The effective technical assistance of Miss Mary Jo Rega is gratefully acknowledged.

1. Jerne, N. K. and Nordin, A. A., Science 140, 405 (1963).

2. Canellas, I. and Intravartola, A., Bacteriol. Proc. 1965, Adstr. No. M84, p. 53,

3. Schwartz, S. A. and Braun, W., Science 149, 209 (1965).

4. Rowley, D. and Wardlaw, A. C., J. Gen. Microbiol. 18, 529 (1958).

5. Michael, J. G. and Braun, W., Proc. Soc. Exptl. Biol. Med. 102, 486 (1959).

6. Michael, J. G. and Braun, W., J. Bacteriol. 87, 1067 (1964).

Received Jan. 8, 1968. P.S.E.B.M., 1968, Vol. 129.

Dose Study of ^{32}P Incorporation into the Nucleic Acids of Rat Lymphoid Tissues* (33457)

JAMES C. PIERCE (Introduced by David M. Hume)

Department of Surgery, Medical College of Virginia, Richmond, Virginia 23219

Radiophosphorus (^{32}P) is widely used to study DNA synthesis in proliferating tissues. Although the toxicity of large doses, especially in lymphoid tissues, is well known (1–3), studies are lacking which demonstrate that the smaller doses usually employed in tracer studies do not also modify the rate of DNA synthesis. Because a number of studies have demonstrated the toxicity of tracer doses of thymidine-^{3}H (4–8), the effect of small doses of ^{32}p on DNA synthesis has been restudied in the lymphoid tissues of rats. The argument of this study is that if ^{32}P does not depress DNA synthesis then the dose incorporation curve of ^{32}P into DNA should be linear and, furthermore, this curve extrapolated to 0 dose should give 0 incorporation.

Materials and Methods. Female, albino, Sprague-Dawley rats weighing 178–207 g were given food and water *ad libitum*. Carrier free ^{32}p (Na_2HPO_4) in 0.45–1.00 ml of 0.9% sterile NaCl was injected intraperitoneally after lightly anesthetizing the rats with ether. At intervals of 4 and 48 hr after injection groups of 5–6 rats at each dosage level were killed with ether. Pooled lymph nodes (axillary, brachial, and mediastinal) and approximately 200 mg of thymus from each rat were placed in tubes of ice-cold (4°) 5% CCl_3COOH and immediately homogenized. At the highest dose of 9.5 μCi/g of body weight there was gross atrophy of the thymus and the lymph nodes at 48 hr and this necessitated the pooling of the lymph nodes into only two samples for the specific activity measurements. The acid soluble phosphate (ASP) fraction, the ribonucleic acid (RNA) fraction, and the deoxyribonucleic acid (DNA) fraction were subsequently isolated (9). The amount of phosphorus in an aliquot of each fraction was measured by the colorimetric method of Fiske and Subbarow (10). The radioactivity of a 1-ml aliquot of each sample was estimated in a Packard Tri-Carb liquid scintillation counter using 9 ml of absolute alcohol as a solvent and 10 ml of scintillation mixture (2.5 diphenyloxazole, 4 g and 1,4-bis-2-(phenyloxazolyl)-benzene, 100 mg in 1 liter of toluene). The specific activity of each sample was calculated as the counts per minute per microgram of phosphorus.

Results. There was linear incorporation into DNA, RNA, and the ASP fraction of doses of ^{32}P ranging from 0.191 to 1.91 μCi/g of body weight in the lymph nodes (Table I) and in the thymus (Table II) at both 4 and 48 hr after injection. This contrasted with the nonlinear dose-incorporation curves observed for tritiated thymidine in a variety of tissues (8). All of the regression lines calculated from the doses 0.191 to 1.91 μCi/g of body weight (Table III) had intercepts at 0 dose which did not differ significantly from 0

* Supported by USPHS Grant HE-08203-05 from the National Heart Institute.

TABLE I. Dose Study of Incorporation *in Vivo* of ^{32}P into DNA, RNA, and ASP of Rat Lymph Nodes.

Fraction	Time after ^{32}P injection (hr)	Specific activities[a] Dose:[b] 0.191	0.48	0.95	1.91	9.5
DNA	4	12 ± 4	30 ± 9	49 ± 19	104 ± 69	559 ± 215
	48	13 ± 6	37 ± 9	62 ± 24	157 ± 49	947 ± 136
RNA	4	46 ± 15	104 ± 19	191 ± 57	389 ± 141	2108 ± 471
	48	72 ± 5	193 ± 33	371 ± 51	769 ± 29	3923 ± 976
ASP	4	314 ±101	789 ±184	1427 ±211	2987 ± 414	13,683 ± 1959
	48	84 ± 9	259 ± 12	425 ± 60	887 ± 57	4761 ± 1959

[a] Mean specific activities (cpm/μg of P) ± SD.
[b] Dosage of ^{32}P injected (μCi/g).

by the *t* test. These data demonstrated that doses of ^{32}P up to 1.91 μCi/g of body weight did not significantly modify DNA synthesis in the lymph nodes and thymus of rats for as long as 48 hr after injection.

At a dose of 9.5 μCi of ^{32}p/g of body weight in the thymus the observed mean specific activity of DNA at 48 hr (983) was far below the 95% confidence limits of the predicted mean at this dose (4483, 5370). This indicated a highly significant depression of DNA synthesis. There was also a slight depression at 4 hr (2391, 2901). On the other hand, the mean specific activities of RNA at this dose level were all within the predicted 95% confidence limits indicating that there was no significant depression of RNA synthesis. This was consistent with the observation that DNA synthesis in the thymus was more sensitive than RNA synthesis to gamma radiation (11). In the lymph nodes there was no depression of ^{32}P incorporation into ASP, DNA, and RNA, in fact, the specific activities at 48 hr slightly exceeded the 95% confidence limits for DNA (633, 943) and ASP (4228, 4685). This highly significant difference in effect on DNA synthesis between the lymph nodes and the thymus probably reflected the difference in the proportion of replicating cells in the two tissues. In the lymph nodes only a small proportion of the total population of cells was actively dividing (12, 13) while in the thymus the entire population of cells was replicating (14, 15).

Summary. There was linear incorporation of doses of ^{32}P ranging from 0.191 to 1.91 μCi/g of body weight into DNA, RNA, and the acid soluble phosphorus fraction of the lymph nodes and thymus in rats at 4 and 48 hr after injection. The intercepts of the regression lines of these dose-incorporation curves extrapolated to 0 dose did not differ significantly from 0. These observations indicated that intraperitoneal doses of ^{32}P up to

TABLE II. Dose Study of Incorporation *in Vivo* of ^{32}P into DNA, RNA, and ASP of Rat Thymus.

Fraction	Time after ^{32}P injection (hr)	Specific activities[a] Dose:[b] 0.191	0.48	0.95	1.91	9.5
DNA	4	53 ± 8	111 ± 20	237 ± 38	520 ± 20	2360 ± 249
	48	105 ± 12	271 ± 24	522 ± 76	993 ± 142	983 ± 183
RNA	4	77 ± 9	175 ± 31	378 ± 39	817 ± 77	3881 ± 477
	48	107 ± 11	257 ± 22	510 ± 66	984 ± 279	4457 ± 2635
ASP	4	357 ± 17	890 ±143	1877 ±105	3653 ± 235	18,397 ± 1501
	48	97 ± 9	259 ± 15	516 ± 82	1137 ± 204	5575 ± 5546

[a] Mean specific activities (cpm/μg of P) ± SD.
[b] Dosage of ^{32}P injected (μCi/g).

TABLE III. Specific Activities of Dose-Incorporation Regression Lines at 0 Dose of ^{32}P.

Fraction	Time after ^{32}P injection (hr)	Specific activity at 0 dose[a]	p
	Lymph nodes		
DNA	4	1.86	>.80
	48	—6.55	>.40
RNA	4	6.4	>.80
	48	—6.7	>.50
ASP	4	10.8	>.90
	48	—10.8	>.40
	Thymus		
DNA	4	—20.4	>.20
	48	17.9	>.50
RNA	4	—23.6	>.10
	48	13.2	>.70
ASP	4	—8.4	>.80
	48	3.6	>.95

[a] Specific activity at 0 dose of ^{32}P dose-incorporation regression line calculated for doses 0.191–1.91 μCi/g; p is the probability that this specific activity does not differ significantly from 0. If there were no radiation effect of ^{32}P upon its incorporation, the specific activity of the regression line at 0 dose should be 0.

1.91 μCi/g of body weight did not significantly modify the rate of DNA or RNA synthesis in the lymphoid tissues of rats. There was, however, a highly significant depression of DNA synthesis in the thymus, but not in the lymph nodes 48 hr after the intraperitoneal injection of 9.5 μCi of ^{32}P/g of body weight. It is concluded that doses of ^{32}P up to 1.9 μCi/g of body weight may be used to study nucleic acid synthesis for time intervals up to 48 hr in the lymphoid tissues of rats.

The author gratefully acknowledges the helpful advice of Dr. David M. Hume.

1. Warren, S., MacMillan, J. C., and Dixon, F. J., Radiology **55**, 375 (1950).
2. Lavik, P. S., Harrington, H., and Buckaloo, G. W., Federation Proc. **9**, 194 (1950).
3. Koletsky, S. and Christie, J. H., Am. J. Pathol. **27**, 175 (1951).
4. Johnson, H. A. and Cronkite, E. P., Radiation Res. **11**, 825 (1959).
5. Grisham, J. W., Proc. Soc. Exptl, Biol. Med. **105**, 555 (1960).
6. Lisco, H., Nishimura, E. T., Baserga, R., and Kisieleski, W. E., Lab. Invest. **10**, 435 (1961).
7. Post, J. and Hoffman, J., Radiation Res. **14**, 713 (1961).
8. Garder, K. H. and Devik, F., Intern. J. Radiation Biol. **6**, 157 (1963).
9. Pierce, J. C., Meeker, W. R., and Varco, R. L., J. A. Med. Assoc. **183**, 866 (1963).
10. Fiske, C. H. and Subbarow, Y., J. Biol. Chem. **66**, 375 (1925).
11. Thomson, J. F., Tourtellotte, W. W., and Carttar, M. S., Proc. Soc. Exptl. Biol. Med. **80**, 268 (1952).
12. Pierce, J. C., Transplantation **5**, 967 (1967).
13. Nossal, G. J. V. and Mäkelä, O., J. Exptl. Med. **115**, 209 (1962).
14. Pierce, J. C., Clinical Res. **16**, 44 (1968).
15. Metcalf, D. and Wiadrowski, M., Cancer Res. **26**, 483 (1966).

Received June 18, 1968. P.S.E.B.M., 1968, Vol. 129

The Effect of Cortisone on Mitotic Activity in the Rat Incisor* (33458)

L. V. DOMM AND MICHAEL L. KIELY[1]

Department of Anatomy, Loyola University Stritch School of Medicine, Hines, Illinois 60141

It is now well known that cortisone stimulates the rate of eruption of the continuously erupting rat incisor. The administration of this steroid has been found to result in an accelerated rate of eruption of the incisor in adults (4) and in a precocious development and eruption in fetal (1, 2) and in newborn

* This investigation was supported in part by NIH Research grant DE-00694 and a NIH General Research Support Grant.

[1] The study was carried out in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Anatomy, Loyola University Stritch School of Medicine, during the tenure of a NIH Predoctoral Fellowship in Anatomy. Present address: Loyola University School of Dentistry, Hines, Illinois 60141.