and 7S gamma-globulins from other species that have been studied previously.

*Discussion and summary.* After a single injection of $10^{8-10}$ bacteriophage $\phi$X 174, the chicken, frog and goldfish were shown to produce approximately the same levels of neutralizing, rapidly sedimenting, $\gamma$-globulin antibodies as those previously obtained in analogously immunized mammals(5,6). Repeated injections of bacteriophage in the frog and goldfish, at intervals of 2-4 weeks, did not elicit an anamnestic antibody response. However, higher levels of antibody, mainly in the slowly sedimenting $\gamma$-globulin fraction were produced after immunization with bacteriophage in complete Freund's adjuvant, and, in the case of the goldfish, after further elevation of the environmental temperature to 32°C. Thus, in 3 classes of non-mammalian vertebrates a change was observed in the sedimentation properties of antibody $\gamma$-globulins produced during immunization. This change appeared similar to the replacement of 19S by 7S antibodies in the circulation of immunized mammals. These findings suggest that the mechanisms responsible for this phenomenon were present in the most recent common ancestors of terrestrial vertebrates and bony fish and that formation of rapidly sedimenting antibody is an integral and important part of the immune mechanism.

The authors wish to acknowledge the excellent technical assistance of Mr. Yuen H. Chinn.

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Received July 9, 1962. P.S.E.B.M., 1962, v111.

## Hemostatic Effects of Heterologous Platelets in Thrombocytopenic Rats.* (27692)

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Transfusions of fresh homologous platelets reduce or eliminate the abnormal diapedesis of red blood cells in thrombocytopenic animals, as determined by studies of lymph composition(1,2). Since the effects of platelets on permeability of small blood and lymph vessels to red blood cells(3) may represent a function separate from the coagulation-promoting and "hemostatic plug" formation activities, the species-specificity of platelets was investigated in this respect. The effect of fresh human platelets on the increased passage of red blood cells into the lymph of x-irradiated thrombocytopenic rats was compared to that of fresh rat platelets.

*Methods and materials.* Female Wistar rats weighing 250 to 300 g were studied. Thrombocytopenia was induced by exposure to 875 r total body x-irradiation.† Seven to

* This investigation was supported by a grant from the Atomic Energy Commission.

† 250kV, 15 m.a., Target-mid-body level distance distance-88cm. 18r/min. 0.25 mm Cu and 1 mm Al filters.

TABLE I. Effects of Infusion of Rat or Human Platelets on Output of Red Blood Cells in Lymph and the Count of Circulating Platelets of Thrombocytopenic (X-Irradiated) Rats.

| | Time after infusion (minutes) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0′ | | 15′ | | 30′ | | 60′ | | 90′ | | 120′ | | 225′ | |
| | R* | P† | R | P | R | P | R | P | R | P | R | P | R | P |
| *Human platelets—no circulation* | | | | | | | | | | | | | | |
| 1 | 119 | 38 | — | 58 | 78 | 94 | 61 | 35 | 145 | 59 | 161 | 26 | 171 | 33 |
| 2 | 395 | 8 | — | 24 | 676 | 9 | 1093 | 5 | 623 | 5 | 315 | 5 | 1073 | 5 |
| 3 | 40 | 38 | — | 67 | 29 | 32 | 24 | 28 | 59 | 22 | 67 | 22 | 53 | 22 |
| Mean | 185 | 28 | — | 50 | 260 | 45 | 393 | 23 | 276 | 29 | 181 | 18 | 432 | 20 |
| ± σ | 152 | 14 | — | 18 | 295 | 36 | 129 | 12 | 248 | 22 | 102 | 8 | 182 | 12 |
| *Human platelets—circulation* | | | | | | | | | | | | | | |
| 4 | 20 | 90 | — | 740 | 13 | 515 | 17 | 130 | 24 | 108 | 22 | 108 | 3 | 75 |
| 5 | 375 | 11 | — | 1215 | 316 | 365 | 179 | 151 | 170 | 130 | 130 | 92 | 63 | 70 |
| 6 | 145 | 10 | — | 780 | 151 | 450 | 123 | 133 | 100 | 66 | 104 | 19 | 112 | 8 |
| 7 | 77 | 6 | — | 1680 | 73 | 1060 | 67 | 170 | 41 | 44 | 16 | 14 | 6 | 8 |
| 8 | 25 | 45 | — | 620 | 35 | 340 | 19 | 26 | 6 | 15 | 13 | 12 | 16 | 9 |
| 9 | 66 | 9 | — | 560 | 9 | 840 | 8 | 700 | 88 | 205 | 88 | 40 | 88 | 40 |
| 10 | 320 | 80 | — | 1560 | 147 | 1750 | 140 | 1580 | 41 | 1290 | 13 | 1200 | 13 | 550 |
| 11 | 281 | 10 | — | 2380 | 248 | 2710 | 166 | 1030 | 119 | 275 | 104 | 118 | 102 | 5 |
| Mean | 163 | 24 | — | 1192 | 124 | 1004 | 85 | 490 | 72 | 267 | 62 | 200 | 50 | 96 |
| ± σ | 133 | 27 | — | 604 | 104 | 787 | 72 | 526 | 54 | 382 | 46 | 380 | 44 | 174 |
| *Rat platelets* | | | | | | | | | | | | | | |
| 12 | 43 | 20 | — | 1150 | .4 | 1090 | .2 | 1200 | .2 | 1110 | .2 | 930 | .1 | 1025 |
| 13 | 248 | 18 | — | 995 | 33 | 1000 | 37 | 985 | 11 | 960 | 17 | 880 | 6 | 846 |
| 14 | 73 | 49 | — | 480 | 62 | 510 | 67 | 970 | 6 | 940 | 3 | 910 | 1 | 850 |
| 15 | 381 | 13 | — | 1960 | 115 | 2245 | 52 | 2270 | 37 | 1900 | 43 | 1540 | 19 | 750 |
| 16 | 42 | 28 | — | 1740 | 16 | 1860 | 17 | 1120 | 26 | 2360 | 18 | 1960 | 4 | 795 |
| 17 | 94 | 26 | — | 770 | 58 | 870 | 13 | 1100 | 16 | 1050 | 10 | 1000 | 5 | 885 |
| Mean | 147 | 26 | — | 1183 | 47 | 1263 | 31 | 1284 | 16 | 1387 | 15 | 1203 | 6 | 855 |
| ± σ | 126 | 15 | — | 525 | 39 | 605 | 23 | 463 | 12 | 536 | 14 | 417 | 6 | 101 |

* 1st figure in each column $\times 10^6$ indicates total output of red blood cells in lymph during 15 min.
† 2nd figure in each column $\times 10^3$ represents No. of circulating platelets/mm³ of blood.

10 days later, the abdominal portion of the main lymph duct was cannulated under Nembutal anesthesia (60 mg/kg) according to the method previously described by Bollman *et al.* (4). The external jugular vein was also cannulated to provide a means for multiple injection of the materials under study. Continuous collection of lymph samples was initiated immediately after cannulation. The lymph was collected in test tubes containing 0.1 ml of 3.8% sodium citrate during successive 15-minute periods. The total number of red blood cells collected with the lymph during each period was determined. Platelet counts were carried out by phase contrast microscopy (5) before injection of the platelets, and at intervals thereafter. Fresh rat platelet concentrates were prepared by differential centrifugation of blood collected from the abdominal aorta of normal animals using ACD as anticoagulant. The platelets separated from 20 ml of platelet rich plasma (an average of $25 \times 10^9$ platelets) were given with each transfusion. The platelets were washed once with 0.85% NaCl prior to resuspension in 2 ml of rat plasma.

The human platelets were separated from 500 ml of fresh human blood using the double plastic bag system(6). The concentrated platelets were washed once with isotonic saline and resuspended in rat plasma as above. The average number of human platelets given was $75 \times 10^9$ platelets.

*Results.* The infusion of fresh rat platelet concentrates to 6 thrombocytopenic rats was followed within 15 to 30 minutes by an increase of the circulating platelets from an average of 26,000/mm³ to normal levels approximating $10^6$/mm³. The total output of red blood cells in the lymph was concurrently

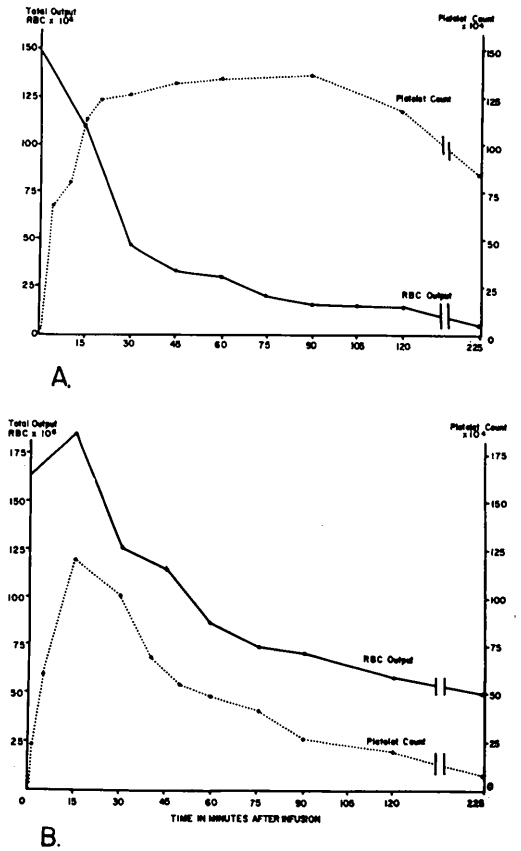


FIG. 1. A. Effect of infusion of fresh rat platelets on the count of circulating platelets and total output of red blood cells in thoracic duct lymph of thrombocytopenic rats. Each point is avg of 6 experiments. B. Effect of infusion of fresh human platelets on circulating platelet count and total output of red blood cells in thoracic duct lymph of thrombocytopenic rats. Each point is avg of the 8 experiments in which significant circulation of platelets was seen.

reduced by an average of 67%. During the remainder of the observation period, the number of platelets increased further or remained stable, while the output of red cells continued to decrease, reaching normal or nearly normal levels about 2 hours after infusion (Fig. 1A, Table I). Eighteen hours later, the circulating platelets still averaged 650,000/mm³.

Fresh human platelets were infused into 11 rats. The number of circulating platelets rose in 8 rats, reaching maximum levels (often higher than normal) within 15 to 30 minutes (Fig. 1B, Table I). A steady decline of the platelet count was then observed until, 2 to 4 hours after infusion, the platelet numbers were again markedly reduced. The output of red blood cells in the lymph, however, was reduced at this time by an average of about 70%. In 3 animals, minimal circulation of human platelets was observed and the effect on red blood cell output in the lymph was only moderate.

*Discussion.* Administration of fresh human platelets to thrombocytopenic rats reduced the output of red blood cells in the lymph. Rat platelets, at comparable levels, were more effective in regard to rate, degree, and duration of the effects. The effects of transfused heterologous platelets were inconsistent. This is in contrast to transfused homologous platelets, which circulated and caused a reduction of red blood cell output in excess of 90% in all 6 experiments.

Although human platelets usually circulated in the early stages following transfusion, they were rapidly removed, in contrast to the transfused rat platelets which persisted in the circulation. The cause of the short *in vivo* survival of the human platelets is not clear. It is known that repeated washing of human(7) and rat(8) platelets reduces the number of platelets capable of circulation and perhaps their *in vivo* survival. The survival observed in our experiments may be due to lesser trauma associated with a single washing or to a minor difference in the technic of handling. It is of interest that washing was not shown to affect the function in addition to the survival of platelets.

Interpretation of these observations is difficult in view of the incomplete information on the mechanisms by which platelets reduce vascular permeability to red blood cells. Should this effect depend on formation of hemostatic "micro-plugs," basically similar to Roskam's "platelet plugs,"(9,10) the lesser reduction of bleeding following infusion of human platelets despite adequate numbers of circulating platelets may suggest inadequate agglutination and perhaps disturbed viscous metamorphosis of the human platelets in a heterologous host. Since draining of extravasated red cells can be very rapid(11), the considerable lapse of time required for complete clearing of the lymph, even after infusion of rat platelets, suggests that other

mechanisms may also be involved in restoring normal permeability of the blood vessels to red cells following platelet transfusion. A direct chemical or physical-chemical interaction between platelets and blood vessel structures could, for example, be involved. Under the conditions of our study it appeared that circulating platelets may be required for such interaction.

It is remarkable that the average output of red blood cells in the lymph of animals treated with human platelets continued to decrease despite the rapid decline of the platelet levels (Fig. 1B). This partial clearing of red cells from lymph in the absence of adequate levels of circulating platelets resembles experiments reported previously(2) on dogs treated with freeze-dried platelets. Reduction of red cell diapedesis into the lymph of thrombocytopenic dogs has also been described following infusions of soybean phosphatides(11). Further studies to determine whether the phospholipids of human platelets disintegrating *in vivo* have contributed to this reduction may, therefore, be warranted.

*Summary.* Fresh human platelets transfused into thrombocytopenic rats are removed from the circulation within 2 to 3 hours. The output of red blood cells into the lymph is reduced (-70%) despite the declining platelet count. The effects of fresh rat platelets are both more marked and more consistent. Species-specificity may perhaps account for the lesser activity of heterologous platelets.

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Received March 30, 1962. P.S.E.B.M., 1962, v111.

## Conversion of Glycine to Fatty Acids by Adipose Tissue.* (27693)

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Whereas the metabolism of carbohydrates and lipids in adipose tissue has been exhaustively studied, the metabolism of amino acids in this tissue has received little attention. Our recent studies have demonstrated that this tissue converts the carbon chain of alanine, isoleucine, leucine, serine and valine to $CO_2$, fatty acids and certain intermediate compounds(1-3). Continuing this program, the present study was undertaken to determine whether glycine can be metabolized by adipose tissue and act as an initial substrate for fatty acid biosynthesis. Carbon-14-labeled glycine, glyoxylate, sarcosine or malate have been incubated with epididymal fat pads and evidence is presented to show that adipose tissue oxidizes glycine as well as utilizes its carbons for biosynthesis.

*Method.* Adult white Swiss mice were fed a diet of Purina laboratory chow *ad libitum* until time of sacrifice. The mice were killed by separation of the cervical vertebrae and the epididymal fat deposit rapidly removed.

* Aided by grants from Nat. Inst. of Arthritis Metab. Dis., U.S.P.H.S. and from Wesson Fund for Medical Research and Education.