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III. Erythrocytes—with Deviations of Observations from Predicted Values

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WITH THE TECHNICAL ASSISTANCE OF
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PARTIAL DOCUMENT

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WHILE a number of reports have appeared describing the radioactive phosphorus levels of erythrocytes over short-time periods, no long-term studies have been noted. The purpose of this investigation is the determination of the time rate of disappearance of radioactive phosphorus from the erythrocyte fraction of the blood of patients with leukemia, and the correlation of this change with the P^{32} levels observed in the plasma of these patients.

This problem has been investigated by Erf, Tuttle, and Lawrence,[3] whose data covered a period of eight days following the oral and intravenous administration of radioactive phosphorus. From their graphic data, it can be determined that the concentration of P^{32} per unit volume of blood decreased uniformly with time at a rate that appeared to be different from that of the plasma. Neither the form of the function describing this curve nor the relationship between these curves can be determined with certainty from their figures. Marinelli and Goldschmidt presented a curve showing a semilogarithmic disappearance curve for P^{32} in erythrocytes and leukocytes for one case, but the ratio to the P^{32} ingested was not given. The results of other investigators[1, 2, 4] on this problem cannot be directly compared with the results reported in the present paper and so will not be discussed.

In the use of P^{32}-labeled erythrocytes for blood-volume determinations, Nylin, and Nieset et al. showed a rapid uptake of P^{32} in vitro with incubation periods of two hours or less. Taylor, Levenson, and Adams showed an uptake of P^{32} in vitro by erythrocytes from P^{32}-labeled plasma in four hours. While our data do not include any short-time studies, the references cited indicate a rapidly attained initial equilibrium between erythrocytes and plasma P^{32}.

The data presented deal primarily with the change of P^{32} concentrations observed in the erythrocytes of patients with leukemia during the intervals between treatments,[5] for periods of from one to six weeks.

METHODS

The method of separation of the erythrocytes from 1.0-ml. samples of whole blood and the techniques of P^{32} assay are described in Parts I and II of this series. The technical errors in the counting procedures were considered to be approximately those of the whole-blood determinations. It was noted that a small fraction (of the order of 10 per cent) of the leukocyte layer sometimes was centrifuged through into the erythrocyte fraction. With a median leukocyte count of 20,000 per cu. mm., after correction for the greater P^{32} activity of leukocytes over erythrocytes, it was found that these additional leukocytes in the erythrocyte layer introduced an error into erythrocyte-P^{32} determinations of the order of 1 per cent, a negligible quantity in comparison with individual variations in P^{32} metabolism shown in Parts I and II.

All data were corrected for the effects of preceding injections, using a curve, uncorrected for the effects of preceding injections, with a biological half-life of 7.4 days.[9]

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Aided by grants from the Medical Research Foundation of Oregon and the Damon Runyon Cancer Research Fund.

The P^{32} used in this investigation was supplied by Clinton Laboratories, and obtained on allocation from the Isotopes Division, United States Atomic Energy Commission.

The authors wish to acknowledge the advisory assistance given them in reviewing the statistical analysis of data by Dr. Carl E. Hopkins, Associate Professor of Public Health and Preventive Medicine, University of Oregon Medical School, Portland, Oregon.

Received for publication, April 18, 1950.

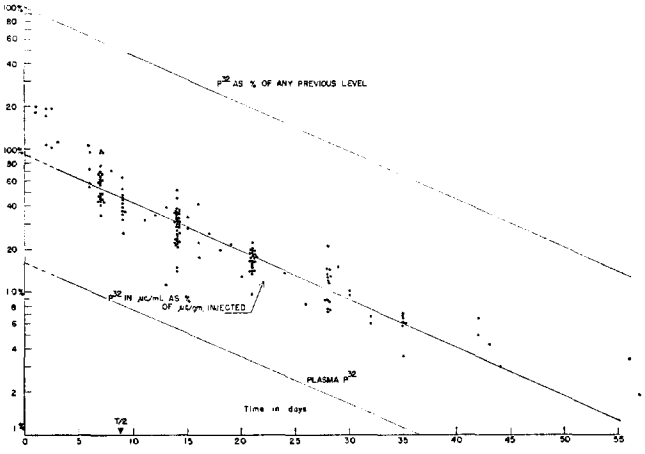

FIG. 1. *The variations of P^{32} per ml. erythrocytes, in terms of the preceding injection, in patients with lymphocytic and granulocytic leukemias.*

RESULTS

The regressions on time of seventy-seven P^{32} levels of erythrocytes from thirteen patients with lymphocytic leukemia and of seventy-four observations from fourteen patients with granulocytic leukemia were plotted; no significant difference was found between the curves for the two groups. The results were combined and the logarithms of 151 observations of P^{32} levels for both lymphocytic and granulocytic leukemias were plotted against time, as shown in Fig. 1. A curve fitted to these logarithmic observations by the method of least squares gave the parameters:

Half-life: 8.9 days (T/2)

Erythrocyte P^{32} in µc. per ml. at the extrapolated zero time: 92 per cent of the injected P^{32} in µc. per gm. of body weight

Equation 1: $\text{Log}_{10} F = 1.961 - 0.033\ T$

$$F = 92\ e^{-0.0779\ T},$$

where $F = P^{32}$ in µc. per ml. erythrocytes as per cent of injected P^{32} in µc. per gm. of body weight

T = time in days

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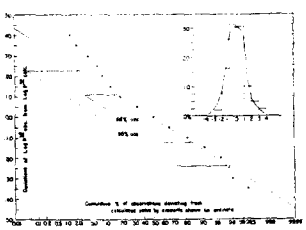

FIG. 2. *Distribution of the deviations of the logarithms of the observed values of erythrocyte P^{32} from the predicted line in Fig. 1. A conventional histogram with the mean line of the probability plot superimposed is shown in the inset.*

The distribution of the deviations of the logarithmic observations from the values predicted by the curve is plotted as Fig. 2. From this curve, it can be seen that the standard error of the estimate is 0.115 and that the central two thirds of the observations lie between 77 and 113 per cent of the predicted values.

Ratios of the P^{32} per ml. of erythrocytes over P^{32} per ml. of plasma were calculated for each of the 151 samples in Fig. 1. Separate distributions for lymphocytic and granulocytic leukemia patients showed no difference in median values. The distribution of these ratios for the combination of lymphocytic and granulocytic leukemia patients is shown in Fig. 3. The median value of erythrocyte P^{32} per ml. of cells over P^{32} per ml. of plasma is 6.1:1.

DISCUSSION

Since the erythrocyte P^{32} is normally the largest fraction of the total P^{32} in a sample of whole blood, and the disappearance curves for P^{32} from whole blood and plasma have been presented in Parts I and II of this series, no curves for the disappearance of the P^{32} from the erythrocyte fraction of the bloods of the individual patients were prepared.

Inspection of Fig. 3 shows that the central 50 per cent of the ratios of P^{32} per ml. of erythrocytes over plasma P^{32} per ml. falls within the fairly narrow limits of 5.2 to 7.0. This is consistent with the normal small variations observed in the determinations of phosphorus levels in blood and its components. Taking the values of Shohl for total "cell" phosphorus as 68 mg. per 100 ml. and total serum phosphorus as 11 mg. per 100 ml. serum (average from twenty-three leukemia patients in this series), the ratio of erythrocyte to plasma phosphorus is 6.2:1.

Reference to Fig. 1 shows that the plasma curve multiplied by the ratio of erythrocyte P^{32} per ml. over plasma P^{32} per ml. (6.1:1) provides a reasonable fit of the observations of P^{32} levels per ml. of erythrocytes. Since both the plasma and the erythrocyte-P^{32} levels were determined from the same blood samples, it may be presumed that an approximate equilibruim exists between total-plasma and erythrocyte P^{32} after seventy-two hours.

In view of the limits of accuracy of prediction shown by Fig. 2, it is evident that the use of the curve in Fig. 1 for the prediction of the erythrocyte-P^{32} level for any particular patient is consistent with the limits of error noted in our series of observations. For convenience in prediction of a future P^{32} level from a known erythrocyte-

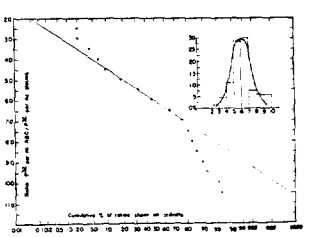

FIG. 3. *Distribution of the ratios of P^{32} per ml. erythrocytes to P^{32} per ml. plasma of the same blood. Data are from the points shown in Figs. 1 and 2.*

P^{32} level, the upper line of Fig. 1 is provided. Equation 1 can be used for the construction of a table. If injections are given at intervals closer than two or three weeks, corrections for the effects of preceding injections, as outlined in Part I of this series of papers, will improve the accuracy of prediction of P^{32} levels.

SUMMARY AND CONCLUSIONS

1. Radioactive phosphorus levels were determined in the erythrocyte and plasma fractions of 151 blood samples divided among thirteen patients with lymphocytic leukemia and fourteen patients with granulocytic leukemia. No significant differences were found between the erythrocyte levels of P^{32} of these two groups, or between the ratios of erythrocyte to plasma P^{32} per unit volume, over the period from three to forty-two days covered by this study.

2. It is shown that the logarithm of the level of P^{32} in the erythrocyte fraction of the blood of leukemia patients, from approximately three to forty-two days after injection of P^{32}, may be represented as a linear function of time. The theoretical zero-time level of P^{32} represented by extrapolation of this curve is 92 per cent of that value predicted by the uniform distribution of the injected phosphorus throughout the body of the patient. The slope of this curve is such that 50 per cent of the initial level is reached in 8.9 days. Two thirds of the observations fall between 77 and 113 per cent of the predicted values.

3. The median ratio of the erythrocyte P^{32} per ml. over the plasma P^{32} per ml. is found to be 6.1:1 with a standard error of ± 0.1. (A value taken from the literature for the ratio of total phosphorus per milliliter "cells" over total phosphorus per milliliter plasma [from twenty-three patients of this series] was found to be 6.2:1.)

4. The curve obtained by multiplication of the curve for the disappearance of P^{32} from the plasma of these patients by the median ratio of the erythrocyte P^{32} over plasma P^{32} (6.1:1) provides a reasonable fit of the erythrocyte-P^{32}–level observations. It is considered that there was no essential difference in the slopes of the curves for the disappearance of P^{32} from the erythrocytes and from the plasma of these patients, and hence an approximate equilibrium between total-plasma and erythrocyte P^{32} exists after the first seventy-two hours.

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