Ca^{45} Uptake in Fracture Callus of Normal and Aminoacetonitrile-Treated Rats.* (27382)

LORENZO BOLOGNANI† AND I. V. PONSETI
Department of Orthopedic Surgery, State University of Iowa, Iowa City

Histological studies have indicated that aminoacetonitrile (AAN) administered to adult rats with experimental fractures induces formation of a voluminous fibro-cartilaginous callus which apparently ossifies well at first, but during the third week becomes severely osteoporotic(1).

Previously we reported a sharp decrease of hexosamines in the fracture callus of AAN-treated rats in comparison with the normal animals, whereas the hydroxyproline values were the same in both groups of animals. The uptake of Ca^{45} in the fracture callus of normal and AAN-treated animals 5, 10, 15 and 20 days following the fracture is here reported. Values for calcium and phosphorus present in the ashed callus are also reported.

Materials and methods. Sixty-eight male Sprague-Dawley rats weighing approximately 160 g at the beginning of experiments were used. Half of the animals received finely ground, laboratory Purina chow. The other half received the same diet containing 0.075% AAN. A fracture in the middle third of both tibiae was produced manually in every rat under ether anesthesia by bending the leg over the dull edge of a large knife held in a vise. The fractures were not immobilized.

Ten, 15 and 20 days after fracture, 6 animals of each group were sacrificed. The fracture callus was dissected, the calluses from the right and left tibiae of the 6 animals were pooled separately, placed in crucibles previously heated in a muffle and cooled in a desiccator. The wet samples were dried at 100°C, weighed and heated at 490°C during 15 hours in a muffle so that the phosphorus would not be volatilized. The crucibles were then brought to room temperature in a desiccator and weighed. The ash was dissolved in 4 N HCl and transferred to a volumetric flask; the volumes were made up to 25 ml with distilled water. The Ca was analyzed according to the method of McCrudden(3), modified by Stearns(4). The phosphorus was analyzed according to the method of Fiske and Subbarow(5). The results are reported in Tables I and II.

Ca^{45} in the form of $CaCl_2$ with a specific activity of 54611 mc/g was obtained from Oak Ridge National Laboratories and diluted with NaCl 0.95% to obtain a solution containing 20 μc of Ca^{45}/ml. Four, 9, 14 and 19 days after fracture, 8 animals (4 normal and 4 lathyric) were injected subcutaneously with 1 ml of this solution. One day later the animals were sacrificed with ether, the callus collected and placed in crucibles, weighed and heated at 1000°C for one hour. After cooling, the crucibles were weighed and the ash dissolved in 4 N HCl and transferred in 10 ml volumetric flasks; the volume was made up with 4 N HCl.

Three 0.5 ml samples of each flask were

TABLE I. Calcium and Phosphorus of Fracture Callus of Normal and Aminoacetonitrile-Treated Rats.

Days after fracture	mg/g dry wt (100°C)				μg/mg ash at (490°C)			
	Calcium		Phosphorus		Calcium		Phosphorus	
	N.	AAN	N.	AAN	N.	AAN	N.	AAN
10	69	131	43	69	236	312	144	164
	81	142	47	74	266	312	149	162
15	113	160	59	80	320	351	166	175
	122	155	62	84	331	344	168	185
20	166	156	80	78	342	345	165	166
	170	175	82	87	333	331	166	175

* Supported by U. S. Public Health Grant.

† On leave from Istituto di Chimica Biologica, Univ. of Pavia, Italy.

TABLE II. Mean Ash Content mg/100 g of Wet Weight of Fracture Callus of Normal and Aminoacetonitrile-Treated Rats.

Days after fracture	Ash (1000°C for 1 hr) Normal*	Ash (1000°C for 1 hr) AAN-treated*	Ash (490°C for 15 hr) Normal*	Ash (490°C for 15 hr) AAN-treated*
5	2.58	8.84		
10	4.09	6.48	3.7	8.2
15	17.40	14.40	11.0	13.73
20	24.17	16.40	20.5	17.45

* Mean of 4 analyses.

desiccated in glass planchets and counted in a Nuclear Chicago Ultrascaler gas flow counter. The times to 10,000 count were registered. After correction for background, the mean referred as count/min/mg of ash at 1000°C is reported in Table III.

Results and discussion. Table I and Fig. 1 give the data on calcium and phosphorus content of the callus of normal and AAN-treated rats, compared to the dry weight of the callus and to total ash. At 10 days, the AAN-treated animals showed nearly twice the amount of calcium per gram of dry substance as did the controls. These differences disappeared by the 20th day. Comparing the calcium content per gram of ash, the value in the AAN-treated animals was about 25% greater at 10 days. Phosphorus values tended in the same direction as the calcium, but the differences were of lesser degree. Increase of calcium content of the dry substances may be due solely to increased deposition of hydroxyapatite. Increase of calcium content of the ash in relation to the phosphorus of the AAN-treated compared to normal animals cannot now be explained. X-ray diffraction patterns of 15- and 20-day-old callus of normal and AAN-treated rats showed hydroxyapatite identical in all samples.‡

Table II gives the mean ash content as mg/100 g of wet weight of the calluses of normal and AAN-treated rats. Data for tissues ashed at 490° and 1000°C are included. At 5 days, the ash content of the wet callus of the AAN-treated animals was more than 3 times that of the normal group. With increased time lapse, the differences disappeared as would be expected from Table I. By 20 days mineralization of the callus appeared somewhat greater in the normal than in the AAN-

TABLE III. Ca^{45} Uptake of Fracture Callus from Normal and Aminoacetonitrile-Treated Rats. (cpm/mg of callus ashed at 1000°C for 1 hr)

Days	Normal Range*	Normal Mean	AAN-treated Range*	AAN-treated Mean
5	1043-2302	1771	841-1894	1341
10	7301-13335	9657	11202-15770	12789
15	5781-6868	6302	4702-7235	5269
20	4412-5889	5316	3304-5614	4434

* 4 analyses.

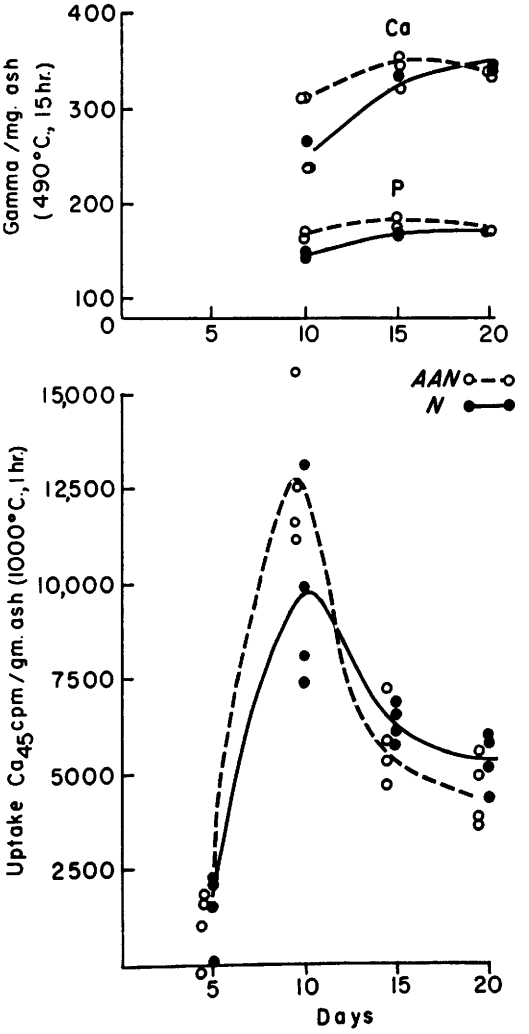

FIG. 1. (Upper graph) Ca and phosphorus of fracture callus of normal and AAN-treated animals. (Lower graph) Uptake of Ca^{45} by fracture callus of normal and AAN-treated rats.

‡ Kindly studied by Dr. Baenziger, Dept. of Chemistry, Univ. of Iowa.

treated animals. Data from the 2 different ashing temperatures showed changes in the same direction, though not always of equivalent magnitude.

The uptake of Ca^{45} measured as cpm per gram of ash (Table III) was about equal for the 2 groups at 5 days. However, as the AAN-treated group showed much greater total mineral content of the callus than the normal (Table II), total Ca^{45} of the AAN calluses was much greater than that of the normal. At 10 days, uptake of Ca^{45} by the AAN callus was considerably higher per gram of ash than by the normal callus. Since at 10 days mineralization of the AAN callus still exceeded that of the normal, total Ca^{45} uptake of the AAN callus sharply exceeded that of the normal. As was noted with the total calcium content of ash and of callus, these differences were lost by the 20th day. In fact, Ca^{45} data exhibited a tendency toward reversal of the earlier position.

Summary. Calcium content and Ca^{45} uptake have been measured in the fracture callus of normal and AAN-treated rats. It appears that total calcium deposition and Ca^{45} uptake are both higher in the young callus, 5 and 10 days after fracture, of the AAN-treated animals. By the 20th day, mineralization of the callus in both groups is similar.

The authors appreciate the help of Dr. James Osborne, Radiation Laboratory, State Univ. of Iowa, in the Ca^{45} uptake studies.

1. Ponseti, I. V., Aleu, F., *J. Bone and Joint Surg.*, 1958, v40A, 1093.
2. Bolognani, L., Submitted for publication.
3. McCrudden, F. H., *J. Biol. Chem.*, 1911, v10, 187.
4. Stearns, G., *J. Lab. Clin. Med.*, 1929, v14, 954.
5. Fiske, C. H., Subbarow, Y., *J. Biol. Chem.*, 1925, v66, 375.

Received September 21, 1961. P.S.E.B.M., 1962, v109.

Corticoid Response to Stress in the Steroid-Inhibited Rat. (27383)

SHAWN SCHAPIRO,* EDWARD GELLER, AND SAMUEL EIDUSON
(Introduced by R. R. Sonnenschein)
Neurobiochemistry Laboratory, Neuropsychiatric Hospital, Veterans Administration Center and Departments of Physiological Chemistry and Psychiatry, School of Medicine, University of California, Los Angeles

It is generally accepted that high circulating levels of certain hormones inhibit the secretion of their respective tropic hormones. As regards pituitary-adrenal function, this relationship has been repeatedly confirmed when ascorbic acid depletion or adrenal gland weight is used as a measure of adrenal activity. However, since proper analytical technics were not available until quite recently, there are only a few reports in the literature concerning direct measurements of adrenal corticoid activity in the steroid-inhibited animal. Peron and Dorfman(1) have recently shown a decrease in rat adrenal gland corticosterone following chronic treatment with cortisone. The work of Holzbauer(2) indicated that, at least in the rat, adrenal corticoid concentration is an accurate reflection of adrenal secretory activity.

Endocrinologists are increasingly aware that different parameters of adrenal function may be influenced by different adrenocorticotropic substances or by central nervous system mechanisms. For example, certain hypothalamic lesions(3) which block the stress response, as measured by ascorbic acid depletion, apparently do not interfere with the capacity to mobilize corticoids.

The present experiments were designed to compare changes occurring in both adrenal ascorbic acid and corticosterone content of the same adrenal gland of the untreated rat and the steroid-inhibited rat in response to stress of unilateral adrenalectomy.

* Present address: San Fernando Valley State College, Northridge, Calif.