seen in serum sickness of rabbits not injected with pressor drugs.

Pressor agents may possibly influence the reactions to foreign serum by causing increased penetration of antigenic materials into intact glomeruli, by damaging glomeruli mechanically while antigens are circulating in the blood or by exerting an adjuvant effect on the antigen-antibody reaction. However, the sera of rabbits given pressor agents following a single large intravenous injection of horse serum had remarkably low titers of precipitating anitbodies on the 11th day as tested by the capillary tube method. They were generally in range of 1:160 or 1:320 and were no different than in rabbits given horse serum and no pressor agents.

No satisfactory explanation can be given for the observation reported from the limited data available. Further investigations may disclose the mechanism involved. However, the use of pressor drugs in association with serum sickness in rabbits provides a convenient technique for producing renal glomerular lesions with considerable regularity.

Summary. Repeated intravenous injections of blood pressure elevating drugs given shortly after a single large intravenous dose of horse serum markedly increased the incidence and severity of diffuse glomerulonephritis in rabbits sacrificed on the 11th day. Only minimal glomerular lesions were observed if the pressor agents were given at a later period. The implication of these findings is that these agents augment the development of renal damage in serum sickness when administered at a time when circulating antigen levels in the blood are high and that they may suppress this reaction when given at a time when antigen levels are rapidly falling and antibody levels rising.

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Received September 9, 1965. P.S.E.B.M., 1965, v120.

A Transplantation Assay for Mouse Cells Responsive to Antigenic Stimulation by Sheep Erythrocytes.* (30678)

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An important part of the immunologic response of normal mice to injected sheep erythrocytes is the generation of large numbers of hemolysin-producing cells which may be enumerated by means of the plaque-formation technique of Jerne and collaborators(1,2). The role of cell proliferation in the production of plaque-forming cells is indicated by the great increase in number of these cells observed in the spleens of mice following immunization with sheep red cells(2) and by our observation that the ability of mice to respond to the injection of sheep erythrocytes by the production of plaque-forming cells exhibits a sensitivity to gamma rays that is of the same order as that found for mammalian cell proliferation(3). These results have been interpreted to mean that plaque-forming cells have progenitors within the spleens of mice, and that these progenitor cells are capable of responding to antigen by

* Supported by the Medical Research Council, Canada (grant MA-1420), National Cancer Inst. of Canada, and the Defense Research Board, Canada (grant 9350-14).

† Research Fellow of Nat. Cancer Inst. of Canada.

proliferating and differentiating to become plaque-forming cells(3,4,5). The name "antigen-sensitive cells" has been applied to these hypothetical precursors of hemolysin-producing cells(3).

In this paper we describe a method for detecting and enumerating antigen-sensitive cells. The assay depends on two postulated properties of these cells: sensitivity to antigenic stimulation by sheep erythrocytes, and ability to respond to this stimulation by proliferating to give rise to cells capable of hemolysin-production. The method is based on the observation that when cells from normal mouse spleen or lymph node are transplanted into heavily-irradiated mice in the presence of sheep red cells, clusters of hemolysin-producing cells arise in the spleens of the irradiated hosts. These clusters of cells, which are assumed to be the progeny of stimulated antigen-sensitive cells, may be detected by their ability to produce large foci of hemolysis in a layer of sheep erythrocytes immobilized in agar. Counts of the number of hemolytic foci produced by spleens of recipients of a given number of transplanted cells may be used to obtain an estimate of the number of antigen-sensitive cells present in the tissue used as the source of the transplanted cells.

Materials and methods. The assay is carried out as follows. C57BL mice 7-10 weeks of age are irradiated either with a single dose of 900 rads of Cs^{137} gamma rays or with 1200 rads delivered as 2 fractions, 500 and 700 rads, separated by one week. Either schedule will eliminate the capacity of the mice to respond to sheep red cells by the production of plaque-forming cells, but the split-dose regimen permits a somewhat better animal survival. Immediately after irradiation, these non-responsive recipient mice are injected with a mixture containing an appropriate number of normal mouse cells and 10^8 washed sheep erythrocytes suspended in 0.5 ml of CMRL-1066(6). Seven to ten days after injection, the spleens of the recipient mice are examined for hemolytic foci as follows: Plastic petri dishes are prepared with a base consisting of 1.5% agar in phosphate buffered saline (pH 7.0) to a depth of about 0.5 cm, and left overnight to dry. An overlay consisting of 2 ml of liquid 0.7% agar in CMRL-1066 at 50°C, 0.1 ml of DEAE-dextran(2), and 0.1 ml of washed packed sheep erythrocytes is poured over the agar base and allowed to form a gel. The plates are ready several hours later and before use are moistened with phosphate buffered saline. Spleens are removed from the mice under test, frozen immediately on the stage of a freezing microtome, and cut longitudinally into serial sections about 300 μ thick. These sections are allowed to thaw briefly in CMRL-1066, placed carefully on the prepared agar surfaces of the petri dishes, and incubated at 37°C for 1 hour. Then 2 ml of guinea pig serum, diluted 1/30-1/50 with phosphate buffered saline, is poured over the surface of the dish, washing loose the sections of spleen which are then removed. The plates are inspected and artifacts caused by bubbles or tears in the agar are marked. The plates are then incubated with the guinea pig serum for a further 30-45 minutes. At this time, discrete areas of lysis can be seen superimposed on the imprints of the sections of spleen. We score as hemolytic foci those areas of lysis that are approximately 0.5-1.0 mm in diameter. Larger areas, up to 3 or 4 mm in diameter, when traced through imprints of serial sections are usually found to consist of two or more foci whose zones of hemolysis have merged on some of the imprints. Scattered about the imprint, especially near the borders of foci, there are usually also some pin-point areas of hemolysis, probably caused by single hemolysin-producing cells that have left the cluster in which they originated. These tiny hemolysed areas are approximately the same size as the minute plaques that result when single hemolysin-producing cells are assayed(1,2) in an agar overlay containing the same high concentration of sheep erythrocytes used in this focus assay. Occasionally these pin-points of hemolysis form a tight cluster on the imprint, and only then are they scored as a focus.

Results. Background. The spleens of unimmunized mice are known to contain hemolysin-producing cells(7) detectable by the plaque-forming techniques of Jerne *et al*(1,2).

TABLE I. Background Hemolytic Foci in the Spleens of 8-Week-Old C57Bl/6JH Male Mice.

Cs^{137} γ-radiation schedule	Antigen*	Isologous transplants†	Avg No. foci/spleen‡ ± 95% confidence limit
0 rads	No	No	32/25 = 1.28 ± .55
900 rads	Yes	No	11/20 = .55 ± .40
500 rads-7 days-700 rads	Yes	No	46/44 = 1.05 ± .60
" " "	No	5×10^6 spleen cells	58/40 = 1.45 ± .75
" " "	No	1×10^7 " "	15/20 = .75 ± .50
" " "	No	2.5×10^7 lymph node cells	2/10 = .20 ± .45

* Antigen is 1×10^8 sheep erythrocytes, injected intravenously one hour after irradiation.
† Single cell suspensions in .5 ml of CMRL-1066, injected intravenously one hour after irradiation.
‡ Assays were carried out on the 8th day after irradiation and injection.

Similarly, an occasional hemolytic focus is produced by the spleens of both normal C57Bl mice that have not been immunized with sheep erythrocytes and those of heavily-irradiated immunized mice. Table I contains the results of experiments in which foci were counted under a number of control conditions. It may be seen from the Table that the spleens of normal unimmunized mice, heavily-irradiated mice given antigen, and heavily-irradiated mice transplanted with isologous spleen or lymph node cells but not given antigen, all produce about the same average number of hemolytic foci. Further, the transplantation of isologous spleen or lymph node cells into irradiated mice in the absence of antigen has no effect on the number of hemolytic foci produced. Thus, the foci observed under the conditions of Table I may be considered to be background. Their number is measured as a control in each experiment, and subtracted from the result.

Linearity. When sheep erythrocytes and a sufficient number of spleen or lymph node cells are injected together into heavily-irradiated recipients, there is a large increase over background in the number of foci per spleen. If these hemolytic foci result from the activity of single antigen-sensitive cells that were randomly distributed in the cell suspension under test, then the relationship between the number of nucleated cells injected and the average number of hemolytic foci that results should be a straight line extrapolating back through the origin. Fig. 1 summarizes the results of a representative experiment in which groups of heavily-irradiated mice were injected intravenously with various dilutions of a spleen cell suspension mixed with 1×10^8 sheep erythrocytes. Eight and ten days later, spleens of mice from each group were assayed for hemolytic foci; on both days the relationship between the average number of foci per spleen and the number of spleen cells originally transplanted is seen to be a straight line extrapolating back through the origin. We conclude from this experiment that hemolytic foci in the spleen result from single entities (presumably antigen-sensitive cells) randomly distributed in the original spleen cell suspension, and that the number of foci is directly proportional to the number of these entities injected. The spleens of mice from a control group, in which spleen cells were injected without sheep erythrocytes, showed only the usual small number of background foci.

Constancy with time. The results given in Fig. 1 indicate that there was no significant difference in the average count of hemolytic

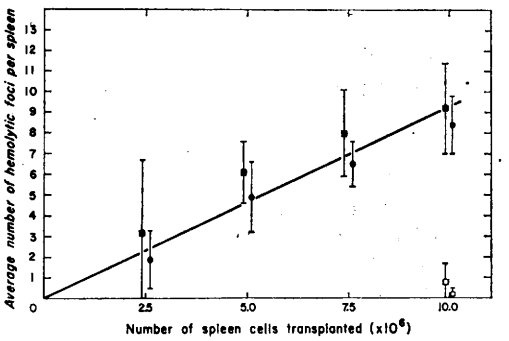

FIG. 1. Relationship between mean number of hemolytic foci per spleen and number of nucleated spleen cells injected. Closed circles: assayed on 8th day after transplantation of spleen cells and 10^8 sheep erythrocytes. Closed squares: assayed on 10th day. Open circles and squares: 10^7 spleen cells injected without sheep erythrocytes. Limits indicated are 95% confidence intervals.

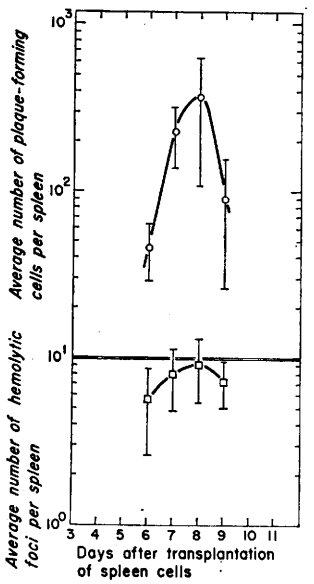

FIG. 2. Assay of spleens of heavily-irradiated mice as a function of time after injection with 5×10^6 spleen cells and 10^8 sheep erythrocytes. Upper panel: results obtained with the plaque formation method of Jerne *et al*(1,2). Corrected for background, which did not on any day exceed an average of 8.4 plaques per spleen. Lower panel: results obtained with the assay for hemolytic foci. Corrected for background, which did not on any day exceed an average of 1.8 foci per spleen. Limits indicated are 95% confidence intervals.

foci in any given group of spleens when assays were carried out on the eighth and on the tenth day after transplantation. A further demonstration of the constancy of the number of hemolytic foci with time is provided by the experimental results presented in Fig. 2. In this representative experiment, heavily-irradiated C57Bl mice were injected with a mixture containing 5×10^6 spleen cells and 10^8 sheep erythrocytes. Beginning on the 6th day after transplantation, spleens from 10 mice were taken daily and assayed individually for hemolytic plaque-forming cells by the method of Jerne *et al*(1,2), or for hemolytic foci by the method described above. In the Figure, the results obtained from the assay for plaque-forming cells are shown in the top panel, in which the logarithm of the mean number of plaques per spleen is plotted on the vertical axis against time after transplantation on the horizontal axis. The results obtained with the focus assay are presented in the lower panel, in which the logarithm of the mean number of foci per spleen is plotted as a function of time. On each day, background values were obtained from recipient mice that received spleen cells without antigen, and have been subtracted from the values obtained in the mice that received both spleen cells and sheep erythrocytes. On no day did average background exceed 1.8 foci per spleen. It is evident from the Figure that no significant change was observed in the number of foci per spleen from the 7th to the 9th day after transplantation. In contrast, during the same period, the number of plaque-forming cells per spleen rose exponentially after the fifth day, reached a peak on the 8th day, and afterwards fell. It is apparent from the Figure that the number of foci per spleen did not change from the 7th to 9th day, although, over the same time interval, large changes were observed in the number of plaque-forming cells per spleen.

Distribution of antigen-sensitive cells. The hemolytic focus method was developed by using cells derived from spleen. It was of interest to determine whethor or not other tissues contain cells capable of forming hemolytic foci. Accordingly, cell suspensions derived from inguinal lymph node, bone marrow, thymus and embryonic liver were tested for their capacity to give rise to hemolytic foci following transplantation into irradiated recipients. In each case, between 5×10^6 and 3×10^7 cells from each tissue were transplanted along with antigen, and the assay was performed 8 days later. The results, and those obtained by using spleen cells, are expressed as the number of foci produced per 10^7 cells injected, and are summarized in Table II. It is evident from the Table that antigen-sensitive cells are found in organs like spleen and lymph node, which are known to be lymphoid in character, and are very rare in marrow and foetal liver, which are primarily hemopoietic organs. The absence of detectable antigen-sensitive cells in the adult thymus supports the view that this organ does not participate directly in immunological responses of adult mice.

Discussion. The enumeration of hemolytic foci forms the basis of the proposed assay

TABLE II. Capacity for Hemolytic-Focus Formation by Cells from Normal Mouse Tissues.

Source of injected cells*	Mean number of foci per 10^7 cells†
Spleen	11.6, 9.2, 8.2, 8.4, 10.8, 7.8, 13.0, 18.2, 11.8, 12.6, 7.2, 9.6, 6.8, 20.1, 19.5
Lymph node	30.6, 26.2, 46.0, 30.4
Bone marrow Thymus Foetal liver	Not significantly different from background

* Single cell suspensions of 5×10^6-3×10^7 isologous cells injected intravenously together with 1×10^8 sheep erythrocytes in .5 ml of CMRL-1066.
† Assays were carried out on 8th day after irradiation and injection. Each number involves a separate experiment, and represents average count of hemolytic foci produced by 7-10 spleens.

for antigen-sensitive cells. From the experimental results presented above we suggest that these hemolytic foci result from the action of hemolysin released by clusters of cells within the spleens of the heavily-irradiated recipients of transplanted spleen or lymph node cells; further, these clusters contain the progeny of single cells that have proliferated and differentiated as a result of antigenic stimulation. The hemolytic foci result from the activity of more than a single cell since foci are larger than the plaques produced by single cells and since the kinetics of production of plaque-forming cells following spleen cell transplantation is different from the kinetics of production of focus-forming ability (Fig. 2). However, the observation that the mean number of foci per spleen is linearly related to the number of nucleated cells injected (Fig. 1) indicates that the sources of the clusters of cells required for focus-formation are single entities, perhaps single cells, present in the injected cell suspension. Since an increase of number of foci over background occurs only when sheep red cells are also present, the transplanted cells of origin are sensitive to this antigen. Thus the hemolytic focus technique provides an assay of the number of antigen-sensitive cells present in the suspension injected into the recipients.

The detection of hemolytic foci is possible because the progeny of antigen-sensitive cells remain clustered for a period of time, permitting them to be observed as a unit. The presence of antigen-sensitive cells in the original suspension is deduced from the activity of their progeny detected many days later. In this regard the formation of hemolytic foci in the spleen may be compared with the formation of hemopoietic colonies in the spleen (8); in the latter case the presence of a hemopoietic stem cell is detected by observing after 9-14 days, the formation of a macroscopic spleen-colony containing in excess of a million cells. However, the results presented in Fig. 2 indicate that each focus probably results from hemolysin released by approximately 10^2 cells, so that antigen-sensitive cells apparently have much less capacity for proliferation than have hemopoietic colony-forming cells. On this basis we have suggested(5) that antigen-sensitive cells are members of a class of "dependent" stem cells, depending for their replenishment on an even more primitive cell type, *i.e.*, on an "independent" stem cell with a much greater capacity for proliferation. Such a cell would be more nearly analogous to the hemopoietic colony-forming cell. From this viewpoint, it is of great interest that Mekori *et al*(9) have recently reported that macroscopic colonies containing more than a million cells develop in the spleens of irradiated mice injected with lymph nodes from mice previously treated with phytohemagglutinin. The relationship between these colonies, spleen colonies of the hemopoietic system, and foci of hemolysin-producing cells in the spleen is yet to be determined.

Recently, Playfair and his collaborators (10) have reported an assay based on a principle similar to that of the assay described in this paper. Their conclusions in respect to the number of progenitor cells and their capacity for proliferation are similar to those presented here.

Summary. When cell suspensions containing normal mouse spleen or lymph node cells mixed with sheep erythrocytes are injected into heavily-irradiated mice, foci of hemolysin-producing cells are formed in the spleens of the recipients. A method is described for demonstration of these foci, and evidence is presented that they result from the proliferation of antigen-sensitive precursors of hemo-

lysin-producing cells. The demonstration of the existence of "antigen-sensitive" cells provides strong support for the view that cell proliferation is involved in the generation of hemolysin-producing cells.

We thank Miss R. Wyncoll, R. Course, R. Kuba and Frank Mallory for their excellent technical assistance.

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Received September 14, 1965. P.S.E.B.M., 1965, v120.

Naturally Occurring Fatal Disease of the Pregnant Golden Hamster.* (30679)

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Eclampsia is a disease which occurs only during or immediately after pregnancy. It is characterized by convulsions on a background of hypertension, edema and albumenuria and it is attended by a high mortality. *Abruptio placentae* is a common accompaniment. Some pre-partum cases develop fibrin thrombi in the renal glomerular capillaries which may lead to bilateral renal cortical necrosis, anuria and uremia(1).

It is believed that eclampsia is peculiar to man and possibly the higher primates since its spontaneous occurrence in other species is not well documented, although syndromes resembling human pre-eclamptic toxemia are known to occur in several animals, most notably the sheep(2).

The generalized Shwartzman reaction (GSR), an experimentally induced disease of certain laboratory animals, bears a striking resemblance to the type of human eclampsia associated with renal cortical necrosis(3). It has recently been shown that during late pregnancy, the Syrian (golden) hamster regularly develops the generalized Shwartzman reaction in response to a single intraperitoneal injection of colchicine(4). A similar reaction sometimes follows devitalization, by arterial occlusion, of the appendix or of a short segment of the small intestine(5).

Since the pregnant golden hamster appears particularly susceptible to such experimentally induced episodes of disseminated thrombosis, the possibility was examined that similar spontaneous disease processes might occur naturally in this species.

Materials and methods. The tissues were examined of hamsters which became ill during late pregnancy or shortly after delivery at a large commercial hamster production establishment, the Lakeview Hamster Colony, Newfield, N. J. The colony consists exclusively of the golden hamster, *Mesocricetus auratus* Waterhouse, 1839. It has been a closed breeding colony since its foundation from 3 breeding pairs in 1949 and a fourth in 1951. Animals are housed in polypropylene cages bedded with white pine shavings.

* Supported by USPHS grant HD 00544.

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