[6] When the boundary values of the variable are finite, it is not sufficient that the square of the absolute value of the eigenfunction should be integrable. The function itself must vanish at the limits. The function $y$ and the integer $v$ are characteristic of the equation in the interval from $-\infty$ to $+\infty$. Since physical significance can only be attached to the interval $-1 \leqslant \xi < +\infty$, they are not the exact solutions of the physical problem. The correct eigenfunction would have the form $F = \bar{y}\exp(-\xi^2/4\alpha)$ with the analytic expression for $\bar{y}$ quite different from that for $y$, since $\bar{y} = 0$ at $\xi = -1$. However, because of the presence of the exponential factor in $F$ even our inexact function involving $y$ is very small at that point, namely, of the order of $\exp(-1/4\alpha)$, or only about $e^{-25}$ times its equilibrium value. The corrections to the eigenvalues are of a correspondingly small order, i.e., very much smaller than any effects considered in this paper. Cf. the discussion in Pauling & Wilson, *Introduction to Quantum Mechanics*, Chap. X §35c, pp. 267–271.

[7] The summation extends to $v/2$ when $v$ is even and to $\frac{1}{2}(v-1)$ when $v$ is odd.

[8] It is to be stressed that the integral is an indefinite one.

[9] The separation of certain expressions into partial fractions is an essential part of the calculations. The following formulas have been used throughout:

$$\frac{1}{\xi(1+\xi)^n} = \frac{1}{\xi} - \sum_{k=1}^{n} \frac{1}{(1+\xi)^k}; \quad \frac{1}{\xi^2(1+\xi)^n} = \frac{1}{\xi^2} - \frac{n}{\xi} + \sum_{k=1}^{n} \frac{n-k+1}{(1+\xi)^k};$$

$$\frac{1}{\xi^3(1+\xi)^n} = \frac{1}{\xi^3} - \frac{n}{\xi^2} + \frac{n(n+1)}{2\xi} - \sum_{k=1}^{n} \frac{(n-k+1)(n-k+2)}{2(1+\xi)^k}$$

$$\frac{1}{\xi^n(1+\xi)} = \sum_{k=0}^{n-1} \frac{(-1)^k}{\xi^{n-k}} + \frac{(-1)^n}{1+\xi}$$

$$\frac{1}{\xi^n(1+\xi)^2} = \sum_{k=0}^{n-1} \frac{(-1)^k(k+1)}{\xi^{n-k}} + \frac{(-1)^n n}{1+\xi} + \frac{(-1)^n}{(1+\xi)^2}$$

$$\frac{1}{\xi^n(1+\xi)^3} = \sum_{k=0}^{n-1} \frac{(-1)^k(k+1)(k+2)}{2\xi^{n-k}} + \frac{(-1)^n n(n+1)}{2(1+\xi)} + \frac{(-1)^n n}{(1+\xi)^2} + \frac{(-1)^n}{(1+\xi)^3}.$$

[10] Whittaker and Watson, *Modern Analysis*, 4th ed., pp. 204–205 (1927).

---

# *THE EFFECT OF RADIOACTIVE PHOSPHORUS UPON THE BLOOD OF GROWING CHICKS*

BY K. G. SCOTT AND S. F. COOK

DIVISION OF PHYSIOLOGY, UNIVERSITY OF CALIFORNIA MEDICAL SCHOOL

Communicated April 9, 1937

*Introduction.*—The recent developments in the field of radiation which are making it possible artificially to prepare certain elements in the radioactive state are of very great significance to the biologist and physician. For entirely new technical procedures may be evolved, utilizing such elements, and representing definite improvement upon and extension of the present standard methods which require the use of x-rays and radium. Since

radioactive elements are identical chemically with their non-active isotopes, they react in biological systems in exactly the same manner.

Of these elements radioactive phosphorus seems to possess particular advantages both from the theoretical and practical points of view. Phosphorus is, of course, present in large amounts in the skeletal structure of animals and hence any effects observed as a result of administering the element in a radioactive form should be of value in the study of the physiology and pathology of bone. Phosphorus also occurs as a necessary constituent of the proteins, lipids and carbohydrates which are concerned with fundamental protoplasmic structure and with many of the more important vital reaction systems.

The value of radioactive phosphorus to the biologist is further enhanced by its comparatively long life (the half life is 14.5 days). This ensures to the investigator adequate time to prepare suitable compounds containing radioactive phosphorus, to administer them to large animals and to observe their effects over periods of relatively long duration. Finally the substance can be prepared in fairly large amounts by means of the cyclotron of Lawrence and Cooksey.[1]

Briefly the theory of this procedure is that when ${}_{15}P^{31}$ is bombarded in the cyclotron with deutrons (${}_{1}H^{2}$) phosphorus ${}_{15}P^{32}$ is formed together with a proton (${}_{1}H^{1}$). The latter form of phosphorus is unstable and decomposes into sulfur ${}_{16}S^{32}$ with the liberation of an electron (${}_{-1}e^{\circ}$). Hence it is this unstable isotope of phosphorus which produces beta rays or the radiation which would be responsible for any biological effects within the animal.

The first biological experiments using radioactive phosphorus were those of Chiewitz and Hevesy[2] who found that when the substance was administered to a white rat a large part of it was deposited in the bones. These investigators apparently fed very small amounts, which were insufficient to produce biological effects. Their findings suggested that when animals are fed radioactive phosphorus there may as a result be a selective irradiation in the region of heavy deposition, that is to say, the bone marrow. Now this is a portion of the animal's body which is exceedingly difficult to reach when the radiation (such as x-rays) is received by the external surface or skin and must then penetrate thick layers of tissue and bone. Hence, with radioactive phosphorus, one would pay particular attention to any disturbances in function on the part of the bone marrow. The latter, of course, would be reflected in distortions of the animal's blood picture, especially the polymorphonuclear leucocytes. There might well be a specific effect exerted on the polymorphonuclear leucocytes rather than on the lymphocytes, which are relatively more affected when animals are treated with x-rays.

In order to test these ideas, a series of experiments was performed in which radioactive phosphorus was administered to growing chicks. These

TABLE 1

RADIOACTIVE PHOSPHORUS

| AGE OF BIRDS WHEN RADIOACTIVE PHOSPHORUS SAMPLES WERE FED | ACTIVITY OF PHOSPHORUS IN BIRDS IN MICROCURIES | RATIO: ACTIVITY IN MICROCURIES / WEIGHT OF BIRDS IN GRAMS | | AGE IN DAYS | LEUCOCYTES NO./CU. MM. | LYMPHOCYTES NO./CU. MM. | POLYMORPHONUCLEARS NO./CU. MM. | BASOPHILES NO./CU. MM. | MONOCYTES NO./CU. MM. | EOSINOPHILES NO./CU. MM. | HEMOGLOBIN 16 GM./100 CC. BLOOD = 100 PER CENT |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 9–11 days | 7 | 7/80 | 0.087 | | | | | | | | |
| | | | | 25 | 38,800 | 31,000 | 4,350 | 2,640 | 660 | 78 | 62 |
| 29–31 days | 42 | 42/175 | 0.004 | 32 | 23,500 | 17,800 | 2,360 | 1,390 | 1,030 | 750 | 66 |
| | | | | 37 | 23,300 | 17,250 | 3,350 | 1,400 | 1,235 | 0 | 66.4 |
| | | | | 44 | 27,400 | 21,500 | 1,580 | 1,120 | 1,730 | 1,560 | 70.2 |
| 50–53 days | 42 | 42/425 | 0.099 | 50 | 32,800 | 25,700 | 1,400 | 1,170 | 1,975 | 2,200 | 72.6 |
| | | | | 57 | 31,800 | 25,000 | 1,560 | 890 | 1,530 | 2,670 | 64 |
| | | | | 58 | 32,600 | 25,200 | 2,300 | 1,085 | 1,470 | 1,920 | 62.8 |
| | | | | 65 | 35,400 | 31,000 | 920 | 950 | 1,100 | 1,900 | 67 |
| 72–73 days | 40 | 40/775 | 0.052 | 72 | 36,000 | 29,000 | 2,000 | 1,100 | 2,200 | 1,100 | 71.4 |
| | | | | 80 | 35,000 | 28,000 | 3,000 | 1,200 | 1,900 | 350 | 71 |
| | | | | 83 | 34,000 | 28,000 | 4,000 | 700 | 1,000 | 750 | 73 |
| 80–81 days | 190 | 190/875 | 0.22 | 84 | 33,000 | 25,000 | 5,000 | 1,100 | 1,700 | 0 | 70 |
| | | | | 87 | 34,000 | 29,000 | 3,500 | 800 | 1,400 | 350 | 68 |
| | | | | 94 | 32,000 | 28,000 | 1,800 | 900 | 650 | 450 | 74 |
| | | | | 101 | 35,000 | 29,000 | 3,100 | 700 | 1,750 | 0 | 78 |
| | | | | 109 | 32,000 | 27,000 | 3,200 | 900 | 1,300 | 0 | 85 |
| | Averages | | | | 32,000 | 26,000 | 2,700 | 1,130 | 1,400 | 900 | 71.5 |

NOTE: With respect to blood measurements each figure in tables 1 and 2 is the average of the values found on all five experimental or control birds at the age given. The averages listed in tables 1 and 2 are derived from all measurements at all ages, or a total of 80 determinations taken from five birds.

animals were chosen because of the authors' previous extended study of the blood pictures in chickens.

*Procedure and Methods.*—White leghorn chicks were selected as experimental animals. Because of their rapid growth, relatively large quantities of phosphorus are deposited in the bones. This should permit fairly complete deposition of the radioactive samples when administered.

Radioactive phosphorus was fed to one group of five birds by mixing it in their diet as phosphoric acid at the intervals stated in table 1. The radioactive phosphoric acid was prepared from red phosphorus, which had been bombarded in the cyclotron. The phosphoric acid was prepared by boiling the active red phosphorus in nitric acid (sp. g. 1.23). After the red phosphorus was converted to phosphoric acid the solution was evaporated to dryness in a steam bath to remove the excess nitric acid. The residue was then dissolved in water and treated with $H_2S$ to remove traces of copper and other metallic salts. The precipitate was filtered off and discarded, and the remaining solution was made up to 500 cc. Of this $^1/_{500}$ was taken for an electroscopic analysis in order to determine the activity. The remainder was mixed with 300 gm. of the ration normally fed, dried on a steam bath and fed to the chicks.

Ordinary red phosphorus was fed to the control group as phosphoric acid prepared in the same manner. The diet given the chicks was composed of:

| | |
|---|---|
| Wheat | 20 parts |
| Corn | 55.5 parts |
| Dried blood meal | 3 parts |
| Casein | 10 parts |
| Alfalfa leaf meal | 5 parts |
| Calcium carbonate | 1 part |
| Sodium chloride | 0.5 part |
| Dried brewer's yeast | 3 parts |
| Sardine oil | 2 parts |

The usual supplement of 1 per cent calcium phosphate was purposely omitted from the diet to enhance the uptake of the radioactive samples of phosphorus when given.

Samples of blood were taken from the control group and the radioactive group at stated intervals for 109 days (see tables 1 and 2). The hemoglobin, leucocytes, lymphocytes, polymorphonuclear leucocytes, basophiles, eosinophiles and monocytes were estimated. The methods may be found in previously published work.[3]

*Results and Discussion.*—The effect of radioactive phosphorus upon the blood constituents of the experimental animals was found to differ in certain aspects from the effect observed when animals are irradiated with x-rays. In particular the lymphocytes of the chicks which received the radioactive

phosphorus were practically unaffected, whereas their number is usually decreased by x-radiation.[4] This difference, as was suggested in the introduction, is of considerable significance since it indicates that a more or less selective irradiation is possible when such elements as radioactive phosphorus are introduced internally by some such method as was here used. There were two periods during the experiment when the number of lymphocytes decreased slightly for a short time. These decreases occurred during and immediately after the feeding of large doses of radioactive phosphorus (after the 29th and 80th days—see table 1). At these times the radioactivity in comparison to the weight of the birds reached its maxima for the experimental period. It will be noted, however, that the number of lymphocytes quickly returned to normal, although the activity of the phosphorus was (minus the decay) approximately

TABLE 2

CONTROLS

| AGE IN DAYS | LEUCOCYTES | LYMPHOCYTES | POLYMORPHONUCLEARS | BASOPHILES | MONOCYTES | EOSINOPHILES | HEMOGLOBIN 16 GM./100 CC. BLOOD = 100 |
|---|---|---|---|---|---|---|---|
| | NO./CU.MM. | NO./CU.MM. | NO./CU.MM. | NO./CU.MM. | NO./CU.MM. | NO./CU.MM. | PER CENT |
| 25 | 28,000 | 20,700 | 5,500 | 820 | 1,000 | 0 | 58.2 |
| 32 | 27,000 | 23,035 | 4,050 | 650 | 365 | 0 | 66 |
| 37 | 34,000 | 27,500 | 3,950 | 950 | 1,835 | 0 | 61 |
| 44 | 29,200 | 22,400 | 4,760 | 560 | 1,340 | 134 | 69 |
| 50 | 30,800 | 24,700 | 3,500 | 990 | 1,350 | 215 | 66.2 |
| 57 | 35,000 | 26,000 | 5,600 | 1,540 | 1,785 | 140 | 62 |
| 65 | 30,400 | 26,000 | 2,500 | 550 | 730 | 850 | 63 |
| 72 | 36,000 | 28,000 | 4,000 | 1,300 | 1,800 | 220 | 65.6 |
| 80 | 39,000 | 32,000 | 4,500 | 1,000 | 1,700 | 150 | 70 |
| 87 | 38,000 | 31,000 | 4,000 | 1,000 | 2,000 | 200 | 68 |
| 94 | 40,000 | 34,000 | 3,500 | 1,000 | 1,600 | 160 | 71 |
| 101 | 39,000 | 32,000 | 4,500 | 800 | 1,500 | 0 | 75 |
| 109 | 35,000 | 30,000 | 3,500 | 700 | 1,400 | 0 | 76 |
| | | | | Averages | | | |
| | 34,000 | 27,500 | 4,100 | 910 | 1,400 | 160 | 68 |

the same as when the effect was initially observed. A reasonable explanation for these observations is that a large portion of the radioactive phosphorus ingested had not as yet been deposited, but was still freely circulating in the blood and other body fluids where lymphocytes are to be found in large numbers. As the phosphorus was progressively withdrawn from the circulating media for deposition, its direction effect on the lymphocytes would diminish and the lymphocyte count would rise correspondingly. In addition to direct action on circulating lymphocytes, radioactive phosphorus dissolved in the body fluids would be freely accessible to the tissue which generates the lymphocytes and therefore might, to some extent, suppress the formation of new cells.

In this connection the effect of x-rays was checked by subjecting three birds to x-radiation (300 r). These birds had been grown under conditions otherwise identical with those which were given radioactive phosphorus. The result was a distinct decrease in lymphocytes, a generally characteristic x-ray effect.

A difference in the effect on polymorphonuclear leucocytes was also observed. When the three birds, mentioned above, were subjected to x-radiation there was first a preliminary increase which is likewise characteristically induced by x-rays. Quickly thereafter, however, the number returned to substantially the normal (see table 3).

On the other hand, when birds were given radioactive phosphorus a profound decrease in the polymorphonuclear leucocytes was the result. The effect on these cells was approximately proportional to the effective dose of phosphorus. The latter may be considered as the ratio:

$$\frac{\text{activity of phosphorus absorbed, in microcuries}}{\text{weight of birds in grams}}.$$

TABLE 3

BLOOD PICTURE AVERAGES OF THREE BIRDS BEFORE AND AFTER TREATMENT WITH X-RAY (DOSE APPROXIMATELY 300 r)

| DATE | LEUCOCYTES | LYMPHOCYTES | | POLYMORPHONUCLEARS | | BASOPHILES | | MONOCYTES | | EOSINOPHILES | | HEMOGLOBIN 16 GM./100 CC. BLOOD = 100 PER CENT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PER CENT | NO./CU. MM. | PER CENT | NO./CU. MM. | PER CENT | NO./CU. MM. | PER CENT | NO./CU. MM. | PER CENT | NO./CU. MM. | |
| 1/27/37 | 47,000 | 86 | 41,000 | 9 | 4,200 | 1 | 470 | 4 | 1,800 | 0 | 0 | 66 |
| 2/19/37 | 47,000 | 85 | 40,000 | 9 | 4,200 | 2 | 900 | 4 | 1,800 | 0 | 0 | 75 |
| GIVEN X-RAY TREATMENT 2/20/37 | | | | | | | | | | | | |
| 14 hrs. after | 18,000 | 32 | 5,700 | 62 | 11,000 | 0 | 0 | 6 | 1,000 | 0 | 0 | 76 |
| 38 hrs. after | 12,000 | 55 | 6,600 | 40 | 4,800 | 0 | 0 | 5 | 600 | 0 | 0 | 79 |
| 62 hrs. after | 13,000 | 61 | 8,000 | 35 | 4,500 | 1 | 130 | 3 | 390 | 0 | 0 | 81 |
| 110 hrs. after | 12,000 | 66 | 8,000 | 25 | 3,000 | 1 | 100 | 5 | 600 | 3 | 300 | 80 |

Since the birds were growing rapidly during the experiment, it is convenient to express the dosage in terms of this ratio as has been done in presenting the data in table 1. Furthermore, it is thus possible to compare the amount of radiation received by the birds during the course of the experiment in terms of unit weight.

From the data in table 1 it will also be observed that whenever a dose of radioactive phosphorus was administered there was a sharp decrease in polymorphonuclear leucocytes. But as the activity of a given sample grew weaker due to natural decay the number of these cells increased at a rate more or less proportional to the rate of decay.

The evidence from polymorphonuclear leucocytes therefore points in the same direction as that from the lymphocytes. That is, the effect in-

duced by radioactive phosphorus on the former cells is apparently the result of the more or less selective deposition of the element in bone. As a consequence the bone marrow, the site of formation of polymorphonuclears, receives relatively much more irradiation than the other tissues of the body, a condition which could not arise were the radiation to come from a source external to the animal. These observations suggest the feasibility of utilizing radioactive phosphorus for the purpose of investigating myelogenous leukemia and other hematopoietic disturbances.

In addition to the effects described above, an increase in eosinophiles was noted. Similar findings have been reported when guinea pigs are treated with x-rays.[5] The basophiles and monocytes did not appear to be much affected. The hemoglobin per cent of the birds fed radioactive phosphorus was for the most part higher than the controls, although we have at present no explanation to offer for this observation. The growth of both groups was normal, and except for a few days after the largest dose was given (81st day) when animals developed temporary diarrhea, there was no evidence of illness. The blood pictures of the control group were similar to those previously described by us and Englebreth-Holm.[3,6]

*Summary.*—1. Because of the importance of phosphorus to living animals, its artificially prepared radioactive isotope ($_{15}P^{32}$) is a valuable tool in biological investigations.

2. The administration of radioactive phosphoric acid (birds were used as experimental animals) gives rise to effects upon the constituents of the blood that are not obtained with x-rays.

3. The lymphocytes which are sensitive to x-irradiation were not particularly affected.

4. The polymorphonuclear leucocytes were greatly decreased in numbers after the administration of radioactive phosphorus.

5. This specific effect upon polymorphonuclear leucocytes is attributed to the selective deposition of radioactive phosphorus in the bones, which allows bombardment of the bone marrow with beta rays.

6. The basophiles and monocytes were not appreciably affected, although an increase in eosinophiles was noted.

7. Birds treated in this manner grew normally and any permanent ill effects resulting from the administration of the radioactive phosphorus were not observed.

*Acknowledgment.*—We are particularly indebted to Professor E. O. Lawrence, Director of the Radiation Laboratory, for his advice and suggestion, and also for furnishing the radioactive phosphorus used in these experiments. We gratefully acknowledge also that the radioactive material was made available through a grant to the Radiation Laboratory from the Josiah Macy, Jr., Foundation. We wish to express our appreciation of the coöperation we received from the staff of the Radiation

Laboratory, and especially Mr. Philip Abelson, who determined the activity of the various phosphorus samples.

We appreciate the interest of Dr. John Lawrence and Mr. Paul C. Abersold and their assistance with x-ray exposures.

The investigation was aided by a grant from the Board of Research of the University of California.

[1] Lawrence, E. O., and Cooksey, P., *Phys. Rev.*, **50**, 131 (1936).

[2] Chiewitz, O., and Hevesy, G., *Nature*, **136**, 754 (1935).

[3] Scott, K. G., and Cook, S. F., *Univ. Calif. Publ. Physiol.*, **8**, 135 (1936).

[4] Taylor, Herbert A., Witherbee, W. H., and Murphy, J. B., *Jour. Exp. Med.*, **29**, 53 (1919).

[5] Aubertin, A., and Beaujard, C. R., *Soc. Biologie*, June 11 (1904).

[6] Englebreth-Holm, J., *Experimentelle Studier over den Overførbare Høseleucose*, Levinn and Munksgaard, 1933.

## *EFFECT OF THE ROOTS ON THE PRODUCTION OF AUXIN BY THE COLEOPTILE*

BY J. VAN OVERBEEK

WILLIAM G. KERCKHOFF LABORATORIES, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated March 16, 1937

Since it had been shown by Skoog (1936, 1937) that removal of the seed (endosperm and scutellum) of Avena seedlings greatly reduces the production of auxin in the coleoptile tip, the question arose whether removal of the root system would have any effect upon the auxin relations of the plant. This was the purpose of the present investigation.

From seedlings of "Victory oats" which were grown in physiological darkrooms under standard conditions (see, e.g., Went 1935), the root system was cut off with a sharp razor blade. At the same time the coleoptile was decapitated by severing 2 mm. of the tip. In order to allow water uptake the level of the water in the zinc trays, from which the plants ordinarily take up their water, was raised until the endosperm was half submerged. The level of the water of the trays in which the roots of the intact control plants were hanging was also raised to the same extent. The plants used were of the same age as required for the standard auxin tests. Three hours after the first decapitation the end of the coleoptiles was cut off again, the primary leaf pulled loose and agar blocks containing indole-3-acetic acid (hetero-auxin) were unilaterally applied to the top of the coleoptile. The resulting curvatures were photographed 110 minutes later. The results are given in table 1 from which it is clear that removal of the