ANTIBODY PROTEIN SYNTHESIS BY LYMPH NODES HOMOTRANSPLANTED TO A HYPOGAMMAGLOBULINEMIC ADULT

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After showing that normal skin homografted upon a congenitally agammaglobulinemic child survived for a prolonged period (1), Good and Varco further demonstrated that within certain limits hypogammaglobulinemic patients might be artificially endowed with a system of active immunity through homotransplantation of a reticuloendothelial tissue (2, 3).

The present study was designed both to explore the therapeutic possibilities of lymph node homotransplantation and to take advantage of the unique antibody-synthesizing defect in hypogammaglobulinemia to quantitate the immune response of human lymphoid tissue.

This paper presents the results of measurements of antibody protein synthesis, during primary and secondary immune responses, by normal lymph nodes homotransplanted to a hypogammaglobulinemic adult, together with qualitative observations of the interplay of recipient and transplant during and after the 150- to 160-day period of survival of the lymph nodes.

MATERIALS AND METHODS

Challenge antigen. Because it is potent, reliable, and rapidly productive of antibodies susceptible to accurate measurement, typhoid vaccine was selected as the antigen with which to test the reactivity of the transplant. The preparation employed[1] to challenge both recipient and donor contained 1,000 million heat-killed organisms of the Panama 58 strain per ml., and was of the type ordinarily stimulating production of H and O (but not V_1) antibodies.

Titration of typhoid H- and O-agglutinins. Agglutinins were measured on coded specimens by one observer at one time, and expressed as the mean of four determinations on recipient sera and two on donor sera, by the slide agglutination method devised by Welch and Stuart (4) and improved by Diamond (5). Single pooled batches of commercial (Lederle) Salmonella Group D somatic antigen and of typhoid H antigen were employed. The test was further refined as follows: the titration was begun at 1 : 10, employing 0.16 ml. of serum, and all subsequent successively smaller serum aliquots were made up to 0.16 ml. with saline prior to the addition of 0.03 ml. of antigen; moreover, dilutions of 1 : 15, 1 : 30, 1 : 60, etc., employing appropriate serum aliquots, were interpolated between the conventional 1 : 10, 1 : 20, 1 : 40, etc. dilutions. Under these more rigorous conditions, end-points (50 per cent agglutination) were sharp and repeated titrations reasonably reproducible.[2] The readings obtained were deemed internally consistent, though not necessarily quantitatively identical with values ordinarily obtained with different batches of tube or slide antigens.

No satisfactory method of accurate titration of low ($< 1 : 10$) titers of H-agglutinins was found. Titers of O-agglutinins as low as 1 : 2.5, however, could be measured satisfactorily by prior 2- or 4-fold concentration of the serum beta$_2$-globulins through the use of zinc-proteinate reactions based on Cohn plasma fractionation Method 12 (6) as follows:

A portion of cold, buffered zinc diglycinate-zinc acetate solution, containing 500 mM Zn^{++} per L., was added to the serum at 0 to 2° C to a final Zn^{++} concentration of 20 mM per L. The mixture was allowed to equilibrate 30 minutes and was centrifuged at 0 to 2° C for 15 minutes at 1,400 *g*. The supernatant was discarded. The precipitate was made up to one-fourth or one-half the original serum volume with 0.3 M sodium citrate at pH 7.2. Recovery of both H- and O-agglutinins appeared to be complete, but the concentrate was suitable for slide agglutination titration of only O-antibody. The presence of citrate ion apparently inhibited the reaction of H-antigen and antibody; the citrate effect could be reversed by dialysis, but only at the expense of reduced concentration.

Titration of tissue antibody. Tissue extracts suitable for titration of H- and O-agglutinins were prepared as fat-free saline suspensions by the method of Mountain (7).

Immunochemical estimation of typhoid O-antibody. To define a ratio between units of serum typhoid O-agglutinin activity (reciprocal of titer) and micrograms of typhoid O-beta$_2$-globulin nitrogen per ml., the method of analysis of specific precipitates (8, 9) was adapted as follows:

The antigen used consisted of a saline suspension of the colonies scraped from a 20-hour agar slant culture

[1] Typhoid Vaccine, Eli Lilly and Co., Lot No. 7289–62783.

[2] See Table III.

of *Salmonella typhi,* H901W strain. The suspension was heated two and one-half hours at 100° C to destroy traces of flagellar antigen, was centrifuged, and was twice washed with saline. Saline was then added to yield a suspension containing approximately 60 μg. N per ml.

Preliminary experiments under conditions of antibody excess and antigen excess indicated a wide equivalence zone, as reported by Landy, Johnson, Webster, and Sagin (10), with prozone and postzone phenomena occurring only under extreme conditions; in addition, the very low antibody N: antigen N ratio in equivalence-point precipitates noted by Gurevitch and Ephrati (11) was confirmed.

Sera taken from donor and recipient were used in 0.2- to 5.0-ml. amounts. Sera were inactivated 30 minutes at 37° C and prior to antigen addition were centrifuged free of traces of particulate debris. Mixtures of serum, saline, and 1.0 ml. antigen were incubated 1 hour, with occasional agitation, in a 37° C bath, and then for 18 hours at 4° C.

The mixtures were spun 30 minutes at 3° C and 1,400 *g*. The supernatants were decanted and checked for residual agglutinating activity, and the precipitates were washed twice with cold saline and transferred quantitatively to Kjeldahl digestion flasks.

Nitrogen was determined by a micro-Kjeldahl procedure employing powdered selenium as a catalyst. The color reaction with Nessler's reagent was developed in the cold and read immediately at 500 mμ in a spectrophotometer.

Immunochemical estimation of typhoid H-antibody. The method described above was used, with the following differences:

Antigen was prepared by adding an equal volume of 0.6 per cent formalinized saline to a 20-hour trypticase-soy broth culture of the H901W strain of *S. typhi,* the motility of which had been increased to a maximum by repeated passage in semi-solid agar. The organisms were centrifuged and washed free of broth protein, and saline was added to yield a suspension containing approximately 50 μg. N per ml. The sera measured were those noted above, from which O-antibody had been completely absorbed.

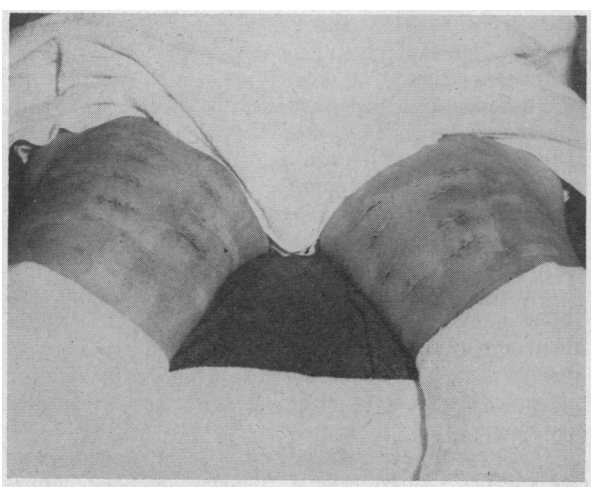

FIG. 1. SITES OF TRANSPLANTATION OF LYMPH NODE SLICES IN MEDIAL THIGHS

Tuberculin testing. Tuberculin tests of the recipient were performed with 0.1-ml. intradermal doses of fresh solutions of a single lot of Sharp and Dohme Second-Strength Tablets Tuberculin, Purified Protein Derivative (0.005 mg. per 0.1 ml.), and 0.1 ml. doses of fresh saline dilutions of that lot equivalent in potency to P.P.D. Intermediate (0.0001 mg. per 0.1 ml.) and P.P.D. No. 1 (0.00002 mg. per 0.1 ml.). Induration was measured by the method of Lovell, Goodman, Hudson, Armitage, and Pickering (12) at 24, 48, and 72 hours, and units of tuberculin reactivity roughly quantitated as millimeters mean maximum induration per log micro-microgram P.P.D., a unit based on the linear relationship between that measurement of the response and the logarithm of the dose, as described in man (12) and in animals (13).[3] Two dilutions were used for each skin test, and on each occasion the two responses agreed within 5 to 10 per cent.

Histological methods. Tissues for histological examination were fixed in 70–30 absolute alcohol-formalin solution and serially sectioned. Alternate sections were stained with hematoxylin-eosin and methyl-green-pyronine, and selected sections were stained with iron-hematoxylin, eosin-methylene blue, and Giemsa stain. Several sections were examined by polaroid and phase microscopy. Attempts to count plasma cells, reticulum cells, and lymphocytes were abandoned, since no one stain differentiated the cell types sufficiently well to insure accuracy on successive counts.

Leukocyte suspensions. Fresh suspensions of viable leukocytes suitable for skin-testing and for agglutinin studies were prepared from whole blood by the dextran-dextrose-sequestrene technique of Brecher, Wilbur, and Cronkite (16). For skin-testing, the .02 ml. of packed leukocytes derived from 10 ml. blood were resuspended in 0.1 ml. normal saline and injected intradermally. The same volume of leukocytes, washed and resuspended in 2.0 ml. saline, served as an antigen for slide agglutinations.

Statistical methods. In the construction of the curve which best fits the observed antibody titers, standard methods of graphic analysis of multiple linear regression curves were employed to resolve the changing slopes.

THE RECIPIENT, THE DONOR, AND THE CLINICAL CONDITIONS

Hypogammaglobulinemic recipient. The recipient of the transplant was a 64-year-old white woman with ac-

[3] Under this schema, maximum responses of 10 × 10 mm. induration to 0.1-ml. doses of P.P.D. No. 2, Intermediate, and No. 1 are equivalent, respectively, to 2.7, 5.0, and 7.7 units of tuberculin reactivity; and the 1+, 2+, 3+, 4+ criteria employed by Lawrence in passive transfer experiments (14, 15) are equivalent, respectively, to 1.4–2.7, 2.7–5.4, 5.4–8.1, and > 8.1 units.

quired hypogammaglobulinemia,[4] whose case history has been reported elsewhere (17). In addition to her basic disease, the following secondary complications and unrelated conditions were present: a stable mediastinal mass (presumably a hyperplastic, now fibrotic, thymus); chronic pyelonephritis (with normal blood urea nitrogen and only mild impairment of renal function); bronchiectasis; hypersplenism (with a moderate hemolytic anemia and a cyclic neutropenia); and arteriosclerotic heart disease, with mild ankle edema despite therapeutic doses of digitoxin.

Throughout the 137-day period preceding the transplantation and the 231-day period following it, the recipient received sulfadiazine sufficient to maintain a near-constant serum sulfonamide level of 5 to 8 mg. per 100 ml. and human gamma globulin,[5] 5.0 gm. intramuscularly every 14 days, a dose which maintained a constant serum gamma-globulin level of 0.30 ± 0.02 gm. per 100 ml. (17) and a constant exogenous typhoid H-agglutinin titer of 1:10. She had previously proved totally unresponsive to primary and repeated booster doses of typhoid-paratyphoid vaccine and to several 0.1-ml. intradermal doses of P.P.D. No. 2.

There began to develop, 30 to 40 days prior to transplantation, a severe neutropenia which, instead of remitting as in the past, persisted until the 257th day after transplantation.

Choice of donor. The following major and minor criteria were formulated for the selection of a donor for safe and successful transplantation:

Major: 1) No evidence of active tuberculosis, of other acute or chronic transmissible infection, or of neoplastic disease; 2) Neither evidence of past or of present hepatitis nor history of transfusions within the past 6 months; 3) Close genetic relationship to the recipient; 4) Requirement of abdominal surgery for other, benign reasons, to which lymph node excision would be incidental.

Minor: 1) Identical blood type (B, Rh positive); 2) Positive tuberculin reaction (or other delayed-type cutaneous hypersensitivity); 3) Age between 20 and 50 years; 4) Absence of typhoid agglutinins and no history of typhoid infection or immunization.

The donor selected was the patient's 62-year-old sister, who had always been in good health and who required an elective hysterectomy and perineal repair for a third-degree uterine prolapse and cystocoele. Studies revealed Type A, Rh positive blood, normal liver function, a positive reaction to P.P.D. No. 1, and X-ray evidence of healed fibrotic disease at the apex of the right lung. There was no history of typhoid fever or of typhoid immunization, and typhoid H- and O-agglutinins were absent in 1:10 and 1:2.5 dilution, respectively. Titers of somatic agglutinins against other Salmonellae were: A: $< 1:5$; B: $< 1:5$; C (C_1, C_2): 1:40; and E (E_1, E_2, E_3): $< 1:5$.

Except in age and blood type, the donor therefore satisfied all the major and minor criteria.

Homotransplantation procedure. On June 22, 1955, a left supraclavicular lymph node was excised from the recipient. One-half was fixed promptly in alcohol-formalin and the other frozen for later antibody studies.

Simultaneously, in an adjacent operating room, the surgeon[6] excised a portion of the fat pad containing the donor's left hypogastric lymph node chain, and immersed the specimen in Ringer's solution. A total of 16 symmetrically placed, 2.0 to 2.5-cm. suboutaneous incisions had meanwhile been made in the inner aspect of the recipient's thighs (Figure 1), and packed with cotton-gauze sponges soaked in Ringer's solution. To minimize tissue trauma, hemostats and ligatures had been used sparingly.

Each of nine small lymph nodes was dissected free of the fat pad, cleaned of traces of pericapsular fat, rapidly and sterilely weighed on a Roller-Smith automatic precision balance, and placed separately in individual screw-cap vials, each vial containing 1.5 ml. of the recipient's serum to which penicillin and dihydrostreptomycin had been added to a concentration of 100 μg. per ml.

The wet weights of the nodes (in mg.) were respectively: 36.9, 41.8, 64.1, 110.1, 120.2, 127.8, 130.1, 213.8, and 82.5. The 82.5-mg. node was divided in halves and grossly examined. One half was placed in alcohol-formalin and the other frozen and saved.

Working rapidly, the operator transplanted each node in turn as follows: with a minimum of trauma, each node was sliced into four strips, each strip no greater than 2 mm. thick. Each strip was briefly checked for gross pathology, and then two strips were placed in an incision in the left thigh and two in the symmetrical incision in the right. No further chemotherapy was given the recipient, and no wound infections occurred. Healing progressed uneventfully, and at no time did sloughing occur.

Antigenic challenge of recipient and of donor. Seven days after transplantation, the recipient was challenged with 0.5 ml. of the previously described typhoid vaccine injected subcutaneously in the left arm. A booster dose of 0.5 ml. was given seven days later, and another, twenty days later.

To avoid problems of interpretation of titers in the donor, however, initial 0.5 ml. challenge was delayed until the ninth day, when the postoperative phase of heightened adrenal cortical activity had presumably passed. Booster doses of 0.5 ml. were given seven and, through an oversight, eighteen (rather than twenty) days later.

Excision biopsies. Nineteen days after transplantation, the strips from the node originally weighing 110.1 mg. were excised from their two symmetrical sites. At the same time, a right supraclavicular node was excised from

[4] Patient referred for study by Stuart O. Foster, M.D., Washington, D. C.

[5] Poliomyelitis Immune Globulin, Squibb and Sons, Lot No. 252–2, kindly supplied by the American National Red Cross.

[6] J. Keith Cromer, Department of Gynecology and Obstetrics, George Washington University School of Medicine, whose cooperation the authors gratefully acknowledge.

the recipient. Half of each of the specimens was fixed in alcohol-formalin; the remainder was subdivided grossly into node fragments, fat, and skin and was frozen and saved.

RESULTS

Immune responses

Tuberculin tests of the recipient two days after transplantation disclosed a powerful passively transferred delayed cutaneous hypersensitivity to tuberculin (Figure 2). Tuberculin reactivity then steadily increased to a plateau which extended from the 68th day to the 149th at a level equivalent to a reaction of 25 × 25 mm. induration to P.P.D. No. 1. Between the 149th and the 217th days reactivity fell off to a plateau at a lower, though still highly reactive, level. Reactivity was essentially unaffected by splenectomy on the 257th day.

In response to early challenges with typhoid vaccine, administered at sites distant from the transplants, the recipient developed low titers of typhoid H- and O-agglutinins (Figure 2) in a manner qualitatively similar to the higher titers observed in the donor (Figure 3). A booster challenge on the 98th day again elicited a secondary response, after which titers began to decline more rapidly. A final challenge on the 168th day elicited no response; instead, measurable H-agglutinins declined exponentially over the next 80 days with a slope statistically indistinguishable from that of passively infused H-agglutinins in other hypogammaglobulinemic patients (described below).

Biopsies

The donor's ninth hypogastric lymph node, which had not been transplanted, was histologically normal and quiescent and contained occasional plasma cells; a saline extract of the node was devoid of typhoid H- and O-antibody ($< 1:20$).

The recipient's left and right supraclavicular nodes, excised before and 19 days after transplantation, were virtually identical; both contained a total of only one or two dubious plasma cells and no antibody ($< 1:20$). Both contained abundant

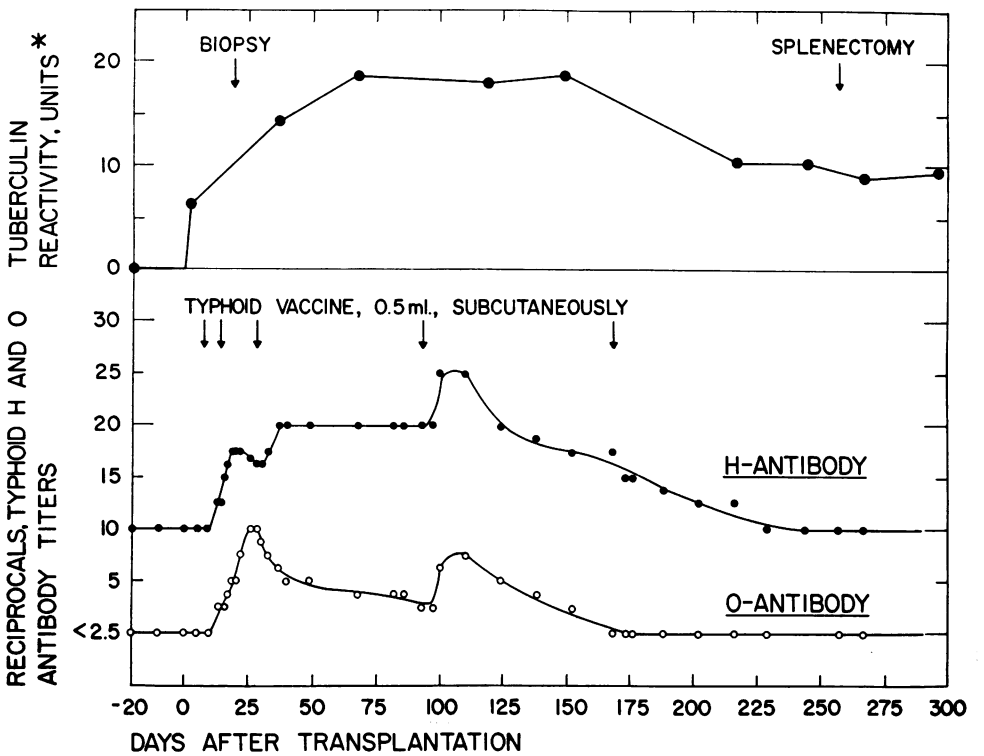

FIG. 2. IMMUNE REACTIONS OF TRANSPLANTED LYMPH NODES

*Tuberculin reactivity units: Millimeters mean maximum induration per log micro-microgram P.P.D. Each point represents the average of responses to two dilutions.

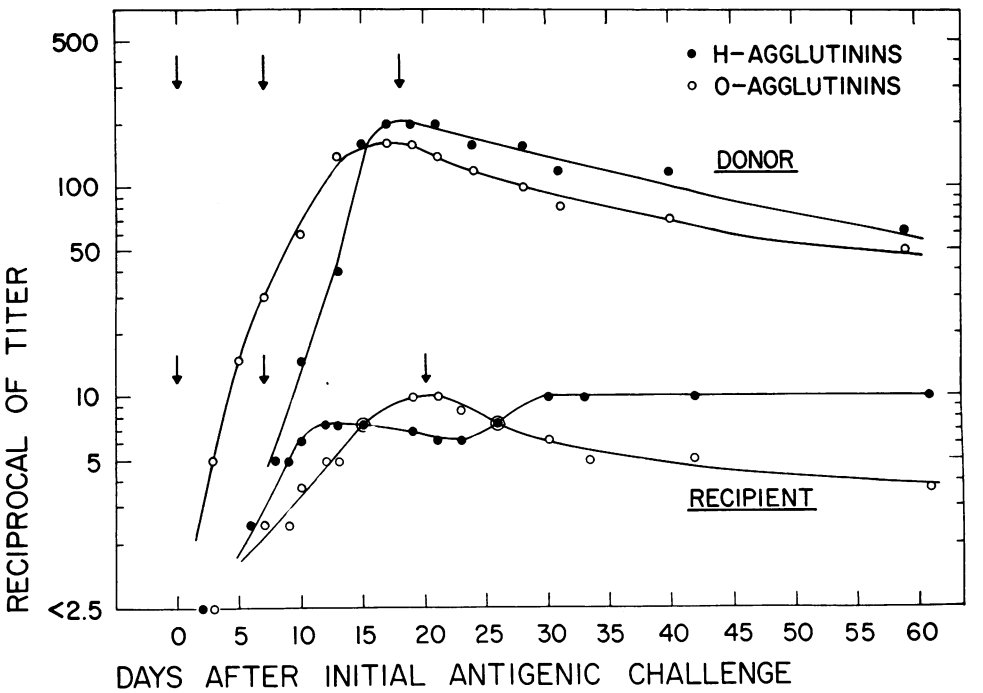

FIG. 3. DONOR AND RECIPIENT ANTIBODY TITERS AFTER TYPHOID IMMUNIZATION
The H-agglutinin titers observed in the recipient (Figure 2) are here corrected for a constant exogenous titer of 1 : 10.

lymphocytes and reticulum cells, but sparse and poorly developed germinal centers.

The 19th day excision biopsy of one of the transplanted nodes disclosed evidence of an initial take; the tissue contained moderate numbers of plasma cells and abundant tissue antibody. Extracts of various fragments grossly identified as node tissue (the bulk of each of which, however, was granulation tissue) titered 1 : 40, 1 : 40, 1 : 80, and 1 : 80 against H-antigen, and 1 : 20, 1 : 40, 1 : 80, and 1 : 200 against O-antigen. Extracts of adjacent specimens of fat and of the overlying skin and scar contained no demonstrable antibody. Serum H and O titers at the time of biopsy were 1 : 17.5 and 1 : 5, respectively.

Microscopically the transplant (Figure 4) contained architecturally disorganized cords of lymphoid tissue intimately penetrated by granulation tissue, with occasional foreign-body giant cells at the periphery. Higher magnification (Figure 5) disclosed intensely vascularized cords and strands of reticulum cells, lymphocytes, and scattered (but not abundant) plasma cells. Study of the giant cells by polarized light suggested that they were not reacting specifically to the donor tissue; each contained a bit of refractile cotton fiber, presumably from the surgical sponges used at the time of transplantation.

Therapeutic effects of transplantation

Despite a severe neutropenia (white blood counts 1200 to 2400, with 2 to 18 per cent neutrophils) the recipient remained free of major infections throughout the life of the transplant. During that interval there occurred episodes of oral moniliasis from the 76th day to the 81st and from the 130th to the 134th; a syndrome of fever, pharyngitis, cervical lymphadenopathy, and atypical lymphocytosis from the 86th day to the 96th; and facial furuncles from the 106th day to the 110th.

In contrast, the 100-day period following the presumed death of the transplant was filled with a succession of increasingly severe infections—ulcerative pharyngitis and stomatitis, recurrent oral moniliasis, facial furuncles, severe acute pansinusitis due to *Staphylococcus aureus,* and mul-

FIG. 4. EXCISION BIOPSY, TRANSPLANTED LYMPH NODE SLICE, NINETEENTH DAY

From left to right are seen granulation tissue, scattered giant cells, cords of lymphoid tissue, and partially necrotic fat (H & E × 85).

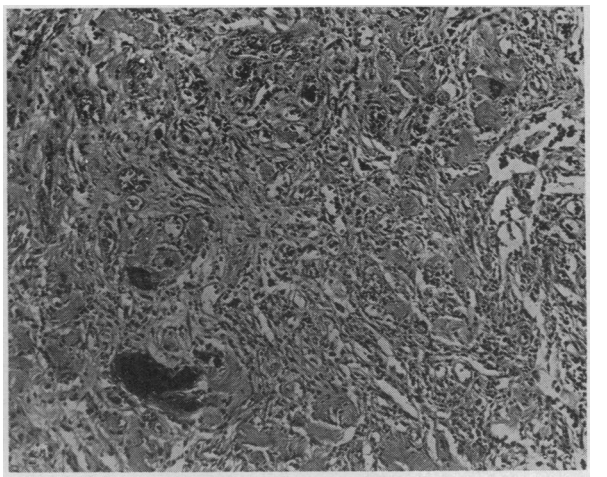

FIG. 5. EXCISION BIOPSY, TRANSPLANTED LYMPH NODE SLICE, NINETEENTH DAY

Within the intensely vascularized, disorganized cords of lymphoid tissue are reticulum cells, plasma cells, and foreign-body giant cells (H & E × 150).

tiple subcutaneous staphylococcal abscesses—which, together with the recipient's first severe thrombocytopenic episode, culminated in splenectomy on the 257th day. Postoperatively there occurred a prompt, sustained hematologic and symptomatic remission.[7]

Also in contrast, during a comparable, non-neutropenic 96-day period before transplantation there occurred only a single mild upper respiratory infection, lasting five days (17).

Neither during the life of the transplant nor after splenectomy did there occur any detectable rise in the recipient's serum gamma-globulin level.

In an attempt to make therapeutic use of the transplant, the patient was immunized, at bi-weekly intervals beginning on the 68th day, with standard parenteral doses of the following antigens: diphtheria toxoid-tetanus toxoid-pertussis vaccine, polyvalent influenza vaccine, and two doses of a combined respiratory bacterial vaccine.[8] In addition, four doses of an autogenous, formalinized *Escherichia coli* (communis) vaccine, prepared from the strain isolated from the recipient during a pyelonephritis and bacteremia fourteen months previously (17) and still present in the urine, were given during this period.

Although there was suggestive evidence of weak responses to several of the administered antigens, extensive titrations of pre- and post-challenge sera failed to demonstrate unequivocal antibody formation.

Immune interactions of transplant and recipient

To explore the possibility that the transplant might be rejecting the recipient, as well as *vice versa,* attempts were made to demonstrate isoimmune phenomena in several systems. Neither clinical nor laboratory evidence of nephritis, encephalitis, or collagen diseases appeared, and no evidence was adduced to implicate the transplanted nodes in the persistence of the recipient's neutropenia. The results of repeated Coombs tests were negative, and the recipient's first episode of thrombocytopenia occurred three months after transplant function had ceased.

[7] Multiple histological sections of the 630-gm. spleen revealed increased numbers of Malpighian follicles, prominent germinal centers, normal numbers of reticulum cells and lymphocytes, and prominent erythrocytophagocytosis by monocytes. Only a very few cells resembling plasma cells were seen, and there was no evidence of lymphoma, granuloma formation, amyloidosis, or collagen disease. A specimen of the liver was histologically normal. Cultures and animal inoculations of organ suspensions were negative for bacteria, mycobacteria, fungi, and *Toxoplasma.*

[8] Immunovac Respiratory Vaccine®, Parke Davis & Co.

Despite the challenge of small numbers of incompatible (Type A) red cells, the recipient failed to develop anti-A isoagglutinins in 1 : 1 dilution, measured by both the saline and the indirect Coombs techniques.

By the use of viable donor leukocytes as a slide agglutination antigen, it was demonstrated that the recipient failed to develop circulating leukocyte agglutinins in 1 : 1 dilution at any time after transplantation.

Skin tests, however, 250 and 266 days after transplantation, demonstrated a reproducible, specific, delayed, tuberculin-like cutaneous hypersensitivity to viable donor leukocytes. Intradermal injection of the leukocytes derived from 10 ml. of fresh donor blood elicited a reaction beginning at three hours and reaching a maximum diameter at 24 to 36 hours of 14 to 16 mm. of erythema and 10 to 12 mm. of tender induration, with a central hemorrhagic spot. Only transient, 3 to 4 mm. diameter erythematous responses were elicited by control injections of approximately equal numbers of leukocytes derived from the recipient and from normal Type O, tuberculin positive; Type B, tuberculin negative; and Type A, tuberculin negative persons, as well as of donor plasma containing dextrose-dextran-sequestrene.[9]

Immunochemical studies

The results of immunochemical studies (Figures 6, 7) indicate that under the conditions of biological assay described above 1 unit of typhoid H-agglutinating activity synthesized by the specific donor (or donor tissue) is equivalent to .126 ± .009 microgram gamma-globulin N per ml. serum and that 1 unit of similarly specific typhoid O-agglutinating activity is equivalent to .102 ± .026 microgram $beta_2$-globulin N per ml. serum. With the whole bacilli antigens employed, the ratios in equivalence-point precipitates of antibody N : antigen N for the H and O systems were, respectively, 1 : 2.85 and 1 : 5.27.

Estimation of rates of antibody synthesis

In the absence of demonstrable recipient antibody synthesis and with the knowledge that extracts only of the transplants contained large amounts of antibody, it was concluded that all observed titers represented antibody synthesized by the transplant. A general equation for calculating daily total synthesis of typhoid antibody by the transplant was therefore formulated as follows:

If S be the weight of H- or O-antibody synthesized and released per gram of lymph node tissue per day; I, the daily increment in serum titer in agglutinating units; D, the units of antibody passively degraded per day; V, the apparent body fluid volume or compartment in which the antibody is diluted; K, the constant that relates units of agglutinating activity to weight of antibody nitrogen; 6.2, the protein : nitrogen ratio in both gamma- and $beta_2$-globulin (18); and W, the weight in grams of transplanted lymph node tissue, then on any given day

$$S = \frac{(I + D)(V)(K)(6.2)}{W}. \qquad (1)$$

Values of I were read from the curve of antibody titer derived from graphic analysis of the observed titers (Figure 3).

Values of D were calculated by substituting each day's average serum titer for C in the equation describing one day's exponential decline of passively acquired antibody from a level of C to a level of C_0,

$$\frac{\text{Log } C - \text{Log } C_0}{1} = \text{Slope} = \text{Constant}, \qquad (2)$$

solving for C_0 and subtracting from C. The appropriate constant for H-antibody was determined, and that for O-antibody was assumed, as follows:

H-antibody: After administration of 50 gm. of human gamma globulin [5] to two other hypogammaglobulinemic patients, the passive rates of decline of serum gamma globulin and of six common antibodies were determined by *in vitro* assays of multiple periodic serum specimens drawn over a 63- to 70-day period (19). The half-life of typhoid H-agglutinin, so determined, was 35.0 ± 3.6 days, corresponding to a slope of 0.0086.

O-antibody: Although the half-life of $beta_2$-globulin in human beings has never been determined, sparse indirect evidence suggests that $beta_2$-globulins are passively degraded considerably faster than gamma globulins. The equations with

[9] Because of the possibility of immunizing the recipient against her prospective donor, the recipient was not skin-tested with donor leukocytes before transplantation.

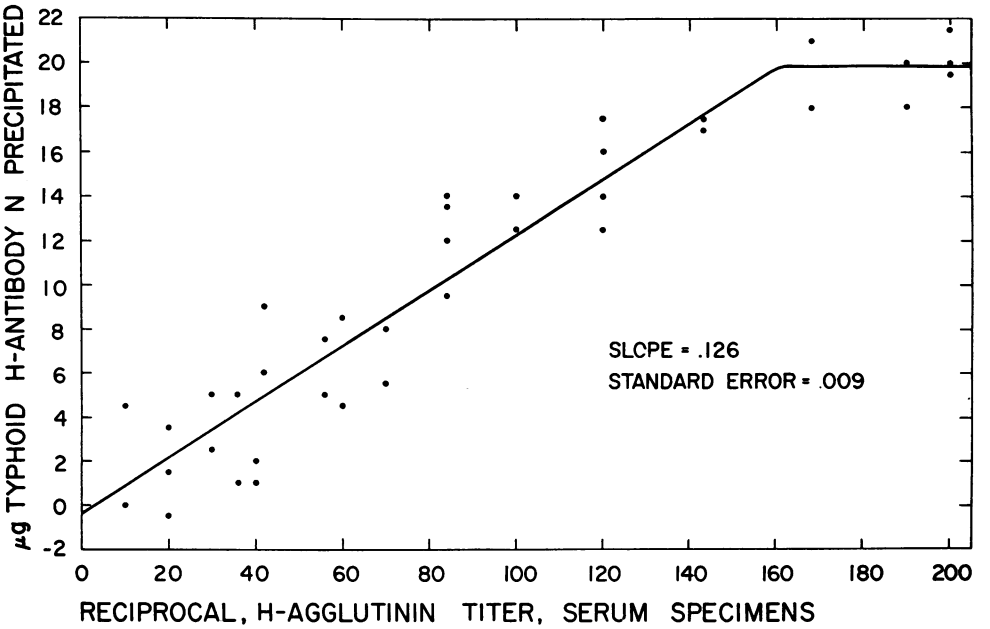

FIG. 6. ANTIBODY NITROGEN PRECIPITATED WITH 56.5 MICROGRAMS TYPHOID H-ANTIGEN NITROGEN

which Wiener demonstrated that newborns passively degrade diphtheria antitoxin at a rate identical with that of adults (20), when applied to the data of Smith on the decline of passively acquired compatible isoagglutinins in newborns (21), indicate that these $beta_2$-globulins have a half-life of 12 to 18 days. Biosynthetic determinations of the half-lives of heterogeneous plasma protein fractions, of which $beta_2$-globulins were only one of many components, have yielded values of 6.2 to 7.8 days for fractions I + III (22) prepared by Cohn Method 10 (23), and 17 days for pooled $alpha_1$-$alpha_2$-beta-globulins (24).

After two transfusions of fresh Type B, Rh positive blood, the recipient's passively acquired anti-A isoagglutinin titers were followed for 28

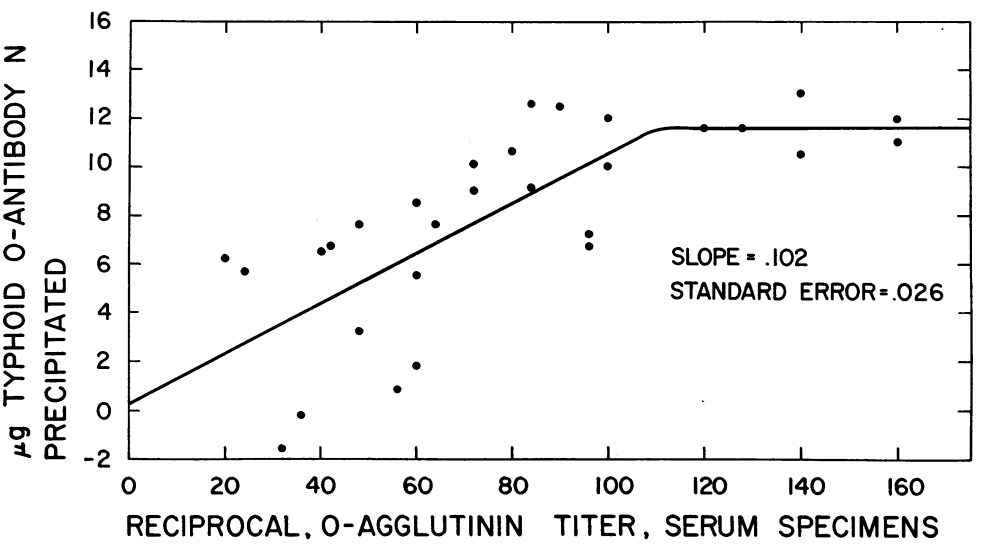

FIG. 7. ANTIBODY NITROGEN PRECIPITATED WITH 61.1 MICROGRAMS TYPHOID O-ANTIGEN NITROGEN

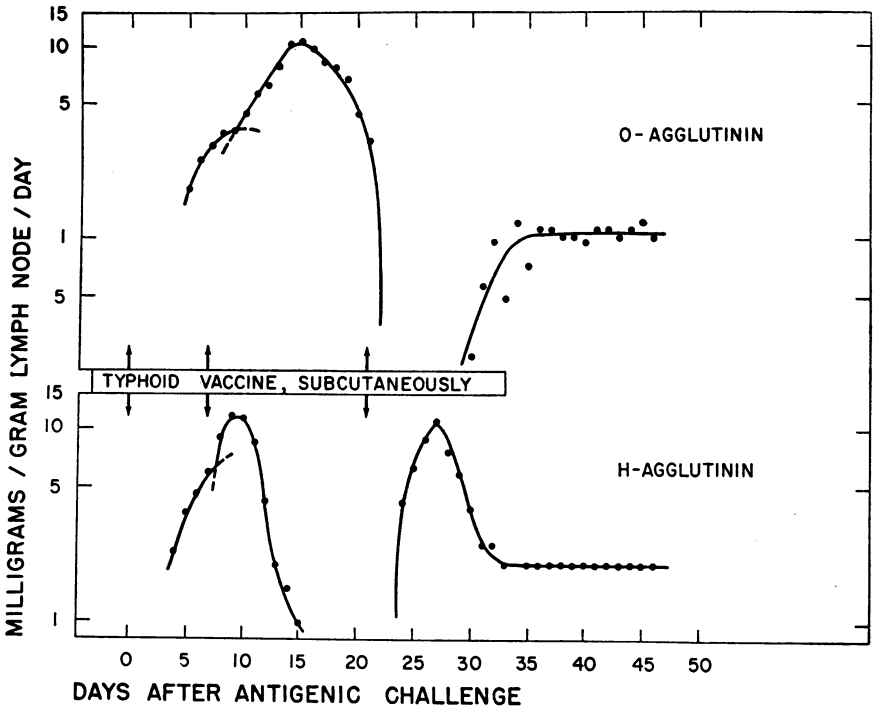

FIG. 8. ANTIBODY PROTEIN SYNTHESIS BY TRANSPLANTED LYMPH NODES

days. Analysis of seven determinations yielded a linear regression curve with a half-life of 16.7 days and a standard error of 2.5 days.

Employing these data and noting that the maximum slopes in the declining phase of the curves of O-antibody titer in both donor and recipient corresponded to half-lives of 15 ± 2 days (Figures 2 and 3), a 15-day half-life of O-agglutinins (or slope of 0.020) was assumed.

Compartment size: Previous studies of the recipient had demonstrated that after equilibration administered gamma globulin (including typhoid H-antibody) behaves as if diluted in a fluid volume equivalent to 20 per cent of body weight (17), *i.e.*, 25 to 30 per cent is found in the plasma, and the remainder, in extravascular compartments. In this study, in which the recipient's weight (47 Kg.) remained essentially constant throughout the period of observation, the volume of distribution V of typhoid H-antibody was therefore equivalent to 9.4 liters.

It was not possible to determine directly V for typhoid O-antibody, since no safe concentrated source of human O-antibody is available for human administration. As a near approximation, the V for anti-A isoagglutinins, which are chemically and electrophoretically similar to typhoid O-agglutinins, was determined. After transfusion of the recipient with fresh blood containing compatible plasma of known isoagglutinin content, anti-A isoagglutinins behaved as if diluted in a volume equivalent to 20 ± 3 per cent of body weight (25). Since this figure is compatible with the known presence of typhoid O-antibody and isoagglutinins in numerous tissues and interstitial fluids (26–29), it appeared reasonable to assume V for typhoid O-antibody to be identical with that of H-antibody.

Employing these data and assuming, further, prompt (24-hour) mixing of newly synthesized antibody and full survival of the transplant (W = 844.8 mg. before excision biopsy and 734.7 after), minimum rates of antibody synthesis were calculated (Table I) and graphed (Figure 8).

DISCUSSION

Clinical observations

The present study confirms the demonstration by Good and Varco (1–3) of the impaired ability of the hypogammaglobulinemic patient to respond

TABLE I

Serum titrations and estimated daily rates of typhoid H- and O-antibody synthesis by transplanted lymph nodes

Days after initial antigenic challenge	Reciprocals of four serum titrations (*units/ml.*)		Reciprocal mean observed endogenous serum titer (*units/ml.*)		Reciprocal estimated graphic titer (*units/ml.*)		Antibody destroyed per day (*units/ml.*)		Total antibody synthesized per day (*units/ml.*)		Milligrams antibody protein synthesized/ gram wet weight lymph node/day	
	H	O	H	O	H	O	H	O	H	O	H	O
2	10, 10, 10, 10		0									
3	10, 10, 10, 10	< 2.5 × 4	0	<2.5	1.0							
4					1.25	1.25	.02		.27		2.32	
5		< 2.5 × 4		<2.5	1.65	1.45	.03	.06	.43	.26	3.70	1.79
6	10, 10, 15, 15		2.5		2.15	1.75	.04	.07	.54	.37	4.64	2.55
7	10, 10, 15, 15	2.5, 2.5, 2.5, 2.5	2.5	2.5	2.80	2.10	.05	.09	.70	.44	6.02	3.03
8					3.80	2.50	.06	.11	1.06	.51	9.14	3.52
9	15, 15, 15, 15	2.5, 2.5, 2.5, 2.5	5.0	2.5	5.05	2.90	.09	.12	1.34	.52	11.52	3.59
10	15, 15, 15, 20	2.5, 3.75, 3.75, 5	6.25	3.75	6.25	3.40	.11	.14	1.31	.64	11.27	4.42
11					7.10	4.05	.13	.17	.98	.82	8.43	5.65
12	15, 15, 20, 20	5, 5, 5, 5	7.5	5.0	7.45	4.75	.14	.20	.49	.90	4.21	6.20
Biopsy												
13	15, 15, 20, 20	5, 5, 5, 5	7.5	5.0	7.50	5.50	.15	.23	.20	.98	1.98	7.77
14					7.50	6.50	.15	.27	.15	1.27	1.48	10.07
15	15, 15, 20, 20	5, 7.5, 7.5, 10	7.5	7.5	7.45	7.50	.15	.32	.10	1.32	.99	10.46
16					7.25	8.35	.14	.36	−.06	1.21	−.59	9.60
17					7.10	9.00	.14	.39	−.01	1.04	−.10	8.25
18					6.95	9.55	.14	.42	−.01	.97	−.10	7.70
19	7.5, 20, 20, 20	10, 10, 10, 10	6.88	10	6.75	9.95	.13	.44	−.07	.84	−.69	6.66
20					6.60	10.05	.13	.45	−.02	.55	−.20	4.36
21	15, 15, 15, 20	10, 10, 10, 10	6.25	10	6.40	10.00	.13	.45	−.07	.40	−.69	3.17
22	15, 15, 15, 20		6.25		6.25	9.35	.12	.44	−.03	−.21	−.30	−1.67
23		7.5, 7.5, 10, 10		8.75	6.20	8.75	.12	.41	.07	−.19	.69	−1.51
24					6.50	8.30	.12	.39	.42	.06	4.16	.48
25					7.00	7.80	.13	.36	.63	−.14	6.24	−1.11
26	15, 15, 20, 20	7.5 × 4	7.5	7.5	7.75	7.40	.14	.34	.89	−.06	8.82	−.48
27					8.70	7.05	.16	.32	1.11	−.03	10.98	−.24
28					9.30	6.70	.18	.31	.78	−.04	7.72	−.32
29					9.70	6.40	.19	.30	.59	0	5.84	0
30	20, 20, 20, 20	5, 5, 7.5, 7.5	10	6.25	9.90	6.15	.19	.28	.39	.03	3.86	.24

TABLE I—*Continued*

Days after initial antigenic challenge	Reciprocals of four serum titrations (*units/ml.*)		Reciprocal mean observed endogenous serum titer (*units/ml.*)		Reciprocal estimated graphic titer (*units/ml.*)		Antibody destroyed per day (*units/ml.*)		Total antibody synthesized per day (*units/ml.*)		Milligrams antibody protein synthesized/ gram wet weight lymph node/day	
	H	O	H	O	H	O	H	O	H	O	H	O
31					9.95	5.95	.20	.27	.25	.07	2.48	.56
32					10.0	5.80	.20	.27	.25	.12	2.48	.95
33	20, 20, 20, 20	5, 5, 5, 5	10	5.0	10.0	5.60	.20	.26	.20	.06	1.98	.48
34					10.0	5.50	.20	.25	.20	.15	1.98	1.19
35					10.0	5.35	.20	.24	.20	.09	1.98	.71
36					10.0	5.25	.20	.24	.20	.14	1.98	1.11
37					10.0	5.15	.20	.24	.20	.14	1.98	1.11
38					10.0	5.05	.20	.23	.20	.13	1.98	1.03
39					10.0	4.95	.20	.23	.20	.13	1.98	1.03
40					10.0	4.85	.20	.22	.20	.12	1.98	.95
41					10.0	4.77	.20	.22	.20	.14	1.98	1.11
42	20, 20, 20, 20	5, 5, 5, 5	10	5.0	10.0	4.70	.20	.21	.20	.14	1.98	1.11

Additional serum titrations

Days after initial antig. challenge		61	75	79	86	90	93	103	117	131	145	161	166	169	181	195	209	222	237
Reciprocals, four serum titrations (*units/ml.*)	H	10	20	20	20	20	20	20	20	15	15	15	15	15	10	10	10	10	10
		20	20	20	20	20	20	20	20	20	15	15	15	15	15	10	10	10	10
		20	20	20	20	20	30	30	20	20	20	20	15	15	15	15	15	10	10
		30	20	20	20	20	30	30	20	20	20	20	15	15	15	15	15	10	10
	O	3.75	3.75	2.5	2.5	2.5	7.5	5	5	3.75	2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
		3.75	3.75	3.75	2.5	2.5	7.5	7.5	5	3.75	2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
		3.75	3.75	3.75	2.5	2.5	7.5	7.5	5	3.75	2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
		3.75	3.75	5	2.5	2.5	7.5	10	5	3.75	2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
Recip. mean endog. titer (*units/ml.*)	H	10	10	10	10	10	15	15	10	8.75	7.5	7.5	5	5	3.75	2.5	2.5	0	0
	O	3.75	3.75	3.75	2.5	2.5	7.5	7.5	5	3.75	2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5

to foreign tissue antigens and of the feasibility of artificially endowing him for a prolonged period with a functional miniature reticulo-endothelial system. The data suggest that the eight transplanted lymph nodes survived with full function for 100 to 110 days, and that destruction of the tissue occurred slowly, culminating in total unreactivity about the 160th day.

The recipient's passively acquired tuberculin hypersensitivity, which reached a peak only gradually, remained constant for three months, and then, coincident with cessation of node function, fell abruptly to a lower level, has continued at that level for a prolonged period. In the absence of evidence for transmission of tuberculosis, this pattern of transferred reactivity conforms to one of those described by Lawrence (15) and interpreted by him and by Chase (30) as suggestive either of two distinct phases—one passive and one "active" —in the recipient's handling of a single transfer substance in intact or disrupted sensitized leukocytes or, alternatively, of two leukocyte substances —one more and one less available—involved in the transfer. If it can be assumed that the transplant was totally destroyed by the 160th day, then the evidence tends to support the first hypothesis, inasmuch as only a portion of the reactivity was lost when the exogenous leukocyte source of hypersensitivity was removed.

The recipient's development of a specific, delayed-type cutaneous hypersensitivity to leukocytes derived from the donor, together with her failure to develop circulating agglutinins to these leukocytes, are in accord with abundant evidence of the pre-eminent role of fixed tissue antibody rather than of circulating antibody mechanisms in the rejection of homografts and homotransplants (31–34). It was not deemed clinically justifiable to test the possibility that the recipient would manifest an accelerated rejection of another lymph node from the specific donor.

All other technical considerations being equal, the limiting factor in the longevity of transplants to patients with hypogammaglobulinemia is thus apparently the degree to which this diffuse disease of the reticulum has impaired the patient's capacity to develop fixed tissue antibody. Though usually markedly impaired, this capacity is well retained in a minority of patients with acquired hypogammaglobulinemia (35); moreover, its presence—though very weak—in congenitally hypogammaglobulinemic children has been demonstrated by Good (3) and Porter (36).

The histological findings in the excision biopsy neither confirm nor deny the evidence for either the plasma cell or the lymphocyte as the major cellular source of circulating antibody (37). The finding of relatively small numbers of plasma cells in microscopic sections may mean only that the pathologist's "half" of the specimen contained a small, unrepresentative portion of the transplant.

Whether or not the transplant ameliorated the recipient's hypogammaglobulinemia is a moot point; her clinical course before, during and after transplantation differed in no striking way from that of progressively severe hypersplenism, or of progressive neutropenia of any etiology. Conversely, it may be that the presence of neutropenia during the life of the transplant precluded effective utilization of any antibody synthesized by the transplant and that transplantation was therefore not given a fair test. Whether the theoretically beneficial effects of lymph node transplantation justify the risks (principally that of contracting serum hepatitis) is thus still conjectural, at least in the case of acquired hypogammaglobulinemia. In congenital hypogammaglobulinemia, on the other hand, in which one may anticipate survival of transplants for years rather than months, lymphoid tissue homotransplantation remains an attractive therapeutic possibility (35).

Laboratory observations

As predicted by Boyd and Hooker (38), who demonstrated an inverse log-log linear relationship between the ratio by weight of antibody to antigen in equivalence-point precipitates and the molecular weight of antigen, the equivalence-point ratios of typhoid H- and O-antibodies to whole bacilli antigens were very low. Consequently, individual measurements of antibody precipitated from most serum specimens fell close to the limits of accuracy of the quantitative agglutinin procedure (9)—just beyond the error of the micro-Kjeldahl technique —resulting in curves the standard error of the slopes of which is relatively high: 7 per cent for H-antibody and 25 per cent for O-antibody.[10]

[10] Because comparably high standard deviations were associated with both repeated determinations of the antibody nitrogen in one serum and single determinations of

TABLE II

Measured rates of protein synthesis by various human and animal tissues, and rates estimated from recent human biosynthetic turnover data

Tissue	Reference	Method	Protein synthesized	Experimental conditions	Rate of synthesis (*mg./gm. wet weight tissue/day*)
			Normal human tissues, *in vivo*		
Lymph node	This paper	Tissue slices homotransplanted to hypogammaglobulinemic adult	Typhoid O-beta$_2$-globulin	Immune response: Peak, primary response	3.6
				Peak, secondary response	10.5
				Artificial acquired immunity, late	1.0
			Typhoid H-gamma-globulin	Peak, primary response	6.0
				Peak, secondary response	11.0, 11.5
				Artificial acquired immunity, late	2.0
Reticulo-endothelial system	(22)	Oral S^{35} and S^{35} labeled, i.v.	Gamma-globulins	Steady state*	1.7–3.9
	(24)	Oral S^{35}			1.7–5.4
	(51)	Oral N^{15}			5.4–5.8
Liver	(22) (53)‡	Oral and i.v. S^{35} and S^{35} labeled, i.v.	Total plasma protein	Steady state†	7–15
	(22)	Oral S^{35} and S^{35} labeled, i.v.			3.4–6.4
	(24)	Oral S^{35}	Albumin	Steady state†	2.9–5.3
	(51)	Oral N^{15}			7–10
	(22)	Oral S^{35} and S^{35} labeled, i.v.	Fibrinogen	Steady state†	1.4–2.4
			Normal animal tissues, *in vitro*		
Liver (rat)	(55)	Perfused whole liver	Total plasma protein	Optimal perfusion conditions; tissue from fasting rats	15–18
	(56)			Tissue from non-fasting rats	50–70§
Liver (chicken)	(58)	Tissue slices	Albumin	Optimal incubation conditions	2.5–3.0
Pancreas (pigeon)	(59)	Tissue slices	Amylase	Tissue depleted of zymogen by carbamyl-choline pretreatment	15–25
Anterior pituitary (rat)	(60)	Tissue slices	Protein hormones	Optimal incubation conditions	45–55

* Estimates assume average adult exchangeable gamma-globulin pool of 75 gm. (22), and average weight of total adult reticulo-endothelial tissue of 500 gm. (52).

† Estimates assume average adult exchangeable total plasma protein pool of 530 gm., albumin of 300 gm., and fibrinogen of 20 gm. (54), and average weight of adult liver of 1500 gm.

‡ Serum protein.

§ Steinbock and Tarver estimate rate of 45 to 65 mg. per gm. wet weight liver per day in intact rat (57).

Although it is not possible to state categorically that the donor and the transplanted nodes underwent a primary immune response to typhoid vaccine, the data suggest that they did. Particularly with respect to H-antibody—ordinarily the most prominent antibody developed in response to immunization—the relatively long initial induction phase in the donor and in the recipient and the

different sera of equal titer (Table IV), it was concluded that the major error in the experiment lay in the immunochemical method rather than in the agglutinin titrations.

relatively low peak titers achieved in the donor (Figure 3) more closely resemble the pattern of the primary than of the secondary immune response to typhoid vaccine (39, 40).

Many investigators have demonstrated and have roughly measured the ability of specifically sensitized human and animal reticulo-endothelial tissue to muster a secondary immune response when brought in contact with the sensitizing antigen in a new normal or irradiated homologous or heterologous host or in the test tube (37, 41, 42). In contrast, because of the lack of an animal counterpart to the syndrome of hypogammaglobulinemia, technical problems associated with inducing varying degrees of immunologic unresponsiveness in animals through barely sub-lethal doses of X-ray or reticulo-endothelial blocking agents (40, 43, 44), and the nature of immunization itself, semiquantitative studies of the entire immune response, including the primary phase, have been accomplished in the intact host only with difficulty (45) and *in vitro,* not at all (40, 46).

The rates of antibody protein synthesis found in the present study (Table I) clearly represent first attempts at reasonable estimates of the order of magnitude of the immune response in man, rather than precise measurements of the reaction. Our estimates are, first, conditioned by the age of the donor, the metabolic state of the recipient, and the relative technical success of the transplantation procedure; second, subject to the cumulative error of the immunochemical measurements, the graphic titers, and the passive half-life values; and third, closely tied to our most critical assumptions and estimates—those concerning pool size and equilibration time. As has been recently emphasized (47, 48), the paucity of information on the last two points constitutes the chief stumbling-block to accurate studies of the biosynthesis and turnover of labeled proteins. Estimates of equilibration time of various plasma proteins range from 4 hours to 6 days (29, 48).

Pool size studies in this laboratory on three other hypogammaglobulinemic patients (19) confirm the results of Gitlin and Janeway (49), who demonstrated that as in the case of albumin (50) approximately half the gamma-globulin pool is intravascular, and the other half extravascular, corresponding to an apparent volume of distribution of 10 to 12 per cent of body weight. The excessively large (20 per cent) pool size in the recipient in the present study presumably reflects expanded plasma and extracellular fluid volumes, caused by her anemia (hematocrit 26 to 28) and moderate congestive heart failure.

With these reservations, the results of the present study are listed with those of several recent studies of protein synthesis in man and in animals (Table II). The comparison emphasizes the striking flexibility and relatively enormous proteosynthetic capacity of human lymphoid tissue. In response to a moderate antigenic stimulus, and with little or no previous experience, human lymphoid tissue is capable of synthesizing highly

TABLE III

Titration of ten sera of known relative typhoid O-agglutinin titer *

Known relative titer (*units/ml.*)	Two-fold plus intermediate dilutions: Four determinations of titer				Observed mean titer (*units/ml.*)	Error, mean titer (*per cent*)	Two-fold dilutions: Four determinations of titer				Observed mean titer (*units/ml.*)	Error, mean titer (*per cent*)
160	160	160	160	160	160	0	160	160	160	160	160	0
152	160	160	160	160	160	5	160	160	160	160	160	5
144	160	160	160	160	160	11	160	160	160	160	160	11
136	160	120	160	160	150	10.5	160	160	160	160	160	18
128	160	120	120	160	140	9.5	160	80	160	160	140	9.5
120	120	120	120	160	130	8.25	160	80	160	160	140	16.5
112	120	120	120	120	120	7	80	160	160	160	140	25
104	120	120	120	120	120	15	80	80	80	80	80	23
96	120	80	120	120	110	14.5	80	80	80	80	80	17
88	120	80	80	60	90	2.25	80	80	40	80	70	20.5
Mean error, mean titers, per cent						8.3						14.6
Standard deviation, individual determinations, per cent						12.9						23.8

* Mixtures in varying proportion of a post-typhoid immunization donor serum and known negative hypogammaglobulinemic serum, made up to encompass one dilution step, in decrements of 5 per cent. Mixtures were coded, scrambled, and titered by the method described.

TABLE IV

Immunochemical estimation of typhoid O-agglutinins: comparison of six determinations of one serum and single determinations of six sera of equal titer *

	One serum						Six sera						Antigen, 1.0 ml.			
Optical density, ppt. N	.148	.141	.144	.140	.141	.153	.148	.139	.139	.144	.153	.151	.136	.132	.137	.133
Ppt. N, μg.	68.7	65.4	66.8	65.0	65.4	71.0	68.7	64.5	64.5	66.8	71.0	70.1	63.1	61.2	63.6	61.7
Agglutinin N, μg., by subtraction	7.1	3.8	5.2	3.4	3.8	9.4	7.1	2.9	2.9	5.2	9.4	8.5				
Agglutinin N:																
Mean, μg.			5.5						6.0							
Stand. Dev., μg.			2.4 (44%)						2.8 (47%)							

* Volume of each serum specimen adjusted to contain 60 agglutinating units.

specific protein at a rate comparable with that at which endocrine and exocrine tissues synthesize such unvarying protein products as enzymes and hormones.

SUMMARY

After subcutaneous homotransplantation to a hypogammaglobulinemic adult, eight unsensitized human lymph nodes survived with full function for 100 to 110 days, and partial function for 50 to 60 additional days. Ultimate rejection by the recipient was associated with a delayed cutaneous hypersensitivity to leukocytes from the lymph node donor.

By the use of appropriate data on the immunochemistry, pool size, and passive half-life of typhoid antibodies, the immune response of the transplant was measured in terms of milligrams of antibody protein synthesized per gram wet weight of lymph node tissue per day.

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