failure in conversion of CO_2 to bicarbonate in the erythrocyte, more being therefore transported in the dissolved and carbamino forms at a higher pressure. The consequences of such increase in venous CO_2 tension are those which have been observed following carbonic anhydrase inhibition in the dog(1). The higher tension of CO_2 in venous blood must result in retention of CO_2, the quantity being dependent upon level of pressure and tissue dissociation constants. This equilibration at a higher level of P_{CO_2} can be accomplished by tissues because they are not dependent upon enzymatic acceleration for conversion of CO_2 to bicarbonate, since the reaction is not temporally limited by circulation. CO_2 is therefore retained until its tissue stores are increased to a new state of equilibrium.

Summary. 1. Venous and tissue gas tensions in the rat have been estimated by use of artificially constructed subcutaneous gas pocket. Inhibition of carbonic anhydrase by acetazolamide results in a rise in venous and tissue CO_2 tension independent of change of oxygen tension. This effect is associated with hyperventilation and an increase in arterial CO_2 content. 2. These findings are interpreted as direct evidence of CO_2 retention resulting from interference in CO_2 transport by carbonic anhydrase inhibition.

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Neutrophil Life Cycle with Tritiated Thymidine.* (24190)

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Patterns of neutrophil balance can be brought into sharper focus than heretofore possible by means of high resolution radioautography with tritium-labeled thymidine. A rather precise localization may be achieved with labeled nucleoside because of its specific incorporation into DNA and the short range of tritium beta particles(1,2). The exogenous precursor is apparently available only for a brief period after administration, which is an added advantage. Neutrophilic leukocytes are part of a highly labile renewal system and the kinetics of their development *in vivo* can be determined from temporal changes in degree of labeling and distribution of the various cells in developmental sequence. The present investigations were initiated to establish the neutrophil life cycle in the normal steady state as a basis for evaluation of various perturbations of the neutrophil system including those induced by irradiation.

Methods. Thus far, 2 normal dogs (1 male and 1 female beagle, 1.5 and 4 yrs. old respectively) have been studied after intravenous injection of tritiated thymidine in dosage of 100 μc/kg body weight. The thymidine, labeled on the pyrimidine moiety, was radiochemically pure as judged by chromatographic and spectrophotometric analyses. Specific activity was 390 mc/mmole.† Bone marrow and peripheral blood studies were carried out at frequent intervals during the first 2 days, and daily thereafter for 2 weeks. Marrow samples (0.8 ml) were aspirated from femur or iliac crest into equal volume of heparinized plasma. The procedure was performed under local anesthesia in trained animals. Peripheral blood studies, which in-

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† Obtained from Schwarz Laboratories, Mount Vernon, N. Y.

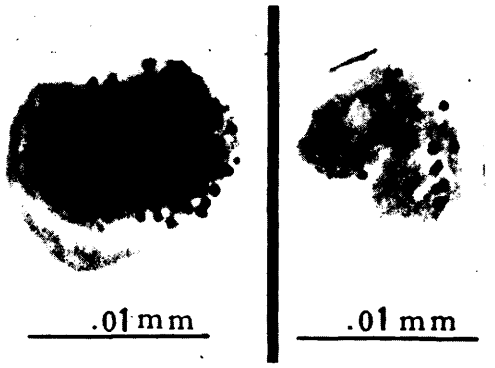

FIG. 1. Left: Radioautograph of myelocyte in marrow 4 hr after inj. of tritiated thymidine. Right: Segmented neutrophil in blood 4 days after inj.

cluded total and differential leukocyte counts, hematocrit, and blood volume, did not reveal any significant changes in neutrophil balance. Thin films of marrow suspension and blood were made on glass slides coated with egg albumin and chrome alum. The preparations were fixed in absolute methyl alcohol and radioautographs were made, using a permeable base stripping emulsion (British Kodak, AR 10)(3). After exposure for 6-8 weeks at 5°C, the preparations were developed, fixed, and treated with Giemsa stain at pH 5.75. Some 300 radioautographs were made for each animal, and, although the various analyses have not been completed, the chronology of neutrophil development *in vivo* is clearly discernible. Counts of labeled cells were based on enumeration of at least 1000 myeloid and associated number of erythroid cells for preliminary analysis of each marrow sample. Since myeloblasts and promyelocytes comprise only a few percent of the total, these cells are included with the myelocytes for the present. In nearly all radioautographs 4 grains over a nuclear area represented a statistically significant increase above background ($p<.01$). Radioautographs of the neutrophil series are shown in Fig. 1.

Results. The tritium label appears first in immature cells which ordinarily contribute to renewal of myeloid and erythroid populations by mitosis. About 15% of myelocytes and 20% of erythroblasts in marrow aspirates are labeled within 2 hours after injection of tritiated thymidine in the dosage employed. Labeled lymphocytes are not seen at this time. Densely labeled telophases of both myeloid and erythroid series are found in marrow by 4 hours. When the number of positive cells is computed in terms of normalized distribution of various cell types, there is a rapid increase with time. Since part of the label remains in the daughter cells of a labeled progenitor, several mitoses may be required, depending upon degree of initial incorporation, before the label is no longer detectable above background. The number of positive cells begins to decrease by 48 hours, owing to dilution of the label by mitosis and, possibly, the beginning of release of labeled cells from marrow. Turnover time of proliferating myeloid elements can be approximated from rate of increase of positive cells and is of the order of 15 hours. Consideration of time relationships between thymidine administration and increment in labeled mitoses, as well as the decrement in grain counts should provide a more complete picture of the life cycle of various proliferating cells.

Distribution of labeled myeloid cells progresses in an orderly manner from the less to the more differentiated forms. There is a comparable progression of the label in nucleated erythrocytes. Only 1-3% of lymphocytes are labeled in marrow samples from 12 hours to 5 days after injection of tritiated thymidine. Labeled metamyelocytes appear in bone marrow within 12 to 24 hours and band cells within 24 to 36 hours. Tagged neutrophils (segmented) can be detected in aspirated marrow within 2 to 3 days and in peripheral blood by 3 to 4 days. The first labeled neutrophils may be released from marrow somewhat earlier without detection in circulation owing to their rapid dilution in the large extravascular pool(4). The peak of labeled cells in peripheral blood (25 to 30%) is attained by 4 to 5 days and this is followed by rapid and, perhaps exponential, decrease in percentage of positive cells with a half time of the order of 2 days. Although the peak of labeled neutrophils in blood appears to correspond with that in marrow, there is an unavoidable dilution of marrow with blood

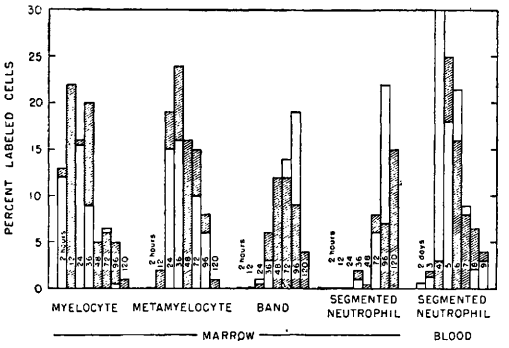

FIG. 2. Time relationship between inj. of tritiated thymidine and appearance of label in the various cells of the neutrophil series. (Hatched bars, male beagle; open bars, female beagle.)

during aspiration, which influences the percentage of labeled segmented neutrophils in marrow. When this factor is considered, it is clear that the true peak in marrow occurs about a day earlier than in the periphery. A similar sequence of segmented neutrophils in marrow and blood has been seen in the mouse with C^{14}-labeled adenine(5). It is of interest to note that labeled myelocytes may be detectable in marrow for several days. It can be inferred from this that some labeled neutrophils are released to the circulation for about an equivalent length of time, although in decreasing numbers. This conclusion is supported by an apparent decrease in grain counts of the peripheral neutrophils with time. The overall sequence of events is depicted in Fig. 2. Comparable results were obtained in both dogs.

There is, in general, a reasonable correspondence between these results and the indirect approximations of neutrophil balance reported previously(6,7). It would appear from these data that the time for differentiation of a myelocyte to a segmented neutrophil in the dog is between 2 and 3 days. Another 2 to 3 days are spent in the segmented form. More detailed counting and more frequent sampling is necessary, however, to establish the precise chronology of neutrophil maturation and the life cycle of the proliferating elements.

Summary. The pattern of neutrophilic granulocyte development has been studied in 2 dogs by high resolution radioautography with tritiated thymidine. Two to 3 days are required for differentiation from the myelocyte to the segmented neutrophil. The half time for disappearance of the latter in the periphery appears to be of the order of 2 days.

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Effectiveness of Gluconate, Chloride, and Other Sodium Solutions in Treatment of Experimental Burn Shock.* (24191)

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There is adequate experimental(1,2,3) and clinical(4,5,6,7) evidence for the effectiveness of oral sodium solutions in prevention and treatment of burn shock. For other types of shock the experimental evidence(8,9,10,11) is just as good, but clinical confirmation is meager. There is relatively little evidence on

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