J. Sedwick — Notes on

Histogenetics

antibody formation

Biopolymers

1958-1961

Survey journals:

Journ 9/57 - 58.

Battisto — progress report any publications?

a disease induced by lymph cells from allergic g. pigs. Was not transmissable. Serum gave skin reactions in some animals.

Normal g.p. serum induced 3 grades of reaction in diffuint animals.

Berenbaum, MC 1958 The antibody content of single cells.
J. Clin. Path. 11: 543 – 547

Glaxo Labs Ltd.,
Greenford, Middlesex

Radioiodinated SA/rabbits/parotid nodes. — on cell smears. — radioautograms to count bound Ag.

Average antigen fixed was 2.1×10^{-13} g.

If all the alcohol-fixed Ab is active, with valence of 2, the cell content was $1.25 - 7.5 \times 10^{-13}$ g Ab. Also assumes that all antigen can be counted, i.e., 2π geometry in fixation as well as development of grains. (This must be gross underestimate)

Note: Humphrey + Dulbecco: Ab turnover is 3 hours.

Note: Matthews formed myeloma to contain ~5 mg/g. globulin. All are calculated at 250×10^{-12} g. ∴ 1.2×10^{-12} is this estimate

10^{-11} ?

Billingham papers on tolerance: (write to Medawar)

1-10.

need to be fully reviewed. But B seems to have rather mystical views on "enhancement" — which is presumably based on desensitization — immunity.
(But cf. Raffel ch.)

~~Grasshopper~~
Bond + Crombie 1957

Rennie of restorative mechanisms.

Raises questions: viability of nuclei & little cytoplasm — or are these preexistent
p. 320 cells with almost no cytoplasm

Concludes that cellular transfer is certain; chemical stimuli may also play a role!

Burnet 1936 et al PRS 1936 146B:1-

discussion on immune theories

CAMERON

Terasaki Cannon + Longmire Jimm 9/58

— Chicken transplant specificity

Quote Cinader on nonspec of tolerance - i.e.,

[Cinader found newborn rabbits treated with HSA sometimes tolerant to $HSO_3\phi N=N-HSA$]

Confused picture of "individual specificity".

4/11

Sutherland & Campbell. 1958 Antigen coated glass as sp. adsorbent for Ab
Jmin 4/58.

purpose – method for non-pptg Ab! (Survey proposals for basic methods as detecting Ab.)

He used pavement marking beads. Washed with HNO_3–H_2SO_4, then treated with "CrCl". (Probably still advantageous to use resin powder.)

See also } Campbell, Leuscher & Lerman, 1951, PNAS 37:575
(Isliker Chem NYAS 57:225 1953.

Congdon, C. C. 1957 Experimental Treatment of Total-body Irradiation Injury: A Brief Review. Blood, J. Hem. 12: 746-753.

In general after 900 r of x-rays one to ten million nucleated bone marrow cells give optimal thirty day survival. However, as few as 50,000 cells will allow a small percentage of mice to survive. (Urso, P., and Congdon, C.C. 1957 The effect of the amount of isologous bone marrow injected on the recovery of hematopoietic organs, survival, and body weight after lethal irradiation injury in mice. Blood 12:251.)

"The effective cell type, or types, in the administered blood-forming tissue has not been clearly determined. Since dividing cells are necessary, the stem cells and reticulum cells of the hematopoietic tissues are implicated."

IV route best, interparitoneal effective, intramuscular and subcutaneous ineffective in mice.

Bone marrow implant may be delayed three to four days.

Billen, D. 1957 Recovery of lethally irradiated mice by treatment with bone marrow cells maintained in vitro. Nature 179:574.- indicated bone marrow cells maintained in culture were affective after 21 days. ? are these proliferating in culture?

Reviews evidence that the peripheral blood elements are of the donor types in theserescued animals.

Maxwell, R. E. and Weston, J. K. 1956 (Abstract). Amelioration of Myleran-induced bone marrow damage in rats with homologous marrow injections. Fed. Proc. 15:457. Chemical injury to bone marrow can also be reversed by implantation.

Secondary reactions noted with bone marrow of homologous origin. Congdon believes this is a reaction of the host to the foreign marrow. This paper does not discuss the use of other tissues from the donor although it is evident that spleen will also work.

Merwin, R.M. and C.C. Congdon, 1957 Repopulation of Hemato-poietic Tissues and Blood in Lethally X-irradiated Mice by Homologous Bone-Marrow Cells. J. of the Nat. Cancer Inst. 19:875-884.

Reviews other evidence that bone marrow repopulated the marrow, produced rbc, and under some conditions repopulated the thymus and lymph nodes of the irradiated host.

They used the sensitization to homografts as a test of the persistence of donor cells. They conclude themselves that there is extensive repopulation.

Proc. of Soc. for Exp. Biol. and Med. 96:797-800.

Schwartz, E. E., A.C. Upton, and C.C. Congdon 1957 A Fatal Reaction Caused by Implantation of Adult Parental Spleen Tissue in Irradiated F_1 Mice.

Fatal reaction caused by implantation of adult parental spleen tissue and so on. This is a rest of the runt disease reaction where parental transplants into hybrid irradiated recipients resulted in runt disease. In this investigation bone marrow rarely and infrequently, 11 out of 59, gave the runt disease whereas spleen cells gave extensive and prompt mortality. The bone marrow from presensitized donors was more regularly mortal. Other investigators have had higher incidences of runt disease with bone marrow and this may depend on the strain.

The disease was not found when isologous hybrid spleen cells were used, and therefore there is apparently competent replacement by such cells. The dose of parent spleen cells was correlated with the time of death of the recipient. Few recipients of less than 75,000,000 spleen cells died in the first thirty days, many others succombed in the second month. Whereas larger doses of bone marrow gave less consistent survival. There is nothing here on homograft receptivity of these chimeras.

Congdon, C.C. and I.S. Ursa,
1957 Homologous Bone Marrow in
the Treatment of Radiation
Injury in Mice. Am. J. Path.
33:767-767.

A discussion of the homologous bone marrow disease which they are unsure whether this develops from host versus graft or visa versa. This may not be the same as homologous spleen disease. It may reflect the total absence of the lymphocytic system. A few animals did survive for prolonged periods.

28-7

~~Ane~~ Dienes + ~~Schoenheit~~
Mallory Am J Path 8:689 (1932)

DHS to albumin injected into a tubercle. which suggests this is a response of characteristic cell type.
I propose DHS as feature of all mimmune responses.

DHS to Shm antigens, Hanks J Immunol 28:105 But rely on Raffel's wax expts →

Raffel 1949 J Exp M 90:53

See Reich for another statement (p. 346-7) of early acceptance of DHS to mmm, preceding antibody formation.

Dixon, F.J. and P.H. Maurer, 1955 Immunologic Unresponsiveness Induced by Protein Antigens. J. Exp. Med. 101:245-257.

Most adult rabbits could not be made lastingly unresponsive and the effect there is undoubtedly due to growth excess of the antigen, heterologous plasma proteins. However, in rabbits infused from the time of birth the induced unresponsiveness lasted throughout the period of observation, ten to eleven months, long after disappearance of all detectable foreign protein in the serum. These were looked for by capillary precipitin ring tests. All rabbits retained their confidence to unrelated protein antigens. Unresponsiveness was not transmitted to the offspring. Antibodies were also being formed against contaminating components of the innocula and therefore large doses are needed to induce unresponsiveness. "Assuming the usual half lives of foreign serum protein non-immune rabbits only a small fraction of a microgram of the infused proteins would be expected in the entire rabbit four to six months after the infusions.

" Whenever the immunologic mechanisms become operative the foreign material is present in considerable quantity and it may be difficult for the rabbit to treat this material as an antigen. In such a situation it is even possible that the foreign protein might be accepted as non-antigenic along with the host's own constituents." p. 255.

" No direct measurements for the last traces of foreign protein in the tissues are feasible."

Passively introduced antibody was not more rapidly eliminated, also tending to rule out the presence of significant amounts of the antigenic proteins. This anitbody was recorded as having a normal half life.

Nothing was determined however as to whether this passive antibody could accelerate the return of tesponsiveness and this is an important experiment to consider.

Apparently Dixon does not believe that persistence of the antigen is obligatory for unresponsiveness but his observations are inconclusive. Find references to the British work on the persistence of such antigens.

Dixon, F.J. and Paul Maurer, 1955 Specificity of the Secondary Response to Protein Antigens. 74:418-431.

When Bsa was followed by HSA a secondary response was found which included excess anti-BSA including BSA not reacting with HSA. On p. 429 several possible explanations are enumerated.

1. Some identical shared determinents present more often in BSA but not so readily detected in a absorption experiments on account of the rarity of the sites. However this should expect to participate in co-precipitation.

2. Storage of pre-formed antibody for which there is no precedent.

3. Inhibition of degredation of anti-BSA, again no precedent.

4. " Some mechanism for production of antibody to the original antigen is stimulated by the subsequent administration of a related antigen as more techniques are being developed by establishing the homogeneity of a protein. It is becoming apparent that there is no absolutely homogeneous protein... purified albumins may have as many as six components... however production of the entire spectrum of antibodies to the original antigen is stimulated by the second antigen bearing only a limited antigenic similarity to the original antigen. Antibodies oriented only to the determinents peculiar to the first antigen also increase. Therefore the second antigen could not be acting merely as a template but rather would be stimulating a pre-existing mechanism oriented toward all the immunological characteristics of the first antigen.

This has serious implications for the question of the range of antibodies that can be produced by the individual responding unit, to the cell. This point has to be tested more directly. The possibility cannot be excluded that there will be stimulative reactions by antigen for antibody containing cells that are not reflected in the precipitation test between antigen and antibody. But if this is the case one should detect a wider spectrum of response to the original antigen. This point has been studied in a paper by Maurer in the JM of Feb. 1944.

Dixon did not try mixed primary with unrelated antigen.

If the second antigen was unrelated to the first, for example, BGG, HSA, there was no effect. The effects seen were not large, the increases being of one order of magnitude only.

Roberts, J.C. and F.J. Dixon.
1955 The Transfer of Lymph Node Cells in the Study of the Immune Response to Foreign Proteins. J. Exp. Med. 102:

379-392.

Primary stimulated lymph node cells from rabbits transferred to X-rayed recipients and then given secondary stimulus. The amount of antibody synthesized by the individual innoculated cells was then calculated. They make the following assumed values:

1. Four billion lymphocytes equal 1 gram.
2. 350,000,000 molecules of rabbit antibody equal 0.1 micro-microgram of protein.

They calculate that each transferred lymphoid cell makes one-third of its weight of antibody during the next five days. This is considered to be a minimum estimate since not all cells can be maximally active. This level of activity is considered to be comparable to that of the entire lymphoid population of the secondary rabbit. He assumes that 20-40 billion lymphoid cells per kilo body weight are available in the rabbit for the immune response, and he used two kilo rabbits. For these estimates - Osgood, E.E., BLOOD, 1954, 9, 1141. Tivey, H. Li, J. G., and Osgood, E.E. BLOOD, 1951, 6, 1013.

total:

5-10 gm/kg. lymphoid cell

80 ml globulin pool ≈ 1 gm. GG.

turnover rate 1/5 days.

∴ lymphoid cells turnover about 1/25 their wt. per day

This is unstimulated total globulin.

Says 1 cell = 250 μ^3

cf. Later: Stimpl's results.

Dixon, F.J., and W.O. Weigle 1957 Antibody Production by Cells of the Neonatal Rabbit. Fed. Proc. 16:411.

"The inability of the neonatal rabbit to make antibody is well recognized. Recently it has been observed that adult rabbit lymphoid cells capable of antibody synthesis after their transfer to X-irradiated adult rabbits do not make antibody after their transfer to neonatal recipients. This suggests that it is the internal environment of the neonatal rabbit and not necessarily the lack of cells potentially capable of antibody production that is responsible for its immunological inadequacy. To test this possibility spenic and thymic cells of neonatal rabbits were exposed to shigella toxin in vitro, washed and transferred to neonatal and X-irradiated adult recipients. No significant agglutinin responses were observed in 14 neonatal recipients of live exposed cells or in 6 adult, X-irradiated recipients of heat killed exposed cells. However, in 9 of 20 adult X-irradiated recipients of live, exposed neonatal cells, significant agglutinin titers developed. This would suggest that lymphoid cells of the neonatal rabbit can, under certain conditions, make an antibody response. Attempts were also made to alter the internal environment of the neonatal rabbit by injecting into them normal adult lymphoid cells prior to the transfer of adult lymphoid cells sensitized to Shigella toxin. Significant antibody responses were observed in 16 or 38 neonatal recipients pretreated with normal lymphoid cells and prior to the injection of sensitized lymphoid cells and 0 of 23 neonatal recipients injected with sensitized lymphoid cells only. It appears from these data that injection of the normal adult lymphoid cells can make neonatal rabbits adequate recipients of antibody producing cells."

**Dixon, F.J. and W.O. Weigle, 1957
The Nature of the Immunologic
Inadequacy of Neonatal Rabbits
as Revealed by Cell Transfer
Studies. J.E.M.**

These are rabbit lymph node cells which were capable of primary response to BSA and BGG as well as Shigella extract. These cells do not produce antibody in newborn recipients. However cells transferred during active ABF do continue to produce antibody.

Dixon F.J. William Weigle,
and J.C. Roberts, 1957
Comparison of Antibody Responses
Associated with the Transfer of
Rabbit Lymph-node, Peritoneal
Exudate, and Thymus Cells.
J. Immunol. 78:56-62.

Peritoneal exudates as well as lymph nodes from pre-sensitized rabbits transferred sizable secondary antibody responses to x-rayed recipients.

Thymic cells transferred much smaller responses. He concludes that either macrophages or lymphocytes can mature presumably into plasma cells in order to make antibody. The exudate cells were as effective as lymph node cells. See reference 6 for other previous work on thymus. "It would be interesting to know whether preparatory injections of antigen directly into or near the thymuses of our donors would have resulted in better transferring of antibody synthesis." Contra these experiments- Roberts, K.B. Brit. J. Exper. Path., 36:199, 1955, did not transfer a response to bacterial antigens but it is impossible to take these two papers side by side.

Nell, A.L. and F.J. Dixon.
1958 Immunohistochemical
Detection of Antibody in Cell
Transfer Studies. Arch Path

Used preimmunized rabbits, to BSA, and lymph nodes. The suspensions contained 90 per cent lymphocytes, 8 per cent macrophages, and 2 per cent plasma cells. Fluorescent antibody technique was used to identify the introduced cells contining antigen and to follow their morphological transformation. Dixon concludes that in the primary response there is a transformation from lymphocytes into plams cells. By the fifth day most of the antibody-containing cells were of mature plasms cells. There was no great amount of mitotic activity but these animal had been intensively stimulated beforehand. "Considering the minimal mitotic activity in the transfer sites it would appear that during their antibody response the transferred lymphocytes metamorphosed to the plasma cells without division via the stages described."

Egdahl, R.H. 1958 Immunological Maturation and Defects in Immunological Capacity Int. Arch. Allergy 12:305-321.

The first part of this review refers to the development of antigenic specificity

"Brephoplastic transplantation applies to situations when the donor is fetal or new-born." There is at least one claim that embryonic parathyroid could be successfully implanted into adolescent patients. (7)(6) He then refers to studies on acquired tolerance an additional reference being (8).

Smith (13)

The mill

~~The~~ period embraces that time span when the animal response to the innoculation of foreign tissue with neither tolerance nor heightened resistance."

Different species mature at different rates with respect to skin rejection. For example, fetal sheep at 100 days reject skin homografts (Schinkel, P.G. and Ferguson, K.A. 1953 Skin transplantation in the foetal lamb, Aust. J. Exp. Biol. Med. Sci. 6: 533-546) and ditto newborn calves. He remarks that newborn sheep lack gamma globulin but nevertheless reject the skin. However, it is not apparent that gamma globulin was absent throughout the entire period of the homograft response.

There is a useful discussion on the maturation of gamma globulin in human children. Despite a high level of globulin, presumably of maternal origin, "the newborn infant is incapable of responding with the usual antibody production during the first few weeks of life." Osborn, J.J., Dancis, J. and Julia, J.F. 1952 Studies on the immunology of the newboen infant, I. Age and antibody production, Pediatrics 9: 736.

Then a review of Good's material, for example a child born from a globulin-negative mother. The child was born without globulin and remained so for six weeks of life. No antibody response was observed in the baby during the first two months. "Between two and four months coincident with the first appearance of gamma globulin in the infant serum there appeared agglutinins against the H, O, and B antigens, diptheria antitoxin and the child became shick negative at four months. Paper electrophoresis demonstrated a steady increase in gamma globulin concentration from 60 days onward, until at 10 months of age the pattern was apparently normal."

Discussion of the immunological immaturity of the newborn. Refers first to Dixon and Weigle but he mentions (p. 313) "They found that the transfer of normal adult lymphocytes prior to transfer of sensitized lymphoid cells into neonates led to antibody production by the transferred cells." Does Gus know about this? Dixon, F.J. and Weigle, W.O. 1957 Antibody production by cells of the neonatal rabbit, Fed. Proc. 16: 411.

The experiments by Sterzl who claimed that subcellular particles from immunized rabbits would transfer abf to 5 day old rabbits, believing that this was due to the mitochondria. Egdahl then refers to various theories of antibody formation. He then turns to hereditary defects in antibody formation giving a history of agammaglobulinemia. The leukocytes of these children are capable of transferring passive hypersensitivity to dinitrofluorobenzene.

Skin homografts have succeeded to globulin negative recipients. Giedion, V.A. und Scheidegger, J. J. 1957 Kongenitale Immunparese bei Fehlen spezifischer β_2-Globuline und quantitativ normalem Gamma-Globulin, Helvet, paed. Acta 12:241-259., is a case of specific lack of certain globulin components with homograft receptivity.

Good transferred homologous lymph node tissue subcutaneously to a globulin negative patient. Continued anti-samlonella antibody was demonstrated for a period up to two months but then disappeared. Author is concerned about possibility of runt disease.

Gengozian, N., I.S. Urso, C.C. Congdon, A.D. Conger, and T. Makinodan 1957 Thymus Specificity in Lethally Irradiated Mice Treated with Rat Bone Marrow. Proc. of Soc. for Exp. Biol. and Med. 96:714-720.

These are x-rayed mice given rat bone marrow. The thymuses were tested by in vitro agglutination with anti-mouse and anti-rat serum. As reported elsewhere there is considerable stimulation of recovery of thymus by the heterologous bone marrow. "Agglutination tests (p. 718) indicate a repopulation of the thymus by rat type cells in lethally irradiated mice treated with rat bone marrow. The agglutination tests showed that repopulation by rat cells was 50 per cent complete on about the 21st day after treatment and by 30 days all the cells in the thymus appeared to be of the rat type." This was confirmed by cytological examination. This result is in contrast to Kaplan's claim where parental bone marrow promoted regeneration of irradiated post-thymus by transplantation tests. Authors refer to experiments by Wolf and Upton where there was a small percentage of takes of regenerated thymus in the parent host.

The question is left upon as to the cellular origin of the thymus cells including the general hypothesis of a multipotent cell.

Germuth, F.G. 1956 The Role of Adrenocortical Steroids in Infection, Immunity, and Hypersensitivity, Pharmacological Reviews 8:1-24.

Ascribes major role to the effect of cortisone on "vascular tone". For example resistence to formation to petechia.

Cortison in Ebert's experiments reduced local reaction of rabbit ear to TB infection. This attributed to direct improvement of vascular response.

Argues against much direct effect on leukocytes.

Survey on these is confusing but concludes that there is no immediate effect on function. Halpin (Benacerraf, B., Halpern, B.N., Biozzi, G. and Benos. S.A. 1954 Quantitative study of the granulopectic activity of the reticulo-endothelial system III: The effect of cortisone and nitrogen mustard on the regenerative capacity of the R.E.S. after saturation with carbon. Brit. J. Exper. Path. 35:97-106.) found that there was no immidiate effect on the uptake of carban partiples by the RE system but that continued capacity to take up carban was inhibited by cortisone. This was attributed to the inhibition of the multiplication of the RE cells.

Cortisone inhibits wound healing and repair, again possibly secondary to vascular function.

Conflicting evidence on the lysis of lymphocytes. Affirmative - Chase, J.H. White, A. add Dougherty, T.F. 1946 The enhancement of circulating antibody concentration by adrenal cortical hormones. J. Immunol. 52:101-112.
Contrary - Eisen, H.N., Mayer, M.M., Moore, D.H., Tarr, R.R. and Stoerk, H.C. 1947 Failure of adrenal cortical activity to influence circulating antibodies and gamma globulin. Proc. Soc. Exper. Biol. and Med. 65:301-306.

This discussion of the effect of cortisone on antibody production is of little use in trying to elucidate the biological basis.

Effective cortisone on hypersensitivity is believed to result both from 1) inhibition of antibody formation 2) the anti-inflammatory effect. For example, " the vascular lesions of experimental serum sickness can be suppressed by doses of cortisone too small to influence antibody response." (Germuth, F.G. Jr. 1953 The mechanism of action of cortisone in experimental hypersensitivity. II. Hypersensitivi of the serum sickness type. J. Exper. Med. 98:1-12.) " However, allergic skin tests in man are not suppressed.

$H\text{-}2^a$	H-2A	CDEFK	A
$H\text{-}2^b$	H-2B	cD^{b}EFk	C57BL, C57L, LP, 129Rr
$H\text{-}2^d$	H-2D	CDE^{d}Fk	BALB/c, DBA/2, C57BL/6KS etc.
$H\text{-}2^{d'}$	H-2D'	CD'E^{d}Fk	YBR/R2, YBR/Wi
$H\text{-}2^k$	H-2K	CdEfK	CBA, C3H, C57BR/a, C57BR/cd, ST
$H\text{-}2^s$	H-2S	C"S"EFk	ASW
$H\text{-}2^q$	H-2Q	C"Q"EFk	DBA/1
$H\text{-}2^p$	H-2P	C"P"E?k	P

Gorer, P. A., 1956 Some Recent Work on Tumor Immunity. Advances in Cancer Research, IV; 158.

Gustafsson and Laurell: 1958 Gamma Globulins in Germ-Free Rats. J. Exp. Med. 102:251-258.

His Summary.

The methods of evaluation of Gamma-Globulin are somewhat dubious and the germ-free animals may have had from none to as much as one-fourth of the globulin level of the controls. The beta globulin was only slightly depressed and the total protein and albumin were normal. They conclude that the gut flora plays some role in stimulating globulin production.

Harris Hanes + Faber

Shigella ab. develops after sensitized cells transferred in rabbits.

pre-sensitization of recipients a week before transfer prevents development of antibody (immune suppression?) Vat agglutinin titres up to 500.
These lymph node cells generally sensitized against with typ specific extract.
Generally transferred 2×10^8 cells; get titres 100-500!
Similar results with donor cells presensitized in vivo

Interpreted as second set rejection of transferred cells.

This is considered corroborative evidence of role of cellular activity.
∴ conclude that primary responding cells are present in lymph nodes.

use sensitized donor cells to destroy homologous cells of host??

(a measure of survival (adoptive immunity).

Law, L.W., and M. Potter, 1958 Further Evidence of Indirect Induction of X-radiation of Lymphocytic Neoplasms in Mice. J. Nat. Can. Inst. 20:489-493.

Review of thymus neoplasms and in particular the work of Kaplan and Brown. According to Law and Potter " the possibility of repopulation of AKR thymic grafts by (C3H x AKR) F_1 host cells. - Law, L.W. 1952 Increase in incidence of leukemia in hybrid mice bearing thymic transplants from a high leukemic strain. J. Nat. Cancer Inst. 12:789-805. Law and Potter had made thymic grafts into irradiated hosts and found that most of the tumors had come from the host and a smaller number of the tumors from the grafted tissue. Later Kaplan had done a similar experiment which indicated " the tumor cells behaved genetically as if they were derived from cells of the implant." The present article is an extension of this study. Perhaps the point of difference is that in some of Kaplan's work the thymus was excised.

A number of thymic grafts were made into the right axillary region after irradiation of the recipient. About half of the tumors were of host, half of recipient origin, thus substantially confirming Kaplan.

The tumors were mainly lymphocytic, but see reference 10. (Dunn, T.B. 1954)

Luzio, N.R., K.A. Simon, B.A. and A.C. Upton. 1957 Effects of X-Rays and Trypan Blue on Reticuloendothelial Cells. A. M.A. Arch. of Path. 64:649-656.

"Exposure of young adult male rats to 400 r of whole-body x-radiation fails to inhibit the hyperplasia of reticuloendothelial cells induced by subsequent administration of trypan blue."

These authors also failed to blockade the res by trypan blue and they criticize the general concept .

McMaster, Philip D, Edwards, Joshua L., and Sturm, Ernest. 1955 Active Anaphylaxis to a Foreign Protein Induced in Mice by the Transfer of Tissue

from Animals Previously Injected with the Protein. J. Exp. Med. 102:119-131.

BGB?

The main point on this is that BSA persisted in rabbit liver for at least six weeks as detected by the capacity to induce anaphylactic sensitivity in mice. By this time the serum was inactive. There is of course circulating antibody in the serum of these animals. There is no reference to persistence in other tissues.

MAK

Halberstadter Jimm 10/57 Radiated mice

delayed reaction in rescue is host vs BM! Jimm 77:430 '56

MEG before X-ray gives substantial protection

950r 180/181 died. Most animals with MEG survived.

ab cells are not completely destroyed in MEG-950r. Anti sheep returns faster than anti mouse on recovery.

① either multiple types of ab cells or

② ab cell has various grades of recognition, the closest being the most sensitive to X-ray.

distinction of homo and heterograft acceptance is explained this way.

Makinodan, T., Gengozian, N. and Shekarchi, I. 1958 Relative Effects of Splenic and Bone-Marrow Cells on Lethally Irradiated Mice. J. Nat. Can. Inst. 20:591-600.

Still arguing about the etiology of runt disease.

Set out to investigate mechanisms of antibody production after irradiation. Irradiated animals immediately after radiation received isologous spleen or bone marrow as well as an immunizing dose of rat blood cells. As one might expect there was a substantial response of the spleem treated animals and not of the bone marrow treated animals. The irradiated untreated mice all died within 13 days but both spleen and bone marrow were however effective. Heterologous spleen caused rapid death and heterologous bone marrow slowly increasing deaths up to a period of 60 days. Two doses of heterologous spleen were used. 12 x 10^6 of [redacted] cells (?) and 10 times this amount. The lower dose gave somewhat slower killing. When I refer to heterologous here I mean homologous strain of mice.

"Only in preimmunized animals has it been reported that antibody-producing cells were detected in the bone marrow. Kolouch, F. Jr. 1938 Origin of bone marrow plasma cell associated with allergic and immune states in the rabbit. Proc. Soc. Exper. Biol. and Med. 39:147-148.
Askonas, B.A., and R.G. White 1956 Sites of antibody production in the guinea pig. The relation between in vitro synthesis of anti-ovalbumin and gamma globulin and distribution of antibody-containing plasma cells. Brit. J. Exper. Path. 37:61-74.

"In an attempt to induce skin-graft tolerance by injection of adult homologous splenic cells into newborn mice, Billingham and Brent obtained comparable results. Billingham, R.E., and Brent, L. 1957 A simple method for inducing tolerance of skin homografts in mice. Transpl. Bull. 4:67-71. These treated newborn mice appeared normal for the first week or two but died soon after.

They criticize the experiment of Uphoff (Uphoff, D.E. 1957 Genetic factors influencing irradiation protection by bone marrow. I. The F_1 hybrid effect. J. Nat. Cancer Inst. 19:123-130.) on the role of graft immunity to the host, but they appear to be back-tracking just a little bit. They feel more controlled experiments are necessary.

Makinodan, T. and Gengozian, N. Primary Antibody Response to a Distantly Related Heterologous Antigen during Maximum Depression Period after Varying Doses X Radiation. J. of Imm. 81: 150.

Mice were irradiated and sensitized to sheep rbc.

The maximum depression of antibody response occurred when antigen was given within 24 hours after X-ray. The main effect was to greatly prolong the induction period. No effect of isolygous bone marrow on the induction period was found. They therefore conclude that normal bone marrow does not produce antibody forming cells. They did not however use bone marrow from sensitized donors.

Gengozian, N. and Makinodan, T. Antigen Injection to Time of Irradiation on Antibody Production in Mice. 1958 J. of Imm. 80: 189.

'Maximum immunologic depression occurred in mice receiving antigens five minutes to one day after x irradiation. Although there was a progressive increase in the response to antigen as the interval between x irradiation and antigen injection was increased the immune mechanism of irradiated mice never returned to a completely normal status.

Irradiation five days after antigen showed total titre above normal.

These results are in conflice with the effects on rabbits which show complete inhibition of antibody formation.

On p. 193 an index "relative immune status" was made using four different measures of antibody effect. They found a reduction at maximum from 4 $\log_2$ down to about 1 $\log_2$ units when the antigen was given immediately after x-ray.

11/2/58 Medawar 1958

skin grafts sensitize more effectively than IV injections!

See H Hellman for adaptive immunity.

any tissue induces complete host/graft tolerance for any other. RBC are not antigenic (for 2° set response).

see p. 163 Medawar also tries to complicate H vs T antigens. Does he imply >1 product of a single Hg gene? Is this justified by studies on isogenic-resistants?

This is importance of using such lines! See 35 54 – Ann NYAS '57
+ M, Thomas + Dube JEM 102:179
Different physical sensitivities. ∴ Thinks that H and T are different substances. & Billingham Nature 178: 514 '56

I suggest using $H^a + T^b$ to provoke anti-T^a, assuming T^b contains adjuvant materials! He is destroying the necessary adjuvants for local response. (write Raffel!)

See also Barry for sensitivity to H antigens after T immunity!

Mitchison 1956

Spleen cells into irradiated mice of the a and the cba standard imbred strains. Effective colonization of the recipients was shown by the iso antigens of the graft in the spleen by virtue of their ability to sensitize new hosts to transplantation.

He also demonstrated considerable but not total destruction of antibody forming ability of irradiated mice as against salmonella typhi h antigen. The immune cell s continued to produce antibody after transfer especially in irradiated as compared to normal recipients. Pre-immunized recipients showed considerably less production of continued antibody.

V A Najjar + Fisher J 1955 Science 122:1272

Nossal, G.J.V., and Lois Larkin 1959 Failure to Induce Immunological Tolerance during Recovery from Irradiation.

He gave mice 850 r of X-rays and rescued them with intravenous isologous bone marrow in large amounts. Subsequently they were given injections twice per week of rat rbc. No tolerance was found although similar treatments of newborn mice will induce tolerance.

He may not have used large enough doses of irradiation, the bone marrow he used may have been responding to the antigen and this is not at all unlikely in view of the large amount, and in any case from Gengozian's results mice do not become completely unresponsive after irradiation.

cf. Dixon and Maurer 1955.

(R)

Odell, T.T. Jr, F.G. Tausche, D.L. Kindsley, and R.D. Owen, 1957 The Hemotransplantation of Functional Erythropoietic Elements in the Rat Following Oc

Total body irradiation
64:811-823
Ann NYAS 3/57.

a) rat chimaeras survived. Many of these are stable for months: established by bone marrow.

b) mice Abf of X-ray, rescued animals not thoroughly investigated. But A x (A·B) Main + Prather: 850 r.

accepted 33/36 grafts from B. 24-30 days later. also, 2/31 A x A accepted B!

Have these animals recovered abf by this time?

The ability of the grafts to abf not studied in either fetal or x chimaeras.

after

Why do they say this is unspecific? Should test on A x B!

[also, bone marrow may reach the few surviving cells of host to induce tolerance in them. Presumably the marrow has a very small population of abf cells!]

~~AB hosts x BC~~

any unit disease with bone marrow?

? Induce tolerance in X-rayed, bm rescued mice? Use tolerant marrow might?

<u>Raffel</u>

Mixture of antigens (pneumo.) gives higher total response. p60

Hektoen + Walker - conditioned reflex p.71 + fragment in infectious mononucleosis.

In rabbit, monkey and man all antibodies are monodisperse. In horse anti SSS has mw = 900,000 instead of 150,000 Anti toxin = 184,000.

In man Wassermann, anti O and isohemagglutinins are ~10^6. Deutsch 1947

allergic antibody is heat labile.

natural antibodies poorly specific p. 84.

Is complement fixed or destroyed? — see Heidelberger.

ablastin is not absorbed by sensitized trypanosomes p.131
ditto anti anthrax factor.

Mechanism of antigen specificity 217
[illegible] 230

Arthus - local vascular damage.
anaphylaxis

Immediate [illegible] [illegible] by [illegible]
Delayed by cells only. Sens. usually three skins + more number

Which cells can be hypersensitized? — cornea, bone marrow. Is it conceivable that every cell has individually responded to the antigen? Or is there a humoral phase? Should look at the pathology of sensitized cornea. See Raffel's reports on waxes as predisposants to HS. albumin + wax → albumin HS. These waxes also provoke local monocyte accumulation. Which <u>cells</u> are hypersensitive

Contacts - in [illegible] - [illegible]

Fundamentally is H.S. a parallel but independent process only inhibited by mature antibody? See Chase.

Raffel's account of anti-flu is weak.

See Merrill Chase in Dubos — p144

generalized reactivity after cell x plant species for some humoral element. He has germ of idea of a special ab produced by a cell type provoked by grafts.

p149 lymphocytes play special role (Ist they do in homograft rxns.)

NOTES

Rosenberg, L. T., Chandler, M.H. Gordon, A. S., and Fischel, E.E. Antibody Production by Guinea Pig Cells Demonstrated by the Passive Cutaneous Anaphylaxis Reaction. 1958 J. of Imm. 81: 136-141.

Authors transferred cells from guinea pigs pre-immunized to egg albumin. After 7 days various organs were removed and cells transferred. Reactions were obtained with "as few as 1,000,000 cells" to sensitize the skin. Authors refer to this as PCA and this seems to be the same experiment as that by Chase on the transfer of hypersensitivity. The reactions were delayed for periods of 1-3 days which they account for on the basis of the amount of antibody persent. I am somewhat confused about the distinction between immediate and delayed hypersensitivity. "In the present study the generation of immediate type hypersensitivity would appear to be dependent upon the structural integrity of the cell."

The effectiveness of cell extracts in transfer is controversial:

Affirmative Waltzer, M., Bowman, K. L., and Stroyman, S., 1957, J. Allergy, 28:206.

p. 17, and negative Friedman (No. 18) Freedman, S. A., Fisher, P., and Cooke, R. A., 1957, J. Allergy, 28:501.

If all cells were active they indicate a rate of production of one molecule antibody per second per cell.

The administration of cortisone to the recipients did not influence the reaction. He states that cortisone is already known to inhibit primary sensitization of the donor.

Spleen cells were consistently effective; thymus occasionally effective in sensitizing recipient skin. Lymph node, bone marrow, circulating leukocytes, and liver did not sensitize.

Santos, G.W., and L.J. Cole 1958
Effects of Donor and Host Lymphoid and Myeloid Tissue Injection in Lethally X-irradiated Mice Treated with Rat Bone Marrow.

J. Nat. Cancer Inst. 21:279-293.

X-rayed mice were treated with rat bone marrow which by itself results in rapid rejection of the rat tissue and death. Claimed that the graft persists longer if isologous liver, lysed or unlysed, and other lysed tissued are also injected. Survival of the mice is significantly shortened when spleen or thymus is injected with the rat bone marrow.

"The most likely explanation is that the injected isologous spleen and thymus cells are capable of initiating an immunological response against the foreign rat tissues."

Trenton has shown that tolerance to homologous skin grafts induced by lethal irradiation and homolous bone marrow can be abolished by the injection of isologous lymph node or of spleen cells from an unirradiated animal of the host strain.

Reviews antibody production by thymus and notes that the evidence for it is meager. This is mainly the work of the Harrises. He fefers however to Dixon and Stoner as affirmative. "It should be emphasized that the search has been for the production of antibodies of the arthis type and not for the delayed tuberculin type of sensitivity." This is presumably in reference to the effect of thymus in accelerating refection of rat bone marrow.

Smith and Bridges: Immunological unresponsiveness in Rabbits Produced by Neonatal Injection of Defined Antigens. J. Exp. Med. 102:227-250. Details in print, as requested.

A single injection of BSA of from 10 to 100 mg induced tolerance lasting 90 to 120 days. Reinjection of antigen during these intervals gave indefinite prolongation of tolerance. Persistance of antigens could not be directly verified but is assumed the basis of these results.

Page 245. The data presented would suggest that the critical antigen may be located intracellularly unavailable to passively administered antibody, (reference 13) since such antibody has no effect upon the unresponsive state. I don't recall that Dixon established this specific point and it should be looked up again. Item: The liver is a known site of prolonged persistance in the course of antibody production. (reference 43). He does not quote McMaster, which may give more data on this point. Item: The authors apparently have a clear picture that persistance of antigen must be considered the mechanism of continuation of tolerance. They feel that these conditions are not accounted for in current theories of immunity. Item: How should one tie in the incompetence of neonatal animals? The fact that even in an adult animal, which is immunological competent, the reappearance of active cells can be surpressed by reinjection of antigen would indicate that neonatal incompetence is not directly relevant. Item: These authors also failed to give tolerance to a number of bacterial antigens but they comment on the possible difficulty of saturating the responding system. Note: They used relatively small quantities to TAB vaccine, no more than ten to the eighth organism.

STAV

Staintelay. J Imm 9/57.

see Fed Proc 1957 Review

Synthesized from 9-9.

1. Rabbit 2. Rat.

Used dyes + tet. toxins & BCG and hemagglutination.

maintains that homologous serum suppresses abf in vitro.

pre-X-rayed + pre-cortisonized animals still serve as recipients for homografts abf falls after interval

---

Schlossman + Stetson J Imm 9/57.

Comroe has become vascularized in his rats . . . lose this support for dimt all toxicity in TB bacilli.

Taliaferro - X-ray effects Journ 66:181

Talmadge 1958

Central arguments for natural selection.
"Harmonty" ± 50,000 species
Ehrlich

eliminate concept of absolute specificity. Antigen can react with many ab and conversely. Reactive sites ± 50,000. "In view of the large amount of info. carried by the chromosomes ... variety of globulin molecules containing substantial fraction of possible ab sites are synthesized in the absence of any information provided by environmental antigens. (Many Ab may have heterospecific multiple sites??)

"Any mechanism of selectivity amplifying those protein molecules with high affinity for antigen will result in the production of 'abs'".

But no detailed model: ?Nature of stimulus. Proposes feedback of Ab — this is not supported by other methods of depressing Ab production. But Specificity of binding is fact. Theory of tolerance? Not developed in detail.

Toolan

3' Host transplantation Conference.

Bacteriostatic xpl. Toolan : (1)

With carefully controlled dosages of cortisone grafts could be maintained over very long periods. But slow rejection when c. was withheld. [Why slow? (a) graft in place (b) residuum of c. and time needed for cells to develop. (c) compare timing with that in adoptive immunity.]

Steps seen : (1) haphazard of ground substance (2) polymorph. invasion ± c/no cytotoxic effect (3) lymphocyte invade + tissue is destroyed.

Much emphasis on (1).

Young bacteriografts take ± cortisone. These never developed adequate connective tissue. Many structural proteins unanswered. Responds to stress or gr. subst. [which may not be the transplant antigen!].

In this case bacteriograft may depend on inhibited development of tissue specificity.

TRE

Tremaine:
Donor rabbits were sensitized to horse serum by intradermal injection & adjuvants.
The exudate cells transferred DHS to rabbit corneas
from such donors.

Nothing was said ^to explain of ab response but "antiserum" inhibited the reaction:
but "the procedure gives ab of titres to 1:100.

∴ presumably exudate cells are generally HS, but not in presence
of ab.

also refers to ~~Tremaine~~ Dienes & states a duality hypothesis.
Dixon et al. find exudate cells are abf.

Trentin, J.J. 1957 The Immunological Basis for Induced Tolerance to Skin Homografts in Irradiated Mice Receiving Bone Marrow Transfusions. Trans. Bulletin, 4:7478.

"The following unpublished data from this laboratory bear on point (d). A suspension of lymphocytes from lymph nodes and/or spleen of normal unimmunized adult CBA mice was injected intr-peritoneally and/or subcutaneously into mice of each of 3 experimental groups: (1) 10 CBA mice bearing skin autografts of 94 to 109 days durations. (2) 9 CBA mice bearing successful homografts of Cb skin (Balb/c without milk factor) of 162 to 182 days duration; the long survival was due to irradiation with 770 r. followed by transfusion with (Cb x CBA)F_1 hybrid marrow, 36 to 56 days prior to skin grafting. (3) 3 CBA mice bearing successful homografts of A skin of 101 to 150 days duration as a result of injection of A strain marrow during fetal life; the skin was grafted at 53 to 66 days of age, as in the experiments of Billingham et al" Billingham, R.E., Brent, L., and Medawar, P.B. Nature 172:603, 1953.

" No change in the skin grafts was noted during the first 2 weeks. By the 18th day however, signs of early breakdown were apparent in all 12 homografts (groups 2 and 3). Of the 9 Cb homografts (groups2) 5 underwent complete breakdown followed by death of the host, with reduced erythrocyte count in 4 cases; one was in early stages of breakdown when the host died of internal hemorrhage; another was in late stages of breakdown when the host died; the 2 remaining mice are alive, one with approximately 25 per cent breakdown, of the graft the other with apparent recovery after only slight initial breakdown.

"Of the 3 A homografts (group 3), one has completely broken down, and 2 have almost completely broken down. No mortality or erythropenia has occurred in any of these 3 mice. Of the 10 autografts, none has broken down and none of the hosts has died."

Trentin seems to believe that in these cases the maintenance of the graft is due to the absence of recovered lymphoid tissue in the host. However, there seems to be other quite good evidence of mixture of cell types so there still is an unsettled controversy here.

c/ Main + Prehm '55

Uphoff, D.E. 1958 Preclusion of Secondary Phase of Irradiation Syndrome by Inoculation of Fetal Hematopoietic Tissue Following Lethal Total-Body X Irradiation. J. Nat. Cancer. Inst. 20: 625-632.

""Under certain, as yet undefined, conditions, a few animals may survive the reaction, whether it is initiated by the graft or the host. The ability of a few animals to survive suggests the possibility that experimental conditions may be found which will preclude this immune response, though to date various attempts to do so have met with little success." Congdon, C.C. and Urso, I.S. 1957 Homologous bone marrow in the treatment of radiation injury in mice. Am. J. Path. 33: 749-767.

Suggested using fetal hematopoetic tissue despite failure by Congdon and Urso just quoted. This is a preliminary report of successes.

Used a BD hybrid and a B donor. Used a dose of 800 r and marrow from four bones on the one hand and homogenate of fetal liver and spleen from 6 or 7 embryos on the other. Implants were about 4,000,000 cells in each case. Adult marrow gives from 11-34 per cent survival at 90 days whereas the fetal marrow gave from 90-100 per cent. She may have induced tolerance in the implant. But there seems to have been no systematic study of the use of smaller doses of bone marrow.

Uphoff, D.E., and L.W. Law
1958 Genetic Factors Influencing Irradiation Protection by Bone Marrow. II. The Histocompatibility-2 (H-2) Locus

J. Nat. Cancer Inst. 20: 617-624.

Uphoff claimed that hybrid marrow affords better protection than marrow of either parental strain in a hybrid recipient. In this study used coisogenic lines. Homozygous DD mice recieved inoculations of BB, DD, or hybrid BD marrow after 700 r total body x-ray. Results of reciprocal implants compatible with the hypothesis of a necessary graft versus host reaction. However the 90 day survivals were 45, 12, 69, 1 per cent for implant of DD, BB, DB, and none respectively into DD mice. Thus on the face of it the hybrid was even better than the isologous implant. With hybrid BD recipients the figures are 0, 44, 88, and o respectively. There is no particular explanation of these differences.

WAKSMAN

10/11/58 Waksman 1958 J Exp Med (I)

Tuberculin as causing hypersens cells.

Note: mild tuberculin reactions are proliferative, not necrotic. Considerable BJEP 35:609
See Progress in Allergy 5 1958.

Parenchymal cells are generally not affected in T/C, though migrating macrophages are.

Stresses lack of quantitative paper data — Uhr JEM 105:11
Cohn JEM 102:61

Apparent conflict with Rich's early data on cellular destruction.
May be a different class of cells:

cells migrating from spleen + marrow — destroyed (Rich)

exudate cells in sterile cavities — "stimulated" (Waksman

"perhaps former are not true fibroblasts or macrophages"

intermediate mononuclears show accelerated differentiation → large macrophages.
also lymphocytes ?

refers to Dienes old hyp. Need to see his review.
Idea of ... series ... - numerous steps.

Peters had shown cutaneous sens. by exudate cells in local implants (i.e. these cells are sensitized)

II Exudate cells transferred local sens. only in skin; spleen cells rarely did. Raises question whether transferred cells react directly with antigen or whether they produce an "antibody" to sensitize the host. Waksman does not clarify this point.
(over)

Does HS due to reactions of another cell type; these cells are not themselves HS but they can transmit HS

? lymphocytes HS
plasmocytes Ab ?

Rich: splen fragments : necrotic response.

Weissman: splen fragments : no transfer of HS

exudate cells : stim response transfer of HS

∴ contradicts Rich's conception of hs.

Did W's method sensitize the splen? (scabe thigh or intra forehead)

D. Hypersensitivity notes.

Most hypersensitivities are "non antigenic" [or is effect blocked by antibody?]

See Raffel for role of lipid in sensitizing to various antigens
does this provide a unique cell type?

Which cells are primarily, specifically altered?

" " secondary targets?

Not transferable to normal animals by HS serum. ds → by cells (lymphocytes?)

[Does specificity depend on serum?

Reagins — skin sensitizing; thermostable; non precipitating or neutralizing.
But unknown in amount or chemical identity.

10/11/58.

DELAYED HYPERSENSITIVITY____

(per Chase and Raffel)

1. Demonstrated by delayed reaction of skin, cornea & fibroblasts in T/C
2. Provoked in Tbc: by protein plus wax. Can also be developed by albumin plus wax best given intracutaneously.
3. Also contact sensnitivity to low mw compounds, exp. picryl chloride, which acc Raffel might interact with normal skin lipid.
4. Transferable only by cells., probably lymphocytes
5. But acg Chase, regional sensitivity of skin argues for induced reactivity via some diffisuble substance.

degree of heritability??

Could all immune cells confer DHS, but have this masked by circulating antibody? If so function of tuberculin wax is to prevent the antibody response.

Controversy on cellular HS to Tb.

Key papers

+ Miller JM + CB Favour 1951 JEM 93:1

+ Rich, Moen JEM 64:943 1936.

+ Raffel

± Waksman Am R Tb 68:746.

– Florio 1958 Jtum 80:12- + renewed.
developed techniques re migration and spindle cell transform of buffy coat in 24 hours. Up to 90% of mononuclears transformed. HS cells inhibited in this hypothesis. Effects are statistically but not individually impressive.

– Poppenheimer JEM 104:321 '56.

Transfer experiments: Lawrence Am J Med 20:428 '56; O'Neill ART 72:577

Leukocytic extracts: ART 73:246 '56., JCI 34:219 1955

Has anything been done with defined substances?

homologous BM cells only some irradiated mice. Has not been studied as a function of dosage. Henry is trying corticosteroid hormones to further reduce the implantation of lymphoid cells.

Runt disease may be chief limitation in protection with homologous BM. No study on f-1 marrow in parental host which should induce tolerance to f-1 skin etc. The host here may have (1) altered or (2) depleted ab. cells. Can this be told? — Trentin did just this!

See Trentin; Parker + Main

· despira — [illegible] [illegible] Tyro

8-612

Wood 9/30/58 letter + papers.

2% of total cells are in primary response. Ditto for number of lymphoblasts.

∴ all cells are reactive & stimulated by any antigen. Contradiction: how can they have kind of response per cell? Don't identify but how account for tolerance?
Presence of antigen in reactive cell suppresses the nucleosome. (Immunological theory doesn't refer to cells but particles.

What status of claims of transduction of immunity?

preselected plasma cells: Clever to add anti-A! All cells tested with both antigens.
(Do simultaneously - add T in ~~and~~ anti A serum). Try g and f factors against

Bj Excellent assays! 70 reactors; 32 anti ad
3 anti 685
35 non-reactors!

Never get spirillae? They would be best + we'll try again!

Dixon + Wright — neonatal environment inhibits def. Incorrect appeal to checkers.

Tried rats and mice

[are heterozygotes systems generally larger than homozygotes?]

P. 7 Secondary response OK ∴ neonatal of environment prevents maturation. Do you conclude tolerance to the host in these grafts? What happens to the injected cells.

9/30/58.

Nossal —

Wants $5,000 — can do. But would be taxable! Forget Rockefeller at $3800 but if they offer further travelling may be best bet. Deducted on training function to your benefit. Travel through the states is knottiest problem. Ask Rockefeller if they will make a travel grant.

You can have 1 month leave c/pay. Trip will cost about $300 fare + expenses. Might earn some on lectures. Better to get a travel grant — say Carnegie. Rockefeller approach may be advisable. Most economical to do travelling en route to England or we might find funds for a meeting trip.

Still applying now — help set up machinery. Pay not bad as reasonably tax free.
What's sordid about all this? Nothing opulent about Stanford — secure base from which to beg for funds.

Yes make preliminary enquiries. Tentative plans for publication:
Stanford: Sept 59 - Sept 60 Travel U.S. Oct 60 (you won't want to be on the road more than a month.

In fact. SFO — Pasadena — Denver — Chicago — Madison — Washington — Phila. —
1 2 3 4 5 6 7 8 9 10 11 12

NY — Yale —
13 14 15

In fact about 3 weeks would be more realistic; you'll be too jaded after that to manage anything.

[Microsomes → non reactive host! to kill a toluene! Transfer of immunity from donor to host cells?]

will omit detailed editorial criticism. But page 5: heated antigen is non immunogenic! Should have explicit control on non immunogenicity of ab treated & heated antigen + ~~[illegible]~~
Control might be antigen + ab added after unstimulated cells. But cells were washed ∴ evidence of uptake. What is sensitivity to the antigen?

What are x's and o's on diagrams? Should be part of legend.
Adjusted serum titre = "Total antibody production." Exaggerates effect; might leave out adjustment or indicate growth scale.

∴ cells in immature host may be hypersensitive?