Concentrations of Radioactive Materials in the Air during 1957

The present concern over the possible hazards associated with the introduction of man-made radioactive materials into the air has been occasioned, in part, by a lack of awareness of the levels of activity of the naturally occurring radioactive materials in the air. It is thought appropriate at this time to present the most recent information obtained from a continuing study conducted by the U.S. Naval Research Laboratory on the radioactivity of the air.

The concentration of radon, thoron, and fission products in the air is obtained from the changes in the rate of decay, over a 16-hour period, of radioactive particulate matter collected on efficient filters (with 98-percent retention of particles as small as 0.3 μ in diameter) through which 900 to 1300 m^3 of air have passed during the previous 24 hours (1). Measurements were made daily on identical equipment located at Washington, D.C.; Yokosuka, Japan; Kodiak, Alaska; and Little America, Antarctica. Calibrated radioactivity standards were counted daily in each unit. The average of the daily measurements covering the full year of 1957, with the exception of short periods when the equipment was undergoing repair, is presented in Table 1. The radon concentration is that occurring in the early afternoon at each site and generally represents the minimum concentration during the 24-hour collection period.

As may be seen, the bulk of the radioactivity is due to the ever-present radon and its decay products, which result from radioactive decay of radium in the soil and the consequent diffusion of the gaseous radon daughter into the air. The concentration of radon and thoron in the air is thus dependent on the location of land masses relative to the prevailing winds passing over the collecting site (*1*). In spite of the fact that the concentration of air-borne fission products in the Washington area was unusually high during 1957, due to the extensive nuclear test series in Nevada, this man-made material amounted to only 1.2 percent of the radon concentration. The other collection sites show similar values for the fission-product–radon ratios. The concentration of thoron is roughly equal to that of the fission products in every case. When one takes into account the series of radioactive products associated with each radon decay, the additional external dose due to the fission products in the air is found to be inconsequential.

Table 1. Geographical distribution of atmospheric radioactivity during 1957 (activity in micromicrocuries per cubic meter).

	Radon	Thoron	Fission Products
Washington, D.C.	172	2.3	2.1
Yokosuka, Japan	54	0.48	0.66
Kodiak, Alaska	7.3	0.042	0.16
Little America, Antarctica	1.5	0.01	0.019

LUTHER B. LOCKHART, JR.
U.S. Naval Research Laboratory
Washington, D.C.

Reference

1. I. H. Blifford, Jr., H. Friedman, L. B. Lockhart, Jr., R. A. Baus, *J. Atmospheric Terrest. Phys.* 9, 1 (1956).

19 May 1958

On the Effect of Inorganic Phosphate on Hexose Phosphate Metabolism

In 1935 Theorell (*1*) showed that inorganic phosphate (P_i) (*2*) inhibited glucose-6-phosphate dehydrogenase, the enzyme catalyzing the first reaction of the hexose monophosphate shunt pathway. It is also well known that P_i plays an essential role in the Embden-Meyerhof glycolytic scheme. These two observations suggest the possibility that the local intracellular P_i level could determine the pathway of glucose metabolism. A high P_i concentration should inhibit the shunt but allow glycolysis to proceed, whereas a limiting P_i concentration should exert the opposite effects. This report provides experimental evidence that the P_i concentration in extracts of ascites tumor cells does produce this effect on hexose phosphate metabolism. The reactions involved are shown diagrammatically in Fig. 1.

Ehrlich mouse ascites tumor cells were removed from the animal, washed two times with 0.1*M* Tris buffer, *p*H 7.4, and then homogenized in 0.01*M* Tris buffer, *p*H 7.4, for 20 seconds with micro glass beads in a Nossal shaker (*3*). The cell debris and mitochondria were removed by centrifugation. The temperature during the preparation of this enzyme extract was maintained at 4°C.

In one series of experiments, 0.2 ml of this extract with added TPN^+ was incubated in the presence or absence of phosphate buffer with (i) G-6-P and (ii) glucose-1-C^{14}. At the end of the incubation period the reaction was stopped by placing the vessels in a boiling water bath or by adding an equivalent volume of 10 percent trichloracetic acid. The G-6-P that remained was determined by treatment of the deproteinized extract with 100-fold purified glucose-6-phosphate dehydrogenase (*4*) and excess TPN^+. In the experiments with glucose-1-C^{14}, the $C^{14}O_2$ produced was collected in KOH in the center well of Warburg vessels and counted as $BaC^{14}O_3$ at infinite thickness. The amount of hexose that was metabolized by way of the shunt pathway was calculated either from the TPN^+ reduction (*5*) or from the $C^{14}O_2$ production. Pentose phosphate formation was not used as a means of estimating the proportion of G-6-P metabolized by the shunt pathway because pentose intermediates are utilized by these enzyme extracts.

A typical experiment is described in the legend of Fig. 2. At the end of a 20-minute incubation period the disappearance of G-6-P was complete in the presence of added TPN^+ whether phosphate was present or not. However, as can be seen on the basis of the increase in absorption at 340 mμ (Fig. 2), the percentage of G-6-P metabolized by way of the shunt in the presence of 0.05*M* phosphate was only 55 percent of that in the presence of Tris. Also recorded in Fig. 2 are the parallel results obtained on the basis of $C^{14}O_2$ production from glucose-1-C^{14}.

A second series of experiments was carried out in a glycolysis medium (*6*) in the presence of either 0.02*M* Tris or 0.02*M* potassium phosphate buffer. Oxidized glutathione was added as an electron acceptor for TPNH (*7*). Under these conditions it was not necessary to add TPN^+ to the incubation mixtures and there was an 85 percent inhibition of $C^{14}O_2$ formation from 0.5 μmole of glucose-1-C^{14} in the phosphate buffer. Lactate was formed in both the Tris and phosphate buffers but was radioactive only in the latter. With glucose-6-C^{14} the lactate was radioactive in both buffers and no $C^{14}O_2$ was formed. These results are in agreement with the view that the glucose is being degraded through both the glycolytic and shunt pathways.

The relative contributions of these two pathways of glucose metabolism in various tissues have been the subject of numerous publications (*8*). Certainly the availability of TPN^+ in the cell may limit glucose oxidation through the

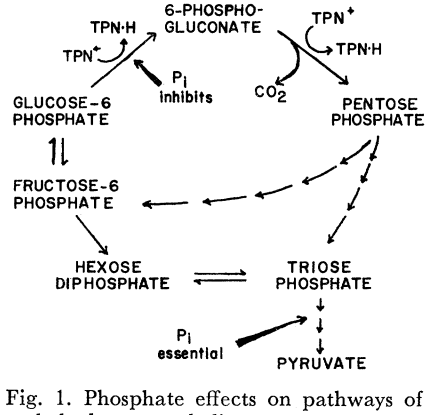

Fig. 1. Phosphate effects on pathways of carbohydrate metabolism.

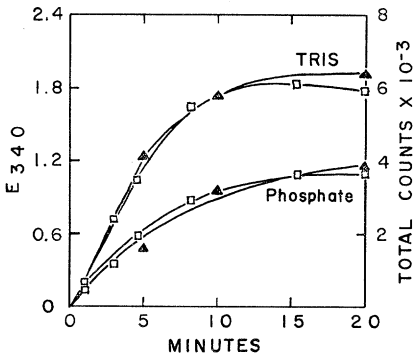

Fig. 2. Inhibition of the shunt in phosphate buffer as measured by TPN reduction and $C^{14}O_2$ formation from glucose-1-C^{14}. For the TPN reduction, reactions were carried out in duplicate vessels either in 0.05*M* Tris buffer, *p*H 7.4, plus 0.09*M* KCl, or in 0.05*M* potassium phosphate buffer, *p*H 7.4. Experimental vessels contained 0.5 μmole of G-6-P, 1.3 μmole of TPN^+, 5.0 μmole of ATP, and 10 μmole of $MgCl_2$ in a final volume of 3.0 ml. In control vessels either G-6-P or TPN^+ was omitted. The reaction was started by addition of 0.2 ml of an enzyme extract prepared as described in the text. The increase in optical density at 340 mμ was followed in a Beckman model DU spectrophotometer at room temperature. For the $C^{14}O_2$ production experimental vessels contained 0.5 μmole of glucose-1-C^{14} instead of 0.5 μmole of G-6-P; otherwise the reactants were the same. Open squares represent optical density at 340 mμ. Solid triangles indicate $C^{14}O_2$ production.

shunt. In intact ascites cells, for example, very little glucose is oxidized by way of this pathway (*9, 10*), most of it proceeding via glycolysis. We have found, however, that with extracts of these cells, the addition of an excess of TPN^+ or of a TPNH electron acceptor allows essentially all of the glucose to follow the shunt route. Methylene blue (*9, 10*) and pyruvate (*10*) have been found to stimulate the direct oxidation of glucose in intact ascites cells, presumably by acting as electron acceptors for TPNH. We have also found that oxidized glutathione will stimulate the shunt pathway in intact cells. These findings certainly implicate the availability of TPN^+ as an important factor in determining how much glucose is metabolized by way of the shunt pathway.

Under our experimental conditions the P_i concentration of the medium has been shown to influence the pathway of glucose breakdown in tissue extracts. It should be noted that the level of phosphate used to produce this effect is about four to ten times the average intracellular P_i concentration of intact Ehrlich ascites tumor cells (*11*). It is not surprising, however, to find that a P_i level greater than physiological is required to alter the pathways. Generally, in experiments designed to test cofactor requirements in cell-free systems, it is necessary to add a higher concentration of cofactor than is actually present in the intact cell. In order to test the effect observed in these studies under more physiological conditions we are at present attempting to devise means of varying the intracellular P_i concentration. It is clear, however, that no matter what the physiological effect of P_i turns out to be, in attempts to compare the relative contributions of the glycolytic and shunt pathways in glucose metabolism (at least in cell free systems), the P_i concentration of the medium should be carefully controlled (*12*).

EDWARD A. KRAVITZ*
ARMAND J. GUARINO
Department of Biological Chemistry, University of Michigan, Ann Arbor

References and Notes

1. H. Theorell, *Biochem. Z.* 275, 416 (1935).
2. The following abbreviations are used in this text: P_i, inorganic orthophosphate; G-6-P, glucose-6-phosphate; TPN^+, oxidized triphosphopyridine nucleotide; TPNH, reduced triphosphopyridine nucleotide; Tris,tris-hydroxymethyl-aminomethane; ATP, adenosine triphosphate.
3. P. Nossal, *Australian J. Exptl. Biol. Med. Sci.* 31, 583 (1953).
4. Yeast glucose-6-phosphate dehydrogenase kindly supplied by Mr. Walter Dempsey.
5. The maximum amount of G-6-P that proceeds by way of the shunt was calculated assuming that 2 μmole of TPNH was formed per micromole of G-6-P utilized.
6. The glycolysis medium consisted of 5 μmole of ATP, 2 μmole of ADP, 10 μmole of $MgCl_2$, 1.4 μmole of DPN^+, 80 μmole of nicotinamide, and 20 μmole of $KHCO_3$ per 3.0 ml of incubation mixture.
7. H. G. Williams-Ashman, *Cancer Research* 13, 721 (1953).
8. H. G. Wood, *Physiol. Revs.* 35, 841 (1955); B. Bloom, M. R. Stetten, D. Stetten, *J. Biol. Chem.* 204, 681 (1953).
9. E. Racker, *Ann. N.Y. Acad. Sci.* 63, 1017 (1956).
10. C. E. Wenner, J. Hackney, J. Herbert, *Proc. Am. Assoc. Cancer Research* 2, 259 (1957).
11. These data were kindly supplied in a personal communication from Dr. R. Wu.
12. A preliminary report of this work was presented at the 132nd meeting of the American Chemical Society. This work was supported in part by a grant from the National Science Foundation (NSF G-2145).

* Public Health Service predoctoral research fellow of the National Cancer Institute.

4 August 1958

On the Role of the Vagus in the Control of Aldosterone Secretion

Abstract. Aldosterone in adrenal venous blood of dogs is increased by constriction and decreased by release of constriction of the inferior vena cava. Section of the cervical vagi did not affect the rise of aldosterone but prevented its fall. The receptors for stimuli to increases of aldosterone secretion may not be the same as those for stimuli to decreases.

Although there is evidence that the control of aldosterone secretion depends in part upon a function of intravascular volume (*1*), no mechanism has been proposed whereby stimuli from volume changes can be transmitted to the adrenal cortex. In view of the known modification of vagal impulses by changes in intrathoracic blood volume (*2*), we sought to determine whether the vagus plays a role in the control of aldosterone secretion.

Acute experiments were carried out in normal dogs anesthetized with Nembutal. Cannulas were placed in the lumbo-adrenal vein (*3*), and blood from the adrenal was collected intermittently for determination of aldosterone. To avoid stimulation of aldosterone secretion by blood loss per se, blood was infused at the rate at which it was lost. Radioactive (C^{14}) cortisone was added to the plasma, which was then extracted with dichloromethane. The dried extract was chromatographed on the following three systems: (i) chloroform:hexane:formamide:water (90:10:5:5); (ii) toluene; methanol:water (10:7:3); (iii) toluene: ethyl acetate:methanol:water (9:1:5:5). The cortisone-plus-aldosterone zone of (i) and (ii) was eluted for transfer to the next system. Aldosterone was determined by measuring soda fluorescence on paper in a fluorimeter (*4*) and corrected for losses as indicated by the C^{14} count on (iii). The mean recovery was 53 ± 10 percent (standard deviation). No other adrenal steroid which produces soda fluorescence has been found to run with aldosterone after passage through these three systems.

Aldosterone secretion was stimulated by constriction of the inferior vena cava above the diaphragm (*5*) by means of an inflatable cuff so as to raise the femoral venous pressure by 10 cm of water. The hemodynamic effects of caval constriction were estimated, in all experiments, from continuous tracings of brachial arterial, right atrial, and femoral venous pressures on a Sanborn 150 eight-channel recorder and from intermittent determinations of hematocrit.

In some experiments the vagi were sectioned; this was done at the level of the thyroid cartilage.

Figure 1*a* shows the results of a "control" experiment, in which constriction was applied twice and released once. By 60 minutes after caval constriction, aldosterone secretion had risen, and by 90 minutes after release of the constriction it had fallen. The temporal relationships were found to be highly reproducible, and these time intervals were used in all subsequent experiments where rises or falls of aldosterone secretion were under investigation.

Table 1*A* shows the effects of caval constriction on 13 occasions; Table 1*C*, the effect of release of constriction on five occasions. Effects of constriction in increasing, and of release of constriction in decreasing, aldosterone secretion are highly significant ($p < .001$ for both effects).

After vagal section, caval constriction was as effective in increasing aldosterone secretion as it was when the vagi were