Effect of Prolactin on Phosphorus Metabolism of Pigeon Crop-Sac: P^{31} and P^{32} Analyses.* (18582)

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Prolactin induces a proliferative hypertrophy of the pigeon crop-sac(1,2). Since tissue growth is associated with an increased synthesis of nucleic acids(3) and probably of other phosphorus-containing compounds, experiments were undertaken to determine the effect of prolactin on the phosphorus metabolism of the crop-sac. Special attention has been given to the quantitative relationship between the dose of prolactin and the phosphorus concentration of the crop-sac because of our interest in the development of a new method for the bioassay of the hormone.

Methods. Prolactin† was administered intramuscularly in equal doses once daily for 4 days to groups of 5 to 15 White Carneau pigeons, 6 to 8 weeks of age. Twenty-four hours after the last injection of prolactin, a solution of inorganic P^{32} which was obtained from Oak Ridge was diluted with 0.9% sodium chloride (100 microcuries per ml) and administered intraperitoneally in a dose of 100 microcuries per kg of body weight. At an appropriate time after the injection of P^{32} the birds were sacrificed by decapitation, blood was collected and two portions of the crop-sac, each weighing 200 to 300 mg, were excised for analysis of P^{31} and P^{32}. One portion of tissue was immediately frozen in solid carbon dioxide for the analysis of various phosphate fractions. The other portion was ashed in 10 N sulfuric acid, the oxidation being completed by the dropwise addition of concentrated nitric acid. Total P^{31} concentration was determined by the Fiske-Subbarow method(4). An aliquot of the ashed material was evaporated in a cupped planchet and the total P^{32} activity measured in a Geiger-Müller counter. The frozen tissue was homogenized in cold 5% trichloracetic acid. Total P^{31} and P^{32} concentrations‡ of the acid-soluble material were determined by the procedures just described. Inorganic P^{31} of the acid-soluble fraction was determined by direct application of the Fiske-Subbarow method(4) to an aliquot of the supernatant fluid. Inorganic P^{32} was measured by the calcium hydroxide method described by Dziewiatkowski and Bodian(5). In the analyses of inorganic P^{31} and P^{32} a small amount of adenosinetriphosphate and phosphocreatine may have been hydrolyzed; the phosphorus so liberated would then be included in the inorganic acid-soluble fraction. Precautions were taken to reduce such hydrolysis to a minimum(6). P^{31} and P^{32} concentrations of the organic acid-soluble fraction were calculated as the difference between total and inorganic acid-soluble concentrations. Phospholipids were extracted from the acid-insoluble residue with an alcohol-ether mixture. The P^{31} concentration of this fraction was determined on an ashed aliquot of the extract by the Fiske-Subbarow procedure(4). An-

* This investigation was supported by a research grant from the National Institutes of Health, Public Health Service. The P^{32} was received on allocation from the Isotopes Division, U. S. Atomic Energy Commission.

1. Riddle, O., and Braucher, P. F., *Am. J. Physiol.*, 1931, v97, 617.

2. Riddle, O., Bates, R. W., and Dykshorn, S. W., *Am. J. Physiol.*, 1933, v105, 191.

3. Caspersson, T., *Symp., Soc. Exp. Biol.*, 1947, no. 1, 127.

† Prolactin (potency, 20 to 25 I.U. per mg) was generously supplied by Dr. R. W. Bates, E. R. Squibb and Co.

4. Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, v66, 375.

‡ P^{32} concentration as used in this paper is the activity in counts per minute per gram of tissue.

5. Dziewiatkowski, D., and Bodian, D., *J. Cell. and Comp. Physiol.*, 1950, v35, 141.

6. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., Manometric Techniques and Tissue Metabolism, 2nd Edition, Burgess Publishing Co., Minneapolis, Minn., 1949.

other aliquot was evaporated in a cupped planchet and radioactivity measured. P^{31} and P^{32} concentrations in the residue fraction were determined by methods described above for total phosphorus. Plasma inorganic P^{31} and P^{32} were determined by the methods described above for the crop-sac.

All radioactivity determinations were compared with a standard P^{32} sample prepared from an aliquot of the material which was injected. The standard was counted simultaneously with the tissue and plasma samples and corrections for P^{32} decay were applied. Specific activity is expressed as P^{32} counts per minute per g tissue divided by mM P^{31} per kg tissue.

Results and discussion. The temporal aspects of P^{32} uptake by the crop-sac were studied in two groups of pigeons. One group received no prolactin and another group received a total of 10 mg of the hormone, a dose capable of producing maximum stimulation of the crop-gland. Both groups were injected with P^{32} and sacrificed 1, 4 and 8 hours after administration of the isotope. The results are shown in Fig. 1. In each group, uptake of P^{32} remained constant over the period studied. The data show that both the rate and the magnitude of uptake of P^{32} increased threefold under the influence of prolactin. From the standpoint of convenience, the pigeons were sacrificed 8 hours after the administration of P^{32} and this interval was employed in all subsequent experiments.

The effect of prolactin on the total P^{31} content of the crop-sac is graphically illustrated in Fig. 2. The average concentration of P^{31} in the crop-sacs of untreated pigeons is 62 mM P^{31} per kg tissue. A total dose of 10 μg of prolactin produced a small but significant increase in the concentration of total phosphorus in the crop-sac ($p = 0.02$). This is a much smaller dose of prolactin than can be detected by other assay technics with the exception of the intracutaneous assay method described by Lyons and Page(7). The specificity of the intracutaneous assay procedure has been challenged(8). A total dose

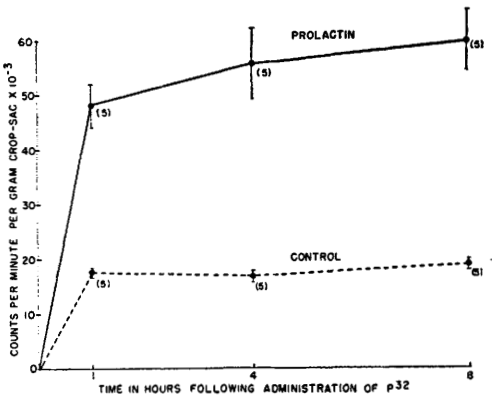

FIG. 1.

P^{32} uptake in control pigeons and pigeons injected with a total dose of 10 mg prolactin. The thin vertical bars represent standard errors. The figures in parentheses represent the number of birds.

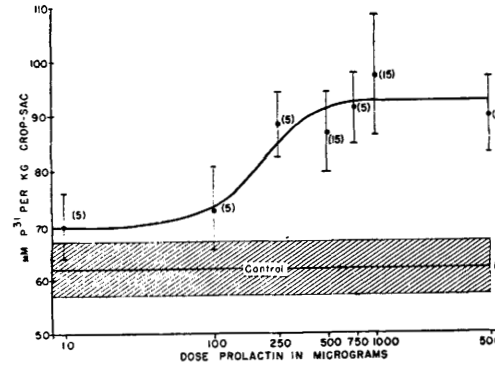

FIG. 2.

Effect of various doses of prolactin on concentration of total P^{31} in pigeon crop-sac. The shaded area above and below the control value and the thin vertical lines about the average values for the prolactin-treated pigeons represent one standard deviation. The doses are plotted on a log scale. The figures in parentheses represent the number of birds.

of 100 μg did not induce a significantly greater increase in the concentration of P^{31} in the crop-sac than did 10 μg. There was a sharp rise in the log-dose response curve between the 100 and 250 μg total dose levels. Maximum response was attained between the 250 and 1000 μg total dose levels; further study is required to determine accurately the maximally effective dose. The 10 and 100 μg total doses produced no macroscopic evidence of

7. Lyons, W. R., and Page, E., PROC. SOC. EXP. BIOL. AND MED., 1935, v32, 1049.

8. Lahr, E. L., Bates, R. W., and Riddle, O., *Endocrinology*, 1943, v32, 251.

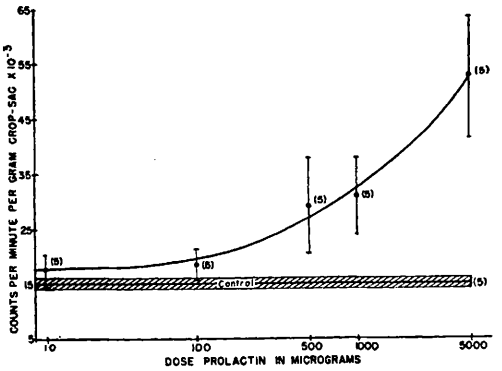

FIG. 3.

Effect of various doses of prolactin on the uptake of P^{32} by pigeon crop-sac. The shaded area above and below the control value and the thin vertical lines about the average values for the prolactin-treated pigeons represent one standard deviation. The doses are plotted on a log scale. The figures in parentheses represent the number of birds.

enhanced vascularization or cellular proliferation, although the total phosphorus concentration of the crop-sac increased. It is probable that metabolic processes are accelerated before there is a detectable increase in the weight of the organ.

The effect of various doses of prolactin on the uptake of P^{32} by the crop-sac is depicted in Fig. 3. A total dose of 10 μg produced no significant increase and 100 μg increased only slightly the uptake of the isotope. However, total doses greater than 100 μg produced marked increases in P^{32} uptake; 5000 μg more than tripled the uptake of P^{32} by the crop-sac. Larger doses must be injected in order to determine the quantity of prolactin which induces a maximum response.

The data suggest that the effect of prolactin on the P^{31} and P^{32} concentrations of the crop-sac is a potentially useful phenomenon for the bioassay of the hormone. Preliminary studies indicate that the results are reproducible. At present, it is not possible to state whether P^{31} or P^{32} determination offers the most useful method of assay. Also the specificity of the methods must be established by the determination of the effect of other adenohypophyseal hormones on the phosphorus metabolism of the crop-sac. The results of the analyses of P^{31} and P^{32} in various phosphate fractions are shown in Fig. 4. Pigeons which received 100 microcuries of P^{32} per kg body weight are compared with birds injected with the same dose of P^{32} plus a total dose of 500 μg prolactin. This dose of prolactin produces gross proliferation of the crop-sac. The following tentative interpretations of the results are offered merely as working hypotheses and are presented chiefly for the purpose of stimulating additional research on the subject.

Prolactin increases the rate of turnover of P^{32} without influencing the inorganic P^{31} concentration of the acid-soluble fraction. Calculations based on the concentrations of plasma inorganic P^{31} and P^{32} (assuming an extracellular space equivalent to 20% of body weight) indicate that the concentration of inorganic P^{31} in cell water is approximately 10 times the extracellular inorganic P^{31} concentration and that the specific activity of the intracellular inorganic fraction is approximately the same as the specific activity of the tissue. (Tissue specific activity represents the sum of the specific activity of the extracellular and intracellular compartments.) This suggests that the overall increase in specific activity of phosphate induced by prolactin can be accounted for almost entirely by changes in the rate of turnover of this element in the intracellular compartment. No change took place in the specific activity of the plasma of prolactin-treated pigeons. However, since a maximally stimulated crop-gland

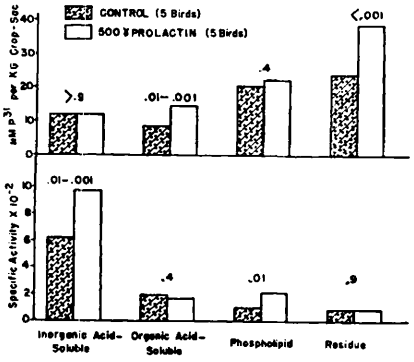

FIG. 4.

Effect of a total dose of 500 μg prolactin on the concentration of P31 and the specific activity of phosphorus in various phosphate fractions of the pigeon crop-sac. The p value immediately above each column expresses the statistical significance of the change induced by prolactin.

represents only 1% of the total body weight of a pigeon, the changes in phosphorus turnover in the crop-sac may not be expected to influence appreciably the large reservoir of phosphate present in the extracellular fluid. The data suggest that inorganic phosphorus enters the cells of the stimulated gland at an increased rate and serves as the source of phosphate for the synthesis of organic phosphorus compounds. This is in conformity with the data to be presented below.

Prolactin produces an increase in the concentration of organic P^{31} in the acid-soluble fraction without influencing the specific activity. This suggests that prolactin increases the rate of synthesis of one or more of the phosphorylated intermediates included in this fraction. Prolactin increases the specific activity in the phospholipid fraction without changing its concentration of P^{31}. The role of phospholipid in the metabolism of fat is uncertain. The data could be interpreted to mean that phospholipid is involved (a) in the transport of fat to the crop-sac [see Chaikoff(9) for convincing evidence against such a role], or (b) in the intracellular synthesis and or oxidation of fat. It is of interest in this connection that both the cells(10) and the milk(11) of the crop-sac contain high concentrations of lipid material.

Prolactin increases the concentration of P^{31} in the residue fraction of the crop-sac without causing an associated change in specific activity. This suggests an increase in the synthesis of nucleoproteins; such an increase is expected in a gland which has undergone proliferation and is in agreement with the demonstration of McShan *et al.*(12) that prolactin increases the concentration of nucleic acids in the crop-sac.

Summary. Prolactin increases the concentration of total P^{31} and P^{32} in the pigeon crop-sac. The applicability of these changes to the bioassay of the hormone is discussed.

Prolactin (a) increases the specific activity without affecting the concentration of inorganic acid-soluble phosphorus in the crop-sac, (b) increases the concentration without affecting the specific activity of the organic acid-soluble fraction, (c) increases the specific activity without affecting the phosphorus concentration in the phospholipid fraction, and (d) increases the concentration without affecting the specific activity of phosphorus in the residue fraction. The significance of these results is discussed.

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Uric Acid Diabetes in the Rat.* (18583)

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An investigation into the susceptibility of the albino rat to uric acid diabetes was prompted by the recent reports of Griffith (1,2) that methionine deficient rabbits exhibited diabetic symptoms following the injection of uric acid. When rabbits, which re-

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