Discussion. By sonic disruption of bacteria, a heterogenous population of macromolecules is probably obtained. Thus, common antigenic and toxic groups might be attached to molecules of various sizes and charges which would be dispersed widely in starch electrophoresis. The antigenic heterogeneity of the fractions and wide dispersal of the active material causing the erythematous eruption substantiate this idea.

Rashes and eruptions are found in many human streptococcal infections. This reaction resembles the eruption seen in human erysipelas. The eruption may be due to a preexisting state of hypersensitivity in some of the rabbits or to induction of a hypersensitive state by the streptococcal injection. Alternately the streptococcal injection may activate release of deleterious host products which in turn produce the reaction. Intravenous injection of trypan blue revealed persisting capillary damage at periphery of the lesion at a time when the secondary eruption normally occurred. The latent period necessary for production of the rash supports either theory. The negative results obtained in this study following both lines of reasoning do not necessarily rule out either hypothesis.

Summary. Intradermal injection of crude sonic extracts and fractions obtained by starch electrophoresis of group A streptococci produced a spreading erythema in a variable percentage of rabbits. Recurrence of the lesion developed in about 10% of rabbits after abatement of primary lesion. The variability in rabbit response made it difficult to characterize the active principle and to determine the underlying mechanism of the reaction.

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Production of Normal-Lived Erythrocytes with Erythropoietin.* (25912)

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Although erythropoietic hormone may be an important factor in normal regulation of erythropoiesis, it can be obtained in significant amount only from animals whose erythropoiesis is markedly disturbed(1). If erythropoietin is part of the normal mechanism of erythropoiesis, one would expect administration of this hormone to result in production of normal erythrocytes. If production of erythropoietin is an emergency measure designed to flood the circulation with hastily or incompletely formed red cells, one might expect to find evidence of this in abnormal morphology, incomplete or abnormal hemoglobinization, increased fragility, or in shortened average survival time in the circulation. Gordon *et al.*(2) investigated the properties of erythrocytes produced under the stimulus

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of human urinary erythropoietin and found normal osmotic fragility and oxygen carrying capacity and no increase in amount of abnormal hemoglobin. However, because it has been suggested(3) that erythrocytes with an abnormally short life span are produced after acute bleeding (a time when erythropoietin is found in high concentration in the circulation), it was considered important to determine the *in vivo* survival of erythrocytes produced under the influence of administered erythropoietin.

Material and methods. Erythropoietin used was obtained by the same method and from the same patient described earlier(4). By direct comparison of methods, it has been determined that 1 mg of human urinary erythropoietin used for the published dose response curves(4) is equivalent to approximately 7 cobalt units as described by White *et al.*(5). Two groups of 10 normal female rats of Long-Evans strain with an average weight of 187 g were used in this study. One group was given the equivalent of 6 cobalt units of human urinary erythropoietin subcutaneously daily for 10 days. On the 5th day (in middle of injection period) both groups were given 10 microcuries of glycine-2-C^{14} intraperitoneally. All animals were weighed periodically so that an accurate correction could be made for dilution of specific activity due to blood volume increase. In order to minimize blood loss due to sampling, only 5 animals of each group were sampled at each interval; 0.25 ml samples were drawn from the tail vein into a heparinized syringe. The cells were washed 3 times with physiological saline and specific activity of $BaC^{14}O_2$ prepared from hemoglobin determined as described previously (6).

Results. A single microhematocrit determination was done the day after last injection of erythropoietin (on the 11th day). The average hematocrit for the injected group was 57.3 $\pm$ 2.1 as compared to 44.3 $\pm$ 0.46 for controls, indicating that erythropoiesis had been effectively stimulated. After 14 days of treatment with the same dose of erythropoietin one would expect approximately a 40% increase in total circulating red cell volume

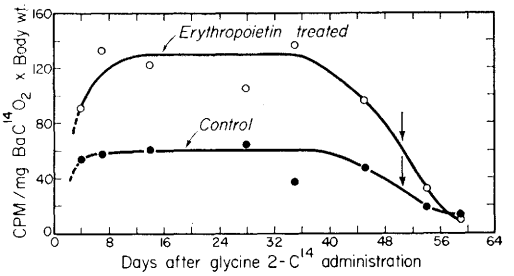

FIG. 1. The shape of both curves is compatible with finite life span and the point at which specific activity falls to ½ the plateau value is the same (51 days) for both groups.

(4). A growth curve was kept during the course of the experiment and the value obtained for each sample was corrected for dilution due to growth. A further small correction was made for loss of activity due to sampling. The results are presented in Fig. 1. The injected group incorporated more than twice as much glycine into red cells as did the controls, substantiating the fact that erythropoiesis had been accelerated at time of glycine administration.

The shape of both curves is compatible with a finite life span and the point of inflection or point at which specific activity falls to ½ is the same for both groups. The time at which level of activity fell to ½ the plateau value was 51 days in both groups. This value is in fair agreement with previously published data from this laboratory(7,8,9) using glycine-2-C^{14}, but is less than the recent 59 day estimate of Belcher and Harriss using Fe^{59} and a Bartonella-free strain of rats(10).

Discussion. Erythropoietic hormone assumes a greater physiological importance if it can be established that it represents part of the normal mechanism by which red cell production is controlled. Cells formed under influence of this hormone are indistinguishable from normal erythrocytes as far as survival time in circulation, pattern of hemoglobin electrophoresis(2), osmotic fragility and oxygen carrying capacity(2). Alpen *et al.*(11, 12) emphasized that both bleeding and human urinary erythropoietin administration cause a greatly increased output of erythrocytes, not by any change in marrow dynamics, but by stimulating stem cell division. An ef-

fect at the stem cell level would be expected to result in production of normal erythrocytes. Thus, there is no evidence to indicate that erythropoietin is part of an abnormal mechanism.

Summary. Erythrocytes produced in rats under stimulus of human urinary erythropoietin have a normal survival time in circulation. This and other evidence suggest that erythropoietin administration results in production of normal erythrocytes. This evidence is compatible with the concept that erythropoietin is part of the normal mechanism controlling erythropoiesis.

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Direct Measurement of Radioactive Sulfate Uptake by Rat Mast Cells.* (25913)

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The *in vivo* uptake of $S^{35}O_4$ by an organic component of mast cells has been demonstrated using autoradiography. By means of intensity grading(1) or grain counting(2) an estimate of rate of uptake and loss of radioactivity was achieved. The grading method is at best semi-quantitative, while the grain counting technic is cumbersome. Therefore we searched for a quantitative method of reasonable simplicity. Millipore filters have proved useful in several types of cytologic study. Collection of free cells on these filters has been employed in clinical cancer cytology (3,4), in *in vitro* studies of cell function(5) and in tissue culture(6). This study was undertaken to extend this method of cell collection to permit direct measurement of incorporation of radioactively labeled substances into cells. The cells chosen for the investigation were free peritoneal mast cells of the rat. Radioacitve sulfate was used as the tracer compound, since sulfate is known to be incorporated into mast cell heparin(7).

Materials and methods. Isotope labeled sulfate obtained as $H_2S^{35}O_4$ in dilute HCl was titrated to neutrality with NaOH and diluted to the desired concentration with .8% aqueous NaCl. This solution was administered intravenously in the tail of an etherized rat. Sprague-Dawley male rats weighing 280-390 g were used in most experiments. Mast cells were obtained from rat peritoneal cavities by the procedure previously described(8). Separation of mast cells from other peritoneal cells was achieved by centrifugation through

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