

$\eta_\alpha$ -set and let  $G = R\{E\}$  be the Hahn group<sup>10</sup> with real coefficients and "exponents" in  $E$ : i.e., let  $G$  be the set of all mappings  $g$  of  $E$  into  $R$  whose support,  $s(g)$ , is either empty or an anti-well-ordered subset of  $E$ , where the addition is point-wise addition and the order is lexicographic. Let  $G_\alpha = \{g \in G : |s(g)| < \aleph_\alpha\}$ . Let  $E_0 \subset E$  and let  $H = \{g \in G : g(e) \in Z \text{ if } e \in E_0\}$ . Finally, let  $H_\alpha = H \cap G_\alpha$ . Then  $G$ ,  $G_\alpha$ ,  $H$ , and  $H_\alpha$  satisfy conditions (i), (ii), and (iii) and hence are  $\eta_\alpha$ -sets. Assume that  $|E| = \aleph_\alpha$ . Then  $|G_\alpha| = |H_\alpha| = \aleph_\alpha$ . To conclude, let  $F$  be the set of formal power series with coefficients in  $R$  and exponents in  $G_\alpha$  whose supports are of power less than  $\aleph_\alpha$ . Then  $F$  is a real-closed field that is an  $\eta_\alpha$ -set of power  $\aleph_\alpha$ .<sup>11</sup>

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<sup>1</sup> Hausdorff, F., *Grundzüge der Mengenlehre* (Leipzig, 1914), pp. 180-185.

<sup>2</sup> These definitions of characters are modifications of Hausdorff's, *ibid.*, pp. 142-147.

<sup>3</sup> Ostrowski, A., "Untersuchungen zur arithmetischen Theorie der Körper," *Math. Z.*, **39**, 269-404 (1935), §11.

<sup>4</sup> Krull, W., "Allgemeine Bewertungstheorie," *J. Reine Angew. Math.*, **167**, 160-196 (1932).

<sup>5</sup> Ostrowski, A., *loc. cit.*

<sup>6</sup> In a totally ordered group  $G$ , the right and left characters of a point  $t \in G$  are the same and equal the right and left characters of 0. The *point character* of  $G$  is this ordinal number.

<sup>7</sup> Alling, N. L., "On the existence of real-closed fields that are  $\eta_\alpha$ -sets of power  $\aleph_\alpha$ " (to appear).

<sup>8</sup> *Ibid.*

<sup>9</sup> *Ibid.*

<sup>10</sup> Hahn, H., "Über die nichtarchimedischen Grössensysteme," *Sitz. der K. Akad. der Wiss. (Vienna)*, **116**, 601-653 (1907).

<sup>11</sup> Alling, N. L., *loc. cit.*

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## INTERCELLULAR TRANSFER OF GAMMA-1 AND GAMMA-2 FORSSMAN HEMOLYSINS\*, †

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Avidity, a general term for the firmness of the antigen-antibody union, may play an important role in hemolysis. Within certain limits, the less avid the hemolysin the more efficient it is, because, when hemolysin can dissociate, it can produce serial injuries to red cells provided an excess of complement is present.<sup>1</sup> On the other hand, high avidity, which is associated with a high antigen-binding capacity, results in an inefficient hemolytic activity<sup>2</sup> but is considered desirable in antitoxic and precipitating serums. Avidity of hemolysins can be studied by the inversely related rate of intercellular transfer.

Rabbits repeatedly injected with heated sheep red cell stromata form two Forssman hemolysins. The earliest to reach peak titer occurs in the  $\gamma_1$  globulins, has a molecular weight of about 1,000,000, and is highly hemolytic, whereas the second one reaches peak titer two weeks or more later, occurs in the  $\gamma_2$  globulins, has a molecular weight of about 160,000, and is poorly hemolytic.<sup>3, 4</sup> In addition, Forssman antigens from different sources, such as guinea pig kidney, stimulate the

production of various hemolysins with different electrophoretic mobilities, and the Forssman antigen of human type A heated red cell stromata engenders very little of the  $\gamma_2$  hemolysin even after long continued immunization.<sup>4</sup>

We<sup>2</sup> recently described a method of titrating the net per cent transfer of hemolysins in terms of 50 per cent units from red cell to red cell by using Cr<sup>51</sup>-labeling of red cells as an indicator. With it, we studied the net intercellular transfer of rabbit hemolysins in normal serums as well as in serums resulting from one injection or reinjection of sheep red cells or heated sheep red cell stromata. Fresh sheep cells with this mild immunization and heated stromata with all types of immunization give rise to the Forssman and not the isophile hemolysin. In general, avidity increased as titer increased, while the per cent transfer decreased as titer increased. Thus, transfer was high (28 to 75%) in normal serums with titers of 15 to 160 units, decreased to values of 3 to 13 per cent by the time peak titers (2,100 to 22,000 units) were attained 7 to 13 days after the initial antigen injection, and then slightly increased to about 15 per cent as hemolysin titers declined (200 to 1,060 units at 4 to 5 months). The sequence after a later antigen injection was similar except that transfer was slightly lower at peak titer (2 to 7 per cent). Furthermore, the hemolysin of both normal and immune serums contained two components, i.e., a nonavid one and an avid one (see *Discussion*).

Subsequent to our work, Goodman and Masaitis<sup>5</sup> essentially confirmed our results using a system which measured the transfer of hemolysin between sensitized sheep red cells and formalized cells—the latter cells unite with antibody and fix complement normally without being lysed. In addition, they studied  $\gamma_1$  and  $\gamma_2$  antisheep hemolysins after electrophoretic separation (see *Discussion*).

The present intercellular transfer experiments deal with Forssman antisheep hemolysins which are produced by immunization with human type A red cells, extend our work with sheep cell stromata to repeatedly injected rabbits, and corroborate, except for the degree of transfer, and amplify Goodman and Masaitis' studies on the  $\gamma_2$  hemolysin.

*Materials and Methods.*—Previously, we have described the methods of titrating hemolysin in 50% units<sup>6</sup> and of titrating intercellular hemolysin transfer in terms of the 50% units of hemolysin which need to be adsorbed on carrier, i.e., unlabeled, red cells (RBC) to give the net transfer of one 50% unit of hemolysin to unsensitized Cr<sup>51</sup>-labeled red cells (RAC).<sup>2</sup> The red cells were labeled with Cr<sup>51</sup> by treating them with Na<sub>2</sub>Cr<sup>51</sup>O<sub>4</sub>, which was obtained from Abbott Laboratories, North Chicago, Illinois, under the commercial name of "Rachromate." In carrying out the *in vitro* intercellular transfer test, one half ( $\approx 1.6 \times 10^8$  cells) of a 100% unit of sheep red cells (RBC) sensitized with known amounts of a given hemolysin was mixed with an equal quantity of unsensitized Cr<sup>51</sup>-labeled sheep red cells (RAC). The mixture, after standing for 30 minutes at 37°C to allow the transfer of hemolysin to take place and after the addition of an excess of complement, was incubated for 30 min at 37°C to allow hemolysis to proceed. Then, the amount of Cr<sup>51</sup> in the supernatant fluid was measured in a  $\gamma$ -ray well-type scintillation counter with a single channel analyzer to determine the amount of hemoglobin liberated from the RAC and, hence, the amount of hemolysin transferred from the sensitized RBC to the unsensitized RAC.

Met peak hemolysin titers during anamnestic responses were obtained by subtracting the titer at the time of the reinjection from the actual peak titer obtained.

Starch zone electrophoresis at pH 8.6 (veronal buffer, 0.1 ionic strength) was carried out on whole serums that had been frozen 2 or 3 months. Just before electrophoresis, each serum was centrifuged 30 minutes at  $500 \times g$  to remove any spontaneous precipitate. The method described by Stelos and Talmage<sup>7</sup> was used with the following slight modifications. The starch block was 50 cm long by 18 cm wide by 0.5 cm thick and was usually divided in half lengthwise

to accommodate two serums. Two or 3 ml of each serum was mixed with potato starch and placed in the 40th and 41st segments. This location is designated the starting point in Figures 5 and 6.

After electrophoresis, aliquots of the eluate of each cm segment of a given serum were tested (1) for protein N by the procedure of Lowry *et al.*,<sup>8</sup> (2) for 50% hemolytic units<sup>6</sup> and (3) for per cent transfer.<sup>2</sup> In graphs 5 and 6, these values are expressed per ml eluate.

*Experimental Results.*—Hemolysin titer and net intercellular transfer were obtained on whole serums from twelve rabbits and from fractions of some of the serums after separation by electrophoresis. Six of the rabbits (1–6) were immunized three times with heated human A red cell stromata (AStr), and the other six rabbits (7–12) were immunized three times with heated sheep red cell stromata (SStr). Each injection was given intravenously and consisted of  $1.6 \times 10^9$  stromata per kg rabbit. Rabbits 1 through 3 were given one injection of AStr; 35 days later, they were given a reinjection of AStr; and 91 days following the reinjection, they were given four reinjections of AStr during the course of eight days and were killed a week later. Rabbits 4 through 6 were repeatedly injected 13 times thrice weekly for 28 days with AStr; 28 days after the last injection, they were given a second similar immunization of AStr; 52 days after the last of the second immunizing injections, they were given four injections of AStr during eight days; and they were killed a week later. Three exactly similar immunizations were given rabbits 7 through 12 except that these six rabbits were given SStr.

*A single injection and reinjection of heated human A red cell stromata (AStr):* As may be seen in Figure 1, the initial hemolysin response varied markedly in rabbits 1 through 3. It reached a peak titer of only 105 units in rabbit 1 on 12.3 days, whereas it reached a peak titer of 5,600 units in rabbit 3 on 5.7 days. The per cent transfer of hemolysin was high at first (31–38%) and decreased more at the time of peak titer in rabbit 3 (12%) than in rabbits 1 and 2 (20%). These results are similar to our previous work<sup>2</sup> involving a single injection of SStr, but the response with SStr more often resembled the response in rabbit 3 than in rabbits 1 and 2. As will be seen in the next section, initial immunization with repeated injections of AStr also gave the more intense hemolysin response and the lower per cent transfer encountered in rabbit 3.

The anamnestic response was moderate in rabbits 1 through 3. Peak titer, when adjusted by subtracting the titer at the time of reinjection, was 380 units for rabbit 1 and 2,600 units for rabbit 3. There was little change in the per cent of transfer. In rabbit 1, a plateau of 20 per cent was maintained throughout the 28 days of observation; in rabbit 2, transfer dropped from 30 to 25 per cent; and in rabbit 3, it rose from 15 to 28 per cent. These results differ from the sharp decrease in percent transfer at peak titer in rabbits reinjected once with SStr.<sup>2</sup>

*Repeated injections of heated human A red cell stromata (AStr):* The initial and anamnestic responses in rabbits 4 through 6 as a result of repeated injections of AStr are shown during the early portion of each immunization in Figure 2. There was an adequate initial hemolysin response and a definite change in per cent transfer in all three rabbits. Thus, peak titers of 13,000 to 47,000 units were attained after induction periods of 2.9 to 3.8 days during antibody rises of 7.7 to 8 days, while the per cent net transfer decreased from normal values of 30 to 45 per cent to values of 13 or 14 per cent. In two of the rabbits, it remained low, and in rabbit 4, it increased to 20 per cent.

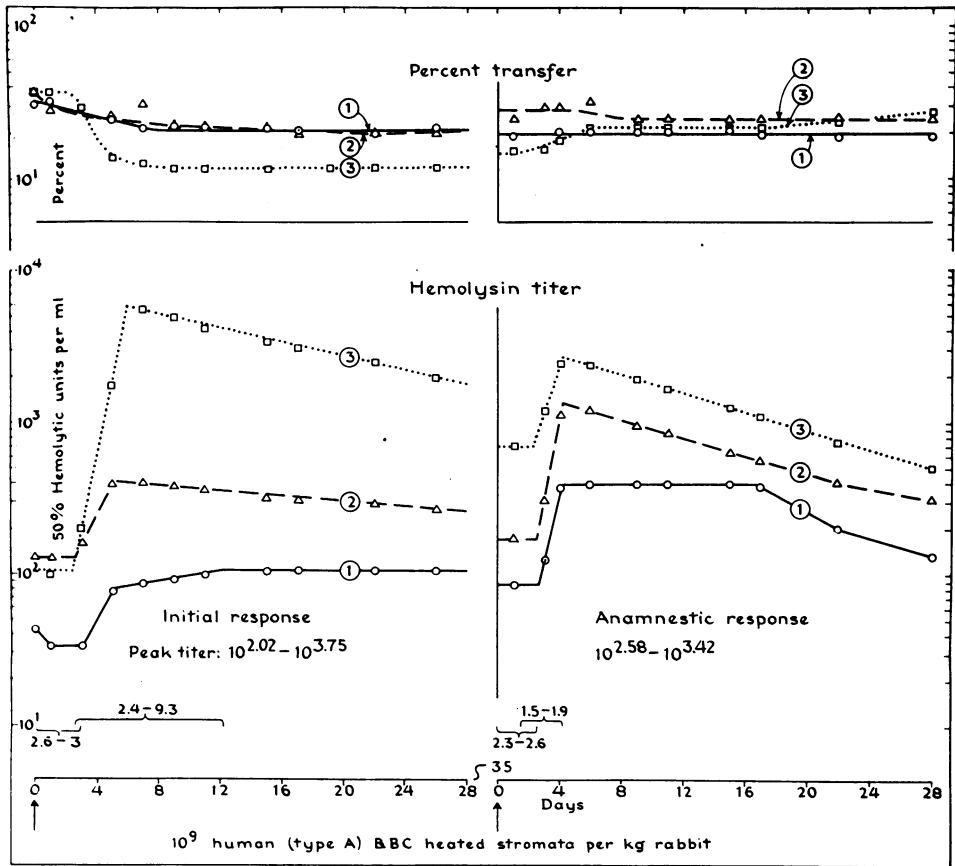


FIG. 1.—Changes in intercellular hemolysin transfer and in hemolytic titer in serums from rabbits 1, 2, and 3 during the initial and anamnestic responses following an intravenous injection and reinjection of heated human A stromata (AStr) as indicated by the arrows.

Hemolysin responses varied, whereas transfer was roughly inversely related to hemolysin titer in the initial response but maintained a plateau during the anamnestic response.

In the first anamnestic response, hemolysin titer and per cent transfer did not change as much as in the initial immunization in spite of the same schedule of injections.

The hemolysin response was slightly more marked in the second than in the first anamnestic immunization but did not equal that of the initial one. Thus, after induction periods of 1.5 to 2 days, titers reached 2,500 to 4,000 units during antibody rises of 3.4 to 9.5 days. The per cent of transfer was little affected. It remained on plateaus of 40 and 29 per cent for rabbits 4 and 5 and rose from 22 to 45 per cent in rabbit 6 during the 14-day period of observation.

Rabbits 1 through 3 had only received one injection and one reinjection of AStr 126 and 91 days previous to their immunization with four injections of AStr. In this second anamnestic response, hemolysin titers of 2,000 to 26,000 units were reached during antibody rises of 7.6 to 8.5 days after induction periods of 1.5 to 2 days, whereas the per cent transfer increased from 31 to 56 per cent in rabbit 1 and maintained plateaus of 39 and 50 per cent in rabbits 2 and 3. Therefore, these anamnestic responses after two single injections of AStr were similar to the anam-

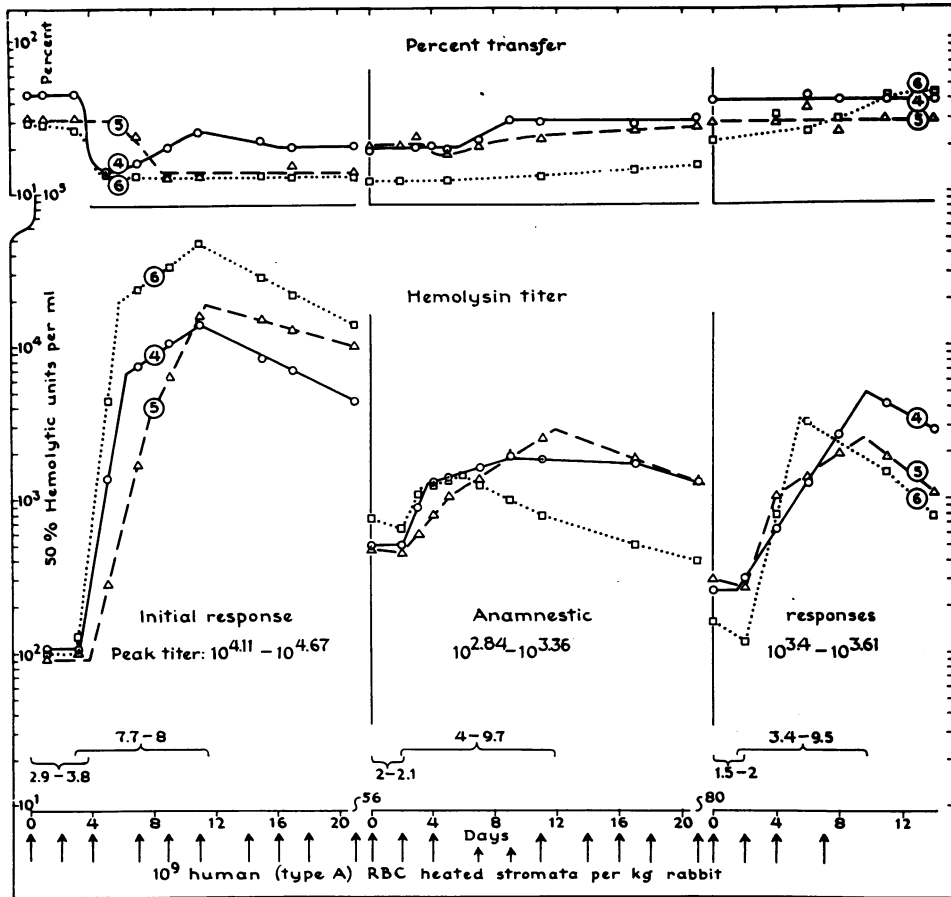


FIG. 2.—Changes in intercellular hemolysin transfer and in hemolytic titer in serums from rabbits 4, 5, and 6 for the first parts of the initial and two anamnestic responses during repeated intravenous injections and reinjections of ASr.

Hemolysin responses were less pronounced and the per cent transfers were higher in the anamnestic responses than in the initial one.

neptic responses after two series of repeated injections of ASr in rabbits 4 through 6.

*Repeated injections of heated sheep red cell stromata (SSt):* Figures 3 and 4 show the early part of the hemolysin response and per cent transfer during the three immunizations in rabbits 7 through 12. The peaks during the first immunization varied from 45,000 units in rabbit 12 to 93,000 units in rabbit 7 and were reached during antibody rises of 6 to 7.5 days after induction periods of 2.5 to 3 days. The high per cent transfer in all rabbits before injection (50–63%) decreased markedly during the antibody rise, reached its lowest point just before peak titer (4 to 7.9%), and increased slightly thereafter until on the 20th day of immunization it varied from 5.6 to 10 per cent. As compared to rabbits 4 through 6, peak titers in these 6 rabbits were much higher and were reached about a day sooner (although the induction periods were equally long) and the per cent transfer decreased more markedly during the antibody rise.

Hemolysin responses during the second immunization, as compared to the primary one, were not as marked, but peaks were reached after shorter induction

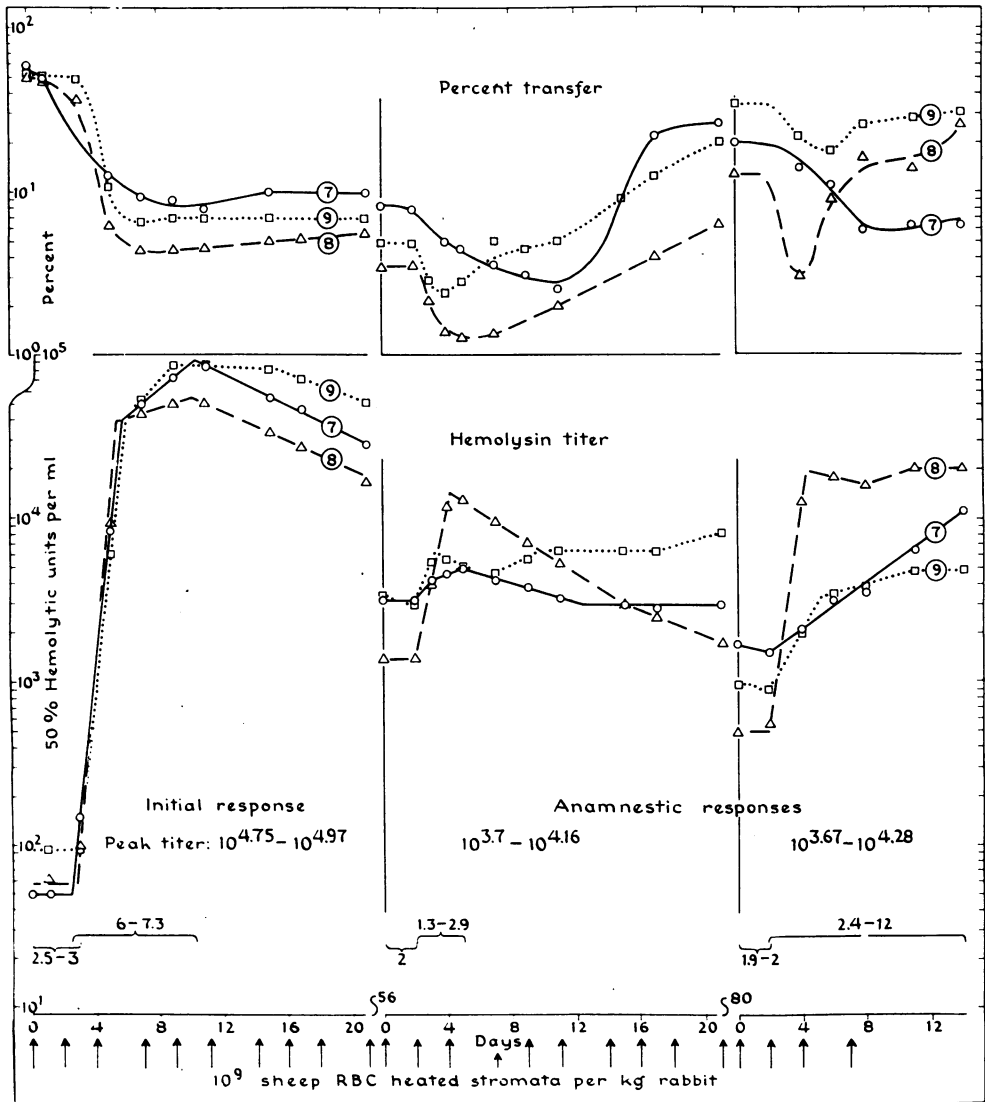


FIG. 3.—Changes in intercellular hemolysin transfer and in hemolytic titer in serums from rabbits 7, 8, and 9 for the first parts of the initial and two anamnestic responses during repeated intravenous injections and reinjections of heated sheep red cell stromata (SStr).

Hemolysin responses were less pronounced and the per cent transfers were higher as immunization was continued.

periods and antibody rises, whereas the per cent transfer decreased to a lower level and rose more rapidly thereafter. As seen in Figures 3 and 4, the per cent transfer varied from 3.6 to 11 on day 56 and decreased at peak titer or shortly thereafter to a low point (1.3 per cent in rabbit 8 to 3.3% in rabbit 11). In rabbit 12, there was a second dip to 2.5 per cent on day 14. The subsequent increase was more marked in rabbits 7, 9, and 11 (12 to 26% on day 20) than in the other 3 rabbits (3 to 7% on day 20). As compared to rabbits 4 through 6, however, peaks were higher and were reached more rapidly, and the per cent transfer decreased more radically.

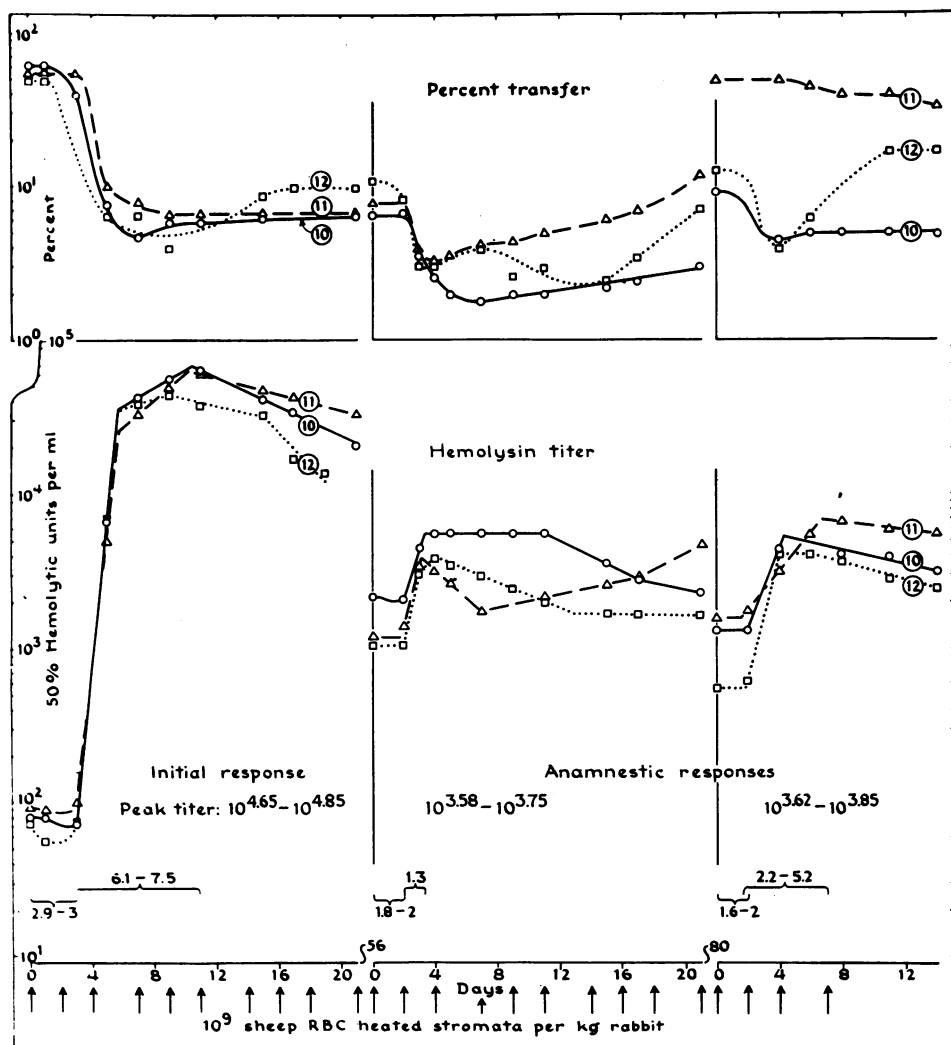


FIG. 4.—Changes in intercellular hemolysin transfer and in hemolytic titer in serums from rabbits 10, 11, and 12 during the same schedule of immunizations with SStr as in Figure 3. Essentially, the same results were obtained as in Figure 3.

At the beginning of the third immunization of rabbits 7 through 12, hemolysin titers varied from 500 to 1,700, and the per cent transfer had reached values of 20 to 50 per cent in three of the rabbits and of 9 to 13 per cent in the other three. The anamnestic hemolysin response was essentially similar to the previous one. The per cent transfer changed more or less characteristically in rabbits 7 and 10, but decreased only briefly in rabbits 8, 9, and 12 and remained practically level in rabbit 11. Changes in transfer with repeated injections of SStr were thus not as pronounced in the third as in the second immunization but were more marked, except for the one in rabbit 11, than during the third immunization with repeated injections of AStr.

*Electrophoretic separation of serums during the course of repeated injections of*

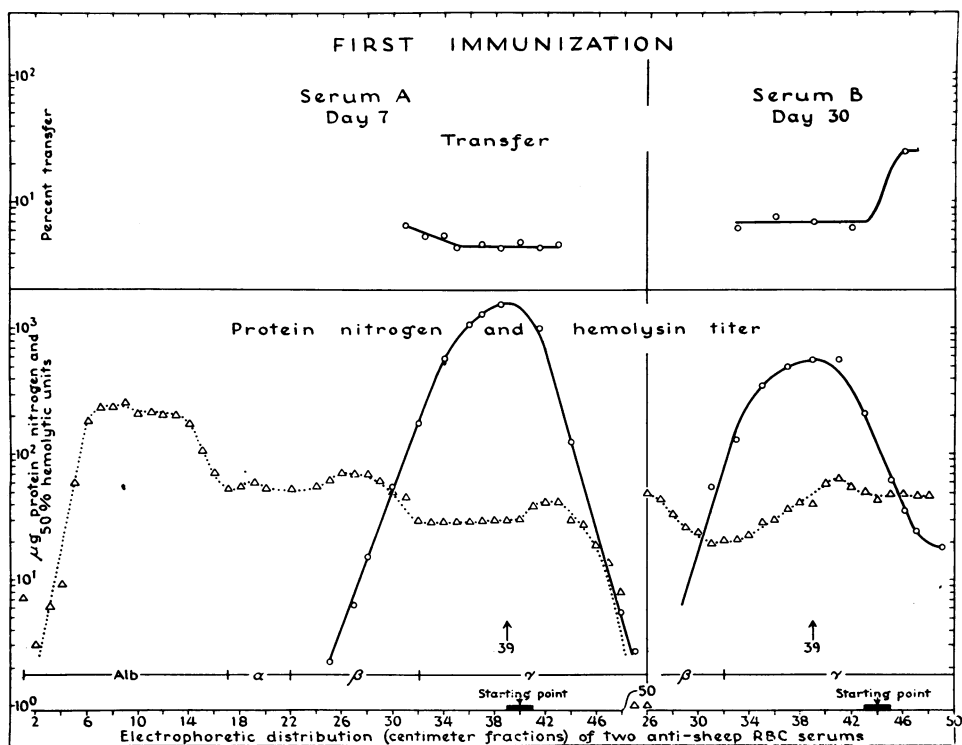


FIG. 5.—Comparison of intercellular hemolysin transfer, hemolytic titer, and protein N (dotted line) in two serums from rabbit 9 separated by starch block zone electrophoresis into 50 one-cm segments.

Serum A contained only  $\gamma_1$  hemolysin as shown by the fact that hemolysin was normally distributed in segments 26 through 48, whereas serum B contained  $\gamma_1$  hemolysin as well as a small amount of  $\gamma_2$  hemolysin as shown by the fact that the hemolysin curve was skewed to the right. Transfer was lower for the  $\gamma_1$  than for the  $\gamma_2$  hemolysin.

*heated sheep red cell stromata (SStr)*: Intercellular transfer and hemolytic titer were then studied in electrophoretically separated fractions of whole serums collected at various times during the three immunizations. Five intervals, A through E, were selected. They were: days 7 and 30 of the initial response, days 5 and 32 of the first anamnestic response, and day 14 of the second anamnestic response. These five intervals were tested in serums from rabbits 7, 9, and 11.

The five whole serums from rabbit 9 gave 6.7, 7, 3, 48, and 31 per cent transfer, respectively. After electrophoretic separation, the results are graphed in Figures 5 and 6. Those of serum A are given for centimeter segments of the whole starch block, but those for the other four serums are given only for the segments of the starch blocks containing the globulins of interest.

The 7-day serum A had a well-defined symmetrical hemolysin distribution with a peak at 39 centimeters and titers of less than 10 units below segment 28 and above segment 46. Intercellular transfer for the segments containing hemolysin varied between 4.5 and 7 per cent. These data indicate that the serum was homogeneous and only contained the  $\gamma_1$  hemolysin with a low per cent transfer.

In the 30-day serum B, hemolysin was present through segment 49, whereas transfer varied from 5 to 7 per cent for segments below 43 and was 25 per cent for a



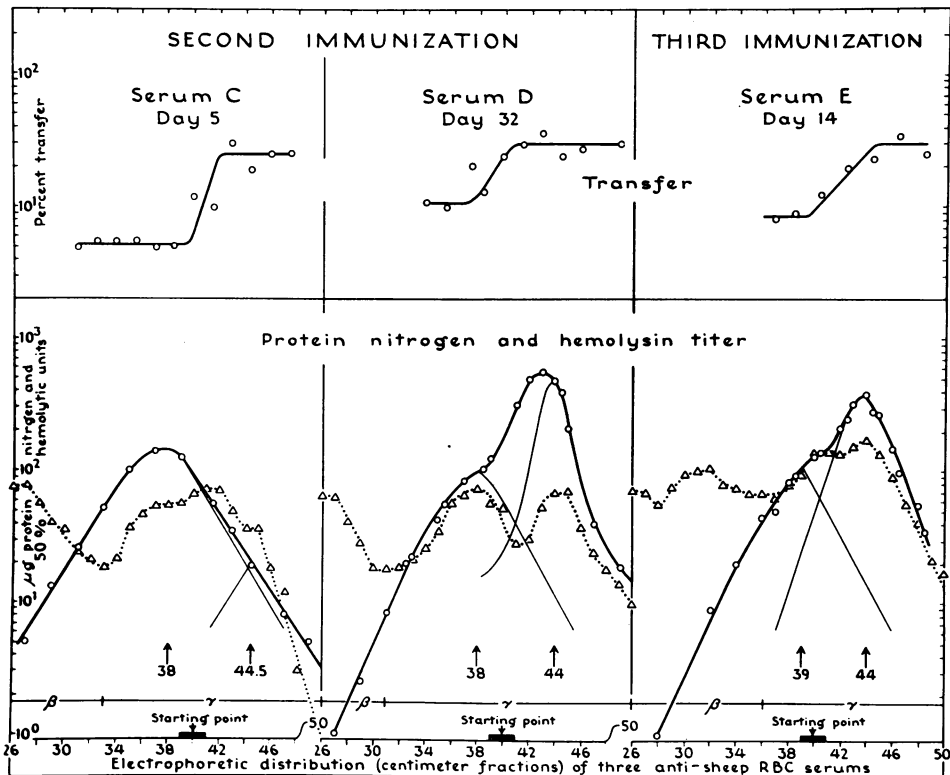


FIG. 6.—Comparison of intercellular hemolysin transfer, hemolytic titer, and protein N (dotted line) in three serums from rabbit 9 separated as in Figure 5.

The  $\gamma_2$  hemolysin increased in amount as immunization proceeded with a peak at segment 44 and with a higher rate of transfer than the  $\gamma_1$  hemolysin. Intermediate rates of transfer were obtained in the transitional segments containing both types of hemolysin.

pool of segments 45 through 47 in which hemolysin titers were low. There was a corresponding increase in the protein content of the end segments. These changes reflected the presence of a small amount of  $\gamma_2$  hemolysin with a high per cent transfer in the  $\gamma_2$  protein fraction. The amount, however, was not sufficient to raise the per cent transfer of the unfractionated serum.

Serums C, D, and E showed a progressive increase in the  $\gamma_2$  protein fraction with peak  $\gamma_2$  hemolysin activity at segment 44. In serum C, the  $\gamma_2$  hemolysin in segments 44 through 50, when present in sufficient quantities to test, gave from 25 to 31 per cent transfer, while the  $\gamma_1$  hemolysin in segments 31 through 39 gave from 5 to 9 per cent transfer. In serums D and E, moreover, the  $\gamma_2$  hemolysin had increased to such an extent that its high per cent transfer masked the low per cent transfer of the  $\gamma_1$  hemolysin in the whole serums before fractionation.

The foregoing results were substantiated by the data from the five fractionated serums of rabbit 7.

The five whole serums A through E from rabbit 11 gave 8, 7, 4, 37, and 33 per cent transfer. After fractionation, the serums behaved as did those from rabbit 9 except that the  $\gamma_2$  hemolysin was more clearly evident in serums B and C. In these two serums, there was a definite hemolysin peak at segment 45 and an increase

in transfer from 4 or 5 per cent at segment 41 to a value of 36 to 50 per cent in segments 43 through 48. There was too little hemolysin in segments 49 and 50 to test for transfer. The disproportionately large amount of  $\gamma_2$  hemolysin in serums D and E, as compared to the  $\gamma_1$  hemolysin, accounted for the high per cent transfer in the whole serums.

As was expected in view of the reported homogeneity of the antisheep  $\gamma_1$  Forssman hemolysin,<sup>4</sup> all of the segments of  $\gamma_1$  hemolysin in a given serum gave essentially the same per cent transfer, as shown in Figure 5. The same statement holds for segments containing only  $\gamma_2$  hemolysin, as shown in Figure 6. Segments containing mixtures of  $\gamma_1$  and  $\gamma_2$  hemolysins gave intermediate values.

The 50 per cent endpoint was used for determining both the hemolysin test and the intercellular transfer test by relating the degree of hemolysis to the dilution of serum in the first case and to the number of hemolysin units needed on the sensitized RBC to cause the transfer of one 50 per cent unit to the unsensitized RAC in the second case. Parallel von Krogh slopes were obtained for these two tests for any given serum and, in general, approximated a 0.4 angle for the  $\gamma_1$  hemolysin and a 0.2 angle for the  $\gamma_2$  hemolysin. In addition, some of our serum fractions, especially in the region of segments 39 through 44, which contained both hemolysins, had slopes intermediate between 0.2 and 0.4. These mixtures of the two fractions with their intermediate slopes may account for the fact that Goodman and Masaitis<sup>5</sup> reported an inverse relation between the von Krogh slope and transfer. In their Figure 4, it may thus be seen that 5 serums with a high rate of transfer had an approximate 0.2 slope, 15 serums with a low rate of transfer had a slope varying between 0.38 and 0.48, and 4 serums with an intermediate rate of transfer had an intermediate slope between 0.3 and 0.35.

*Discussion.*—The most striking result found in our earlier work<sup>2</sup> was that serums containing normal and immune (mostly  $\gamma_1$ ) antisheep hemolysins contained a non-avid component that transferred rapidly and an avid component that transferred slowly. The avid component of normal serums was less avid than the one in immune serums. Moreover, a lapse of time after the first antigen injection and especially after a second antigen injection resulted in an increase in the mean avidity of the whole serum that was largely accounted for by an increase in amount and/or avidity of the avid component. That avidity increased after a second antigen injection was shown by the extremely small amount of transfer in the avid component, even after a two-hour transfer period. The present work indicates that, in addition to such changes, the appearance of  $\gamma_2$  hemolysin lowers the average avidity.

The present paper extends our previous results<sup>2</sup> on intercellular transfer to the  $\gamma_1$  Forssman hemolysin made in the rabbit by immunizing with heated human type A red cell stromata. As might be expected, the antiAStr hemolysins behave essentially as do the  $\gamma_1$  Forssman antiSStr hemolysins. Certain differences, however, were found. For example, as compared to the antiSStr hemolysins, more transfer was obtained in the antiAStr hemolysins after an initial injection of antigen and especially after a second injection. Thus, 12 to 20 per cent and 16 to 31 per cent were the lowest transfers obtained after one injection and one reinjection, respectively, with AStr, whereas 5 to 19 per cent and 1.3 to 4 per cent were reached during similar immunization with SSstr (cf. the per cent transfer in the initial and anamnestic responses in Figure 1 with those in Figures 2 and 3 in our earlier work).<sup>2</sup>

Similarly, 12 per cent was the lowest transfer obtained during both immunizations with repeated injections of AStr, whereas 4 to 6 per cent transfer and 2 to 3 per cent were often reached during initial and secondary immunizations, respectively, with SSstr (cf. the per cent transfer in Fig. 2 with those in Figs. 3 and 4).

These higher transfers, i.e., lower avidity, of AStr hemolysins cannot be explained by the presence of  $\gamma_2$  hemolysins because even hyperimmune antiAStr serums contain very little  $\gamma_2$  hemolysins.<sup>4</sup> They can probably be accounted for by the fact that less antibody is formed after immunizing with AStr than with SSstr or possibly by a difference in specificity. In the latter case, a greater avidity might be found if human type A cells were used for the transfer.

It is of interest to point out here that the hemolysin response after peak titer in rabbits 4 through 6, which were repeatedly injected with AStr, declined during all three immunizations and was less intense during the second and third than during the initial immunization. The second and third series of injections were started 28 and 52 days after the last of the preceding injections, respectively. The rabbits thus entered a period of partial immunologic unresponsiveness during their initial immunization (see our work<sup>9</sup> for a discussion of what we originally designated a partial refractory stage during immunization) and had probably not recovered from it when the anamnestic responses were initiated according to unpublished experiments on SSstr.

The per cent of transferable antibody, as reported by Goodman and Masaitis,<sup>5</sup> should correspond to our per cent transfer although they used a different system and a 60-minute instead of a 30-minute transfer time. Thus, their range of transfer for 5 to 7 days after a single injection of sheep cells (6 to 20%) is similar to the mean ( $10.6 \pm 1.5\%$ ) previously published by us.<sup>2</sup> On the other hand, other transfer rates do not agree. Thus, 14 to 20 per cent transfer 5 to 9 days after one reinjection of sheep cells appears high as compared to the mean ( $4 \pm 0.7\%$ ) previously published by us.<sup>2</sup> In addition, the low rates of transfer (0 to 4%) for the  $\gamma_1$  hemolysin and the high rates of transfer (98 to 100%) for the  $\gamma_2$  hemolysin during late first or second immunizations with repeated injections of sheep cells or stromata are not substantiated by our present results. We obtained 4 to 13 per cent for  $\gamma_1$  hemolysin and 25 to 50 per cent for  $\gamma_2$  hemolysin, as exemplified in serums B and D of Figures 5 and 6. In other words, in their electrophoretic fractionation, they obtained an essentially nondissociable  $\gamma_1$  hemolysin and a readily dissociable  $\gamma_2$  hemolysin, whereas our nonavid  $\gamma_2$  hemolysin was only about 4 to 6 times more dissociable than the avid  $\gamma_1$  hemolysin.

*Summary and Conclusions.*—The per cent net intercellular transfer of hemolysin from red cell to red cell was studied during the initial and anamnestic hemolysin responses in rabbits given repeated injections of  $1.6 \times 10^9$  heated sheep red blood cell stromata (SSstr) three times a week for a month, or given similar repeated or single injections of heated human A red blood cell stromata (AStr). Intercellular transfer was also studied in some of the antiSSstr serums after electrophoretic separation.

In general, during corresponding immunizations, changes in the hemolysin response and in the percent net transfer were greatest with multiple injections of SSstr (Figs. 3 and 4), were intermediate with multiple injections of AStr (Fig. 2), and were least with single injections of AStr (Fig. 1). Moreover, with a given antigen, the changes were more marked in the initial immunization than in the

anamnestic responses. Thus, with both antigens, hemolysin titer was, in general, reciprocally related to per cent transfer during the initial immunization, but transfer sometimes remained high at peak titer during the second and especially during the third immunization (Figs. 1-4). This result, with respect to transfer in the rabbits immunized with SSr, was associated with a progressive increase in the  $\gamma_2$  hemolysin with a high transfer rate and a decrease in the  $\gamma_1$  hemolysin with a low transfer rate (Figs. 5 and 6). Inasmuch as little or no  $\gamma_2$  hemolysin has been found by various investigators in response to ASr, the high rates of transfer in the anamnestic responses to ASr were possibly associated with a relative increase in normal hemolysins as production of immune hemolysins decreased.

Transfer was ascertained in terms of the number of 50 per cent units of hemolysin needed to be adsorbed on unlabeled red cells to give the net transfer of one 50 per cent unit of hemolysin to unsensitized Cr<sup>51</sup>-labeled red cells. The slope of the von Krogh graphs in the tests was usually 0.4 for the  $\gamma_1$  hemolysin and 0.2 for the  $\gamma_2$  hemolysin.

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<sup>1</sup> Bowman, W. M., M. M. Mayer, and H. J. Rapp, *J. Exper. Med.*, **94**, 87-110 (1951); and Weinrach, R. S., and D. W. Talmage, *J. Infect. Dis.*, **102**, 74-80 (1958).

<sup>2</sup> Taliaferro, W. H., L. G. Taliaferro, and A. K. Pizzi, *J. Infect. Dis.*, **105**, 197-221 (1959).

<sup>3</sup> Review by Taliaferro, W. H., *J. Cell. and Comp. Phys.*, **50**, Suppl. 1, 1-26 (1957).

<sup>4</sup> Stelos, P., *J. Infect. Dis.*, **102**, 103-113 (1958); Stelos, P., and W. H. Taliaferro, *J. Infect. Dis.*, **104**, 105-118 (1959); and Stelos, P., L. G. Taliaferro, and P. D'Alesandro, *J. Infect. Dis.*, **108**, 113-119 (1961).

<sup>5</sup> Goodman, H. S., and L. Masaitis, *J. Immunol.*, **85**, 391-397 (1960).

<sup>6</sup> Taliaferro, W. H., and L. G. Taliaferro, *J. Infect. Dis.*, **87**, 37-62 (1950) and *idem.*, **99**, 109-128 (1956).

<sup>7</sup> Stelos, P., and D. W. Talmage, *J. Infect. Dis.*, **100**, 126-135 (1957).

<sup>8</sup> Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265-275 (1951).

<sup>9</sup> Taliaferro, W. H., and L. G. Taliaferro, *J. Infect. Dis.*, **89**, 143-168 (1951).

## NEGATIVE ENTROPY AND PHOTOSYNTHESIS

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As was suggested by E. Schrödinger,<sup>1</sup> the maintenance of the high organization of living beings is due to a continuous influx of negative entropy. Animals get this negative entropy by eating plants (as well as one another) while plants get their negative entropy, along with energy, from the sun's rays. The point is that solar radiation arriving on the earth is not in a state of thermodynamical equilibrium. Indeed, using Wien's formula  $\lambda \max T = \text{const.}$  for the maximum of the energy in the spectrum, we obtain  $T = 6,000^\circ\text{K}$ , i.e., the surface temperature of the sun.