Postirradiation Changes in the Levels of Organic Phosphorus in the Blood of Patients with Leukemia


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(Received for publication July 23, 1941)

INTRODUCTION

Tracer doses of radioactive phosphorus, P32, have been used by Lawrence and co-workers (4) to study the metabolism of phosphorus in leukemic mice. Lawrence was interested primarily in the distribution of P32 among the various groups of phosphorus compounds in the animal tissues. It seemed desirable, however, to extend his experiments to the leukemic patient and to measure, after the administration of small quantities of P32, the concentration of both radioactive and nonradioactive phosphorus in the various phosphorus-containing fractions of the patient's blood cells. These fractions included the inorganic and organic acid-soluble (O.A.S.P.) compounds of the erythrocytes, of the leukocytes, and of whole blood.

It was noted that the ingestion of a subtherapeutic dose of approximately 1.5 mc. of radioactive phosphorus, P32, by the first of the patients investigated was followed by a rapid increase in the concentration of the organic acid-soluble phosphorus compounds of the erythrocytes. Subsequently, an increased concentration in both erythrocytes and leukocytes as a sequel to the administration of P32 was observed in 4 other leukemic patients, but it has not followed the administration of nonradioactive phosphorus to the same individuals.

It was desirable to establish whether the observed change in the organic acid-soluble phosphorus levels of the blood cells was due to the radioactivity of the P32 or to some chemical property of this isotope. If to the former, similar changes would be expected after the administration of comparable doses of x-rays. Accordingly, such doses were administered to several patients by two methods of external irradiation.

The first method used was whole body irradiation. This was chosen because it distributes the radiation throughout the entire body, a distribution comparable to that effected by the administration of P32. The second method was the direct exposure of the cardiac area, and was employed to ascertain the change in organic acid-soluble phosphorus levels which followed irradiation essentially limited to the circulating blood.

METHODS

The concentrations of inorganic phosphorus and of organic acid-soluble phosphorus compounds of whole blood, erythrocytes, and leukocytes were measured by the method of Fiske and Subbarow (2). The erythrocytes and the leukocytes were readily separated from the whole blood of leukemic patients. The cells from 20 cc. of whole oxalated blood were allowed to settle for 15 minutes. The sedimentation rate of the red cells of leukemic blood is much more rapid than that of the leukocytes, which thus remained suspended in the supernatant plasma. The plasma, with its contained leukocytes, was pipetted carefully into a centrifuge tube, and then centrifuged at 3,000 r.p.m. for 15 minutes. The supernatant plasma was removed as completely as possible, and the packed white cells resuspended in 5 cc. of isotonic saline. The concentration of white cells in this suspension was determined by its hematocrit value. Similarly, a sus-
To test the reliability of the technic used to separate the red and white cells, the inorganic and total acid-soluble phosphorus concentrations were determined in samples of whole blood and in the erythrocytes, leukocytes, and plasma of those same samples. Four such experiments were done, and the sum of the amounts of phosphorus found in the erythrocytes, leukocytes, and plasma in each of the four equalled the amount found in an equivalent amount of whole blood.

Phosphorus determinations were made daily while the patients were fasting, and also 3 hours after their evening meal.

The nonradioactive phosphorus used in this investigation was an aqueous solution of Na$_2$HPO$_4$, containing from 15 to 30 mgm. per cc. This was administered to each of the 6 patients studied.

The radioactive phosphorus used was an aqueous solution of Na$_2$HPO$_4$ which contained from 15 to 30 mgm. per cc. and had an activity of from 100 to 200 $\mu$C per cc.

The whole body radiation was administered by a 185 kv. machine at a distance of 500 cm. and at a rate of 0.4 r per hour.

The cardiac radiation was administered by a 150 kv. machine with 1.0 mm. Cu. filter, at a distance of 60 cm. through a 10 cm. cone. The rate of output was from 2 to 4 r per min. Under these conditions, it has been calculated that the delivery of 20 r (air) to the precordial area would administer an effective dose of not more than 2 r to the entire volume of blood as it circulated through the heart during the irradiation period.

**Clinical Material**

The clinical material comprised 6 patients with chronic leukemia. These were all adults; 5 males and 1 female.

Of the 6 patients, 4 had myelogenous and 2 lymphatic leukemia. One of the latter was in an aleukemic phase of his disease. The diagnosis in each case was established by the usual criteria of physical findings and hematologic picture. Marrow aspiration biopsies confirmed the diagnosis in all instances.

All of the 6 patients remained afebrile during the course of this investigation. In no case was any significant hematologic change noted.

Four (G.V., S.G., J.L.P., P.S.) of the 6 patients were on a constant diet of 180 gm. of carbohydrate, 100 gm. of fat and 80 gm. of protein. This diet was not modified during the period of observation. Before any form of radiation was administered to these 4 patients, they had taken a standard diet for from 10 to 15 days in order that the concentration of the organic acid-soluble phosphorus compounds in the blood cells might reach a stable level.

**Results**

The results obtained, while qualitatively uniform, varied quantitatively from case to case. Hence, it is impossible to draw a curve to show the average effect of the ingestion of P$^{32}$ or of the radiation on the intracellular organic phosphorus. It is necessary, therefore, to consider separately the results found in each patient.

G.V. This patient had been on a standard diet for 10 days when he was given nonradioactive phosphorus. For the 5 days previous to the administration, the organic acid-soluble phosphorus (O.A.S.P.) concentration of his blood cells ranged from 60 to 88 mgm. per 100 cc. of leukocytes, and from 46 to 62 mgm. per 100 cc. of erythrocytes (Fig. 1).

No rise in the O.A.S.P. levels followed the administration of 450 mgm. of nonradioactive phosphorus. For the next 4 days the O.A.S.P. levels of the leukocytes ranged from 65 to 88 mgm. per 100 cc. of cells, and of the erythrocytes from 55 to 60 mgm. per 100 cc. of cells.

The oral administration of 1.4 mc. of P$^{32}$ in a total of 450 mgm. of Na$_2$HPO$_4$ was followed by a rise of the O.A.S.P. in the leukocytes from 82 mgm. per 100 cc. to 146 mgm. per 100 cc. in 60 hours. During the next 13 days the value gradually returned to its original level. No significant change in the concentration of the O.A.S.P. fraction was noted in the erythrocytes during this period.

At this time, 2 weeks after the administration of P$^{32}$, the patient was exposed to 3.1 r of whole body x-radiation. Within 3 hours the O.A.S.P. of the leukocytes rose from 68 mgm. per 100 cc. to 80 mgm. per 100 cc., reached a peak value of 220 mgm. per 100 cc. in 4 days, and did not return to its original level until 12 days later. The O.A.S.P. of the erythrocytes also rose within 3 hours from a level of 44 mgm. per 100 cc. to 56 mgm. per 100 cc., reached a peak of 94 mgm. per 100 cc. within 4 days, and remained about 65 to 75 mgm. per 100 cc. even after 17 days.

Seventeen days after the whole body irradiation, the blood of this patient was irradiated, 20 r of x-ray being administered to a field over the heart. The exposure was of 10 minutes' duration, and was followed within 2 hours by a sharp rise in the O.A.S.P. of the leukocytes from 72 to 125 mgm. per 100 cc., but by a fall from 75 to 42 mgm. per 100 cc. in the red blood cells. During the next 11 days, the concentrations of the O.A.S.P. fraction in both white cells and red cells fluctuated over wide ranges from day to day (Fig. 2).

S.G. During the last 4 of 7 days on which this patient was taking the standard diet, the blood cell O.A.S.P. ranged from 60 to 89 mgm. per 100 cc. of leukocytes, and from 52 to 72 mgm. per 100 cc. of erythrocytes. The values obtained during the 7 days after the oral administration of 450 mgm. of nonradioactive Na$_2$HPO$_4$, ranged from 52 to 78 mgm. per 100 cc. of white blood cells, and from 40 to 72 mgm. per 100 cc. of red blood cells (Fig. 3).

X-ray was first administered to this patient in the form of local cardiac irradiation; 20 r were delivered in 5.5 minutes. In both the erythrocytes and leukocytes a rise in the O.A.S.P. resulted. That of the leukocytes rose from 52 to 120 mgm. per 100 cc. and returned to its original level within 48 hours; that of the erythrocytes rose from 62 to 108 mgm. per 100 cc.
but then fell to the low level of 3 mgm. per 100 cc. within the succeeding 24 hours.

Ten days after cardiac irradiation was administered, the patient was fed 1.4 mc. of P³². Within 12 hours after the ingestion, the O.A.S.P. of the leukocytes rose from 60 to 82 mgm. per 100 cc. This rise was succeeded by a prompt fall to 28 mgm. per 100 cc. in the next 12 hours. The O.A.S.P. fraction of the Rbc. rose from 62 to 82 mgm. per 100 cc. within 12 hours.

Twelve days subsequent to the administration of P³², this patient was subjected to whole body irradiation. Five r were delivered in 18 consecutive hours on each of 3 succeeding days; a total of 15 r thus were delivered over a 72-hour period. This irradiation was followed each day by a rise in the O.A.S.P. fraction from 70 to about 96 mgm. per 100 cc. in the leukocytes, and from 50 to about 90 mgm. per 100 cc. in the erythrocytes. In both cells the peak of the rise occurred during the administration of the x-ray.

It should be noted that in this case the changes of O.A.S.P. were observed shortly after the administration of x-ray, and Na₂HPO₄. However, 48 hours after the oral administration of 1.4 mc. of P²⁴ in a total of 450 mgm. of Na₂HPO₄, the O.A.S.P. rose in the white cells from 63 to 114 mgm. per 100 cc. and in the red cells, from 50 to 70 mgm. per 100 cc.

Ten days after the administration of P³², the patient was exposed to 3.0 r whole body x-radiation. This was followed by an increase in the concentration of both red and white blood cell O.A.S.P. The increase in concentration first was noted 6 hours after the exposure, and reached its maximum in from 48 to 72 hours, when the white cell O.A.S.P. had risen from 50 to 110 mgm. per 100 cc., and the red cell O.A.S.P. from 40 to 86 mgm. per 100 cc.

P.S. Cardiac x-ray was the only type of radiation administered to this patient.

Previous to this irradiation, the O.A.S.P. of his white cells ranged from 66 to 76 mgm. per 100 cc., and that of his red cells from 58 to 82 mgm. per 100 cc. (Fig. 5).

After 20 r of x-ray were delivered over the precordium through a 10 cm. cone in 5.5 minutes, the O.A.S.P. of the

That the O.A.S.P. levels after radioactive phosphorus persisted for about 72 hours (Fig. 3).

J.L.P. This patient had been on the standard diet for 15 days before he was given nonradioactive phosphorus. During the last 5 days of the control period, the organic acid-soluble phosphorus (O.A.S.P.) concentration of his blood cells ranged from 72 to 92 mgm. per 100 cc. of leukocytes, and from 52 to 62 mgm. per 100 cc. of erythrocytes (Fig. 4).

The organic acid-soluble phosphorus (O.A.S.P.) levels of the leukocytes and erythrocytes did not change during the 3 days following the oral administration of 450 mgm. of nonradioactive phosphorus. However, 48 hours after the oral administration of 1.4 mc. of P²⁴ in a total of 450 mgm. of Na₂HPO₄, the O.A.S.P. rose in the white cells from 63 to 114 mgm. per 100 cc. and in the red cells, from 50 to 70 mgm. per 100 cc.

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followed by a rise in the O.A.S.P. of his leukocytes from 90 to 220 mgm. per 100 cc. The maximum rise was reached in 12 days. At the time when the maximum leukocyte O.A.S.P. concentration was noted, 450 mgm. of nonradioactive Na2HPO4 was administered to the patient, but no further rise in the leukocyte concentration of O.A.S.P. was observed (Fig. 7).

A second rise in the cell O.A.S.P. fraction was effected after another dose of 1.98 mgm. of P32 in 450 mgm. of Na2HPO4. In this instance, the level rose from 60 to 166 mgm. per 100 cc. within 24 hours.

Throughout the study in this patient, no significant change was noted in the erythrocyte level of O.A.S.P.

**Discussion**

This investigation has been confined to a single organ in a single form of malignant neoplastic disease—the blood in leukemia. The group of patients studied was small and the results obtained have varied quantitatively from patient to patient. The one characteristic, however, which binds the group together is that in all patients there was a definite alteration of the organic acid-soluble phosphorus levels in the leukocytes, and usually of the erythrocytes, after all types of radiation.

The technical difficulties in the measurement of the phosphorus content of the small amounts of leukocytes which could be obtained from permissible samples of normal blood, for the present, prevented the study of a control group of normal subjects. Likewise, the phosphorus metabolism of other organs or tissues subjected to irradiation as yet has not been studied. Therefore, it must be remembered that the observed changes may represent a radiation effect seen only in the blood of patients with leukemia.

As a rule, the evening values of organic acid-soluble phosphorus measured during both the control period and after the ingestion of nonradioactive phosphorus were somewhat higher than were the fasting levels. This was probably due to the ingestion of food. However, after the administration of any form of radiation, the variations between the fasting and nonfasting levels were markedly accentuated. The presentation, both of the fasting and nonfasting levels, was not done under the impression that they are truly comparable values, but to show the increase in the variation between these levels after radiation.

Evidence has been presented to show that the alterations of the organic acid-soluble phosphorus of the
blood cells following the administration of $^{32}$P are probably due to the radioactivity of the isotope, since no similar alteration followed the ingestion of non-radioactive phosphorus. Furthermore, external x-irradiation produced alterations similar to those which resulted from the administration of the radioactive isotope.

At the present time, a great number of investigations on metabolism are being conducted with various radioactive elements. It is quite possible that the results of some of these studies may not represent normal physiology, but rather the metabolism existing during irradiation. It is not suggested that all metabolic experiments conducted with radioactive isotopes are at fault because radiation has affected the results, but the possibility that some of them have been affected by the radiation must be considered. This possibility warrants particular attention in those investigations in which radioactive phosphorus was used to study phosphorus metabolism.

The objection may be advanced that larger than tracer doses of the isotope were used in this investigation. However, from 1 to 5 $\mu$g in a 30 gm. mouse is a frequent tracer dose. This amount may be considered equivalent to from 2 to 30 mc. in a 60 kg. adult human being. In each instance, the amount of radioactive phosphorus administered to the patients studied was less than 1.5 mc. It is believed, therefore, that while the amount of radioactive isotope used is more than is necessary for tracer work with humans, it is quite comparable to the amount used in animals.

This fact is brought out more clearly by the calculation that if 1 mc. of radioactive phosphorus is retained by a 70 kg. adult for 24 hours, 0.6 "equivalent roentgens" (3) of the whole body radiation is administered. In the case of a 30 gm. mouse, it is found that retention of 1 mc. for 24 hours will administer to that mouse about 1.3 "equivalent roentgens" of whole body radiation. Hence, 5 mc., a not uncommon tracer dose, would, in 24 hours, deliver 6.5 "equivalent roentgens" to the animal. This is almost twice the whole body dose of radiation administered to the patients in the present investigation, which has been shown to cause the marked effects on the phosphorus metabolism of the blood cells. Obviously, then, the radiation accompanying a 5 $\mu$g. dose of $^{32}$P to a mouse, or any comparable amount of the isotope to other animals, cannot be regarded as negligible in metabolism studies.

In all likelihood, the alterations of total acid-soluble phosphorus levels in leukemic leukocytes and erythrocytes are not the primary effect of the irradiation. Probably these alterations are only an indication of a disturbance of one or more of those systems which control phosphorylation. It is those factors which must be studied carefully before any interpretation of the radiation effect on phosphorus metabolism is possible.

The organic acid-soluble phosphorus compounds are connected intimately with the cellular enzymes which control respiration and carbohydrate metabolism. The alteration of the organic acid-soluble phosphorus values suggests that the radiation has in some degree affected these enzyme systems. Such a disturbance of these systems might prove detrimental to the normal functioning of the cell. It is possible, therefore, that when therapeutic amounts of radiation are administered, these enzyme systems are so profoundly disturbed that no recovery to the normal state is possible, and, as a result, the cell is permanently damaged or killed.

It is the opinion of the authors that the biochemical effects after very small doses of irradiation, as noted in this paper, are the first of that nature to be observed. It would appear that the alterations of phosphorus metabolism are, at present, the most sensitive index of exposure to radiation.

**SUMMARY AND CONCLUSIONS**

1. The administration of subtherapeutic amounts of radioactive phosphorus to 5 patients with leukemia has been followed by an alteration of the organic acid-soluble phosphorus fraction of their blood cells.

2. The administration of nonradioactive phosphorus to 6 patients never was followed by any significant alteration of the organic acid-soluble phosphorus of the blood cells.

3. These same alterations were observed after the administration of very small doses of whole body x-irradiation to 3 patients, and after irradiation of the blood through a precordial port to 3 patients.

4. The amount of radiation delivered by the tracer doses of radioactive isotopes used in metabolism studies cannot be regarded as negligible. It is possible that some of these studies have measured the metabolic changes consequent upon the radiation.

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Cancer Res 1941;1:771-775.

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