lead metal were determined by placing absorbers of these materials in front of a Victoreen thimble ionization chamber (25 r) situated within an 8-cm square lucite block (*2*) at a distance of approximately 100 cm from the betatron target. A well-collimated and uniform 6-cm-diameter beam of 23.5-Mev maximum x-ray energy was employed.

The values for water and zinc bromide were measured in a lucite tank. A small ion chamber was moved along the axis of the beam by remote control (*3*). The instantaneous ionization current at any point was amplified and recorded. The field size was 10×12 cm at 100 cm from the target. Inverse square correction was applied to all readings. The characteristics of the different materials are shown in Table 1 and Fig. 1. Measured values for lead are given for comparison.

It should be noted that the data show only the rate of absorption due to ionization after the electronic equilibrium has been reached, which is at approximately a depth of 4 cm for water.

References

1. FERGUSON, K. R. *Nucleonics,* **10** (1952).
2. HARVEY, R. A., HAAS, L. L., and LAUGHLIN, J. S. *Radiology,* **58**, 23 (1952).
3. LAUGHLIN, J. S., *et al. Am. J. Roentgenol. Radium Therapy,* **65**, 787 (1951).

Manuscript received May 29, 1953.

The Dynamic Equilibrium between Circulating and Extravascular Plasma Proteins[1]

David Gitlin and Charles A. Janeway[2]

Department of Pediatrics, Harvard Medical School, and the Children's Medical Center, Boston, Massachusetts

It has been demonstrated that after intravenous administration of native (*1*) and labeled heterologous (*1, 2*) and homologous (*3–6*) plasma proteins to animals and labeled (*6*) and native (*7, 8*) homologous plasma proteins to humans, approximately half of the administered plasma protein, exclusive of that amount catabolized, leaves the circulation within the first few days. The importance of the nature of the label, the manner in which labeling has been performed, and, in the case of heterologous proteins, the activity of the reticuloendothelial system and the influence of antibody production in these experiments has been discussed (*1, 6*).

The extravascular distribution of native heterologous plasma proteins in animals (*9, 10*) and native homologous plasma proteins in humans (*7, 11*) has also been investigated; specific plasma proteins were found in the connective tissues and in the cells of many organs. In instances of a marked deficiency of a specific circulating plasma protein in humans, γ-globulin in agammaglobulinemia (*8*) and fibrinogen in congenital afibrinogenemia (*7*), it has been found that extravascular sites in these patients are also depleted of the specific plasma protein (*7, 11*). Extravascular deficits of the given plasma protein in these patients could be quickly rectified by intravenous administration of the protein (*7, 11*).

Although it has been demonstrated that an excess of a given plasma protein in the circulation will shift extravascularly, for a true equilibrium to exist between the intravascular and extravascular pools of plasma protein, it must also be shown that an excess in the extravascular compartment will result in movement of the protein into the circulation. It was the purpose of this investigation to demonstrate, if possible, the reversible nature of the shift of protein between the intravascular and extravascular compartments.

Rabbit antisera vs. pneumococcus type III polysaccharide were employed in this study since the antibody could be detected by its immunochemical reactivity with specific antigen without additional labeling. The antisera were passively transferred to normal rabbits, thus avoiding the question of synthesis by the host animal of the protein being measured. The levels of antibody in the circulation were determined by the method of Heidelberger *et al.* (*12*), the specific precipitates were measured spectrophotometrically (*13*). The antisera used were pooled, fractionated with ammonium sulfate at half (0.5) saturation, and the precipitate was dialyzed exhaustively against saline buffer ($\Gamma/2$ NaCl = 0.1, $\Gamma/2$ phosphate = 0.05) and then against 0.15 *M* NaCl. The filtered concentrated solution contained 16.9 mg antibody/ml and represented a 10% solution of protein. Prior to intravenous administration, a volume of blood equal to the amount of antiserum to be injected was removed from an ear vein. The following experiments were performed and the results are recorded in Fig. 1.

Expt. 1. Effect of exchange transfusion. Four rabbits were given 20 ml antiserum intravenously. At the end of the 4th day of equilibration, the right femoral vein in each of the rabbits was exposed, and a No. 19 polyethylene catheter was inserted through an incision in the vein wall.

A. Two of the rabbits were then given exchange transfusions of 300 ml of normal rabbit blood, with removal of blood and its replacement in 10-ml aliquots over a period of an hour. The exchanged rabbits demonstrated the expected fall in circulating antibody immediately following the procedure; a rapid rise in circulating antibody soon occurred.

B. The 2 control rabbits were not treated and they showed no significant alteration of circulating antibody other than a continued logarithmic decay over this same period.

Expt. 2. Effect of injection of specific antigen. A. Four rabbits were given 18 ml of antiserum intravenously, and at the end of the 2nd day were given 2.2 mg of pneumococcus polysaccharide III intra-

[1] Supported by a grant (RG-346) from the National Institutes of Health, U. S. Public Health Service, and a grant (No. 28) from the Playtex Park Research Institute.

[2] The authors wish to thank Lederle Laboratories, Inc., for the generous supply of rabbit antiserum and pneumococcus polysaccharide used in these experiments.

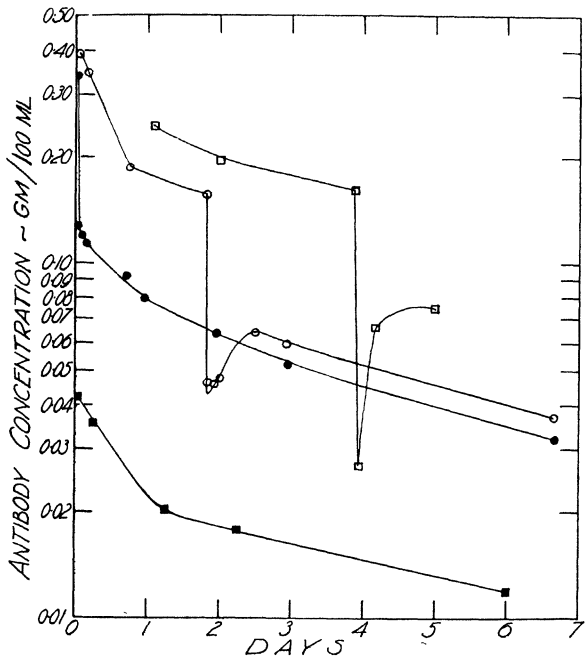

FIG. 1. All individual points on these curves represent the average values for the rabbits within a given experimental group. □ Expt. 1 A; ○ Expt. 2 A; ● Expt. 2 B; ■ Expt. 3.

venously in 1.0-ml volume, slowly. This amount of polysaccharide was calculated as enough to remove most but not all of the antibody circulating at that moment, the complex presumably being removed from the circulation in a short period of time. As can be seen, the level of circulating antibody fell immediately after administration of the specific antigen. A secondary rise soon occurred, however, and the circulating antibody then resumed its previous rate of logarithmic decline.

B. Two rabbits were given 15 ml of the same antiserum intravenously and then, after 20 min, 2.2 mg of polysaccharide III were injected. An immediate fall in circulating antibody occurred; the antibody then assumed a logarithmic rate of decline proportionate and parallel to the curve for the 4 rabbits in Expt. 2 A, and closely approximating it after the secondary rise in circulating antibody had taken place in the latter rabbits.

Expt. 3. Effect of reinfusion of antibody removed after equilibrium reached. To determine if the initial rapid fall in circulating antibody, observed in the rabbits of Expt. 2 A before the decline became exponential, was dependent upon the disappearance of antibody molecules with markedly shorter half-lives, serum derived from blood removed from the rabbits of Expt. 1 A during exchange transfusion was injected into 2 other rabbits after 3-fold concentration by negative pressure dialysis and centrifugation twice at 4000 rpm at 0° C for 30 min. Twenty-five milliliters of this serum were given intravenously to 2 rabbits after removal of 25 ml blood. The same initial rapid fall was observed. Since the donor rabbits had been in equilibrium with respect to circulating antibody, the derived serum presumably should have contained little or no antibody of very short half life. Hence it seems reasonable to conclude that the initial rapid fall following infusion of a plasma protein is, as has been previously postulated, an equilibration of the circulating protein with the extravascular protein pool.

The half life of the rabbit antibodies in these experiments varied between 5 and 7 days, which agrees with the values already reported (*14, 15*).

It would appear from the data that preformed plasma protein present in the extravascular pool can move rapidly into the circulation upon depletion of the specific protein in the latter compartment. Thus, the extravascular plasma protein is in dynamic equilibrium with the intravascular plasma protein; once equilibrium is established, a decrease in the mass of a specific plasma protein in one compartment results in the movement of that plasma protein to that compartment until equilibrium is again attained.

References

1. GITLIN, D., *et al. J. Immunol.*, **66**, 451 (1951).
2. DIXON, F. J., BUKANTZ, S. C., and DAMMIN, G. J. *Science*, **113**, 274 (1951).
3. MILLER, L. L., *et al. J. Exptl. Med.*, **90**, 297 (1949).
4. ABDOU, I. A., and TARVER, H. *J. Biol. Chem.*, **190**, 769 (1951).
5. ———. *Proc. Soc. Exptl. Biol. Med.*, **79**, 102 (1952).
6. STERLING, K. *J. Clin. Invest.*, **30**, 1228 (1951).
7. GITLIN, D., and BORGES, W. H. *Blood.* In press.
8. JANEWAY, C. A., APT, L., and GITLIN, D. In preparation.
9. COONS, A. H., LEDUC, E. H., and KAPLAN, M. H. *J. Exptl. Med.*, **93**, 173 (1951).
10. GITLIN, D., *et al.* In preparation.
11. GITLIN, D., LANDING, B., and WHIPPLE, A. *J. Exptl. Med.*, **97**, 163 (1953).
12. HEIDELBERGER, M., and KENDALL, F. E. *Ibid.*, **62**, 697 (1935).
13. GITLIN, D. *J. Immunol.*, **62**, 437 (1949).
14. HEIDELBERGER, M., *et al. J. Biol. Chem.*, **144**, 555 (1942).
15. GERMUTH, F. G., JR., OYAMA, J., and OTTINGER, B. *J. Exptl. Med.*, **94**, 139 (1951).

Manuscript received May 14, 1953.

A Homogeneous Cell Preparation from Soybean Leaves[1, 2]

D. W. Racusen and S. Aronoff

Institute for Atomic Research and the *Department of Botany, Iowa State College, Ames*

A typical dicotyledonous leaf is composed of three major types of tissue: epidermis, minor venation, and mesophyll. From the data of Wylie (*1*) these tissues appear to be present in roughly equal amounts. The specific biochemical contributions of the individual tissues to the general physiology of the leaf are essentially unknown (except, of course, for the photosynthesis of the mesophyll). Whereas epidermal cells may be obtained frequently merely by stripping, no procedure exists for obtaining mesophyll cells in

[1] Contribution No. LR-144.

[2] This work was supported by a grant from the Atomic Energy Commission and a fellowship to one of us (DWR) by the Oak Ridge Institute for Nuclear Studies.