Uptake of P^{32} by Pigeon Crop-Sac as Index of Lactogenic Hormone.* (26873)

HENRY C. DAMM,† G. W. PIPES, R. VON BERSWORDT-WALLRABE AND C. W. TURNER
(With technical assistance of Mary E. Powell)
Department of Dairy Husbandry, University of Missouri, Columbia

It has long been known that lactogenic hormone will produce a proliferative hypertrophy in crop-sac of pigeons with concomitant production of crop-milk(1). It has been shown that resultant growth and secretory processes appear to be associated with increased synthesis of nucleic acids and other phosphorous containing compounds(2,3). More specifically it has been demonstrated that crop-sac proliferation induced by systemic injection of lactogenic hormone rapidly incorporates P^{31} and P^{32}(4).

Recently improvements have been made in method of measurement of diametric response in pigeon crop-sac produced by intradermal injection of lactogenic hormone(5). Development of an improved, more sensitive and objective technic for bioassay of lactogen would be facilitated by correlation of this index of lactogenic activity in pigeons with technics involving incorporation of P^{32} into crop-sac.

Materials and methods. In first experiment, 2.8 μg lactogenic hormone in .1 ml distilled H_2O was administered once daily over one side of crop-sac for 4 days to common pigeons. Twenty-four hours after last injection, a solution of inorganic P^{32} having an activity of 100 μc per ml was administered intraperitoneally in dose of 10 μc per bird. Pigeons were sacrificed and weighed 4 hours after P^{32} injection and blood was collected. Crop-sacs were removed, dissected free of fat, cut approximately in half, and weighed on an analytical balance.

Crop-sac halves were prepared for P^{31} and P^{32} determination by digestion with nitric and perchloric acids and diluted to a volume of 100 ml. An aliquot was taken for P^{31} determination by photometric method, employing vanadomolybdate as color developing reagent. A 10 ml aliquot of undiluted acid digest was mixed with 10 ml scintillation solution containing POPOP, PPO and dioxane and placed in a Packard tricarb spectrometer for determination of P^{32}. Counting period was 30 to 50 minutes depending upon radioactivity of the samples. Conventional corrections were made for background and decay.

In the second experiment, 6 groups of rat pituitaries obtained from groups of animals given various treatments were macerated and diluted to 10 ml. Injections of .1 ml were injected intradermally over one side of crop-sac of pigeon daily for 4 days. Twenty-four hours after last injection of pituitary homogenate, 10 μc inorganic P^{32} solution were injected intraperitoneally. Pigeons were sacrificed and weighed 4 hours after P^{32} injection, crop-sacs removed, halved, and weighed on analytical balance. Diametric responses were estimated using standard calibrated disks for comparison(5) and P^{32} determined.

Results. In first experiment (Table I) specific activity (counts per minute of P^{32} per mg P) was increased in treated crop-sac halves as compared to control halves or blood. Total P per g tissue was significantly higher in crop-sac halves treated with lactogen than in control halves ($P<.05$), although lower than in blood ($P<.02$). P^{32} concentration (counts per minute per g tissue) was significantly higher in treated crop-sac halves than in control halves ($P<.01$).

In second experiment, lactogen equivalents (total amount injected per bird over 4 days) were calculated on basis of diametric response comparison to standard lactogen curve(6) (Table II). Comparisons of individual crop-sac weights and body weights *vs* per cent P^{32} uptake yielded correlation coefficients of .248

* Contribution of Mo. Agr. Exp. Sta. Journal Series No. 2336. Approved by the Director.

† Postdoctoral fellow of Nat. Cancer Inst. Supported in part by a grant from U. S. Atomic Energy Commission. Lactogenic hormone kindly supplied by N.I.H.

TABLE I. Effect of Lactogenic Hormone on P^{31} and P^{32} in Crop-Sac and Blood.

	No. of birds	Total P/mg	Wt of crop-sac (g)	Counts/min. /mg P^{31}	mg P/g tissue	Counts /min./g tissue
1. Crop-sac, control	10	1.389 ±.148	1.287 ±.218	1353 ± 147	1.210 ±.113	1662 ± 282
2. Crop-sac, injected	10	2.008 ±.111	1.501 ±.132	1628 ± 168	1.418 ±.104	2324 ± 321
3. Blood	10	2.854 ±.898	1.446 ±.366	1017 ± 108	1.809 ±.114	1772 ± 587
		Significances*			1-2, P <.05	1-2, P <.01
					1-3, P <.01	1-3, not sig.
					2-3, P <.02	2-3, " "

* Computed by paired comparison "t" test.

TABLE II. Relationship of P^{32} Uptake to Diametric Response in Pigeon Crop Gland after Intradermal Injection of Rat Pituitaries.

Group	No. of birds	Wt of bird (g)	Wt of crop-sac	Diametric response (cm)	Lactogen equivalent (μg)	% P^{32} uptake $\times 10^4$	Adjusted % P^{32} uptake $\times 10^4$
1	14	328 ± 6	1.314 ±.092	2.89 ±.10	3.57	3.974 ± .314	4.57 ±.55
2	14	331 ± 6	1.672 ±.094	2.66 ±.16	2.69	4.528 ± .388	6.77 ±.08
3	16	306 ±12	.929 ±.044	3.09 ±.11	4.59	12.128 ±3.233	7.56 ±.79
4	13	303 ±14	1.040 ±.035	3.75 ±.13	10.32	10.165 ± .612	8.27 ±.91
5	16	314 ± 8	1.038 ±.060	3.09 ±.11	4.59	7.138 ± .426	6.34 ±.57
6	16	320 ± 9	1.260 ±.060	3.87 ±.11	12.05	9.179 ± .922	9.47 ±.99

and – .082 respectively. After adjustment of per cent uptake for these two factors(7), means of adjusted % P^{32} uptake were correlated with mean diametric response with a resultant coefficient correlation of .79.

Discussion. In first experiment, it would appear that P^{32} in blood had passed its specific activity peak, and hence had a faster equilibration rate than in crop-sac halves. Since specific activity is higher in injected crop-sac halves than in control halves, it would appear also that rate of incorporation of P^{32} at this time interval is considerably enhanced by local intradermal pretreatment with small amount of lactogenic hormone.

Previous study(4) has shown that P^{32} is more rapidly incorporated into tissue products of crop-sac after larger systemic doses of lactogenic hormone were administered. Present first experiment indicates that P^{32} incorporation into crop-sac half may be markedly influenced by local intradermal injection of small quantity of lactogenic hormone, and indicates possibility of use of this incorporation as index of lactogenic activity.

Diametric response has been used commonly for sensitive determination of lactogenic activity in crude and purified anterior pituitary extracts(5). Since mean diametric responses were shown to be highly correlated with % P^{32} uptake adjusted for crop and body weights, it would appear that such uptake is primarily dependent on lactogenic activity of crude pituitary homogenate injected. From standpoint of lactogen assay, adjusted P^{32} uptake as index of lactogenic activity not only retains sensitivity of diametric response technic, but adds element of objectivity.

Summary. Studies were conducted on ability of purified and crude lactogenic preparations to stimulate incorporation of P^{31} and P^{32} in crop-sac of pigeon. Lactogenic hormone injected intradermally over one side of crop-sac stimulated significant P^{31} and P^{32} incorporation as compared to control sides. Diametric responses were correlated with % P^{32} uptake after injection of crude pituitary homogenates with a resultant correlation coefficient of .79, indicating that P^{32} incorporation is primarily a function of lactogenic activity of homogenate injected.

1. Bergman, A. J., Meites, J., Turner, C. W., *Endocrinology,* 1940, v26, 716.

2. Caspersson, T., *Symp. Soc. Exp. Biol.,* 1947, No. 1, 127.

3. McShan, W. H., Davis, J. S., Soukup, S. W., Meyer, R. K., *Endocrinology,* 1954, v47, 274.

4. Brown, R. W., Woodbury, D. M., Sayers, G.,

PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 639.

5. Grosvenor, C. E., Turner, C. W., *Endocrinology,* 1958, v63, 530.

6. Berswordt-Wallrabe, R. v., Turner, C. W., unpublished data.

7. Snedecor, G. W., *Statistical Methods,* 5th Ed., Iowa State College Press, Ames, 1956, p. 130.

Received July 14, 1961. P.S.E.B.M., 1961, v108.

In vivo Transfer of a Thrombopoietic Factor.* (26874)

BERTRAM SPECTOR (Introduced by Marjorie B. Zucker)

Clotting Mechanism Section, Division of Experimental Surgery and Physiology, Sloan-Kettering Institute for Cancer Research, New York City

The fact that the platelet count remains remarkably constant for months(1) although survival time of individual platelets is less than 2 weeks(2,3,4,5,6) suggests that there is a regulatory system or thrombocytostatic mechanism. Regulation implies feedback control in which unbalance acts as a stimulus to restore balance. According to this hypothesis, thrombocytopenia induced in an animal should stimulate thrombopoietic† activity to compensate for decreased platelet concentrations in the circulation.

Craddock *et al.*(7) and Matter and associates(8) reported increased thrombopoietic activity in dogs and rats, respectively, 4 days after production of severe thrombocytopenia by thrombopheresis. Both groups assumed that the thrombocytopenia was the sole stimulus for the thrombopoietic activity. However, recent unpublished experiments of the author showed that relatively moderate thrombocytopenia induced in rabbits by thrombopheresis resulted in pronounced thrombocytosis. Control experiments involving anesthesia and sham operations also produced very marked increases in platelet counts 3 to 4 days later. Because results were variable, the contribution of thrombocytopenia alone as a thrombopoietic stimulus was not amenable to quantitative analysis when thrombocytopenia was produced in rabbits by thrombopheresis.

These results indicated the importance of minimizing stress and suggested that a thrombopoietic factor in blood plasma might be demonstrable by intravenous transfer of plasma from thrombocytopenic donors to unstressed recipients. Provided that transfer of normal plasma was ineffective, a rise in platelet count could be interpreted as a response to a thrombopoietic factor induced by the thrombocytopenia.

Methods. Female albino rabbits (Blue Spruce) were used. Platelet counts were taken by puncture of an ear vein, using the direct method of Brecher and Cronkite(9). Although duplicate counts were not taken routinely, critical changes were often verified by a second technician. *A. Daily variations in circulating platelets.* Platelet counts of 9 rabbits were taken for 4 consecutive days and again on the seventh day. Three of the rabbits had been splenectomized more than a month prior to tests, but platelet levels had returned to pre-operative values within a week. *B. Transfer of plasma from normal donors to normal recipients.* Plasma was obtained from rabbits which showed steady platelet levels for at least one week prior to sacrifice. Blood was collected by heart puncture, performed under Nembutal anesthesia. Each 7.5 ml was put in a Becton and Dickson 3200 KA tube which provides a large excess of heparin. Within 2 hours of bleeding, 25 ml plasma were injected intravenously into each of 15 unanesthetized recipients, 4 of

* This study was supported by contract with Atomic Energy Commission and was submitted in partial fulfillment of the requirements for the Ph.D. degree from Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell Univ. Med. Coll. An abstract appeared in *Fed. Proc.,* 1961, 20, 67.

† It is recognized that the proper term is thrombocytopoietic but for convenience it is contracted to thrombopoietic.