunological competence as determined by humoral antibody formation or skin allograft rejection. Under special circumstances, however, the adult thymus is important to the restoration of immunological competence. Thus, Claman and Talmadge (*10*) rendered mice tolerant of bovine γ-globulin by injections of this antigen from birth to adulthood and found that thymectomy of such mice as adults impaired, but did not abolish, their recovery from the tolerant state. Similar results with this antigen and also with bovine serum albumin have been reported by Taylor (*11*). Normal adult mice given a potentially lethal dose of ionizing irradiation followed by infusion of isogeneic bone marrow recover normal immune responses 4 to 10 weeks after irradiation and bone-marrow therapy. However, adult mice thymectomized prior to irradiation and bone-marrow therapy failed to respond to skin allografts and sheep erythrocytes 2 months after irradiation (*6, 12*). The recovery of

the immune response after total-body irradiation and bone-marrow therapy is apparently thymus-dependent.

Rabbit antiserum to mouse lymphocytes is a potent immunosuppressive agent (*13, 14*). Such serum contains lymphocyte cytotoxins and agglutinins (*14*). Adult mice given daily serum injections for 1 week show peripheral lymphopenia and depletion of lymphocytes in lymphoid tissues for approximately 1 month after initiation of treatment. During this period of serum-induced peripheral and tissue lymphopenia, mice fail to reject skin allografts and xenografts and show an impaired ability to form humoral antibody (*14*). After 1 month, however, the numbers of lymphocytes in the blood and lymphoid tissues return to normal and the animals regain immunological competence.

Our study was undertaken to determine if the recovery from immunological depression induced in mice by antiserum to lymphocytes was thymus-dependent. The degree and duration of peripheral and tissue lymphopenia were compared in groups of adult mice thymectomized or sham-operated prior to serum treatment. Immune responses were tested by rejection of skin allografts and by the capacity to form antibody to sheep erythrocytes.

Rabbit antiserum to mouse lymphocytes was prepared as previously described (*13*). White New Zealand rabbits were immunized by injecting the four foot-pads with a saline suspension (100×10^6 cells per rabbit) of A/Jax lymph-node cells incorporated into Freund's adjuvant. One month later, rabbits were given booster injections, intravenously, of the same total dose of saline-suspended cells; the dose was given over a period of 4 consecutive days, and the animals were bled 1 week after the last injection. Throughout the experiment repeated courses of intravenous booster injections were given as required for maintenance of appropriate antibody titers. Serum was harvested, pooled, heated to 56°C (30 min) to remove the complement, and stored at −20°C until used. The lymphocyte agglutinin titer of each batch of serum was determined (*15*), a titer of 1:128 to 1:256 usually being obtained. The same pool of serum was used throughout each group of experiments.

Inbred mice of the C57BL/6, C3H/He, and A/Jax strains were used. Skin grafts were performed according to the method of Billingham and Medawar (*16*). Plaster casts were removed 6 days after operation, and the state of the grafts was followed by visual and tactile inspection until destruction was complete. The median survival times (MST) of C57BL/6 skin grafts on A/Jax mice, a combination differing at the strong H-2 (histocompatibility) locus, was 9.8 days, with a standard error of 0.2 days (30 animals). The MST for the C3H/He to A/Jax combination, representing a somewhat lesser immunogenetic difference, was 10.2 ± 0.3 days (30 animals). Thymectomy of A/Jax mice, aged 8 to 10 weeks, was carried out under Nembutal anesthesia by a modification of Miller's method (*17*). Hemagglutinins to sheep erythrocytes, after primary and secondary challenge by the intraperitoneal injection of 0.1 ml of a 20-percent suspension of washed sheep erythrocytes, were determined by a standard method (*18*). In experiments in which the degree and duration of peripheral lymphopenia and tissue lymphocyte depletion were studied, total (standard hemocytometer) and differential (Wright's stain) counts of white blood cells were made at weekly intervals, and the total lymphocyte counts were obtained. Mice were killed at weekly intervals, and their lymphoid tissues were fixed in Formalin and stained with hematoxylin and eosin.

At the end of each experiment all thymectomized animals were killed and examined at autopsy for completeness of thymectomy; serial sections of all suspicious mediastinal tissue were made. Those few mice with residual thymic tissue were not included in the tabulation of the experimental results.

The A/Jax mice (age 8 to 10 weeks) were divided into three groups: one was thymectomized, one received a sham operation, and one was left intact. Half of each group was injected intraperitoneally with 0.25 ml of rabbit antiserum to A/Jax lymphocytes for seven consecutive days. The mean total peripheral lymphocyte counts, obtained at weekly intervals beginning the day after the first serum injection, are shown in Fig. 1 for the various groups (12 to 16 animals per group). Of the mice receiving no serum, the intact and sham-thymectomized animals showed the same lymphocyte counts, whereas the thymectomized animals showed a slight fall in peripheral lymphocyte counts for the first 2 to 3 weeks, after which normal levels returned. Of the groups receiving serum, intact or sham-thymectomized mice showed a rapid and significant decrease in the total peripheral lymphocyte counts, and the mice did not recover until 3 weeks after cessation of serum treatment. Animals thymectomized prior to serum treatment showed the same immediate reduction in peripheral blood lymphocytes but failed to regain normal values until 6 weeks after termination of serum treatment. All serum-treated groups showed profound depletion of small lymphocytes in the lymphoid tissues, particularly in the lymph nodes, but depletion was most pronounced in the group thymectomized prior to serum treatment. Furthermore, the lymphoid tissues remained depleted for a much longer

Table 1. Survival of C57BL/6 skin allografts in various groups of intact (no operation), sham-thymectomized (ST), thymectomized (T), or splenectomized (SP) adult A/Jax mice treated or untreated with rabbit antiserum to A/Jax lymphocytes. One group of serum-treated, thymectomized mice received subcutaneous implantation (STI) of normal, isogeneic A/Jax thymus at the time of skin grafting. Mice with incomplete thymectomy at autopsy are not included in the results.

Operation	Days of graft survival			
	< 12	13–24	25–36	37–67
	No Serum			
None or ST	24	0	0	0
T	22	0	0	0
	Serum			
None	0	26	0	0
ST	0	16	0	0
T	0	3	10	11
T and STI	0	9	9	0
SP	0	16	0	0

Table 2. Survival of C3H/He grafts in various groups of intact (no operation), sham-thymectomized (ST), or thymectomized (T) adult A/Jax mice treated or untreated with rabbit antiserum to A/Jax lymphocytes. Mice with incomplete thymectomy are not included in the results.

Operation	Days of graft survival				
	< 14	15–28	29–42	43–100	> 100
	No serum				
None or ST	24	0	0	0	0
T	16	0	0	0	0
	Serum				
None	0	3	25	0	0
ST	0	1	13	0	0
T	0	1	2	18	9

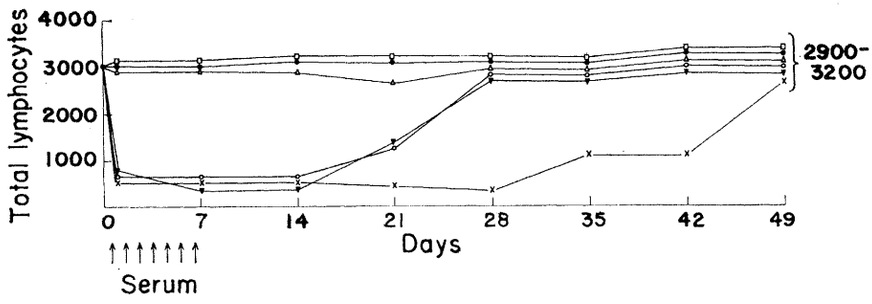

Fig. 1. Mean total lymphocyte counts of intact (open circle), sham-operated (closed triangle), and thymectomized (cross) A/Jax mice treated for 7 days with rabbit antiserum to A/Jax lymphocytes. Control groups received no serum. Blood lymphocytes (intact, closed circle; sham-thymectomy, open square; thymectomy, open triangle) in the serum-treated intact and sham-thymectomized groups returned to normal count after 1 month, whereas the count was not normal in serum-treated thymectomized animals until much later.

time in the group thymectomized prior to treatment, and this delayed recovery was correlated with the prolonged peripheral lymphopenia observed.

Similar groups of A/Jax mice were treated as above with antiserum for 7 days. Two weeks after the last serum injection the animals were challenged primarily with sheep erythrocytes. The mice were bled a week later, challenged a second time, and bled again 7 days later (Fig. 2). Thymectomy or sham thymectomy alone had no effect on the primary or secondary hemagglutinin response. Intact serum-treated or sham-operated serum-treated animals had depressed primary responses, but all animals had detectable hemagglutinin titers. The primary response of mice thymectomized prior to serum treatment was, however, much more depressed, and 9 of 16 animals had no demonstrable hemagglutinins. The secondary responses of normal and sham-thymectomized mice treated with serum were essentially the same as those of normal, sham-thymectomized, or thymectomized mice receiving no serum. The secondary responses of serum-treated thymectomized mice were still significantly depressed, 3 of 16 mice still showing no detectable hemagglutinin formation. Thymectomized serum-treated mice did not respond with normal secondary titers until challenged a third time 6 weeks after serum treatment.

Intact, sham-operated, or thymectomized A/Jax mice were given 0.25 ml antiserum to lymphocytes intraperitoneally for 7 days and then grafted with C57BL/6 skin the day after the last serum injection (Table 1). Control groups of intact, sham-operated, or thymectomized mice which received no serum rejected skin grafts in the usual time for a first skin graft; that is, all grafts were rejected in less than 12 days. Skin allografts in normal or sham-operated groups in which the mice received serum survived up to two weeks beyond the expected time of rejection (all grafts rejected between 12 to 24 days). However, mice thymectomized before receiving the same serum treatment did not as readily reject the C57BL/6 skin grafts. Of 24 grafts, 21 survived longer than the longest graft surviving on animals given serum alone, and 7 of 24 grafts survived beyond 50 days. All of these grafts grew full, luxuriant coats of hair. Once signs of rejection began, the process was slow and protracted. A group of adult A/Jax mice were thymectomized, given serum for 7 days, and then grafted the following day with C57BL/6 skin. At the time of skin grafting, two lobes of normal A/Jax thymus were implanted subcutaneously. The subcutaneous implantation of isogeneic thymus significantly hastened the recovery of immunological competence in previously thymectomized, serum-treated mice (Table 1). The possibility that removal of the thymus, a lymphoid organ, prior to serum treatment resulted in a greater serum dose relative to lymphoid mass, in thymectomized as opposed to intact mice, was considered as a possible explanation for the results obtained. However, splenectomized A/Jax mice treated with serum, as described, rejected skin grafts at the same rate as intact or sham-thymectomized serum-treated mice (Table 1). Removal of the spleen, therefore, a lymphoid organ twice the weight of the thymus in this species, failed to augment the effect of serum treatment.

The effect of prior thymectomy on adult A/Jax mice given the same serum treatment as described, but grafted with C3H/He skin, is shown in Table 2. In this strain combination, in which a weaker genetic histocompatibility difference exists, intact or sham-operated recipients given serum allowed longer survival of C3H/He grafts (majority of grafts rejected in 29 to 42 days) than comparable animals receiving C57BL/6 grafts (Table 1). Skin grafts on 27 of 30 mice thymectomized prior to serum treatment survived longer than the longest graft surviving in the unthymectomized serum-treated groups. In 9 of the 30 serum-treated thymectomized mice, C3H/He grafts survived beyond 100 days in perfect condition. These nine mice were subsequently grafted with C57BL/6 skin. All C57BL/6 skin grafts were rejected in the usual time for a first skin graft (9 to 13 days). Of the nine long-tolerated C3H/He grafts, five were

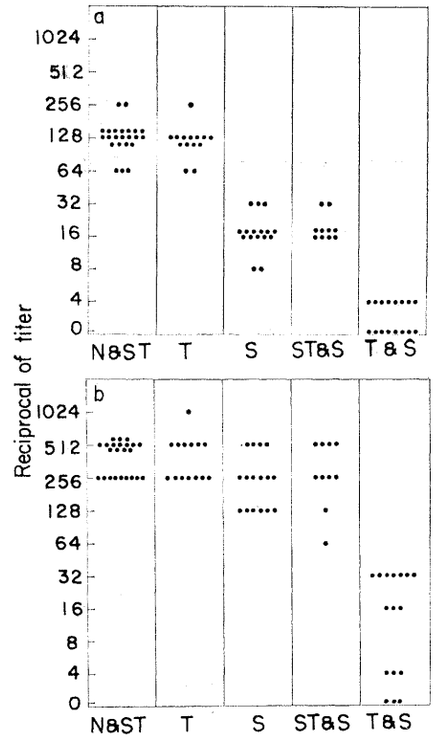

Fig. 2. *a*, Primary hemagglutinin titers (day 7) in various groups of adult A/Jax mice given rabbit antiserum to A/Jax lymphocytes for 7 days and challenged with sheep erythrocytes 2 weeks after termination of serum treatment. *b*, Secondary hemagglutinin titers (day 7) of the same groups after repeat injection of sheep erythrocytes 1 week after primary challenge. All control groups received no serum. Each dot represents one mouse. *N*, normal; *S*, serum; *T*, thymectomized; *ST*, sham-thymectomized.

subsequently slowly rejected over the 30 days following the rejection of the C57BL/6 grafts while the other four remained unaltered.

Finally, it has been previously shown that treatment with antiserum to lymphocytes prolongs the survival of second grafts in A/Jax mice which have rejected C57BL/6 or C3H/He skin grafts (*14*). Our experiments show, however, that thymectomy of mice previously sensitized to such grafts and then treated with the antiserum in doses already mentioned does not prolong the survival of second grafts beyond that seen in sensitized animals given the antiserum alone.

The data suggest that the adult thymus functions in the recovery from a state of immunologic depression associated with lymphocyte depletion induced by antiserum to lymphocytes. Of significance is the fact that recovery is delayed in most of the animals but not permanently prevented. In this regard the few mice with long-surviving C3H/He grafts are of interest. The possibility that specific tolerance of the C3H/He grafts was induced, perhaps maintained by release of antigen from the graft, is to be considered. More experimentation is required to clarify this point. No conclusion can be drawn as to the mechanism by which the adult thymus functions in the recovery from treatment with antiserum to lymphocytes. The thymus glands of intact mice treated with antiserum in these and other experiments (*14*) show a marked depletion of lymphoid elements, but epithelial-reticular components are preserved. This observation suggests to some extent that a humoral factor may be responsible for the action of the adult thymus. This is in keeping with the observations that implants of thymus in Millipore chambers restore immunologic competence in neonatally thymectomized mice (*18, 19*).

Adult thymectomy prior to hydrocortisone treatment in mice (*20*) or sublethal irradiation in rats (*8*) fails to augment skin allograft survival beyond that seen in unthymectomized animals. Neither treatment alone induces prolongation of skin allograft survival comparable to that achieved with antiserum to lymphocytes alone. The degree of immunosuppression induced may determine whether the adult thymus participates in restoration of immune responses. Lethally irradiated mice protected by isogeneic spleen cells recover immune responses at the same rate whether thymectomized or unthymectomized (*12*). Lethally irradiated thymectomized mice given bone marrow, however, fail to recover immune responses at the same rate as their unthymectomized litter mates. Apparently, bone marrow does not contain large numbers of immunologically competent cells, and bone marrow cells attain competence only in the presence of the thymus. Therefore, the degree to which a given immunosuppressive regimen destroys or depletes lymph node or spleen cells, with concomitant necessity for repopulation of lymphoid tissues dependent on cells from the bone marrow, may in turn determine the importance of the thymus in restoration of immunologic competence.

ANTHONY P. MONACO
MARY L. WOOD
PAUL S. RUSSELL
Department of Surgery, Massachusetts General Hospital, Boston

References and Notes

1. J. F. A. P. Miller, *Lancet* **1961-II**, 748 (1961).
2. D. M. V. Parrott, *Transplant. Bull.* **29**, 102 (1962); ——— and J. East, *Nature* **195**, 347 (1962).
3. J. F. A. P. Miller, *Ann. N.Y. Acad. Sci.* **99**, 340 (1962); A. P. Dalmasso, C. Martinez, R. A. Good, *Proc. Soc. Exptl. Biol. Med.* **111**, 143 (1962).
4. R. A. Good, A. P. Dalmasso, C. Martinez, O. K. Archer, S. C. Pierce, B. W. Papermaster, *J. Exptl. Med.* **116**, 773 (1962); C. Martinez, J. Kersey, B. W. Papermaster, R. A. Good, *Proc. Soc. Exptl. Biol. Med.* **109**, 193 (1962).
5. J. F. A. P. Miller, *Proc. Roy. Soc. London, Ser. B* **156**, 415 (1962); A. H. E. Marshall and R. G. White, *Advan. Immunol.* **2**, 111 (1962).
6. J. F. A. P. Miller, S. M. A. Doak, A. M. Cross, *Proc. Soc. Exptl. Biol. Med.* **112**, 785 (1963).
7. T. N. Harris, J. Rhoads, J. A. Stokes, *J. Immunol.* **78**, 27 (1948); L. D. MacLean, S. J. Zak, R. L. Varco, R. A. Good, *Transplant. Bull.* **4**, 21 (1957).
8. P. M. Braunstein, H. H. Zwick, L. D. MacLean, *J. Surg. Res.* **5**, 137 (1965).
9. K. E. Fichtelius, G. Laurell, L. Philipsson, *Acta Pathol. Microbiol. Scand.* **51**, 81 (1961).
10. H. N. Claman and D. W. Talmadge, *Science* **141**, 1193 (1963).
11. R. B. Taylor, *Immunology* **7**, 595 (1964).
12. A. M. Cross, E. Leuchars, J. F. A. P. Miller, *J. Exptl. Med.* **119**, 837 (1964).
13. J. G. Gray, A. P. Monaco, P. S. Russell, *Surg. Forum* **15**, 142 (1964).
14. J. G. Gray, A. P. Monaco, M. L. Wood, P. S. Russell, unpublished observations.
15. D. B. Amos and N. Peacocke, in *Proc. Congr. Europ. Soc. Haemat. 9th Congr.* Lisbon (Karger, New York, 1963), pp. 1132–1140.
16. R. E. Billingham and P. B. Medawar, *J. Exptl. Biol.* **28**, 385 (1951).
17. J. F. A. P. Miller, *Brit. J. Cancer* **14**, 93 (1960).
18. D. Osoba and J. F. A. P. Miller, *J. Exp. Med.* **119**, 177 (1964).
19. L. W. Law, N. Trainin, R. H. Levey, W. F. Barth, *Science* **143**, 1 (1964); R. H. Levey, N. Trainin, L. W. Law, *J. Natl. Cancer Inst.* **31**, 199 (1963).
20. H. O. Zunker and H. A. Azar, *Proc. Soc. Exptl. Biol. Med.* **118**, 423, 1965.
21. Supported in part by grants AM 07055, AI 06320, T4 CA 5018, and CRTY 5018 from the USPHS and by the William F. Milton Fund of Harvard University.

23 April 1965

Human Transferrins C and D_1: Chemical Difference in a Peptide

Abstract. *Peptide analyses have been carried out for chymotryptic digests of transferrin C and transferrin D_1. There is a difference in one peptide, and amino acid analyses indicate that an aspartic acid residue in transferrin C is replaced probably by a glycine residue in transferrin D_1.*

Transferrin (siderophilin) is an iron-binding protein. It has a molecular weight of about 90,000 and appears to be composed of a single polypeptide chain (*1*). It binds two atoms of ferric iron per molecule (*2*) and has four sialic acid residues in each molecule (*3, 4*).

Some 15 electrophoretic variants of human transferrins are known, each resulting from simple Mendelian inheritance. They are named according to their electrophoretic mobility in starch gel at alkaline *p*H (*5*). Transferrin C is the most frequent form, whereas transferrin D_1 is a slower-moving variant common in Negroes.

By analogy with hemoglobin variants, one or more amino acid exchanges would be expected to account for the differences among the transferrin variants, although no such exchanges have been reported. The object of our research was the elucidation of the structural differences between transferrins C and D_1.

Transferrins were isolated by a combination of rivanol precipitation and starch-block electrophoresis (*6*). About 500 ml of blood was collected from persons who were homozygous for transferrin C and for transferrin D_1. One part of plasma, to which $FeCl_3$ was added (about 1 mg/100 ml) to saturate the transferrin, was diluted with three parts buffer (tris-hydroxymethylaminomethane, 0.005*M*, *p*H 8.8), and an equal volume of 0.6 percent rivanol (2-ethoxy-6,9-diaminoacridine lactate, California Foundation for Biochemical Research) dissolved in the same buffer was added. The supernatant was passed through potato starch to remove the rivanol, and the proteins in the filtrate were concentrated by adsorption onto diethylaminoethyl-Sephadex (A-50, coarse). After elution with 0.1*M* NaCl and dialysis against water (*p*H 8.0), the material was lyophilized. The lyophilized material was dissolved in barbital buffer, *p*H 8.6, ionic strength 0.05, and subjected to